Glyphosate

DOCUMENT M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Version history[1]

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013, Chapter 4 "How to revise an Assessment Report"

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CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Commission Directive 2001/99/EC included glyphosate as an active substance in Annex I to Council Directive 91/414/EEC. Following a peer review organised by the European Commission, glyphosate was included in Annex I of Council Directive 91/414/EEC with Commission Directive 2001/99/EC, entering into force on 01st July 2002. According to Regulation (EU) No 540/2011, glyphosate was deemed for approval under Regulation (EC) No 1107/2009 as well.

In agreement with Article 4 of Regulation (EC) No 1141/2010 Monsanto Europe S.A./N.V. (now Bayer Agriculture BV) on behalf of the then European Glyphosate Task Force submitted an application to Germany as RMS and Slovakia as Co-RMS notifying the intention to renew the existing approval of glyphosate on 24th March 2011 during the AIR 2 process. A collective supplementary dossier from the Glyphosate Task Force comprising 24 applicants was submitted on 25th May 2012.

On 12th November 2015, the European Food Safety Authority (EFSA) published its conclusions on the peer review of the pesticide risk assessment of the active substance glyphosate in the framework of the renewal of the approval under Commission Regulation (EU) No 1141/2010 (EFSA Journal 2015;13(11):4302)[1].

EFSA was requested by the European Commission (EC) to consider available information on the potential endocrine activity of the pesticide active substance glyphosate in accordance with Article 31 of Regulation (EC) No 178/2002. The assessment concluded that the weight of evidence indicates glyphosate does not possess endocrine disrupting properties via oestrogen, androgen, thyroid or steroidogenesis modes of action based on a comprehensive database available in the toxicology area.

On 17th March 2016, the rapporteur Member State, Germany, submitted a dossier to the European Chemical Agency for harmonised classification and labelling of the substance glyphosate. The proposal document was prepared in accordance with Article 37 of Regulation (EC) No 1272/2008 of the European Parliament and of the Council.

The Committee for Risk Assessment (RAC) assessed the hazards presented by glyphosate against the criteria in the Classification, Labelling and Packaging Regulation[2]. The RAC concluded that the available scientific evidence did not meet the criteria in the CLP Regulation and that glyphosate would not be classified as possessing STOT (specific target organ toxicity), carcinogenicity, mutagenicity or reproductive toxicity.

The AIR 2 process at EU level, concluded that it has been established with respect to one or more representative uses of at least one plant protection product containing the active substance glyphosate that the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 are satisfied. Thus, the approval criteria of demonstrating a safe use were deemed to be satisfied. It was therefore appropriate to renew the active substance glyphosate[3]. Glyphosate was renewed (date of approval) on 16th December 2017 with the expiration of approval set up for 15th December 2022.

Bayer Agriculture BV[4] submits the dossier on behalf of the Glyphosate Renewal Group (GRG) for the AIR 5 process.

In the frame of the pre-submission meeting held between the GRG and the Assessment Group on Glyphosate (AGG) on 27th September 2019, the AGG provided a reference document to GRG on the

[1] Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate in the framework of the renewal of the approval under Commission Regulation (EU) No 1141/2010; EFSA Journal 2015;13(11):4302, 107 pp; doi:10.2903/j.efsa.2015.4302.
[2] RAC Opinion proposing harmonised classification and labelling at EU level of glyphosate (ISO); N (phosphono-methyl)glycine. CLH-O-0000001412-86-149/F. Adopted 15 Mar 2017.
[3] COMMISSION IMPLEMENTING REGULATION (EU) 2017/2324.
[4] Due to the Bayer-Monsanto acquisition in 2018, the legal entity name Monsanto Europe S.A. / N.V. has been changed to Bayer Agriculture BV.

process to be considered when summarizing studies from past submissions in the June 2020 renewal dossier[5].

In 1995, glyphosate active substance dossiers were submitted by both task force and individual companies comprising a total of 19 applicants. The majority of applicants of the 1995 submissions did not join the 2012 Glyphosate Task Force (GTF) nor the GRG submitting the AIR 5 dossier in 2020. The GRG was not able to get access to a total of 46 study reports from three companies that were part of the submissions in 1995 (for details please refer to the Document B, Doc ID: 110054-B-GRG_Jun_2020), because some of the companies involved in the submissions in 1995 have subsequently been acquired by/merged with other companies or have since exited the market. Therefore, the GRG contacted Germany as the former RMS for glyphosate to discuss options available in order for AGG to get access to all said 46 study reports. A list of all these studies was sent to BVL (letter from 03rd March 2020). BVL replied to this request on 24th March 2020, advising the AGG to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009)" to the BVL. Then, BVL will forward the respective studies directly to the AGG. In the present AIR 5 Dossier, information on those inaccessible studies has been summarised based on the 2000 monograph documents[6] and are identified (as Category 4a and 4b) in the present AIR 5 dossier[7]. In these cases, GRG was unable to provide updated Appendix E summaries due to lack of access to these studies.

A number of new regulatory studies, generated after the previous EU renewal process and/or not previously submitted at EU level, are presented as part of the data package of this AIR 5 dossier. To date, those new studies have not been peer-reviewed at EU level (please refer to the Application document Rev 2 Dated May 2020 – Document F, Doc ID: 110054-F-GRG_Jun_2020).

A literature search for the active substance glyphosate and metabolites was performed in accordance with the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and according to the updated Appendix to this Guidance document[8]. The scientific literature review was performed for the period of 01st January 2010 until 31st December 2019, and total of 98 relevant and reliable articles were identified for the environmental fate section. The identified relevant and reliable articles are presented as appendix E summaries in this M-CA section. For further detailed information on the Literature Review Report (LRR) and the corresponding evaluation, please refer to M-CA Section 9 "Literature". In the frame of the pre-submission meeting held on 27th September 2019, the AGG provided a reference document to GRG on the process to be considered when presenting literature in the June 2020 submission dossier[9].

During the former EU processes, public literature data was evaluated, listed and reported by the RMS. An annex, containing information about all previously submitted and/or included public literature articles from the former EU process is presented, for sake of completeness, as Annex to this M-CA Section 7.

From the previous EU evaluations several studies are available which were performed with glyphosate-trimesium as test item. Since the glyphosate-trimesium salt dissociates quickly, the behaviour of the glyphosate anion (phosphonomethyl-glycine anion, PMG) is independent from the related cation used. Thus, all variants of the active substance are equivalent. For evaluation and further assessment, only the results for the glyphosate (PMG) anion are considered.

[5] AGG_Advice to GTF2_Literature search_Final Oct 2019 "HOW TO SUMMARISE STUDIES IN DOSSIERS FROM 1998 AND 2012 IN THE DOSSIER TO BE SUBMITTED JUNE 2020"
[6] Monograph and Addendum to the monograph EU 2001: Glyphosate monograph
[7] In the AIR 5 dossier, in each M document, a category has been assigned to each regulatory study included in the AIR 5 dossier (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020).
[8] Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances approved 27 March 2019 (doi: 10.2903/sp.efsa.2019.EN-1612)
[9] AGG_Advice to GTF2_Literature search_Final Oct 2019 "ADVICE TO GTF2: HOW TO PRESENT THE LITERATURE SEARCH IN THE DOSSIER TO BE SUBMITTED JUNE 2020"

Assessment against Cut-Off Criteria as defined in Regulation (EC) 1107/2009

Glyphosate does not meet the criteria for persistence (see below) or potential for long-range atmospheric transport (see CA 7.3).

Persistence Criteria according to Regulation (EC) 1107/2009:

- persistent organic pollutant (POP)
  DT_{50} in water > 2 months
  or
  DT_{50} in soil or sediment > 6 months

- persistent, bioaccumulative and toxic (PBT)
  DT_{50} in water > 40 days
  or
  DT_{50} in sediment or soil > 120 days

- very persistent and very bioaccumulative (vPvB)
  DT_{50} in water >60 days
  or
  DT_{50} in sediment or soil >180 days

Persistence assessment

Water
Glyphosate was well degraded in water, paralleled by partitioning to the sediment. The DT_{50} values serving as indication for persistence (trigger endpoints) for degradation and dissipation from water are all clearly below the triggers set by POP, PBT and vPvB conventions (60 or 40 days), with a range of DT_{50} values in water of 1.1-7.9 days (n = 4, trigger values) and a geomean if 3.1 days (n = 4, trigger values), coming from the water-sediment studies. Two trigger values suggested for the OECD 309 study were 12.3 and 21.8 days (geomean of 16.4 days, n = 2).

Consequently, glyphosate is considered to be not persistent in water.

Soil/sediment:
For assessment of persistence of glyphosate in aerobic soil, the persistence trigger values were compared against the DT_{50} values derived from 15 relevant laboratory data sets (temperature of 20 to 25 °C, viable soil, adequate soil moisture) and 14 individual field trials. The trigger values were in the range of 0.6 to 147 days (n = 29) including the maximum value of 147 days being estimated for one field trial conducted in Iowa, USA. Without the exceptional maximum value, trigger values of DT_{50} in soil range from 0.6 to 60.2 days. Geomean overall of the trigger values laboratory and field in soil is 9.4 days, with a geometric mean in the acidic soils of 13.6 days (geomean of modelling endpoints in acidic soils of 26.8 days). All degradation endpoints in soil are <180 days and more than 95 % of degradation values are <120 days (weight of evidence), with all laboratory degradation data <120 days.

Hence, glyphosate is considered not persistent in soil.

For sediment, two DT_{50} values are available from two laboratory data sets: 33.9 and 158.7 d, with a geomean of 73.3 days (n = 2). Though glyphosate adsorbs strongly to the sediment, it is clearly degraded in the following as indicated by mineralisation. The available values are <180 days, the geomean of both values of 73.3 days is also <120 days. Glyphosate is considered not persistent in sediment.

The triggers of DT_{50} in water, soil or sediment were not exceeded regarding the persistence assessment of POP and vPvB.

Regarding the persistence critiera for a PBT substance, 95th percentile/means of DT_{50} in soil or sediment indicate no persistence.

CA 7.1 Fate and Behaviour in Soil

CA 7.1.1 Route of degradation in soil

CA 7.1.1.1 Aerobic degradation

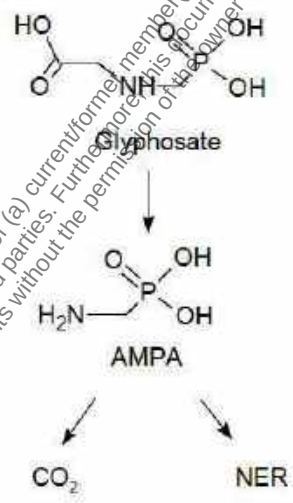
The fate of glyphosate in soil under aerobic laboratory conditions was investigated in five studies to result in data sets from eight soils that are considered valid to address the data point ([redacted], 2010, CA 7.1.1.1/001, [redacted] 1996, CA 7.1.1.1/003, [redacted], 1996, CA 7.1.1.1/004, [redacted] 1995, CA 7.1.1.1/005 and [redacted], 1993, CA 7.1.1.1/006).

Apart from this, there are six existing studies (Table 7.1.1.1-1) potentially serving as information while the design and conduct resulted in their evaluation as invalid. The studies were therefore not used in environmental risk assessment.

Glyphosate was rapidly degraded in aerobic soil as indicated by the high extent of mineralisation to ^{14}C-carbon dioxide at a maximum amount of 70.6 % applied radioactivity (AR) after 121 days of incubation. As a consequence of fast degradation till mineralisation, formation of non-extractable residues (NER) was moderate to occur at a maximum of 20.4 % AR after 90 days. The metabolite aminomethylphosphonic acid (AMPA) was observed as the major degradation product in aerobic soil to occur at a maximum of 50.1 % AR in laboratory studies.

The proposed degradation pathway in soil is presented below.

Figure 7.1.1.1-1: Proposed degradation pathway of glyphosate in soil



Within the Literature Review Report performed for glyphosate on peer reviewed publications (2010-2019), one publication was identified that could provide information potentially relevant to this data point ([redacted] *et al.*, 2019, CA 7.1.1.1/012). The reliability assessment resulted in the classification "reliable with restrictions". Consequently, this information did not result in a contribution to the endpoints derived.

In summary, the publication describes a soil degradation experiment performed with stable-labelled and non-labelled glyphosate where the formation of AMPA as metabolite was confirmed. However, the main focus is on investigations of transformation of glyphosate-derived phosphorous in soil. The publication is therefore considered as supportive information.

Table 7.1.1.1-1: Studies on aerobic soil degradation with glyphosate (route)

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.1.1/001	[redacted] 2010	Route and rate	Glyphosate	Valid	Updated kinetic evaluation in CA 7.1.2.1.1/001
CA 7.1.1.1/002	[redacted] 1996	Route and rate	Glyphosate	Invalid	
CA 7.1.1.1/003	[redacted], 1996	Route and rate	Glyphosate	Valid	Updated kinetic evaluation in CA 7.1.2.1.1/001
CA 7.1.1.1/004	[redacted] 1996	Route and rate	Glyphosate	Valid	Updated kinetic evaluation in CA 7.1.2.1.1/001
CA 7.1.1.1/005	[redacted], 1995	Route and rate	Glyphosate	Valid	Updated kinetic evaluation in CA 7.1.2.1.1/001
CA 7.1.1.1/006	[redacted], 1993	Route and rate	Glyphosate	Valid	Updated kinetic evaluation in CA 7.1.2.1.1/001
CA 7.1.1.1/007	[redacted], 1993	Route and rate	Glyphosate	Invalid	
CA 7.1.1.1/008	[redacted], 1991	Route and rate	Glyphosate	Invalid	
CA 7.1.1.1/009	[redacted], 1992	Route and rate	Glyphosate	Invalid	Addendum to [redacted], 1991
CA 7.1.1.1/010	[redacted] 1985	Route and rate	Glyphosate-Trimesium	Invalid	
CA 7.1.1.1/011	[redacted], 1972	Route and rate	Glyphosate	Invalid	

Table 7.1.1.1-2: Aerobic route of degradation - relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.1.1/012	[redacted] *et al.*, 2019	Aerobic degradation	Glyphosate	Reliable with restrictions	

An overview on maximum occurrences of AMPA in the various tests is provided in Table 7.1.1.1-3. Besides the studies on route of degradation in soil, also the results from studies on rate of degradation (see CA 7.1.2.1) were considered. For comparison, maximum amounts of AMPA found in terrestrial field dissipation studies are additionally summarized in Table 7.1.2-5**:** (see CA 7.1.2.2.1).

Table 7.1.1.1-3: Summary of maximum occurrence of AMPA in aerobic laboratory studies

Study	Soil	Soil type (USDA)	Soil origin	pH (H_2O)	AMPA (% AR)	DAT
[redacted], 2010 CA 7.1.1.1/001	Gartenacker	Loam	Switzerland	7.1	14.7	55
[redacted], 1996 CA 7.1.1.1/003	Soil B	Sandy Loam	Japan	6.7	21.0	30
[redacted] 1996 CA 7.1.1.1/004	Speyer 2.1	Sand	Germany	5.9 [1]	**50.1**	90
	Speyer 2.2	Loamy Sand	Germany	5.6 [1]	42.4	60
	Speyer 2.3 (20 °C)	Loamy Sand	Germany	6.4 [1]	32.0	7
	Speyer 2.3 (10 °C)	*Loamy Sand*	*Germany*	*6.4 [1]*	*34.3*	*60*
[redacted], 1995 CA 7.1.1.1/005	Arrow	Sandy loam	United Kingdom	5.9 [1]	27.3	120
[redacted] 1993 CA 7.1.1.1/006	Les Evouettes	Silt loam	Switzerland	6.1	29.3	84
[redacted] 2010 CA 7.1.2.1.1/002	Drusenheim	Loam	France	7.4	21.2	8
	Pappelacker	Loamy Sand	Switzerland	7.0	14.5	48
	18-Acres	Sandy clay loam	United Kingdom	5.7	13.3	91
[redacted] 1993 CA 7.1.2.1.1/003 Addendum: [redacted] 2002 CA 7.1.2.1.1/004	Speyer 2.1	Sand	Germany	6.1 [2]	41.2	14
	Speyer 2.2	Sand	Germany	6.0 [2]	42.4	7
	Speyer 2.3	Loamy Sand	Germany	6.9 [2]	25.1	14
[redacted] 1992 CA 7.1.2.1.1/005	Speyer 2.1 (Dose group A: 20 C, 40 % ´MWHC)	Sand	Germany	6.9	31.8	64
	Speyer 2.1 (Dose group B 20 °C, 20 % MWHC)	*Sand*	*Germany*	*6.9*	*27.55*	*104*
	Speyer 2.1 (Dose group C 8 °C, 40 % MWHC)	*Sand*	*Germany*	*6.9*	*23.19*	*104*
	Speyer 2.1 (Dose group D sterile, 20 °C, 40 % MWHC)	*Sand*	*Germany*	*6.3*	*20.35*	*70*
	Speyer 2.1 (Dose group E: lower rate, 20 C, 40 % ´MWHC)	Sand	Germany	6.9	31.42	64
	Beedon manor (Dose group F: 20 C, 40 % ´MWHC)	Clay Loam	United Kingdom	7.8	13.54	8

Italic font: experiments were conducted under non-standard conditions, i.e. lower temperature, lower soil moisture or sterile soil.

[1] Measured in $CaCl_2$

[2] Method of pH determination not reported, H_2O assumed

1. Information on the study

Data point:	CA 7.1.1.1/001
Report author	[redacted]
Report year	2010
Report title	Rate and route of degradation of [14C]glyphosate in one soil incubated under aerobic conditions
Report No	1923W
Document No	
Guidelines followed in study	US EPA OPPTS 835.4100 OECD Guideline 307
Deviations from current test guideline	From OECD 307: - the duration of experiment slightly exceeded the recommended period of 120 days (132 days)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate was investigated in one soil under aerobic conditions in the dark in the laboratory at 20°C and 50 % of the water holding capacity at pF 2.5 for 132 days.

The soil used was the loam Gartenacker from Switzerland. The amount of organic carbon was 2.0 % and the pH in water was 7.1.

The test was performed in flow-through systems, purged with moistened, CO_2-free air and connected to one ethylene glycol trap to collect volatile organic compounds followed by two 1 N aqueous NaOH traps to collect carbon dioxide.

The nominal application rate was 3.8 mg/kg soil (dry weight), corresponding to a single field application rate of 2.88 kg glyphosate/ha, based on a soil density of 1.5 g/cm^3 and a penetration depth of 5 cm.

Duplicate soil samples were removed and processed at 0, 3, 6, 10, 20, 34, 55, 90, 112 and 132 days after treatment (DAT). Sodium hydroxide traps were exchanged for fresh ones as needed. Approximately once every 10-20 days, trapping solutions for all remaining samples were exchanged for fresh ones.

The mean material balance per sampling interval ranged from 91.4 to 99.7 % of applied radioactivity (% AR) (mean of two replicates).

The maximum of ^{14}C-carbon dioxide was reached at study end (132 DAT) to account for 60.0 % AR (mean of two replicates). There were no organic volatiles determined at all sampling points.

The radioactivity extractable from soil decreased from 0 DAT to 132 DAT from 97.6 to 13.8 % AR (mean of two replicates).

The non-extractable radioactivity (NER) increased from 0 DAT to 90 DAT from 2.1 to 20.4 % AR and then slightly decreased to 18.9 % AR at 132 DAT (mean of two replicates).

^{14}C-Glyphosate extracted from soil decreased from 0 DAT to 132 DAT from 96.2 to 2.5 % AR. Besides ^{14}C-carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was detected at a maximum of 14.7 % AR by 55 DAT to further decrease to 8.3 % AR by 132 DAT. No other components were detected at or above 5 % AR at any time.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C-phosphonomethyl]-glyphosate
Lot No.: 53463-3-23
Specific activity: 10.28 MBq/mg (47 mCi/mmol)
Radiochemical purity: 99.8 %

2. Soil:

The soil was collected freshly in Switzerland, no fertilizers or pesticides have been applied to the soil for 5 years. Following arrival at the testing facility the soil was sieved to ≤ 2 mm and stored refrigerated in the dark in a container with free access to air for less than three months. Characteristics of the test soil are presented in the table below.

Table 7.1.1.1-4: Characteristics of test soil

Parameter	Results
Soil	Gartenacker
Country	Switzerland
Textural Class (USDA)	Loam
Sand (50 µm – 2 mm) (%)	49
Silt (2 µm – 50 µm) (%)	38
Clay (< 2 µm) (%)	13
pH (water)	7.1
Organic carbon (%)	2.0
Organic matter (%)	3.5
Cation exchange capacity (meq/100 g)	13.6
Maximum Water Holding Capacity (%)	52.1
Water Holding Capacity at 0.33 bar (%)	21.4
Water Holding Capacity at 15 bar (%)	6.1
Bulk Density (disturbed) (g/cm^3)	0.91
Microbial biomass (µg C/g)	
Experimental Start (prior to dosing)	37.5
During Incubation Period	71.7
Study end (132 DAT)	59.2

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

The individual soil samples were connected to form flow-through test systems, purged with moistened, CO_2 free air. After leaving the test vessels, the air was passed through a trap containing ethylene glycol to trap volatile organic compounds followed by two traps containing 1 N aqueous NaOH to collect carbon dioxide.

Each test vessel consisted of 50 g of sieved soil (dry weight equivalents) and soil moisture was adjusted to 50 %±10 % of the water holding capacity at pF 2.5. The samples were acclimated for one week at test conditions.

The study application rate corresponded to a single field use rate of 2.88 kg a.s./ha. [^{14}C]-glyphosate was diluted by [^{13}C]- and [^{12}C]glyphosate to result in a concentration of 0.38 mg glyphosate/mL in water. To each soil sample, 0.5 mL of this solution was applied to result in a final test concentration of 3.8 mg/kg soil.

Test systems were incubated under aerobic conditions in the dark at 20 °C and 50 % of the water holding capacity at pF 2.5 for 132 days in maximum.

2. Sampling

Duplicate test systems were removed 0, 3, 6, 10, 20, 34, 55, 90, 112 and 132 days after treatment (DAT). All samples were processed the same day. NaOH traps were exchanged for fresh ones as needed. Approximately once every 10-20 days, trapping solutions for all remaining samples were exchanged for fresh ones.

3. Analytical procedures

At each sampling interval, soil samples were extracted 3 to 4 times successively with 0.5 M NH_4OH solution by shaking for one hour. The extracts were pooled and and a aliquot removed for radioactivity determination by LSC.

Combined soil extracts were acidified to pH 2 to 3 by adding concentrated phosphoric acid (H_3PO_4) prior to further workup. Soil extracts were concentrated and cleaned up before HPLC analysis: For extracts from 55 to 132 DAT, 0.01 M EDTA was added prior to concentration to breakdown any potential chelates formed from the interaction of glyphosate with metal ions in soil. The average workup-recovery was 99.3 ± 6.1 %. The LOD each for glyphosate and metabolites observed in the HPLC radio chromatograms was 0.003 µg/g soil (3 µg/kg soil).

All samples were extracted at the day of removal from the test system, followed by initial HPLC analysis performed within 7 days of removal. All samples and standard solutions were stored frozen (<0°C) when not in use. Traps from the samplings and monthly trap changes were stored at room temperature.

Identification and quantitation of glyphosate residues was done by cation-exchange HPLC analysis. Confirmatory HPLC analysis with anion-exchange HPLC method was carried for representative extracts. Peak assignment for glyphosate was based on co-elution with the reference standard injected with each sample. Peak assignment for AMPA was by comparison of retention time with a [^{14}C]-AMPA reference standard using the corresponding HPLC method.

The non-extractable radioactivity in soil post-extraction was determined by combustion/LSC.

For the two replicates of 90 DAT, NER were fractionated into fulvic acid, humic acid and humins. The extracted soil sample was treated with 0.1 M aqueous NaOH. The extract was acidified with 12 N aqueous hydrochloric acid (HCl). After precipitation overnight, the precipitated humic acid fraction was separated by centrifugation, and the fulvic acid fraction (supernatant) was decanted. The humic acid fraction was re-dissolved in aqueous 0.1 M NaOH. The two fractions were analysed by LSC.

Radioactivity in trapping solutions was determined by LSC. The confirmation of identity of ^{14}C-CO_2 in the NaOH trapping solution traps was performed by precipitation as $Ba^{14}CO_3$.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts as well as results from fractionation of NER are summarised in the tables below.

Table 7.1.1.1-5: Distribution of radioactivity in soil Gartenacker following incubation of [^{14}C]glyphosate under aerobic conditions (expressed as percent of applied radioactivity)

		DAT									
Compound	**Replicate**	**0**	**3**	**6**	**10**	**20**	**34**	**55**	**90**	**112**	**132**
Glyphosate	A	96.6	71.1	58.1	44.4	33.3	17.6	10.5	4.5	3.0	2.3
	B	95.8	69.2	56.6	43.4	29.2	18.0	9.3	4.7	3.4	2.7
	Mean	96.2	70.2	57.4	43.9	31.3	17.8	9.9	4.6	3.2	2.5
AMPA	A	0.6	4.3	7.0	8.2	11.0	11.5	14.9	12.1	9.9	8.8
	B	0.6	4.6	7.2	8.0	13.7	12.7	14.5	12.3	10.2	7.8
	Mean	0.6	4.5	7.1	8.1	12.4	12.1	14.7	12.2	10.1	8.3
Unknown D-1 [1]	A	0.7	0.9	1.5	1.8	1.2	4.0	1.9	2.2	2.0	1.9
	B	0.4	0.9	1.5	1.7	2.2	3.0	2.1	2.2	2.0	1.9
	Mean	0.6	0.9	1.5	1.8	1.7	3.5	2.0	2.2	2.0	1.9
Other unknowns	A	0.1	0.2	0.7	0.6	0.6	2.3	1.0	0.7	0.6	1.0
	B	0.3	0.1	0.1	0.8	0.5	1.8	1.5	0.5	0.6	1.2
	Mean	0.2	0.2	0.4	0.7	0.6	2.1	1.3	0.6	0.6	1.1
Carbon Dioxide	A	NS	9.5	16.6	22.3	34.0	43.4	48.8	55.4	58.3	60.4
	B	NS	9.5	15.1	23.6	33.7	40.6	46.8	52.9	54.9	59.5
	Mean	NS	9.5	15.9	23.0	33.9	42.0	47.8	54.2	56.6	60.0
Volatile organic compounds	A	NS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	NS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	NS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total extractable residues	A	98.0	76.5	67.3	55.0	46.0	35.4	28.3	19.4	15.6	14.0
	B	97.1	74.8	65.4	54.0	45.6	35.5	27.4	19.7	16.2	13.5
	Mean	97.6	75.7	66.4	54.5	45.8	35.5	27.9	19.6	15.9	13.8
Non-extractable Residues	A	2.1	11.8	13.4	14.2	16.8	18.8	18.1	19.9	19.7	19.1
	B	2.1	11.7	12.9	13.6	17.2	17.2	18.1	20.8	19.7	18.7
	Mean	2.1	11.8	13.2	13.9	17.0	18.0	18.1	20.4	19.7	18.9
Total mass balance	A	100.1	97.8	97.3	91.5	96.8	97.6	95.2	94.7	93.6	93.5
	B	99.2	96.0	93.4	91.2	96.5	93.3	92.3	93.4	90.8	91.7
	Mean	99.7	96.9	95.4	91.4	96.7	95.5	93.8	94.1	92.2	92.6

DAT: days after treatment
NS: not sampled
[1] Secondary HPLC analysis of the D-1 isolate showed multiple peaks demonstrating the presence of multiple degradates but none represented >1.8 % applied radioactivity.

Table 7.1.1.1-6: Soil organic matter fractionation of day 90 post extracted soil (in percent of applied radioactivity)

Experiment	Replicate	Fulvic acid	Humic acid	Humin
Gartenacker	A	5.8	5.0	9.1
	B	5.9	5.1	9.8
	Mean	5.9	5.1	9.5

B. MASS BALANCE
The material balance ranged from 91.4 to 99.7 % of applied radioactivity (% AR) for soil Gartenacker (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
The radioactivity in the soil decreased from 0 DAT to 132 DAT from 97.6 to 13.8 % AR. Non-extractable residues (NER) increased from 0 DAT to 90 DAT from 2.1 to 20.4 % AR to then slightly decrease to 18.9 % AR at 132 DAT (mean of two replicates).

Following partitioning of NER for extracted 90 DAT samples, the insoluble humin fraction was the largest portion representing 9.5 % AR on average. The fulvic and humic acid fractions represented 5.9 and 5.1 % AR, respectively.

D. VOLATILE RADIOACTIVITY
The maximum radioactivity found as carbon dioxide in traps was 60.0 % AR at study end (132 DAT, mean of two replicates). There were no organic volatiles determined (<0.1 % AR) at all sampling points. Results of barium precipitation confirmed the identity of volatile radioactivity as ^{14}C-carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM
The portion of glyphosate extractable from soil decreased from 0 DAT to 132 DAT from 96.2 to 2.5 % AR. Besides carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was identified to occur at a maximum of 14.7 % AR at 55 DAT to decrease to 8.3 % AR at 132 DAT. No other radioactive components were detected at or beyond 5 % AR at any point in time.

F. KINETICS
The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020, CA 7.1.2.1.1/001).

III. CONCLUSIONS

An aerobic soil metabolism study was conducted on a loam soil from Switzerland using [^{14}C]glyphosate at a dose equivalent to a field application rate of 2.88 kg/ha at 20 °C for 132 days. The material balance averaged 94.8 ± 2.8 % of the applied dose. Glyphosate degraded rapidly and represented 2.5 % AR at 132 DAT. The main degradate observed in the study was $^{14}CO_2$, with a maximum average of 60.0 % AR at 132 DAT. The metabolite AMPA, which represented a maximum average of 14.7 % AR at 55 DAT, and subsequently declined to 8.3 % AR at 132 DAT. No other metabolites were detected above 1.8 % of the applied glyphosate. Bound residues represented up to 20.4 % AR at 90 DAT.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted according to the current guideline. The study duration was 132 days compared to a standard maximum duration of 120 days. This minor deviation is regarded to have no influence on the outcome of the study.
Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.1/002
Report author	[redacted]
Report year	1996
Report title	[P-Methylene-14C]glyphosate acid: aerobic soil metabolism
Report No	548W-1
Document No	
Guidelines followed in study	US EPA 162-1
GLP	Yes
Previous submission	Not accepted in RAR (Final Addendum, 2015)
Short description of study design and observations:	Study type: aerobic soil metabolism Test item: [^{14}C] glyphosate, phosphonomethyl-label (97.4 % radiochemical purity) Test soil: Visalia (CA, USA) Soil type: sandy loam pH (water?): 8.3 Organic matter: 0.60 % Application rate: 4.74 mg/kg Test design: semi-static system with biometer flasks kept slightly pressurized with oxygen Volatiles trapping: CO_2: 10 % KOH solution in trap Organic volatiles: foam plug Incubation: 25±1 °C (incubator, temperature controlled), soil moisture adjusted to 75 % of water holding capacity at 0.33 bar Sampling: 0, 1, 2, 3, 4, 8, 11, 14, 18, 24 and 31 days after treatment (DAT), duplicate samples Workup: threefold extraction with 1 M KH_2PO_4 (pH 2.0) at ambient temperature Analysis of radioactivity: Extracts: LSC (combined extracts) NER: combustion/LSC Volatiles: LSC; foam plug extracted with dichloromethane Identification of radioactive residues: HPLC/radiodetection and TLC/radiodetection co-chromatography with reference items.
Short description of results:	Recovery of radioactivity (mean values): 85.8 – 96.8 % AR Mineralization: 65.2 % AR at 24 DAT Other volatiles: not detected (< 2 x background) Extractable radioactivity (mean values): 94.8 % AR at 0 DAT, 22.8 % AR at 31 DAT Non-extractable radioactivity (mean values): 2.0 % AR at 0 DAT, 7.5 % AR at 8 DAT, 5.9 % AR at 31 DAT Transformation of test item (HPLC analysis): Glyphosate: 93.0 % AR at 0 DAT, 1.3 % AR at 31 DAT AMPA: 1.6 % AR at 0 DAT, 20.2 % AR at 31 DAT, max. 24.4 % AR at 11 DAT

	No unidentified metabolites >5 % AR. The half-life for glyphosate was estimated to be 5.4 days.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered to be invalid due to the following deficiencies: - Test systems were purged with oxygen instead of air. The actual influence of this difference in design on the overall outcome of the study cannot be evaluated.
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.1.1/003
Report author	[redacted]
Report year	1996
Report title	(14C)-Glyphosate: aerobic soil metabolism
Report No	1413/1-1015
Document No	
Guidelines followed in study	EPA Pesticide Assessment Guidelines, Subdivision N Paragraph 162-1 (October 1982); Japanese MAFF Guidelines (January 1985)
Deviations from current test guideline	From OECD 307: - so information about soil history - Soil A is not representative for European agricultural soils since it is a humus volcanic ash loam soil; additionally it has a very high organic carbon content (6.8 %)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate was investigated in two soils under aerobic conditions in the dark in the laboratory at 25 ± 2 °C and 75 % of the water holding capacity at 0.33 bar for 121 days.

The following two soils from Japan were used: a humus volcanic ash loam soil (soil A) with an organic carbon content of 6.8 % and a pH in water of 5.9, and a non-volcanic inorganic sandy loam soil (soil B) with an organic carbon content of 0.7 % and a pH in water of 6.7.

The test was performed in flow-through systems flushed with moistened carbon-dioxide free air. The outlets of the systems were connected to an empty trap for security, a trap containing ethanediol for collection of polar organic volatiles, a trap containing 2 % paraffin in xylene to collect non-polar organic volatiles and two traps containing 0.1 M sodium hydroxide to trap carbon dioxide.

The study application rate corresponded to the rate of 3 kg a.s./ha.

Duplicate test vessels were processed and analysed 0, 1, 3, 7, 14, 30, 63, 90 and 121 days after treatment (DAT). The trapping reagents associated with the 0, 1, 3 and 7 day incubation units were removed at these sampling intervals. From the 14 DAT sampling point onwards, the trapping reagents from all remaining test systems were sampled and replenished with fresh reagent at the time of sampling.

Material balances ranged from 98.0 to 105.8 % of applied radioactivity (% AR) for soil A and from 98.0 to 102.2 % AR for soil B (mean of two replicates).

Maximum amounts of carbon dioxide reached at study end (121 DAT) were 4.6 % AR in soil A and 70.6 % AR in soil B (mean of two replicates). No organic volatiles were detected for both soils at all sampling points.

The amount of radioactivity extractable from soil decreased from 0 DAT to 121 DAT from 34.9 to 18.8 % AR in soil A and from 96.2 to 18.1 % AR in soil B (mean of two replicates).

The amount of non-extractable residues (NER) in soil A was in the range from 64.1 (immediately after application) to 81.4 % AR (at 90 DAT). In soil B it increased from 3.6 % AR at 0 DAT to 15.6 % AR at 14 DAT and then slightly decreased to 12.3 % AR at 121 DAT (mean of two replicates).

The results of analysis of extractable residues with HPLC and TLC were found to be very similar at each sampling interval. Therefore, further discussion refers to average values of HPLC and TLC analysis.

In soil A, glyphosate was recovered with an amount of 32.4 % AR at 0 DAT and decreased to 16.2 % AR at 121 DAT. In soil B, it was detected with an amount of 92.9 % AR at 0 DAT and decreased to 2.0 % AR at 121 DAT (mean of two replicates, average values of HPLC and TLC analysis).

Besides carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was detected. In soil A, it reached a maximum amount of 1.8 % AR already at 0 DAT. In soil B, amounts of AMPA reached a maximum of 21.0 % AR at 30 DAT and decreased until the end of the experiment to 13.3 % AR. No other metabolites were detected above 5 % AR at any time. All values refer to mean of two replicates, average values of HPLC and TLC analysis.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C-phosphonomethyl]-glyphosate
Lot No.:	CFQ 8910
Specific activity:	55 mCi/mmol
Radiochemical purity:	99.2 %

2. Soil:

The soils were received 76 days before use and stored refrigerated at 4 °C in the dark in loosely tied plastic bags. Soils were sieved to ≤ 2 mm. Characteristics of the test soils are presented in the table below.

Table 7.1.1.1-7: Characteristics of test soils

Parameter	Results	
Soil	A (Humus volcanic ash)	B (Non-volcanic inorganic)
Country	Japan	Japan
Textural Class (USDA)	Loam	Sandy loam
Sand (50 µm – 2 mm) (%)	46.0	68.3
Silt (2 µm – 50µm) (%)	44.9	16.6
Clay (< 2 µm) (%)	9.1	15.2
pH (water)	5.9	6.7
pH (KCl)	5.5	6.1
Organic carbon (%)	6.8	0.7
Organic matter (%)	11.7	1.2
Cation exchange capacity (meq/100 g)	65.9	11.7
Water Holding Capacity at 0.33 bar (%)	72.1	14.2
Microbial biomass (µg C/g)		
Study begin	442	214
Study end	546	229

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of Erlenmeyer flasks filled with soil. The flasks were purged with moist carbon-dioxide free air. After leaving the test vessels the air was passed through a series of traps: an empty trap for security, a trap containing ethanediol for collection of polar organic volatiles, a trap containing 2 % paraffin in xylene to collect non-polar organic volatiles and two traps containing 0.1 M sodium hydroxide to trap carbon dioxide.

25 g of sieved soil (dry weight equivalents) were weighed into each test vessel and the test systems were acclimated for 5 days at test conditions.

The study target application rate corresponded to a field rate of 3 kg a.s./ha. A test solution of [^{14}C]glyphosate was prepared by dissolving 6.552 mg glyphosate in 12 mL water, and the final concentration of the application solution was determined by LSC. 76.9 µg of glyphosate were applied to each test system, resulting in a final concentration of about 3 mg/kg dry soil.

Test systems were incubated under aerobic conditions in the dark for 121 days at 25 °C and 75 % of moisture holding capacity at 0.33 bar.

2. Sampling

Duplicate test vessels were processed and analysed 0, 1, 3, 7, 14, 30, 63, 90 and 121 days after treatment (DAT). The trapping reagents associated with the 0, 1, 3 and 7 day incubation units were removed at these sampling intervals. From the 14 DAT sampling point onwards, the trapping reagents from all remaining test systems were sampled and replenished with fresh reagent at the time of sampling.

3. Analytical procedures

At each sampling interval, soil samples were extracted four times with 0.5 M aqueous ammonia solution and once with acetone. Extracts and soil were separated by centrifugation and decantation. The ammonia and acetone extracts were each analysed by LSC.

The ammonia extracts were directly analysed by HPLC/radio-detection. The acetone extracts contained less than 1.0 % of the applied radioactivity and were not analysed further. The limit of detection (LOD) was defined as a signal correlating to 0.05 % AR. The amount of radioactivity in volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

Glyphosate and metabolites were identified by radio HPLC and TLC co-chromatography with reference standards. The identity of glyphosate and the major metabolite AMPA was further confirmed by HPLC-MS for selected samples.

The non-extractable residues in the two replicates of each soil system from days 1, 14 and 90 were further fractionated into fulvic acid, humic acid and humin fractions. The previously extracted soil sample was shaken with of 0.5 M NaOH for five hours at 50 °C. The samples were centrifuged and separated into the solids (humin fraction) and the supernatant. The supernatant was adjusted to pH 2 and centrifuged. The precipitate, containing the humic acid fraction was separated and redissolved in 0.1 M NaOH. The supernatant, containing the fulvic acid fraction was partitioned against dichloromethane into organo-soluble and aqueous soluble fractions. Solid subsamples (humic acid fraction) were combusted and analysed by LSC. The solutions (fulvic and humic acid fraction) were analysed by LSC.

The identification of CO_2 in the sodium hydroxide traps was confirmed by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised in Table 7.1.1.1-8 and Table 7.1.1.1-9 for the respective soils.

Soil extracts were analysed by HPLC and TLC but it is not reported which method was used as primary method. The results of analysis of extractable residues with HPLC and TLC were found to be very similar at each sampling interval. Therefore, further discussion and kinetic evaluation refers to average values of HPLC and TLC analysis.

Fractionation of non-extractable residues into fulvic acid, humic acid in humin fractions is presented in Table 7.1.1.1-10.

Table 7.1.1.1-8: Degradation of [^{14}C]glyphosate in volcanic ash soil A under aerobic conditions (expressed as percent of applied radioactivity)

		DAT								
Compound	**Replicate**	**0**	**1**	**3**	**7**	**14**	**30**	**63**	**90**	**121**
HPLC Results [1]										
Glyphosate	A	30.5	25.3	27.5	26.0	24.0	19.2	18.6	19.0	17.2
	B	33.7	26.7	25.7	25.3	23.8	18.6	19.9	18.7	17.7
	Mean	32.1	26.0	26.6	25.7	23.9	18.9	19.3	18.9	17.5
AMPA	A	3.2	2.3	1.0	1.2	0.7	0.8	1.2	1.1	1.3
	B	2.0	1.2	0.8	1.1	0.7	0.7	1.5	0.9	1.1
	Mean	2.6	1.8	0.9	1.1	0.7	0.8	1.4	1.0	1.2
Unknowns	A	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 7.1.1.1-8: Degradation of [^{14}C]glyphosate in volcanic ash soil A under aerobic conditions (expressed as percent of applied radioactivity)

		DAT								
Compound	**Replicate**	**0**	**1**	**3**	**7**	**14**	**30**	**63**	**90**	**121**
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND
Background	A	0.2	0.8	0.1	0.2	ND	0.2	0.1	ND	0.1
	B	0.1	0.2	0.4	0.2	ND	0.1	0.1	ND	0.1
	Mean	0.2	0.5	0.3	0.2	ND	0.1	0.1	ND	0.1
	TLC Results [1]									
Glyphosate	A	32.0	27.1	27.2	25.9	22.8	18.0	18.0	17.9	14.5
	B	33.3	26.6	25.6	24.7	23.0	17.6	19.0	17.2	15.3
	Mean	32.6	26.8	26.4	25.3	22.9	17.8	18.5	17.5	14.9
AMPA	A	0.9	0.5	0.8	0.6	0.8	1.0	0.9	1.2	1.7
	B	1.2	0.6	0.6	0.9	0.6	0.9	1.1	1.2	1.6
	Mean	1.1	0.5	0.7	0.8	0.7	0.9	1.0	1.2	1.6
Unknowns	A	ND	ND	ND	ND	ND	ND	ND	ND	1.7
	B	ND	ND	ND	ND	ND	ND	ND	ND	1.2
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	1.5
Background	A	0.1	0.2	ND	ND	0.1	0.2	0.1	0.1	0.1
	B	ND	0.2	0.2	0.2	0.2	0.1	0.3	0.2	0.1
	Mean	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.1
	Mean of HPLC and TLC Results									
Glyphosate	A	*31.3*	*26.2*	*27.4*	*26.0*	*23.4*	*18.6*	*18.3*	*18.5*	*15.9*
	B	*33.5*	*26.7*	*25.7*	*25.0*	*23.4*	*18.1*	*19.5*	*18.0*	*16.5*
	Mean	*32.4*	*26.4*	*26.5*	*25.5*	*23.4*	*18.4*	*18.9*	*18.2*	*16.2*
AMPA	A	*2.1*	*1.4*	*0.9*	*0.9*	*0.8*	*0.9*	*1.1*	*1.2*	*1.5*
	B	*1.6*	*0.9*	*0.7*	*1.0*	*0.7*	*0.8*	*1.3*	*1.1*	*1.4*
	Mean	*1.8*	*1.2*	*0.8*	*1.0*	*0.7*	*0.9*	*1.2*	*1.1*	*1.4*
Unknowns	A	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*0.9*
	B	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*0.6*
	Mean	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*0.7*
Background	A	*0.2*	*0.5*	*0.1*	*0.2*	*0.1*	*0.2*	*0.1*	*0.1*	*0.1*
	B	*0.1*	*0.2*	*0.3*	*0.2*	*0.2*	*0.1*	*0.2*	*0.2*	*0.1*
	Mean	*0.1*	*0.4*	*0.2*	*0.2*	*0.2*	*0.2*	*0.2*	*0.2*	*0.1*
	Recovery									
Carbon Dioxide	A	NA	0.5	0.9	1.5	2.1	3.0	3.7	4.5	4.5
	B	NA	0.4	0.9	2.2	2.2	3.0	4.0	4.4	4.8
	Mean	NA	0.5	0.9	1.9	2.2	3.0	3.9	4.5	4.6
Volatile organic compounds	A	NA	ND	ND	ND	ND	ND	ND	ND	ND
	B	NA	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	NA	ND	ND	ND	ND	ND	ND	ND	ND
Total extractable residues [2]	A	*33.9*	*28.4*	*28.7*	*27.5*	*24.8*	*20.2*	*20.0*	*20.2*	*18.7*
	B	*35.9*	*28.3*	*27.0*	*26.7*	*24.6*	*19.6*	*21.6*	*19.6*	*18.9*
	Mean	*34.9*	*28.4*	*27.9*	*27.1*	*24.7*	*19.9*	*20.8*	*19.9*	*18.8*
Non-extractable Residues	A	65.1	72.2	68.8	69.9	71.7	77.5	72.6	80.3	76.6
	B	63.1	71.1	70.8	72.3	73.5	78.2	74.3	82.6	77.2
	Mean	64.1	71.7	69.8	71.1	72.6	77.8	73.5	81.4	76.9
Mass balance	A	99.0	101.1	98.3	98.8	98.7	100.8	96.3	105.0	99.8
	B	98.9	99.8	98.6	101.3	100.3	100.7	99.8	106.6	100.9
	Mean	99.0	100.4	98.5	100.0	99.5	100.8	98.0	105.8	100.3

[1] Analysis of ammonia extracts
[2] Total extractable residues were calculated as sum of radioactivity in acetone and ammonia extracts, the maximum amount in acetone extracts was 0.1 % AR
DAT: days after treatment
NA: Not applicable
ND: Not detected (defined as less than 0.05 % AR)
Values calculated in the course in writing this summary are given in *italics*.

Table 7.1.1.1-9: Degradation of [^{14}C]glyphosate in Japanese non-volcanic soil B under aerobic conditions (expressed as percent of applied radioactivity)

		DAT								
Compound	**Replicate**	**0**	**1**	**3**	**7**	**14**	**30**	**63**	**90**	**121**
	HPLC results [1]									
Glyphosate	A	92.8	44.7	34.4	18.6	11.4	6.0	13.3	2.9	1.8
	B	92.9	45.6	34.0	18.6	13.5	5.0	2.6	2.6	2.0
	Mean	92.9	45.2	34.2	18.6	12.5	5.5	7.9	2.7	1.9
AMPA	A	3.6	20.6	17.4	22.6	19.7	21.9	7.3	14.4	13.0
	B	2.3	19.1	17.3	22.1	19.7	21.3	16.3	13.9	15.8
	Mean	3.0	19.9	17.3	22.4	19.7	21.6	11.8	14.1	14.4
Unknowns	A	ND	2.7	2.7	1.9	3.1	2.3	ND	1.4	1.6
	B	ND	1.7	2.0	2.3	3.0	2.0	1.3	1.1	1.4
	Mean	ND	2.2	2.3	2.1	3.0	2.2	0.7	1.3	1.5
Background	A	0.4	1.3	0.4	0.2	1.0	0.3	0.2	ND	0.1
	B	0.3	0.5	0.4	0.1	ND	0.5	0.6	0.5	ND
	Mean	0.4	0.9	0.4	0.2	0.5	0.4	0.4	0.3	0.1
	TLC results [1]									
Glyphosate	A	93.6	56.2	38.8	21.4	11.2	6.6	2.2	1.3	1.9
	B	92.4	55.3	38.7	20.5	13.6	5.7	2.1	1.5	2.1
	Mean	93.0	55.7	38.7	20.9	12.4	6.1	2.1	1.4	2.0
AMPA	A	2.7	12.7	15.7	18.9	21.2	20.8	15.6	14.5	10.8
	B	2.5	11.6	14.9	19.4	19.5	20.0	16.0	13.3	13.5
	Mean	2.6	12.2	15.3	19.1	20.4	20.4	15.8	13.9	12.1
Unknowns	A	ND	ND	ND	2.1	2.4	2.3	2.0	1.5	3.3
	B	ND	ND	ND	2.6	2.4	2.5	1.9	1.6	3.4
	Mean	ND	ND	ND	2.4	2.4	2.4	2.0	1.5	3.3
Background	A	ND	0.3	0.4	0.2	ND	0.2	0.1	ND	0.3
	B	0.1	0.1	0.1	0.1	0.2	0.1	ND	0.1	0.2
	Mean	0.1	0.2	0.2	0.2	0.1	0.2	ND	0.1	0.2
	Mean of HPLC and TLC Results									
Glyphosate	A	*93.2*	*50.5*	*36.6*	*20.0*	*11.3*	*6.3*	*7.8*	*2.1*	*1.9*
	B	*92.7*	*50.5*	*36.4*	*19.6*	*13.6*	*5.4*	*2.4*	*2.1*	*2.1*
	Mean	*92.9*	*50.5*	*36.5*	*19.8*	*12.4*	*5.8*	*5.1*	*2.1*	*2.0*
AMPA	A	*3.2*	*16.7*	*16.6*	*20.8*	*20.5*	*21.4*	*11.5*	*14.5*	*11.9*
	B	*2.4*	*15.4*	*16.1*	*20.8*	*19.6*	*20.7*	*16.2*	*13.6*	*14.7*
	Mean	*2.8*	*16.0*	*16.3*	*20.8*	*20.0*	*21.0*	*13.8*	*14.0*	*13.3*
Unknowns	A	ND	*1.4*	*1.4*	*2.0*	*2.8*	*2.3*	*1.0*	*1.5*	*2.5*
	B	ND	*0.9*	*1.0*	*2.5*	*2.7*	*2.3*	*1.6*	*1.4*	*2.4*
	Mean	ND	*1.1*	*1.2*	*2.2*	*2.7*	*2.3*	*1.7*	*1.4*	*2.4*
Background	A	*0.2*	*0.8*	*0.4*	*0.2*	*0.5*	*0.3*	*0.2*	*ND*	*0.2*
	B	*0.2*	*0.3*	*0.3*	*0.1*	*0.1*	*0.3*	*0.3*	*0.3*	*0.1*
	Mean	*0.3*	*0.6*	*0.3*	*0.2*	*0.3*	*0.3*	*0.2*	*0.3*	*0.2*
	Recovery									
Carbon Dioxide	A	NA	19.2	31.6	40.8	51.2	58.9	68.1	70.5	73.4
	B	NA	19.0	30.1	38.8	45.7	60.2	68.7	70.7	67.6
	Mean	NA	19.1	30.9	39.8	48.5	59.6	68.4	70.6	70.5
Volatile organic compounds	A	NA	ND	ND	ND	ND	ND	ND	ND	ND
	B	NA	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	NA	ND	ND	ND	ND	ND	ND	ND	ND
Total extractable residues [2]	A	*96.8*	*70.0*	*55.0*	*43.5*	*35.3*	*30.6*	*21.0*	*18.8*	*16.7*
	B	*95.6*	*68.1*	*54.0*	*43.3*	*36.4*	*29.1*	*21.2*	*18.1*	*19.4*
	Mean	*96.2*	*69.1*	*54.5*	*43.4*	*35.9*	*29.9*	*21.1*	*18.5*	*18.1*
	A	3.5	11.1	14.3	14.6	15.3	13.0	12.6	12.3	11.7
	B	3.6	11.9	14.7	15.1	16.0	12.6	12.1	12.3	13.1

Table 7.1.1.1-9: Degradation of [^{14}C]glyphosate in Japanese non-volcanic soil B under aerobic conditions (expressed as percent of applied radioactivity)

		DAT								
Compound	**Replicate**	**0**	**1**	**3**	**7**	**14**	**30**	**63**	**90**	**121**
Non-extractable Residues	Mean	3.6	11.5	14.5	14.8	15.6	12.8	12.3	12.3	12.4
Mass balance	A	100.3	100.3	101.0	98.8	101.8	102.5	101.7	101.4	101.9
	B	99.2	98.9	98.8	97.2	98.1	101.9	102.0	101.1	100.3
	Mean	99.8	99.6	99.9	98.0	100.0	102.2	101.8	101.3	101.1

[1] Analysis of ammonia extracts
[2] Total extractable residues were calculated as sum of radioactivity in acetone and ammonia extracts, the maximum amount in acetone extracts was 1.1 % AR
DAT: days after treatment
NA: Not applicable
ND: Not detected (defined as less than 0.05 % AR)
Values calculated in the course in writing this summary are given in *italics*.

Table 7.1.1.1-10: Fractionation of post extracted soil (in percent of applied radioactivity)

	Fulvic acid fraction	Humic acid fraction	Humin fraction
Soil A			
1 DAT	2.4	29.2	17.8
14 DAT	17.4	22.9	19.1
90 DAT	2.9	21.5	20.2
Soil B			
1 DAT	8.6	0.4	2.5
14 DAT	11.0	0.8	3.8
90 DAT	7.7	0.5	3.5

B. MASS BALANCE

Material balances ranged from 98.0 to 105.8 % of applied radioactivity (% AR) for soil A and from 98.0 to 102.2 % AR for soil B (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from soil decreased from 0 DAT to 121 DAT from 34.9 to 18.8 % AR in soil A and from 96.2 to 18.1 % AR in soil B (mean of two replicates).

The amount of non-extractable residues (NER) was in the range from 64.1 to 81.4 % AR in soil A for all sampling points. In soil B, it increased from 0 DAT to 14 DAT from 3.6 to 15.6 % AR and then slightly declined to 12.4 % AR until 121 DAT (mean of two replicates).

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (120 DAT) were 4.6 % AR in soil A and 70.5 % AR in soil B (mean of two replicates). No organic volatiles were detected for both soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The results of analysis of extractable residues with HPLC and TLC were found to be very similar at each sampling interval. Therefore, further discussion refers to average values of HPLC and TLC analysis.

In soil A, Glyphosate was recovered with an amount of 32.4 % AR at 0 DAT and decreased to 16.2 % AR at 121 DAT. In soil B, it was detected with an amount of 92.9 % AR at 0 DAT and decreased to 2.0 % AR at 121 DAT. Besides carbon dioxide, the metabolite AMPA was detected. In soil A, the maximum amount

of 1.8 % AR occurred already at 0 DAT, then decreased to around 0.8 % AR at 3 DAT and remained stable until the end of the experiment. In soil B, AMPA was detected with a maximum amount of 21.0 % AR at 30 DAT and decreased to 13.3 % AR at 121 DAT. All values presented are the mean of two replicates, average values of HPLC and TLC analysis. No other metabolites were detected above 5 % AR at any time.

In the fractionation of non-extractable residues of soil A 2.4 to 17.4 % AR were found in the fulvic acid fraction, 21.5 to 29.2 % AR were found in the humic acid fraction and 17.8 to 20.2 % AR were found in the humin fraction. In soil B, 7.7 to 11.0 % AR were found in the fulvic acid fraction, 0.4 to 0.8 % AR were found in the humic acid fraction and 2.5 to 3.8 % AR were found in the humin fraction.

F. KINETICS
The kinetic evaluation of results was updated according to latest guidances and is provided in Sachers (2020, CA 7.1.2.1.1/001).

III. CONCLUSIONS

Glyphosate is degraded in soil under aerobic conditions in the dark in the laboratory. Formation of carbon dioxide was up to a maximum of 70.6 % AR in soil B. Besides carbon dioxide, the major metabolite AMPA was detected with a maximum amount of 21.0 % AR at 30 DAT in soil B. Formation of non-extractable residues in soil B was up to 15.6 % AR.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted consistent with the current guideline, showing minor deviations. These include that no information on the soil history and storage conditions are provided. The deviation is considered to not influence the results and overall outcome of the study.
Therefore the study is considered valid to address the data point.
Results for volcanic ash soil A are excluded from use in risk assessment since the soil is not representative for the EU.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.1/004
Report author	[redacted]
Report year	1996
Report title	[14C]-Glyphosate: determination of soil degradation, bio-transformation and metabolism under aerobic conditions
Report No	96-120-1020
Document No	
Guidelines followed in study	SETAC – Procedures for Assessing the Environmental Fate and Exotoxicity of Pesticides, 1995; Annex of FAO revised guidelines on environmental criteria for the registration of pesticides; BBA Guideline Part IV, 4-1
Deviations from current test guideline	From OECD 307: - One replicate was analysed per sampling point
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation and metabolism of [^{14}C]-glyphosate was investigated in three soils under aerobic conditions in the dark in the laboratory at 20°C and 45 % of the maximum water holding capacity for up to 120 days. Additionally, one of the soils was incubated at 10°C.

The following three soils were used: the sand soil Speyer 2.1; the loamy sand soil Speyer 2.2 and the loamy sand soil Speyer 2.3. The amount of organic carbon of the soils ranged from 0.62 to 2.32 % and the pH in $CaCl_2$ ranged from 5.6 to 6.4.

The test was performed in flow-through systems purged with moistened, CO_2-free air and connected to an ethylene glycol trap to collect volatile organic compounds and a 0.5 M NaOH trap to collect carbon dioxide.

The application rate was 3.11 mg/kg dry soil, corresponding to the anticipated use rate of 2.3 kg glyphosate/ha.

Soil samples were processed and analysed at 0, 1, 2, 4, 7, 15, 29 and 60 days after treatment (DAT), and additionally at 90 DAT for soil Speyer 2.1 and at 90 and 120 DAT for soil Speyer 2.2. The volatile traps were assayed at each sampling interval to determine the amount of carbon dioxide and volatile organic compounds.

Material balances ranged from 90.7 to 100.8 % of applied radioactivity (% AR) for soil Speyer 2.1, from 97.0 to 104.1 % AR for soil Speyer 2.2, from 90.9 to 112.3 % AR for soil Speyer 2.3 incubated at 20 °C and from 92.4 to 102.4 % AR for soil Speyer 2.3 incubated at 10 °C.

Maximum amounts of carbon dioxide reached at the end of the experiment were 43.0 % AR in soil Speyer 2.1 at 90 DAT, 36.5 % AR for soil Speyer 2.2 at 120 DAT and 63.4 % AR for Speyer 2.3 at 20 °C and 60 DAT. For soil Speyer 2.3 at 10 °C the maximum amount of carbon dioxide was 48.2 % AR at 60 DAT. Organic volatiles determined were <0.1 % AR for all soils at all sampling points.

For the soils incubated at 20°C, the amount of radioactivity in soil decreased from 0 DAT to 90 DAT from 97.2 to 52.3 % AR in soil Speyer 2.1, from 102.9 to 59.9 % AR at 120 DAT in soil Speyer 2.2 and from 98.1 to 21.5 % AR at 60 DAT in soil Speyer 2.3. For soil Speyer 2.3 incubated at 10 °C it decreased from 98.3 to 41.8 % AR at 60 DAT.

For the soils incubated at 20 °C, the amount of non-extractable residues (NER) increased from 0 DAT to the end of the study from 0.5 to 2.5 % AR in soil Speyer 2.1, from 0.9 to 4.9 % AR in soil Speyer 2.2 and from 1.0 to 7.8 % AR in soil Speyer 2.3. For soil Speyer 2.3 incubated at 10 °C it increased from 1.1 to 2.4 % AR in soil Speyer 2.3 at 10 °C.

For the soils incubated at 20 °C, the amount of glyphosate in soil extracts decreased from 0 DAT to study end from 96.0 to 2.2 % AR in soil Speyer 2.1, from 99.2 to 19.0 % AR in soil Speyer 2.2 and from 91.1 to 3.0 % AR in soil Speyer 2.3. For soil Speyer 2.3 incubated at 10 °C it decreased from 93.6 to 7.5 % AR.

Besides carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was detected in soil Speyer 2.1 with a maximum amount of 50.1 % AR at the end of the experiment (90 DAT). In soil

Speyer 2.2 it was found with a maximum amount of 42.4 % AR at 60 DAT and showed a slight decrease to 40.9 % AR towards the end of the experiment (120 DAT). In soil Speyer 2.3 at 20 °C, AMPA was found with a maximum amount of 32.0 % AR at 7 DAT and decreased to 18.5 % AR at the end of the experiment (60 DAT). In soil Speyer 2.3 incubated at 10 °C AMPA was found with a maximum amount of 34.3 % AR at the end of the experiment (60 DAT). No other metabolites were detected above 5 % AR at any time.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: [^{14}C- phosphonomethyl]-glyphosate
Lot No.: D1
Specific activity: 316 µCi/mg
Radiochemical purity: 99.6 %

2. Soil:
Soils were sampled from the fields and placed outside in the Springborn soil holding area. There, the soils were kept in wooden boxes underlying barley grass and seeded with Phacellia plants to provide natural conditions. The plots were irrigated if natural rainfall did not provide enough moisture. After four months of storage, soil was collected from the Springborn soil holding area and sieved to ≤ 2 mm. The soil moisture content was determined and adjusted to the approximate incubation moisture. No pesticides or fertilizers were applied for at least four years. Characteristics of the test soils are presented in the table below.

Table 7.1.1.1-11: Characteristics of test soils

Parameter	Results		
Soil	Speyer 2.1	Speyer 2.2	Speyer 2.3
Country	Germany	Germany	Germany
Textural Class (DIN)	Sand	Loamy Sand	Loamy Sand
Sand (>63 µm) (%)	88.4	81.2	60.9
Silt (2 µm – 63 µm) (%)	9.8	13.4	29.6
Clay (< 2 µm) (%)	1.9	5.5	9.5
pH ($CaCl_2$)	5.9	5.6	6.4
Organic carbon (%)	0.62	2.32	1.22
Organic matter (%)[1]	1.07	3.99	2.10
Cation exchange capacity (meq/100 g)	5.0	10.9	10.2
Maximum Water Holding Capacity (%)	31	48	39
Microbial biomass (mg C/100g)			
Before application (acclimated for 2 days at 45 % moisture at 0 bar)	90	71	89 (20 °C) 89 (10 °C)
Study end (90 DAT)	210	246	173 (20 °C) 123 (10 °C)

DAT = days after treatment
[1] Calculated from organic carbon according to OM = OC x 1.72

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used. Soil samples were incubated in 500 mL glass metabolism flasks. The flasks per experimental part were equipped with a trapping system: one washing bottle containing ethylengylcol was used to trap organic volatiles, three washing bottles containing 0.5 M NaOH solution were used to trap $^{14}CO_2$. The metabolism flasks were continuously ventilated with CO_2 free and moistened air at a flow rate of about 30 to 60 mL per minute.

100 g of sieved soil (dry weight equivalents) were weighed into each test vessel, soil moisture was adjusted to 45 % of the maximum water holding capacity (MWHC) and the test systems were acclimated for 3 days at test conditions.

The study application rate corresponded to an anticipated use rate of 2.3 kg a.s./ha. A test solution of [^{14}C]glyphosate, mixed with unlabelled glyphosate was prepared in water. 0.2 mL of this solution were applied to each test system, resulting in a final concentration of 3.11 mg/kg dry soil.

Test systems were incubated under aerobic conditions in the dark for 90 days at 20 °C and 45 ± 2 % MWHC for soil Speyer 2.1 and 2.2 and for 60 days at 20 °C and at 10 °C at 45 ± 2 % MWHC for soil Speyer 2.3.

2. Sampling

One test system was processed and analysed 0, 1, 2, 4, 7, 15, 29 and 60 days after treatment (DAT), and additionally at 90 DAT for soil Speyer 2.1 and at 90 and 120 DAT for soil Speyer 2.2. All soil samples were processed on the designated sampling day. The ethylene glycol and NaOH traps were assayed at each sampling point.

3. Analytical procedures

The analytical procedure was confirmed prior to the experimental start of the definitive test by extractability tests, which showed recoveries of 99 to 101 %.

At each sampling interval, soil samples were extracted consecutively three times with 125 mL portions of 0.35 M aqueous H_3PO_4/0.09 M aqueous $CaCl_2$ per 50 g dry weight of soil by shaking the samples in an overhead shaker at about 60 rpm at ambient temperature. After centrifugation of each individual extract, extraction efficiency was determined by LSC. After exhaustive solvent extraction, extracts were pooled and the extraction efficiency was determined. Extracts were analysed qualitatively and quantitatively by HPLC via direct injection. The amount of volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

Glyphosate and metabolites were identified by radio-HPLC and radio-TLC co-chromatography with reference items.

The identity of CO_2 in the sodium hydroxide traps was confirmed by the addition of barium hydroxide to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised in the tables below for the respective soils.

Table 7.1.1.1-12: Degradation of [^{14}C]glyphosate in soil Speyer 2.1 under aerobic conditions (expressed as percent of applied radioactivity) at 20 °C

	DAT								
Radioactive Residues	**0**	**1**	**2**	**4**	**7**	**15**	**29**	**60**	**90**
Glyphosate	96.0	84.8	74.3	59.2	53.9	38.2	21.0	8.5	2.2
AMPA	1.3	12.1	12.9	25.1	27.3	27.5	37.9	42.3	50.1
Total extractable residues	97.2	97.0	87.2	84.4	81.2	65.7	58.9	50.8	52.3
Carbon dioxide	ND	5.1	7.4	12.8	17.9	23.2	32.4	39.4	43.0
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	0.5	0.7	1.0	1.1	1.7	1.7	2.0	1.9	2.5
Mass balance	97.7	102.7	95.6	98.2	100.8	90.7	93.3	92.1	97.8

DAT: days after treatment
ND: not determined

Table 7.1.1.1-13: Degradation of [^{14}C]glyphosate in soil Speyer 2.2 under aerobic conditions (expressed as percent of applied radioactivity) at 20 °C

	DAT									
Radioactive Residues	**0**	**1**	**2**	**4**	**7**	**15**	**29**	**60**	**90**	**120**
Glyphosate	99.2	96.1	84.2	77.1	71.8	60.3	41.7	26.7	25.9	19.0
AMPA	3.7	4.3	7.9	12.9	15.7	21.0	34.5	42.4	39.0	40.9
Total extractable residues	102.9	100.5	92.1	89.9	87.5	81.3	76.2	69.1	64.9	59.9
Carbon dioxide	ND	2.8	3.8	7.2	10.5	16.3	22.5	30.6	33.9	36.5
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	0.9	0.9	1.2	1.2	1.7	1.6	2.2	1.6	3.7	4.9
Mass balance	103.8	104.1	97.0	98.3	99.7	99.1	100.9	101.4	102.4	101.2

DAT: days after treatment
ND: not determined

Table 7.1.1.1-14: Degradation of [^{14}C]glyphosate in soil Speyer 2.3 under aerobic conditions (expressed as percent of applied radioactivity) at 20 °C

	DAT							
Radioactive Residues	**0**	**1**	**2**	**4**	**7**	**15**	**29**	**60**
Glyphosate	91.1	76.2	63.9	34.2	18.4	13.3	<0.1	3.0
AMPA	7.0	13.0	27.0	25.7	32.0	25.3	31.1	18.5
Total extractable residues	98.1	89.3	90.9	60.0	50.4	38.6	31.1	21.5
Carbon dioxide	ND	12.9	18.6	30.5	38.1	48.4	55.4	63.4
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	1.0	2.0	2.8	2.3	3.6	4.1	4.4	7.8
Mass balance	99.1	104.2	112.3	92.8	92.2	91.0	90.9	92.7

DAT: days after treatment
ND: not determined

Table 7.1.1.1-15: Degradation of [^{14}C]glyphosate in soil Speyer 2.3 under aerobic conditions (expressed as percent of applied radioactivity) at 10 °C

	DAT							
Radioactive Residues	**0**	**1**	**2**	**4**	**7**	**15**	**29**	**60**
Glyphosate	93.6	87.3	80.0	62.2	54.9	35.9	21.7	7.5
AMPA	4.7	8.7	9.2	19.3	22.1	25.8	28.7	34.3
Total extractable residues	98.3	96.1	89.2	81.6	76.9	61.7	50.4	41.8
Carbon dioxide	ND	4.8	6.6	12.6	18.9	30.0	40.4	48.2
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	1.1	1.5	1.6	1.7	2.3	3.6	3.9	2.4
Mass balance	99.3	102.4	97.4	95.9	98.2	95.3	94.7	92.4

DAT: days after treatment
ND: not determined

B. MASS BALANCE
Material balances ranged from 90.7 to 102.7 % of applied radioactivity (% AR) for soil Speyer 2.1, from 97.0 to 104.1 % AR for soil Speyer 2.2, from 90.9 to 112.3 % AR for soil Speyer 2.3 at 20 °C and from 92.4 to 102.4 % AR for soil Speyer 2.3 at 10 °C.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
The amount of radioactivity extractable from soil decreased from 0 DAT to 90 DAT from 97.2 to 52.3 % AR in soil Speyer 2.1, from 102.9 to 59.9 % AR at 120 DAT in soil Speyer 2.2 and from 98.1 to 21.5 % AR at 60 DAT. In soil Speyer 2.3 at 10 °C it decreased from 0 DAT to 60 DAT, from 98.3 to 41.8 % AR.

The amount of non-extractable residues (NER) increased from 0 DAT to the end of the study from 0.5 to 2.5 % AR in soil Speyer 2.1, from 0.9 to 4.9 % AR in soil Speyer 2.2 and from 1.0 to 7.8 % AR in Soil Speyer 2.3 at 20 °C. In soil Speyer 2.3 at 10 °C it increased from 1.1 to 2.4 % AR.

D. VOLATILE RADIOACTIVITY
Maximum amounts of carbon dioxide reached at study end (120 DAT, 90 DAT or 60 DAT) were 43.0, 36.5 and 63.4 % AR in soils Speyer 2.1, Speyer 2.2 and Speyer 2.3 at 20 °C, respectively. In soil Speyer 2.3 at 10 °C the maximum amount was 48.2 % AR at the end of the study. Organic volatiles determined were <0.1 % AR for all soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM
For all incubations at 20 °C, residues of glyphosate decreased quickly. In soil Speyer 2.1 it was detected with 96.0 % AR at 0 DAT and decreased to 2.2 % AR at 90 DAT. In soil Speyer 2.2 it was found with 99.2 % AR at 0 DAT and decreased to 19.0 % AR at 120 DAT. In soil Speyer 2.3, it was found with 91.1 % AR at 0 DAT and decreased to 3.0 % AR at 60 DAT.
In soil Speyer 2.3 at 10 °C, glyphosate was degraded slightly slower compared to the experiment at 20 °C with 93.6 % AR at 0 DAT and 7.5 % AR at 60 DAT.

Besides carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was detected in all soils. In soil Speyer 2.1 it reached a maximum amount of 50.1 % AR at the end of the study (90 DAT). In soil Speyer 2.2 it was found with a maximum amount of 42.4 % AR at 60 DAT and showed a slight decrease to 40.9 % AR at the end of the study (120 DAT). In soil Speyer 2.3 at 20 °C AMPA was found with a maximum amount of 32.0 % AR at 7 DAT and decreased to 18.5 % AR at the end of the study (60 DAT). In soil Speyer 2.3 incubated at 10 °C AMPA was found with a maximum amount of 34.3 % AR at the end of the experiment (60 DAT). No other metabolites were detected above 5 % AR at any time.

F. KINETICS

The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020, CA 7.1.2.1.1/001).

III. CONCLUSIONS

[^{14}C]glyphosate showed a similar degradation behaviour in the three soils after treatment with 3.11 mg/kg. The main degradation product was carbon dioxide: Between 36.5 and 63.4 % of the applied radioactivity was mineralized, depending on the soil type and the incubation temperature. Glyphosate was degraded quickly in all incubation systems at 20 °C with amounts of 2.2 % AR at 90 DAT in the soil Speyer 2.1, 19.0 % AR at 120 DAT in the soil Speyer 2.2 and 3.0 % AR at 60 DAT in the soil Speyer 2.3 at the end of the study. At 10 °C, the decrease of glyphosate residues was slightly slower with 7.5 % AR in soil Speyer 2.3 at the end of the study at 60 DAT. AMPA was identified as the only major metabolite with a maximum amount of 50.1 % AR. Non-extractable residues amounted to maximum 7.8 % AR.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study conduct was consistent with the current guideline, showing minor deviations.
One replicate sample was processed and analysed per sampling point while the standard is work-up of duplicates. Instead, the number of sampling points was increased to i.e. eight being well beyond the minimum of five to six to allow for robust for kinetic analysis. The deviations are considered to not influence the overall outcome of the study.
Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.1/005
Report author	[redacted]
Report year	1995
Report title	HR-001: Aerobic soil metabolism and route of degradation
Report No	SNY-333/951445
Document No	
Guidelines followed in study	US EPA pesticide Assessment Guidelines, Subdivision N, 162-1; German BBA Guidelines for the Official Testing of Plant Protection Products; Part IV, 4-1, Stage 1; Japanese MAFF, 59 NohSan No. 4200, January 1985; Draft Guidelines Concerning the Inclusion of Active Substances in Annex I to Council Directive 91/414/EEC part 7.1.1.2.
Deviations from current test guideline	From OECD 307: - No information about soil history and storage conditions are reported - Study duration was 180 days
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate (HR-001) was investigated in soil under aerobic conditions in the dark in the laboratory at 20 ± 1°C and 40 % of the maximum water holding capacity for 180 days.

The soil used was the sandy loam Arrow from the United Kingdom. The amount of organic carbon of the soil was 2.2 % and the pH in $CaCl_2$ was 5.9.

The test was performed in flow-through systems flushed with moistened air. The outgoing air was first passed through polyurethane foam bungs to trap neutral, volatile organic compounds and further through a trap containing 2-(ethoxyethoxy)ethanol to collect volatile organic compounds, one trap containing 1 M KOH aqueous solution and a trap containing ethanolamine/2-ethoxyethanol (1/3, v/v), both to collect carbon dioxide.

The application rate was 487 µg/50 g soil (dry weight), corresponding to 9.7 mg/kg soil.

Duplicate samples were processed and analysed at 0, 3, 7, 14, 30, 60, 90, 120 and 180 days after treatment (DAT). The volatile traps were assayed and changed at each sampling time or at approximately two weeks intervals to determine the amount of carbon dioxide and volatile organic compounds.

Mass balance ranged from 90.2 to 97.1 % of applied radioactivity (% AR, mean of two replicates).

Maximum amounts of carbon dioxide reached at study end (180 DAT) were 23.6 % AR (mean of two replicates). No organic volatiles were detected at any sampling point.

The amount of extractable radioactivity decreased from 0 DAT to 180 DAT from 94.6 to 57.3 % AR (mean of two replicates).
The amount of non-extractable residues (NER) increased from 0 DAT to 90 DAT from 2.6 to a maximum level of 10.9 % AR, stayed at a constant level until 120 DAT and slightly decreased to 9.4 % AR at 180 DAT (mean of two replicates).

The amount of glyphosate in soil extracts decreased from 0 DAT to 180 DAT from 91.9 to 27.1 % AR (mean of two replicates).

Besides carbon dioxide, the major metabolite aminomethy lphosphonic acid (AMPA) was detected with a maximum amount of 27.3 % AR at 120 DAT, which slightly decreased to 25.6 % AR by the end of the experiment (mean of two replicates). No other metabolites were detected above 5 % AR at any time.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C-phosphonomethyl]-glyphosate
Lot No.:	CFQ8432
Specific activity:	327.7 µCi/mg
Radiochemical purity:	>99 %

2. Soil:

The soil was sieved to ≤ 2 mm. Characteristics of the test soil are presented in the table below.

Table 7.1.1.1-16: Characteristics of test soil

Parameter	Results
Soil	Arrow
Country	United Kingdom
Textural Class (DIN)	Sandy loam
Sand (63 µm – 2 mm) (%)	68.61
Silt (2 µm – 63 µm) (%)	19.22
Clay (< 2 µm) (%)	12.18
pH ($CaCl_2$)	5.9
Organic carbon (%)	2.2
Organic matter (%) [1]	3.8
Cation exchange capacity (meq/100 g)	10.0
Maximum Water Holding Capacity (%)	37.95
Microbial biomass (µg C/g soil)	
At application	337
Intermediate (120 DAT)	337
Study end (217 DAT)	256

DAT = days after treatment
[1] Calculated from organic carbon according to OM = OC x 1.72

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of glass columns of 10 cm inner diameter where the individual test vessels were stored on a rack. The columns were connected to a set of washing bottles. Air entering the system was passed through a water bottle to moisten incoming air. After leaving the glass column, the air was passed through a polyurethane foam bung to collect neutral, volatile organic compounds followed by a trap system. It consisted of an empty trap to prevent suck back into the system, a trap containing 2-(ethoxyethoxy)ethanol to collect volatile organic compounds, one trap containing 1 M KOH aqueous solution laced with phenolphthalein indicator and another trap containing ethanolamine/2-ethoxyethanol (1/3, v/v), both to collect carbon dioxide.

50 g of sieved soil (dry weight equivalents) with a soil moisture slightly above 40 % of the maximum water holding capacity were weighed into each test vessel and the test systems were acclimated for 7 days at test conditions.

The study application rate was 9.7 mg a.s./kg dry soil. An aqueous application solution containing a mixture of [^{14}C]-labelled and unlabelled glyphosate with a concentration of 1 mg/mL was prepared. 0.450 mL of this solution were applied to each test system, resulting in a final concentration of 487 µg/50 g dry soil.

Test systems were incubated under aerobic conditions in the dark for 180 days at 20 °C and 40 % MWHC.

2. Sampling

Duplicate samples were processed and analysed 0, 3, 7, 14, 30, 60, 90, 120 and 180 days after treatment (DAT). Samples were extracted on the day of sampling. Extracts were stored at <-15 °C prior to analysis. Extracts were generally analysed within 6 weeks of sampling. The trapping solutions were assayed and changed at each sampling time or at approximately two weeks intervals.

3. Analytical procedures

Duplicate soil samples were analysed separately at each sampling time. Each soil sample was extracted three times with 150 mL of an aqueous solution containing $NH_4(OH)$ (0.25 M) and KH_2PO_4 (0.1 M) by treatment in an ultrasonic bath at ambient temperature for 15 min followed by shaking for 15 min at ambient temperature. A fourth extraction was conducted with 100 mL of the same extraction solution by sonication at 50 °C for 60 minutes and followed by shaking for 15 min at ambient temperature. After each extraction step, the solvent was separated by centrifugation and the radioactivity of the extracts was determined by LSC.

Prior to analysis by HPLC and TLC, the soil extracts were pooled. The combined extracts were cleaned-up by strong cation exchange solid phase extraction. The column was eluted with 0.5 M HCl, the eluate was concentrated to dryness and reconstituted in 5 mM KH_2PO_4 and 4 % methanol (v/v) adjusted to pH 2.1 with phosphoric acid. Recovery of radioactivity from this procedure was quantitative.

The extracted soil residue was allowed to air dry and then combusted. The combustion products were analysed by LSC.

The polyurethane foam bungs removed at sampling (0 DAT to 60 DAT inclusive) were individually extracted with an aqueous solution containing $NH_4(OH)$ (0.25 M) and KH_2PO_4 (0.1 M), and the extracts were analysed by LSC. The volatile trapping solutions were also analysed by LSC.

Glyphosate and metabolites were identified and quantified by HPLC. The presence of glyphosate and AMPA was confirmed by TLC co-chromatography with reference items.

The identification of CO_2 in the KOH traps was determined by the addition of sodium carbonate to aliquots of the trap solution. The mixture was added to saturated barium chloride, the barium carbonate precipitate formed was separated by centrifugation and the supernatant was analysed by LSC.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised in the table below.

Table 7.1.1.1-17: Degradation of [^{14}C]glyphosate in soil Arrow under aerobic conditions, Results of HPLC measurements (expressed as percent of applied radioactivity)

		DAT								
Compound	**Replicate**	**0**	**3**	**7**	**14**	**30**	**60**	**90**	**120**	**180**
Glyphosate	A	92.6	87.0	74.0	64.2	54.0	41.1	32.5	28.1	26.5
	B	91.2	82.2	73.9	69.5	54.6	38.4	35.5	29.0	27.6
	Mean	*91.9*	*84.6*	*74.0*	*66.9*	*54.3*	*39.8*	*34.0*	*28.5*	*27.1*
AMPA	A	1.0	3.9	6.9	10.4	14.4	22.1	27.5	28.0	25.8
	B	1.1	3.1	6.6	8.3	13.7	22.3	25.4	26.6	25.3
	Mean	*1.1*	*3.5*	*6.8*	*9.4*	*14.1*	*22.2*	*26.5*	*27.3*	*25.6*
Polar compounds	A	0.6	0.7	1.4	1.8	2.6	3.3	2.7	2.8	4.0
	B	0.8	0.8	1.5	1.2	3.0	3.0	2.3	3.0	3.9
	Mean	*0.7*	*0.8*	*1.5*	*1.5*	*2.8*	*3.2*	*2.5*	*2.9*	*4.0*
Others	A	0.7	0.6	1.1	0.8	1.2	1.4	0.8	0.2	0.8
	B	1.2	0.6	0.9	1.1	1.4	1.4	0.8	0.4	0.6
	Mean	*1.0*	*0.6*	*1.0*	*1.0*	*1.3*	*1.4*	*0.8*	*0.3*	*0.7*
Carbon Dioxide	A	ns	2.3	4.2	7.1	11.7	16.6	19.4	21.3	23.9
	B	ns	2.1	4.3	7.0	11.0	15.8	18.7	20.7	23.3
	Mean	*ns*	*2.2*	*4.3*	*7.1*	*11.4*	*16.2*	*19.1*	*21.0*	*21.6*

Table 7.1.1.1-17: Degradation of [^{14}C]glyphosate in soil Arrow under aerobic conditions, Results of HPLC measurements (expressed as percent of applied radioactivity)

		DAT								
Compound	**Replicate**	**0**	**3**	**7**	**14**	**30**	**60**	**90**	**120**	**180**
Volatile organic compounds	A	ns	nd	nd	nd	nd	ns	ns	ns	ns
	B	ns	nd	nd	nd	nd	ns	ns	ns	ns
Total extractable residues	A	94.9	92.1	83.3	76.7	72.0	67.9	63.5	59.1	57.1
	B	94.2	86.2	82.8	79.4	72.6	64.9	63.9	59.0	57.4
Non-extractable Residues	A	2.7	5.9	9.0	7.6	7.6	9.6	11.3	10.9	9.4
	B	2.4	5.3	8.2	8.4	9.0	9.7	10.5	11.8	9.3
Mass balance	A	97.6	100.3	96.5	91.4	91.3	94.1	94.2	90.4	90.4
	B	96.6	93.6	95.3	94.8	92.6	90.4	93.1	91.5	90.0
	Mean	*97.1*	*97.0*	*95.9*	*93.1*	*92.0*	*92.3*	*93.7*	*91.0*	*90.2*

DAT: days after treatment
Values calculated in this summary are given in italics
nd: below limit of accurate determination (two times background noise)
ns: not sampled

B. MASS BALANCE
Material balances ranged from 90.2 to 97.1 % AR (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
The amount of extractable radioactivity decreased from 0 DAT to 180 DAT from 94.6 to 57.3 % AR (mean of two replicates).

The amount of non-extractable residues (NER) increased from 0 DAT to 90 DAT from 2.6 to a maximum level of 10.9 % AR, stayed at a constant level until 120 DAT and slightly decreased to 9.4 % AR at 180 DAT (mean of two replicates).

D. VOLATILE RADIOACTIVITY
The maximum amount of carbon dioxide reached at study end (180 DAT) was 23.6 % AR (mean of two replicates). No organic volatiles were detected at any sampling point. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM
Glyphosate residues decreased from 91.9 % AR at 0 DAT to 27.1 % AR at 180 DAT. Besides carbon dioxide, the metabolite aminomethylphosphonic acid (AMPA) was detected with a maximum amount of 27.3 % AR at 120 DAT and a further slight decrease to 25.6 % AR by the end of the experiment (mean of two replicates). No other metabolites were detected above 5 % AR at any time.

F. KINETICS
The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020, CA 7.1.2.1.1/001).

III. CONCLUSIONS

The aerobic degradation of glyphosate in soil Arrow at 20 °C in darkness and 40 % maximum water holding capacity has been studied. Under these conditions, fast degradation of glyphosate occured in the soil. The two most important degradation products were identified as AMPA (aminomethylphosphonic acid, the only significant soil metabolite) and carbon dioxide due to mineralization.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted consistent with the current guideline, showing minor deviations.
The test duration of 180 days was prolonged in comparison to 120 days recommended. No information on the soil history and storage conditions are reported. These deviations are considered to not have influenced the results and overall outcome of the study.
Therefore, it is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.1/006
Report author	[redacted]
Report year	1993
Report title	Degradation and metabolism of 14C-Glyphosate in soil incubated under aerobic conditions
Report No	246486
Document No	
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision N: Chemistry: Environmental Fate Section 162-1 (October, 1982); BBA Richtlinie Part IV, 4-1 (December, 1986)
Deviations from current test guideline	From OECD 307: - Study duration was 364 days instead of recommended 120 days - LOD/LOQ was not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The degradation of [^{14}C]glyphosate was investigated in soil under aerobic conditions in the dark in the laboratory at 20 ± 1 °C and 40 % of the maximum water holding capacity for 364 days.

The soil used was a silt loam from Les Evouettes. The amount of organic carbon in the soils was 1.40 % and the pH in water was 6.1.

The test was performed in flow-through systems flushed with moistened air and connected to an ethylene glycol trap to collect volatile organic compounds and a 2 N NaOH trap to collect carbon dioxide.

The application rate was 240 µg/100 g soil (dry weight), corresponding to a field rate of 1.8 kg glyphosate/ha.

Duplicate samples from each system were processed and analysed at 0, 3, 7, 14, 28, 56, 84, 112, 168, 252 and 364 days after treatment (DAT). All soil samples were processed on the designated sampling day. The ethylene glycol and NaOH traps were assayed and changed at 3 DAT, then on a weekly basis for the first four weeks. After 42 DAT the ethylene glycol trap was removed and the NaOH trap was changed and analysed every two weeks.

Mass balances ranged from 91.8 to 103.2 % of applied radioactivity (AR, mean of two replicates).

Glyphosate was detected with 78.3 % AR at 0 DAT and decreased to 6.7 % AR at 364 DAT (mean of two replicates).

The amount of carbon dioxide reached at study end (364 DAT) was 41.6 % AR. Organic volatiles determined were ≤0.1 % AR at all sampling points (mean of two replicates).

The amount of extractable radioactivity decreased from 0 to 364 DAT from 92.2 to 36.3 % AR (mean of two replicates).

The amount of non-extractable residues (NER) increased from 0 to 364 DAT from 6.4 to 19.8 % AR (mean of two replicates).

Besides carbon dioxide, the major metabolite AMPA was detected with a maximum amount of 29.4 % AR at 84 DAT. Two unknown highly polar radioactive fractions (M2 and M5), which were observed with amounts above 10 % AR, can be attributed to substances bound to fulvic or humic acids which were co-extracted at the high pH. Other unknown compounds were below 5 % AR at any time.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]-phosphonomethyl-glyphosate
Lot No.: CFA. 745 C4
Specific activity: 11.2 MBq/mg (304 µCi/mg)
Radiochemical purity: 96.6 %

2. Soil:

About one month prior to application, the soil was sampled from outdoor containers, where it was stored after retrieval from the field, and acclimated at room temperature. No pesticides or fertilizers were applied for at least one year. Soils were sieved to ≤ 2 mm. Characteristics of the test soil are presented in the table below.

Table 7.1.1.1-18: Characteristics of test soil

Parameter	Results
Soil	Les Evouettes
Country	Switzerland
Textural Class (USDA)	Silt loam
Sand (50 µm – 2 mm) (%)	38.0
Silt (2 µm – 50µm) (%)	50.7
Clay (< 2 µm) (%)	11.3
pH (water)	6.1
Organic carbon (%) [1]	1.40
Organic matter (%) [2]	2.41

Table 7.1.1.1-18: Characteristics of test soil

Cation exchange capacity (meq/100 g)	15.5
Maximum Water Holding Capacity (MWHC) (%)	55.3
Field Capacity (FC) (%)	40.2
Bulk Density (40 % MWHC) (g/cm^3)	0.913
Microbial biomass (mg C/100 g)	
Before application	58.5
Study end (364 DAT)	22.0

DAT = days after treatment, USDA: United States Department for Agriculture
[1] Referring to soil dry weight
[2] Calculated from organic carbon according to OM = OC x 1.72

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of glass jars filled with soil and connected to washing bottles. Air entering the system was moistened by a water-filled gas-washing bottle. After leaving the test vessels, the air was passed through a trap containing 50 mL 2N NaOH aqueous solution to collect carbon dioxide and a trap containing 50 mL of ethylene glycol to trap volatile organic compounds. Airflow was controlled by a flow meter.

100 g of sieved soil (dry weight equivalents) were weighed into each test vessel.

The study application rate corresponded to the anticipated use rate of 1.8 kg a.s./ha. 900 µL of an aqueous test solution, containing a mixture of labelled [^{14}C]glyphosate and unlabelled glyphosate with a specific activity of 14.8 µCi/mL were applied to each test system, resulting in a final concentration of 240 µg/100 g dry soil. After application, the soil moisture was adjusted to 40 % of the maximum water holding capacity (MWHC, corresponding to 55 % of the field capacity), the test vessels were closed with trap attachments and the airflow was set to 60 mL/minute.

Test systems were incubated under aerobic conditions in the dark for 364 days at 20 °C and 40 % MWHC.

2. Sampling

Duplicate test systems were processed and analysed at 3, 7, 14, 28, 56, 84, 112, 168, 252 and 364 days after treatment (DAT). At 0 DAT one replicate was processed and analysed. All soil samples were processed on the designated sampling day. The ethylene glycol and NaOH traps were assayed and changed at 3 DAT, then on a weekly basis for the first four weeks. After 42 DAT, the ethylene glycol trap was removed and the NaOH trap was changed and analysed every two weeks.

3. Analytical procedures

At each sampling interval, soil samples were extracted four to five times with 0.5 N NH_4OH solution for 30 minutes followed by one or two extractions with water. The respective extracts were combined and the radioactivity was measured by liquid scintillation counting (LSC). The day 0 sample was extracted five times with methanol/water (8/2, v/v), three times with water and four times with 0.5 N NH_4OH.

Aliquots of the combined extracts were filtered and concentrated by evaporation under reduced pressure. The aqueous phase was further analysed by thin layer chromatography (TLC), using three different stationary phases, and high performance liquid chromatography (HPLC). The amount of volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

Test item and metabolites were identified by radio-HPLC-UV and TLC co-chromatography with reference items. As well as high voltage electrophoresis (HVE) of selected samples.

The stability of the extracts for at least two years was demonstrated by repeated TLC analysis of selected extracts.

The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium hydroxide solution to aliquots of the trap contents. The presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised in the table below.

Table 7.1.1.1-19: Degradation of [^{14}C]glyphosate in soil Les Evouettes under aerobic conditions (values expressed as percent of applied radioactivity), Results of TLC analysis

		DAT										
Compound	**Replicate**	**0**	**3**	**7**	**14**	**28**	**56**	**84**	**132**	**168**	**252**	**364**
Glyphosate	A	78.3	65.6	49.5	48.9	36.7	24.3	19.4	16.3	9.4	8.3	7.4
	B	ND	69.0	58.6	38.7	36.1	25.4	19.6	21.8	10.8	8.4	6.0
	Mean	ND	67.3	54.0	43.8	36.4	24.8	19.5	19.1	10.1	8.3	6.7
AMPA (M1)	A	4.0	6.2	14.9	12.2	19.7	21.1	28.3	28.3	16.6	17.7	21.2
	B	ND	6.4	11.5	13.5	21.9	22.7	30.4	26.9	21.7	18.8	21.4
	Mean	ND	6.3	13.2	12.8	20.8	21.9	29.3	27.6	19.2	18.3	21.3
Unknown 1 (M2)	A	9.9	8.5	11.9	9.1	8.7	8.1	3.7	5.4	0.0	0.0	0.0
	B	ND	10.5	13.2	21.2	4.8	6.1	5.0	2.0	0.0	0.0	0.0
	Mean	*5.0*	*9.5*	*12.6*	*15.2*	*6.8*	*7.1*	*4.4*	*3.7*	*0*	*0*	*0*
Unknown 2 (M3)	A	0.0	0.0	0.0	6.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	ND	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unknown 3 (M4)	A	0.0	0.0	0.0	0.0	0.0	0.0	4.9	0.0	0.0	0.0	0.0
	B	ND	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unknown 4 (M5)	A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.0	14.0	7.8
	B	ND	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.2	12.5	8.7
	Mean	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*13.6*	*13.3*	*8.3*
Carbon Dioxide	A	ND	5.9	10.5	15.2	22.0	29.2	32.2	34.0	36.6	39.1	41.6
	B	ND	5.7	10.9	15.8	22.2	28.4	32.5	31.3	36.9	38.3	41.6
	Mean	ND	5.8	10.7	15.5	22.1	28.8	32.3	32.7	36.8	38.7	41.6
Volatile organic compounds	Mean	ND	<0.1	<0.1	<0.1	<0.1	ND	ND	ND	ND	ND	ND
Total extractable residues	Mean	92.2	83.1	79.7	75.3	63.9	53.8	55.7	50.4	42.8	39.9	36.3
Non-extractable Residues	A	6.4	10.5	9.9	13.8	14.6	13.4	13.6	14.1	15.7	13.4	16.1
	B	NA	9.3	10.2	11.3	12.7	14.9	12.1	13.6	18.0	13.0	23.5
	Mean	ND	9.9	10.0	12.5	13.7	14.1	12.8	13.9	16.9	13.2	19.8
Mass balance	Mean	98.6	98.8	100.5	103.2	99.7	96.7	100.8	96.9	96.4	91.8	97.7

DAT: days after treatment
Values calculated in this summary are given in italics.
ND: not determined
NA: not applicable

B. MASS BALANCE

Material balances ranged from 91.8 to 103.2 % AR (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
The amount of extractable radioactivity decreased from 0 DAT to 364 DAT from 92.2 to 36.3 % AR.

The amount of non-extractable residues (NER) increased from 0 DAT to 364 DAT from 6.4 to 19.8 % AR (all values mean of two replicates). The rather low and stable amount of NER was explained by the high pH value of the extraction medium (0.5 N NH_4OH). It was concluded that fulvic and humic acids were extracted at the same time.

D. VOLATILE RADIOACTIVITY
The maximum amount of carbon dioxide reached at study end (364 DAT) was 41.6 % AR. Organic volatiles determined were ≤0.1 % AR at all sampling points (all values mean of two replicates). The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM
Glyphosate was detected with 78.3 % AR at 0 DAT and decreased to 6.7 % AR at 364 DAT (mean of two replicates). Glyphosate concentrations were confirmed by HPLC analysis. Besides carbon dioxide, the major metabolite AMPA was detected with a maximum amount of 29.4 % AR at 84 DAT and decreased to 21.3 % AR at 364 DAT (mean of two replicates).

Two highly polar radioactive fractions (M2 and M5) were observed with amounts exceeding 10 % of the applied radioactivity. Fraction 1 (M2) was detected with a maximum amount of 15.2 % AR at 14 DAT and decreased to zero from 168 DAT onwards. Fraction 4 (M5) was detected from 168 DAT onwards with a maximum amount of 13.6 % AR at 168 DAT and decreased to 8.3 % AR at 364 DAT.

Next to parent and AMPA (reference B), another nine reference items were analysed by TLC, however did not overlap with the two fractions above (M2 and M5). These reference items were sarcosine (reference A), N-methyl-AMPA (reference C), N-methyl-glyphosate (reference D), hydroxymethyl phosphonic acid (reference E), methylamine, hydrochloride (reference F), dimethylamine hydrochloride (reference G), N-carboxylmethyl-N-phosphonomethyl)glycine (reference H), methylphosphonic acid (reference I) and N-N-dimethalymino-methylphosphonic acid (reference J). M2 was present at day 0 as TLC start radioactivity in all chromatographic systems. From day 168, the start radioactivity (M2) changed its properties towards a more mobile behaviour and could be differentiated as M5. In conclusion, it is most likely that M2 and M5 represent radiolabelled substances bound to fulvic or humic acids which were co-extracted at the high pH.

Other unknown compounds were below 5 % AR at any time.

F. KINETICS
The kinetic evaluation of results was updated according to latest guidances and is provided in [redacted] (2020, CA 7.1.2.1.1/001).

III. CONCLUSIONS

After incubation of soil Les Evouettes with [^{14}C]-glyphosate, 6.4 % of the radioactivity applied was rapidly and irreversibly bound to the soil. The main metabolic product found in the soil was aminomethyl-phosphonic acid (AMPA).

As expected, the extent of mineralization of glyphosate was significant, i.e. 41.6 % of the radioactivity applied in 364 days.

The non-extractable radioactivity remained low, i.e. 6.4 % to 19.8 % of the radioactivity applied. This was an expected result, because of the high pH value of the extracting solutions. The extraction method applied allowed the relatively easy detection of parent and AMPA, but co-extractions complicated the analyses.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is conducted consistent with the current guideline, showing minor deviations. The study duration is 364 days instead of the proposed 120 days to fulfill US data requirements. No LOD or LOQ is reported. These deviations do not influence the results and outcome of the study.
Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.1/007
Report author	[redacted]
Report year	1993
Report title	Rate of degradation and metabolism of [14C]-Glyphosate in soil under aerobic conditions
Report No	IWM-R93/047
Document No	
Guidelines followed in study	Dutch Guideline for Biocides, section G.1.1
Deviations from current test guideline	From OECD 307: - the work-up procedure was not suitable to result in adequate procedural recoveries. Soil extracts were concentrated by freeze-drying to result in high losses, i.e 15.3 to 57.6 % of extracted radioactivity could not be re-constituted for analysis, presumably due to high portions of test item bound to humic substances. In addition, high variability in results was observed between the duplicates per sampling interval investigated - soil history not reported - no limit of detection reported for TLC analytical method - for two soils Drovendaal & Lisse, no full material balance was established - for two soils representativeness as agricultural soil unknown
Previous evaluation	Yes, accepted in RAR (2015)
GLP	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary
The degradation of [^{14}C]glyphosate was investigated in three soils under aerobic conditions in the dark in the laboratory at 20 ± 2 °C and 0.32 bar soil moisture for 15 weeks (100 days).

The soils used were a humic sand (Droevendaal), a sandy loam (Maasdijk) and a low humic-content (lhc) sand (Lisse) from the Netherlands. The amount of organic carbon in the soils was between 0.64 % and 2.32 % and the pH in KCl was between 5.2 and 7.5.

The test was performed in static systems topped with a glass tube containing oil covered quartz wool for collection of organic volatiles and CO_2-absorbing soda lime.

The application rate was 3.8 mg/kg soil (dry weight).

Duplicate soil samples were processed and analysed 0, 1, 2, 4, 8 and 15 weeks after treatment. According to the tables in the report this corresponds to 0, 7, 14, 35, 70 and 100 days after treatment (DAT).

Material balances ranged from 91.8 to 95.4 % AR (each mean of two replicates) for the Maasdijk soil. No material balances were determined for the two soils Droevendaal and Lisse.

The maximum amount of carbon dioxide reached at study end (100 DAT) was 79.6 % AR. Organic volatiles were found with a maximum amount of 0.3 % AR at 100 DAT (all values mean of two replicates). Volatiles were only determined for sandy loam soil.

The amount of radioactivity extractable with 0.5 M NH_4OH decreased from 0 DAT to 100 DAT from 73.9 to 56.2 % AR with an intermediate minimum of 47.1 % AR at 14 DAT in the Droevendaal soil. In the Maasdijk soil, extractable radioactivity decreased from 90.4 at 0 DAT to 4.1 % AR at 100 DAT. In the Lisse soil, extractable radioactivity decreased from 98.1 at 0 DAT to 55.9 % AR at 100 DAT.

The amount of radioactivity extractable from freeze-dried residues, which is considered to be glyphosate, decreased from 23.9 % AR at 0 DAT to 28.2 % AR at 100 DAT in the Droevendaal soil. In the Maasdijk soil, it decreased from 41.1 % AR at 0 DAT to 4.3 % AR at 70 DAT and in the Lisse soil it decreased from 67.4 % AR at 0 DAT to 30.4 % AR at 100 DAT.

The amount of radioactivity not extractable from freeze-dried residues, which is considered to be glyphosate complexly bound to humic substances, decreased from 50.0 % AR at 0 DAT to 28.0 % AR at 100 DAT in the Droevendaal soil. In the Maasdijk soil it decreased from 49.3 % AR at 0 DAT to 4.3 % AR at 70 DAT and in the Lisse soil it fluctuated between 16.4 and 39.0 % AR.

Non-extractable radioactivity (NER) increased from 0 DAT to 70 DAT from 3.6 to 9.1 % AR and decreased to 8.7 % AR at 100 DAT (mean of two replicates) for the Maasdijk soil. NER were not determined for the Droevendaal and Lisse soil.

All radioactivity extracted was considered to be glyphosate. According to the TLC, no known metabolites of glyphosate were found in the extracts after freeze-drying.

I. MATERIALS AND METHODS

A. MATERIALS
1. Test Material:
Identification: [^{14}C-phosphonomethyl]-glyphosate
Lot No.: CFA.745 batch 17
Specific activity: 12.3 MBq/mg
Radiochemical purity: ≥98.9 %

Identification: glyphosate (non-radiolabelled)
Lot No.: F92/-/086
Chemical purity: 99 %

2. Soil:
About one to two months prior to application, the soils were sampled from experimental stations (Droevendaal and Lisse) or an apple orchard (Maasdijk). Until the start of pre-incubation, soils were stored at 3 ± 2 °C for a maximum of 68 days. Soils were partly air-dried and sieved to ≤ 2 mm. The moisture adjusted to 0.32 bar and soil pre-incubated at 20 °C for seven days before application. Characteristics of the test soils are presented in the table below.

Table 7.1.1.1-20: Characteristics of test soils

Parameter	Results		
Soil name	Droevendaal	Maasdijk	Lisse
Soil	Humic sand	Sandy loam	Low humic-content (lhc) sand
Origin	Experimental farming station Droevendaal	Apple orchard located at the Maasdijk	Laboratory for Bulb-Research at Lisse
Country	Netherlands	Netherlands	Netherlands
Textural Class	Humic sand	Sandy loam	Low humic-content sand
Sand (>50 µm) (%)	88.6	64.0	96.7
Silt (2 µm – 50µm) (%)	8.1	24.1	0.5
Clay (< 2 µm) (%)	3.3	11.8	2.9
pH (KCl)	5.2	7.5	7.2
Organic carbon (%) [1]	2.32	1.10	0.64
Organic matter (%)	4.0	1.9	1.1
Moisture at pF 2.5 (g/100 g dry soil)	13.7	12.7	4.3
Microbial biomass (mg C/kg)			
Start of the study (2 DAT)	102	196	22
Study end (107 DAT)	70	136	13

DAT = days after treatment
[1] Calculated from organic carbon according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions
The rate of degradation was determined in two soils (Droevendaal and Lisse) by measuring the extractable radioactivity in the soils and by characterisation of the glyphosate present in the extracts. The metabolism of glyphosate was determined in one soil (Maasdijk soil) by monitoring the evolution of ^{14}C-carbon dioxide as a measure of mineralisation of the labelled carbon, by determining the extractable radioactivity in the soils and by characterisation of the radioactive compounds present. The amount of non-extractable residues was also determined.

Static test systems were used, consisting of glass flasks filled with 50 g of sieved soil (dry weight equivalents) and topped with a glass tube containing oil covered quartz wool for collection of organic volatiles and CO_2-absorbing soda lime.

The study application rate was 3.8 mg/kg. The test item was applied to each test system as a mixture of radiolabelled and unlabelled glyphosate in 500 µL aqueous solution, resulting in 157 kBq [^{14}C]-glyphosate and 0.18 mg unlabelled glyphosate per test system.

Test systems were incubated under aerobic conditions in the dark for 15 weeks at 20 ± 2 °C and a soil moisture of 0.32 bar. About every five weeks the loss of water was compensated.

2. Sampling
Duplicate test systems were processed and analysed 0, 1, 2, 4, 8 and 15 weeks after treatment. According to the tables in the report this corresponds to 0, 7, 14, 35, 70 and 100 days after treatment (DAT).

3. Analytical procedures
Before opening the Maasdijk soil test vessels, test systems were blown through with moist air to force volatiles into the traps.

At each sampling interval, soil samples of all soils were extracted with 0.5 N NH_4OH solution for 5 minutes several times until the last extract contained < 5 % of the applied radioactivity. Extracts and soil were separated by centrifugation for 5 minutes. All extracts were pooled and freeze-dried. The residue after freeze-drying was extracted with 18 % HCl solution. A considerable amount of radioactivity was not extractable from the residue after freeze-drying. This occurred already immediately after adding glyphosate to soil. It was assumed in the report that this fraction could partly be explained by glyphosate complexly bound to humic substances, which had been extracted from the soils at very alkaline conditions (NH_4OH).

The amounts of glyphosate and its metabolites were determined by thin layer chromatography TLC in concentrated extracts. Plates were developed in isobutyric acid:water:1-propanol:concentrated ammonium hydroxide:2-propanol:1-butanol (500:95:70:20:15:15) with 0.24 g of sodium-EDTA.

The test item and it metabolite aminomethyl phosphonic acid (AMPA) were identified by co-chromatography with reference items.

For the Maaskijk soil, the amount of volatiles (soda lime and and oil-covered glass wool) non-extractable residues was determined by LSC and combustion/LSC, respectively.

II. RESULTS AND DISCUSSION

A. DATA
Distribution of residues of [^{14}C]-glyphosate in the tested soils are summarised in the tables below.

Table 7.1.1.1-21: Degradation of [^{14}C]glyphosate in Droevendaal soil under aerobic conditions (values expressed as percent of applied radioactivity)

		DAT					
Compound	**Replicate**	**0**	**7**	**14**	**35**	**70**	**100**
NH_4OH extract [1]	A	73.7	62.7	46.4	56.6	58.5	56.7
	B	74.0	61.1	47.8	58.3	58.5	55.7
	Mean	*73.9*	*61.9*	*47.1*	*57.5*	*58.5*	*56.2*
Glyphosate [2]	A	16.1	26.0	31.1	25.1	20.7	25.4
	B	31.7	22.1	28.4	32.0	25.0	31.0
	Mean	*23.9*	*24.1*	*29.8*	*28.6*	*22.9*	*28.2*
Complexed Glyphosate [3]	A	57.6	36.7	15.3	31.5	37.8	31.3
	B	42.3	39.0	19.4	26.3	33.5	24.7
	Mean	*50.0*	*37.9*	*17.4*	*28.9*	*35.7*	*28.0*

[1] Radioactivity extractable with 0.5 M NH_4OH
[2] Radioactivity extracted with 18 % HCl after freeze-drying, considered to be glyphosate according to TLC ("free glyphosate")
[3] Radioactivity not extractable from freeze-dried residues, considered to be glyphosate complexly bound to humic substances
Values in *italics* are calculated in the course of summary preparation
DAT: days after treatment

Table 7.1.1.1-22: Degradation of [^{14}C]glyphosate in Maasdijk soil under aerobic conditions (values expressed as percent of applied radioactivity)

		DAT					
Compound	**Replicate**	**0**	**7**	**14**	**35**	**70**	**100**
NH_4OH extract [1]	A	89.4	37.2	24.9	16.1	8.7	4.2
	B	91.3	36.7	25.2	15.5	8.2	3.9
	Mean	*90.4*	*37.0*	*25.1*	*15.8*	*8.5*	*4.1*
Glyphosate [2]	A	36.8	13.0	9.6	8.4	4.6	ND
	B	45.4	15.5	10.5	7.5	3.9	ND
	Mean	*41.1*	*14.3*	*10.1*	*8.0*	*4.3*	*ND*
Complexed Glyphosate [3]	A	52.6	24.2	15.3	7.7	4.2	ND
	B	45.9	21.1	14.7	8	4.3	ND
	Mean	*49.3*	*22.7*	*15.0*	*7.9*	*4.3*	*ND*
Carbon Dioxide	A	ND	47	59.6	67.4	77.3	79.9
	B	ND	48.4	59.4	65.9	72.5	79.3
	Mean	*ND*	*47.7*	*59.5*	*66.7*	*74.9*	*79.6*
Volatile organic compounds	A	ND	0.1	0.4	0.1	0.1	0.3
	B	ND	0.1	0.4	0	0.3	0.2
	Mean	*ND*	*0.1*	*0.4*	*0.1*	*0.2*	*0.3*
Non-extractable Residues	A	3.3	7.2	7.8	8.1	9.1	7.5
	B	3.8	6.9	10.1	7.6	9.1	9.8
	Mean	*3.6*	*7.1*	*9.0*	*7.9*	*9.1*	*8.7*
Mass balance	A	92.7	91.4	92.7	91.7	95.2	91.9
	B	95.1	92.1	95.1	99	90.1	93.2
	Mean	*93.9*	*91.8*	*93.9*	*95.4*	*92.7*	*92.6*

[1] Radioactivity extractable with 0.5 M NH_4OH, not used for material balance
[2] Radioactivity extracted with 18 % HCl after freeze-drying, considered to be glyphosate according to TLC
[3] Radioactivity not extractable from freeze-dried residues, considered to be glyphosate complexly bound to humic substances
Values in *italic*s are calculated in the course of summary preparation
DAT: days after treatment
ND: not determined

Table 7.1.1.1-23: Degradation of [^{14}C]glyphosate in Lisse soil under aerobic conditions (values expressed as percent of applied radioactivity)

		DAT					
Compound	**Replicate**	**0**	**7**	**14**	**35**	**70**	**100**
NH_4OH extract [1]	A	98.2	83.3	80.3	68.5	55.7	58.2
	B	97.9	84.5	81.1	68.7	56.4	53.6
	Mean	*98.1*	*83.9*	*80.7*	*68.6*	*56.1*	*55.9*
Glyphosate [2]	A	61.7	54.6	36.4	40.6	40.2	30.3
	B	73.1	56.1	47.0	36.9	39.2	30.4
	Mean	*67.4*	*55.4*	*41.7*	*38.8*	*39.7*	*30.4*
Complexed Glyphosate [3]	A	27.7	28.7	43.8	28.7	15.5	27.9
	B	24.8	28.4	34.1	31.7	17.2	23.2
	Mean	*26.3*	*28.6*	*39.0*	*30.2*	*16.4*	*25.6*

[1] Radioactivity extractable with 0.5 M NH_4OH
[2] Radioactivity extracted with 18 % HCl after freeze-drying, considered to be glyphosate according to TLC ("free glyphosate")
[3] Radioactivity not extractable from freeze-dried residues, considered to be glyphosate complexly bound to humic substances
Values in *italic*s are calculated in the course of summary preparation
DAT: days after treatment

B. MASS BALANCE
Material balances ranged from 91.8 to 95.4 % AR (mean of two replicates) for the Maasdijk soil. Material balances were not established for the other two soils (Droevendaal and Lisse).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
The amount of radioactivity extractable with 0.5 M NH_4OH decreased from 0 DAT to 100 DAT from 73.9 to 56.2 % AR with an intermediate minimum of 47.1 % AR at 14 DAT in the Droevendaal soil. In the Lisse soil, extractable radioactivity decreased from 98.1 at 0 DAT to 55.9 % AR at 100 DAT. In the Maasdijk soil, extractable radioactivity decreased from 90.4 at 0 DAT to 4.1 % AR at 100 DAT.

The amount of radioactivity extractable from freeze-dried residues, which is considered to be glyphosate, decreased from 23.9 % AR at 0 DAT to 28.2 % AR at 100 DAT in the Droevendaal soil. In the Lisse soil it decreased from 67.4 % AR at 0 DAT to 30.4 % AR at 100 DAT and in the Maasdijk soil it decreased from 41.1 % AR at 0 DAT to 4.3 % AR at 70 DAT.

The amount of radioactivity not extractable from freeze-dried residues, which is considered to be glyphosate complexly bound to humic substances, decreased from 50.0 % AR at 0 DAT to 28.0 % AR at 100 DAT in the Droevendaal soil. In the Lisse soil it fluctuated between 16.4 and 39.0 % AR and in the Maasdijk soil it decreased from 49.3 % AR at 0 DAT to 4.3 % AR at 70 DAT.

The amount of non-extractable residues (NER), which was only determined for the Maasdijk soil, increased from 0 DAT to 70 DAT from 3.6 to 9.1 % AR and decreased to 8.7 % AR at 100 DAT (mean of two replicates).

D. VOLATILE RADIOACTIVITY
The maximum amount of carbon dioxide reached at study end (100 DAT) was 79.6 % AR. Organic volatiles were found with a maximum amount of 0.3 % AR at 100 DAT (all values mean of two replicates). Volatiles were only determined for sandy loam soil.

E. TRANSFORMATION OF THE TEST ITEM
All radioactivity extracted was considered to be glyphosate. According to TLC no known metabolites of glyphosate were found in the extracts after freeze-drying.

F. KINETICS
The DT_{50} value for the degradation of glyphosate was calculated by first order kinetics (not according to current guidance) to be < 7 days in the Maasdijk soil, 180 days for Droevendaal soil and 110 days for Lisse soil. As the study is considered invalid, kinetic evaluation was not updated.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study shows major deficiencies. Problems probably resulting from inadequate work-up procedures caused high portions of radioactivity that remained sticking to the soil residues thus not allowing to re-constitute the formerly water soluble radioactive residues after freeze-drying into a solvent for analysis. Radioactivity lost during freeze-drying was assigned to glyphosate complexly bound to humic substances (co-)extracted. For two soils, no full material balance was established, i.e. non-extractable residues and volatiles were not determined.
Therefore, the study is considered invalid and was not used for endpoint derivation.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.1/008
Report author	[redacted]
Report year	1991
Report title	Aerobic metabolism of [14C]Glyphosate in sandy loam and silt loam soils with biometer flask
Report No	368
Document No	
Guidelines followed in study	U.S. EPA 162-1
Deviations from current test guideline	From OECD 307: - samples aerated with oxygen instead of air - procedural recoveries were rather variable (soil Kickapoo: 86.2 to 136.7 %, soil Dupo: 87.7 to 143.8 %); recoveries corrected for values below 90 % or above 110 % - mass balance <90 % for some sampling points, probably due to loss of carbon dioxide - high variation of recoveries between the two replicates of day 0 - duration of study 12 months following US data requirements
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

Data point:	CA 7.1.1.1/009
Report author	[redacted]
Report year	1992
Report title	Review of the Aerobic Metabolism of [^{14}C]Glyphosate in Soil - Addendum to Monsanto Report No. PTRL 368
Report No	368
Document No	
Guidelines followed in study	see CA 7.1.1.1/008
Deviations from current test guideline	see CA 7.1.1.1/008
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The degradation of [^{14}C]-glyphosate was investigated in two soils under aerobic conditions in the dark in the laboratory at 25 ± 0.1 °C and 75 ± 10 % of the field capacity for 12 months.
The following two soils were used: a sandy loam soil from Kickapoo, Kentucky, USA and a silt loam soil from Dupo, Missouri, USA. The amount of organic carbon of the soils was 1.6 and 0.6 % and the pH was 7.3 and 7.5, respectively.

The test was performed in flow-through systems, kept under pressure with pure oxygen and connected to a 1 N NaOH trap to collect carbon dioxide and a foam plug to collect volatile organic compounds.

The application rate of glyphosate was 4 mg/kg soil (dry weight).

Duplicate samples from each system were processed and analysed at 0, 1, 3, 7, 14 days after treatment (DAT) and 1, 2, 3, 4, 6, 9 and 12 months after treatment. The foam plug and NaOH trap were assayed and changed at all sampling points up to month 4 and changed monthly afterwards.

Material balances ranged from 71.3 to 94.8 % of applied radioactivity (% AR) for soil Kickapoo, from 84.0 to 103.2 % AR for soil Dupo.

Maximum amounts of carbon dioxide reached at 9 months of incubation were 71.8 and 82.9 % AR in soils Kickapoo and Dupo, respectively. At the end of the study a slight decrease to 70.8 and 78.3 % AR, for soils Kickapoo and Dupo, respectively (each value as mean of two replicates). No organic volatiles were determined for both soils at all sampling points.

The amount of radioactivity in soil decreased from 0 DAT to 12 months after treatment from 69.4 to 6.5 % AR in soil Kickapoo and from 89.5 to 4.7 % AR in soil Dupo.

In soil Kickapoo, NER increased from 3.5 % AR at 0 DAT to 8.8 % AR 6 months after treatment and decreased to 7.4 % AR 12 months after treatment. In soil Dupo, NER increased from 4.1 % AR at 0 DAT to 6.1 % AR 3 months after treatment and decreased to 4.2 % AR 12 months after treatment (each value as mean of two replicates).

The amount of glyphosate in soil extracts decreased from 0 DAT to 12 months of incubation from 47.6 to 0.5 % of the applied radioactivity in soil Kickapoo and from 73.3 to 0.6 % of the analysed radioactivity in soil Dupo (mean of two replicates).

Besides carbon dioxide, one major metabolite was detected. AMPA was detected with a maximum amount of 26.3 % AR at 14 DAT in soil Kickapoo and decreased to below 2 % of the applied radioactivity after 12 months of incubation. In soil Dupo, AMPA was detected with a maximum amount of 28.7 % AR at 14 DAT and decreased to below 2 % of the applied radioactivity after 12 months of incubation (all values mean of two replicates). No other metabolites were detected above 5 % AR at any time.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]-phosphonomethyl-glyphosate
Lot No.:	C-927.45
Specific activity:	3.98 mCi/mmole
Radiochemical purity:	98.8 %

2. Soil:
Soils were sieved to ≤2 mm and air-dried. Characteristics of the test soils are presented in the table below.

Table 7.1.1.1-24: Characteristics of test soils

Parameter	Results	
Soil	Kickapoo	Dupo
Country	Kentucky, USA	Missouri, USA
Pesticide use history	Soils had not been treated with pesticides during the past five years	
Textural Class	Sandy Loam	Silt Loam
Sand (%)	68	24
Silt (%)	22	68
Clay (%)	10	8
pH [1]	7.3	7.5
Organic carbon (%) [2]	1.6	0.6
Organic matter (%)	2.8	1.0
Cation exchange capacity (meq/100 g)	9.0	10.7
Water Holding Capacity at 0.33 bar (%)	21.0	18.0
Microbiological characteristics (before study initiation) (total colony forming units , CFU)		
Aerobic Bacteria	6.2×10^5	4.0×10^6
Actinomycetes	5.8×10^5	3.2×10^6
Fungi	1.1×10^3	4.2×10^4

DAT = days after treatment, USDA: United States Department for Agriculture
[1] Medium/method not reported
[2] Calculated from organic matter according to OC = OM × 0.58

B. STUDY DESIGN

1. Experimental conditions
Flow-through test systems were used, consisting of biometer flasks filled with 50 g of sieved soil (dry weight equivalents). The biometer flasks had a side arm to which 50 mL of 1 N NaOH was added to trap CO_2. A pre-extracted (acetone) polyurethane foam plug was placed in the side arm connector to trap volatile organic compounds. An equilibrium was established in the biometer flasks by way of the humidified oxygen passed through the system which maintained a positive pressure on flasks to accommodate pressure differences realized by the adsorption of $^{14}CO_2$ into the NaOH upon its formation.

A test solution of [^{14}C]-glyphosate with a concentration of 2.4 µCi/mL was prepared in water. 2 mL of this solution were applied to each test system, resulting in a final concentration of 4 mg/kg. After application the soil moisture was adjusted to 75 % of the field capacity by addition of water.

Test systems were incubated under aerobic conditions in the dark for 12 months at 25 ± 0.1 °C and 75 ± 10 % of the field capacity.

Sterilised (autoclaved) samples were incubated in parallel and sampled 1, 3 and 6 month after treatment.

2. Sampling
Duplicate test systems were processed and analysed 0, 1, 3, 7, 14 days after treatment (DAT) and 1, 2, 3, 4, 6, 9 and 12 months after treatment. The foam plug and NaOH trap were assayed and changed at all sampling points up to month 4 and changed monthly afterwards.

3. Analytical procedures

At each sampling interval, soil samples were extracted three times with 0.5 N KOH. After centrifugation the pooled extracts were radioassayed by liquid scintillation counting (LSC). Selected samples were furthermore extracted with 1 N KOH and all extracts of a respective sample were pooled. Prior to HPLC analysis the pooled extracts were cleaned up by solid phase extraction and eluted with 0.1 N ammonium hydroxide. Recovery of radioactivity was investigated for the applied clean-up procedure. Recoveries were in the range from 86.2 to 136.7 % for soil Kickapoo and in the range from 87.7 to 143.8 % for soil Dupo. If recoveries were below 90 % or above 110 % calculations were corrected for the respective recovery.

The cleaned-up extracts were concentrated under reduced pressure using a rotary evaporator and then analysed by HPLC/radiodetection. The limit of detection (LOD) for the HPLC/radiodetection method was two times the background noise. The amount of volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

Glyphosate and metabolites were identified by radio-HPLC co-chromatography with reference items using a different HPLC system as used for separation.

The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents. The formed precipitate was titrated with acid.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised in the tables below for the respective soils.

Table 7.1.1.1-25: Degradation of (^{14}C)glyphosate in soil Kickapoo under aerobic conditions (expressed as percent of applied radioactivity)

		Time after treatment											
		Days					Months						
Compound	Replicate	0	1	3	7	14	1	2	3	4	6	9	12
Glyphosate	A	50.6	32.8	18.9	10.6	5.4	2.8	1.6	2.0	2.1	1.9	0.9	0.5
	B	44.5	32.0	15.9	7.7	6.4	2.8	2.1	1.1	1.3	0.8	0.5	0.6
	Mean	47.6	32.4	17.4	9.2	5.9	2.8	1.9	1.6	1.7	1.4	0.7	0.5
AMPA	A	12.9	21.6	31.1	28.5	23.9	17.3	9.9	7.7	5.4	3.7	2.8	1.7
	B	19.0	25.0	20.0	18.0	28.6	17.0	12.1	5.5	4.1	3.5	0.1	2.1
	Mean	16.0	23.3	25.6	23.3	26.3	17.2	11.0	6.6	4.8	3.6	1.5	1.9
Unknown A	A	1.8	2.7	2.8	3.5	2.2	2.3	2.9	1.7	2.6	2.3	2.0	1.7
	B	2.5	2.5	2.1	2.4	1.8	2.7	3.0	2.0	2.4	1.9	1.4	1.3
	Mean	2.2	2.6	2.5	3.0	2.0	2.5	3.0	1.9	2.5	2.1	1.7	1.5
Unknown B	A	2.0	2.5	0.7	3.6	1.2	2.4	1.8	0.8	1.0	0.9	1.2	0.9
	B	2.6	2.3	2.4	2.4	1.3	2.2	2.0	1.3	1.4	1.0	0.7	0.5
	Mean	2.3	2.4	1.6	3.0	1.3	2.3	1.9	1.1	1.3	1.0	1.0	0.7
Unknown C	A	ND	ND	ND	0.5	ND	0.6	0.3	0.2	0.4	0.3	0.4	0.2
	B	ND	ND	ND	ND	ND	ND	ND	0.3	ND	0.3	0.1	0.2
	Mean	NA	NA	NA	NA	NA	NA	NA	0.3	NA	0.3	0.3	0.2
Others	A	ND	ND	ND	ND	0.4	1.3	ND	0.2	0.1	ND	0.2	ND
	B	0.8	0.7	0.4	ND	ND	ND	0.5	0.1	0.3	ND	0.1	0.2
	Mean	NA	NA	NA	NA	NA	NA	NA	0.2	0.2	NA	0.2	NA
Carbon Dioxide	A	NA	23.5	33.6	38.8	47.2	61.0	59.8	68.7	64.0	66.8	69.6	71.0
	B	NA	24.6	38.3	44.4	47.3	54.1	59.1	67.5	67.1	72.5	74.0	70.5
Volatile organic compounds	A	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total extractable radioactivity	A	68.1	54.2	47.8	40.1	30.3	22.9	19.2	14.4	11.5	9.5	6.6	6.7
	B	70.6	62.5	45.7	39.0	38.6	28.2	17.9	12.8	11.6	9.1	6.6	6.3

Table 7.1.1.1-25: Degradation of (^{14}C)glyphosate in soil Kickapoo under aerobic conditions (expressed as percent of applied radioactivity)

		Time after treatment											
		Days					Months						
Compound	Replicate	0	1	3	7	14	1	2	3	4	6	9	12
Non-extractable radioactivity	A	3.2	4.9	7.8	7.1	9.5	7.5	8.5	7.8	8.1	11.9	6.1	7.8
	B	3.7	7.7	5.7	6.0	6.5	7.9	6.1	9.4	8.6	5.6	6.9	7.0
Mass balance	A	71.3	82.6	89.3	86.0	87.0	91.5	87.6	91.0	83.6	88.2	82.3	85.4
	B	74.3	94.8	89.6	89.4	92.4	90.2	83.1	89.6	87.4	87.3	87.5	83.9

NA: not applicable; ND: not detected

Table 7.1.1.1-26: Degradation of (^{14}C)glyphosate in soil Dupo under aerobic conditions (expressed as percent of applied radioactivity)

		Time after treatment											
		Days					Months						
Compound	Replicate	0	1	3	7	14	1	2	3	4	6	9	12
Glyphosate	A	64.3	49.7	27.3	13.9	7.0	2.7	1.5	1.7	1.1	0.7	0.8	0.4
	B	82.2	61.5	23.9	11.7	5.6	3.3	1.5	1.4	1.3	0.7	0.6	0.7
	Mean	73.3	55.6	25.6	12.8	6.3	3.0	1.5	1.6	1.2	0.7	0.7	0.6
AMPA	A	14.3	22.0	26.3	28.0	34.6	22.3	14.7	10.0	5.1	2.8	2.1	1.6
	B	18.7	19.1	21.7	23.4	22.7	26.3	14.9	11.4	8.8	4.3	2.2	1.5
	Mean	16.5	20.6	24.0	25.7	28.7	24.3	14.8	10.7	7.0	3.6	2.2	1.6
Unknown A	A	1.4	2.0	1.7	1.7	1.4	1.8	1.6	1.7	1.6	0.8	1.0	1.7
	B	0.9	2.4	1.3	1.6	2.8	2.2	1.4	1.7	1.8	1.3	1.1	0.9
	Mean	1.2	2.2	1.5	1.7	2.1	2.0	1.5	1.7	1.7	1.1	1.1	1.3
Unknown B	A	0.9	0.9	0.9	1.3	0.6	0.8	0.9	1.0	1.0	0.4	0.4	0.8
	B	0.9	1.0	1.2	1.3	3.1	1.3	0.7	0.9	0.9	0.7	0.6	0.3
	Mean	0.9	1.0	1.1	1.3	1.9	1.1	0.8	1.0	1.0	0.6	0.5	0.6
Unknown C	A	ND	ND	ND	ND	ND	ND	ND	ND	0.1	0.2	0.1	0.2
	B	ND	ND	ND	ND	ND	ND	ND	ND	0.2	0.1	0.1	0.2
	Mean	NA	NA	NA	NA	NA	NA	NA	NA	0.2	0.2	0.1	0.2
Others	A	ND	ND	1.0	ND	ND	ND	ND	ND	ND	0.1	ND	0.4
	B	4.6	ND	ND	ND	ND	ND	ND	ND	0.6	0.1	ND	ND
	Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.1	NA	NA
Carbon Dioxide	A	NA	16.9	32.2	36.9	46.5	58.7	65.2	71.7	76.4	79.7	81.9	78.0
	B	NA	16.3	32.8	38.4	47.2	57.4	63.8	72.1	75.4	80.4	83.8	78.6
Volatile organic compounds	A	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total extractable radioactivity	A	81.3	75.5	65.2	45.5	39.9	28.2	21.4	11.4	8.8	6.9	5.4	4.4
	B	97.9	67.9	48.1	38.6	39.6	25.8	18.7	15.7	11.9	7.2	5.4	4.9
Non-extractable Radioactivity	A	2.7	7.0	7.0	5.0	7.8	5.7	7.0	6.2	4.7	5.0	5.3	3.7
	B	5.4	3.0	3.9	4.4	6.8	5.2	4.7	5.9	5.4	5.2	4.9	4.7
Mass balance	A	84.0	99.5	104.4	87.4	94.1	92.6	93.5	89.3	89.8	91.7	92.6	86.1
	B	103.2	87.1	84.8	81.4	93.6	88.4	87.2	93.6	92.8	92.9	94.1	88.3

NA: not applicable; ND: not detected

B. MASS BALANCE

Material balances ranged from 71.3 to 94.8 % of applied radioactivity (% AR) for soil Kickapoo, from 84.0 to 103.2 % AR for soil Dupo.

The total recoveries of applied in sterilized samples was radiocarbon were 100.0 ± 1.5 and 102.0 ± 5.3 % for the Kickapoo and Dupo soils, respectively.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in soil decreased from 0 DAT to 12 months after treatment from 69.4 to 6.5 % AR in soil Kickapoo and from 89.5 to 4.7 % AR in soil Dupo.

In soil Kickapoo, NER increased from 3.5 % AR at 0 DAT to 8.8 % AR 6 months after treatment and decreased to 7.4 % AR 12 months after treatment. In soil Dupo, NER increased from 4.1 % AR at 0 DAT to 6.1 % AR 3 months after treatment and decreased to 4.2 % AR 12 months after treatment (each value as mean of two replicates).

D. VOLATILE RADIOACTIVITY

In both test soils formation of $^{14}CO_2$ increased steadily during the experimental period. Maximum amounts of carbon dioxide reached at 9 months of incubation were 71.8 and 82.9 % AR in soils Kickapoo and Dupo, respectively. At the end of the study a slight decrease to 70.8 and 78.3 % AR, for soils Kickapoo and Dupo, respectively (each value as mean of two replicates). No organic volatiles were determined for both soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

In the sterilized samples, radiolabelled CO_2 accounted for 42.5 and 38.2 % AR after 6 months for Kickapoo and Dupo soils, respectively.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in soil extracts decreased from 0 DAT to 12 months of incubation from 47.6 to 0.5 % of the applied radioactivity in soil Kickapoo and from 73.3 to 0.6 % of the analysed radioactivity in soil Dupo (mean of two replicates).

Besides carbon dioxide, one major metabolite was detected. AMPA was detected with a maximum amount of 26.3 % AR at 14 DAT in soil Kickapoo and decreased to below 2 % of the applied radioactivity after 12 months of incubation. In soil Dupo, AMPA was detected with a maximum amount of 28.7 % AR at 14 DAT and decreased to below 2 % of the applied radioactivity after 12 months of incubation (all values mean of two replicates). No other metabolites were detected above 5 % AR at any time.

In the sterilized flasks, degradation of glyphosate was significantly less rapid. In soil Kickapoo, glyphosate accounted to 46.3 % AR and AMPA to 12.9 % AR after 6 months. In soil Dupo, glyphosate accounted to 44.6 % AR and AMPA to 17.3 % AR after 6 months.

F. KINETICS

The DT_{50} values for glyphosate were calculated using a non-linear, first-order kinetic model, not according to current guidance. Degradation of glyphosate was very fast with are DT_{50} values of 1.85 and 2.06 days for Kickapoo sandy loam and Dupo silt loam soils, respectively. As the study is considered invalid, kinetic evaluation was not updated.

III. CONCLUSIONS

Glyphosate is rapidly degraded in soil under aerobic conditions. The primary degradation products in both soils are CO_2 and aminomethyl phosphonic acid (AMPA). Several low-level unidentified metabolites are also produced. However, none of these unidentified products constitute greater than 3.6 % of the initial glyphosate concentration and, therefore, are considered insignificant. The proposed metabolic pathway involved the initial conversion of glyphosate to AMPA, followed by further metabolism of AMPA to CO_2.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study conduct had a major deficiency compared to current guidelines by pressing pure oxygen for aeration through the samples rather than to allow a gentle stream of air to pass through.
In addition, a number of minor deviations occurred including recoveries to be below 90 %, often for soil Kickapoo and occasionally for soil Dupo, presumably due to a loss of volatiles/CO_2.
Due to the major deficiency in conduct the study is considered invalid and was not used for endpoint derivation.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.1/010
Report author	[redacted]
Report year	1985
Report title	Metabolism of SC-0224 in soil: Fate of the anion moiety
Report No	PMS-186
Document No	
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision N: Chemistry: Environmental Fate Section 162-1 (October, 1982)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: aerobic soil metabolism Test item: [^{14}C] glyphosate, phosponomethyl-label (96.5 % radiochemical purity) Test soil: Sorrento (origin not reported) Soil type: loam pH: 6.8 (medium not stated) Organic matter: 3.1 % Two separate experiments were conducted: a “large-scale” experiment with a duration of 376 days and a “small-scale” experiment with a duration of 21 days for identification of glyphosate and its metabolite. <u>Large-scale experiment:</u> Application rate: 30 mg/kg Test design: flow-through system with biometer flasks flushed with pure oxygen Volatiles trapping: CO_2: NaOH solution in flow-through trap Organic volatiles: polyurethane foam plug Incubation: 23 °C (temperature-controlled), soil moisture adjusted to field capacity Sampling: 0, 5, 9, 30, 76, 150, 310, 344 and 376 DAT, duplicate samples

	Workup: twofold extraction with 0.5 M NH_4OH solution (only samples up to 150 DAT) Analysis of radioactivity: Extracts: LSC (combined extracts) NER: combustion/LSC Volatiles: LSC; presence of $^{14}CO_2$ confirmed by $BaCl_2$ precipitation. Identification of radioactive residues: none <u>Small-scale experiment</u> Application rate: 30 mg/kg Test design: static system with sealed Wheaton serum bottles; pure oxygen was supplied under positive pressure Volatiles trapping: CO_2: 10 % KOH solution in small tubes lined with filter paper, placed in test vessels Organic volatiles: none Incubation: ambient temperature (between 18 and 26.7 °C), soil moisture adjusted to field capacity Sampling: 0, 4, 9 and 21 DAT, duplicate samples Workup: twofold extraction with 0.5 M NH_4OH solution Analysis of radioactivity: Extracts: LSC (individual extracts) NER: combustion/LSC Volatiles: LSC Identification of radioactive residues: TLC with reference standards
Short description of results:	<u>Large-scale experiment:</u> Recovery of radioactivity: 90.5 – 103.1 % AR Mineralization: 83.1 % AR after 376 days Other volatiles: none Extractable radioactivity (mean values): 57.6 % AR at 0 DAT, 6.9 % AR at 150 DAT for later samplings, no extraction performed. Non-extractable radioactivity (mean values): 40.3 % AR at 0 DAT, 16.6 % AR at 376 DAT Transformation of test item: not analysed <u>Small-scale experiment:</u> Recovery of radioactivity (mean values): 91.6 – 99.9 % AR Mineralization: 37.2 % AR after 21 days Other volatiles: not analysed Extractable radioactivity (mean values): 37.2 % AR at 0 DAT, 27.3 % AR at 21 DAT Non-extractable radioactivity (mean values): 17.3 % AR at 0 DAT, 31.5 % AR at 21 DAT Transformation of test item (TLC analysis): Glyphosate: 78.0 % AR at 0 DAT, 8.2 % AR at 21 DAT AMPA: 0.4 % AR at 0 DAT, 15.4 % AR at 21 DAT The half-life of glyphosate was estimated from the small-scale experiment to about 3 days.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies in study conduct: - Test systems were aerated with pure oxygen instead of air. - For the main ('large-scale') degradation experiment, glyphosate and its metabolites were not identified. - For the 'small-scale' experiment, incubation temperature was not controlled or reported.
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.1.1/011
Report author	[redacted]
Report year	1972
Report title	The degradation and metabolism of MON-0573 in soil
Report No	269
Document No	
Guidelines followed in study	U.S. Department of Agriculture (ARS, Pesticides Regulation Division): Pesticide Registration (PR) Notice 70-15 "Guidelines For Studies to Determine the Impact of Pesticides on the Environment," June 23, 1970
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (Final Addendum, 2015)
Short description of study design and observations:	Study type: aerobic/anaerobic soil metabolism, degradation in water Test item: [^{14}C]-glyphosate, phosphonomethyl-label (97 % radiochemical purity), 1-glycine label (96 % radiochemical purity), 2-glycine label (99 % radiochemical purity) Test soils (soil type): Ray (silt loam), Drummer (silty clay loam), Lintonia (sandy loam), Norfolk (sandy loam) pH: 6.5, 7.0, 6.0, 5.7 (method not stated) Organic matter: 1.0 %, 6 %, 1 %, 1 % The total study included various tests including aerobic and anaerobic degradation (samples water-logged) in non-sterile and sterilized soil (soil Ray only). Tests with exaggerated application rates performed for identification of metabolites (soil Ray). This summary focuses on the results of aerobic degradation tests. Application rate: 109 to 126 mg/kg for the different labels, 1000 mg/kg for metabolite identification with test substance applied to water phase, i.e. not applied directly to soil Test design: 5 g soil suspended in 100 mL water, continuously agitated by shaking; 100 g soil and 1000 mL for large scale tests Volatiles trapping: CO_2: ascarite trap Organic volatiles: no trapping Incubation: 30 °C, continuous shaking, soil flooded/suspended Sampling: 0, 1, 3, 7, 14, 21, 28 days after treatment (DAT) for soil Ray, 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 66, 77, 84, 91, 105 and 112

	DAT for soil Norfolk, 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 66, 77 and 84 DAT for soil Drummer, 0, 1, 3, 7, 14, 21, 28 and 35 DAT for soil Lintonia, single samples collected per soil and sampling interval Workup: taking of an aliquot of the soil-water suspension, centrifugation, washing of soil with water, lyophilisation of soil, threefold extraction with 0.5 N aqueous NH_4OH solution at ambient temperature Determination of radioactivity: Extracts: LSC NER: combustion/LSC Volatiles: ascarite treated with HCl, trapping in 0.25 N NaOH, LSC Identification of radioactive residues: TLC/radiodetection co-chromatography with reference items, ^{1}H and ^{31}P-NMR
Short description of results:	Recovery of radioactivity: 68.7 – 109.8 % AR for all glyphosate labels and soils at the day of experiment termination Mineralization: 46.8 to 55.3 % AR for soil Ray, 5.8 to 9.3 % AR for soil Norfolk, 34.7 to 41.4 % AR for soil Drummer, 14.3 % AR for soil Lintonia (for all soils at termination) Other volatiles: not measured Extractable radioactivity: 2.7 to 22.9 % AR at 28 DAT for soil Ray, 65.4 to 81.8 % AR at 112 DAT for soil Norfolk, 12.0 to 19.6 % AR at 84 DAT for soil Drummer, 18.3 % AR at 35 DAT for soil Lintonia Non-extractable radioactivity: 8.5 to 40.3 % AR at 28 DAT for soil Ray, 4.6 to 13.5 % AR at 112 DAT for soil Norfolk, 16.7 to 33.9 % AR at 84 DAT for soil Drummer, 2.6 % AR at 35 DAT for soil Lintonia Transformation of test item (TLC analysis): Glyphosate: 0.2 to 7.4 % AR at 14 DAT and not detected at 28 DAT for soil Ray, 45.6 to 80.1 % AR at 14 DAT and 0.8 to 16.3 % AR at 112 DAT for soil Norfolk, 12.5 to 25.5 % AR at 14 DAT and 7.6 to 15.7 % AR at 84 DAT for soil Drummer, 69.5 % AR at 14 DAT and 59.5 % AR at 35 DAT for soil Lintonia AMPA: 8.5 % AR at 14 DAT and 4.4 % AR at 28 DAT for soil Ray; 0.5 % AR at 14 DAT and 1.7 % AR at 28 DAT for soil Norfolk, 1.8 % AR at 14 DAT, 8.4 % R at 56 DAT and 8.3 % AR at 84 DAT for soil Drummer, 6.9 % AR at 14 DAT and 6.6 % AR at 35 DAT for soil Lintonia (phosphonomethyl-label only for all soils) No unknown metabolites were observed at > 5 % AR.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: - mixed aerobic/anaerobic design in conduct strongly beyond actual standards and guidelines in soil degradation testing, i.e. soil suspended in aqueous solution during incubation followed by application of the test substance - work-up of aliquots only instead of complete soil sample - closed system without aeration during incubation - incubation at 30 °C - soil history, sampling and storage not reported
Category study in AIR 5 dossier (L docs)	Category 3b

Relevant articles from literature search

1. Information on the study

Data point:	CA 7.1.1.1/012
Report author	[redacted]
Report year	2019
Report title	Degradation of glyphosate and bioavailability of phosphorus derived from glyphosate in a soil-water system
Document No	Water Research 163 (2019) 114840
Guidelines followed in study	None
Deviations from current test guideline	Many details are missing in the report to evaluate against OECD 307: - e.g. soil properties, exact soil water content during incubation, the size/mass of soil samples, procedures of work-up including procedural recoveries for glyphosate and AMPA (except for figure)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

In this study, liquid chromatography mass spectrometry (LC-MS) and electrospray ionization (ESI) source Q Extractive Orbitrap mass spectrometry (ESI-Orbitrap MS) was used to identify non-labelled/stable-labelled glyphosate degradation products and combined with sequential extraction and stable isotopes to investigate the degradation of glyphosate and transformation of phosphorous (P) product in a soil-water system. The LC-MS and ESI-Orbitrap MS results showed that glycine formed during the early stage but was rapidly utilized by soil microorganisms. AMPA started to accumulate at the late stage and was found to be 3-6 times more resistant than glyphosate against degradation; while no sarcosine was formed. The ^{18}O labeling and phosphate oxygen isotope results allowed a clear distinction of the fraction of inorganic P (P_i) derived from glyphosate, about half of which was then rapidly taken up and recycled by soil microorganisms. Our results provide the first evidence of the preferential utilization of glyphosate-derived P_i by microorganisms in the soil-water system. The rapid cycling of P_i derived from this disregarded source has important implications on nutrient management as well as water quality.

Materials and methods

Reagents and chemicals
Glyphosate (≥96 %), (Aminomethyl) phosphonic acid (≥98 %) and 9-Fluorenyl-methoxycarbonyl chloride (FMOC-Cl) (≥97 %) were obtained from Sigma-Aldrich. Isotope labeled compounds including glyphosate-2-^{13}C, ^{15}N, glycine-d_5 and sarcosine-d_3 (methyl-d_3) were purchased from Sigma-Aldrich. Other chemicals including glycine (≥99 %) and sarcosine (≥98 %) were purchased either from Acros Organics or Fisher Scientific. All the reagents were of analytical grade and stock solution were prepared with DI water.

Soil collection and incubation
A typical silt loam soil (0-15 cm depth) from the Agricultural Experiment Station research farm at the University of Delaware was used in this study. The detailed information about the soil characterization has been reported in a previous publication. After removing any plant residues and granular rock particles, the soil samples were air-dried, homogenized, passed through a 2 mm sieve, and stored until analyses.

A flowchart of the experimental and analytical approach used is shown in Figure 7.1.1.1-2. The first

degradation experiment was run to identify glyphosate and its degradation products in soil as well as to determine the degradation kinetics and half-lives of major products. The soil was incubated with 1 µmol/g unlabeled glyphosate at 20ºC in the dark with 60 % water content for 175 d. A separate experiment with dual isotope (^{13}C and ^{15}N) labeled glyphosate (1 µmol/g) spiked in soil was performed for 35 d to accurately identify degradation products. The control experiment was performed under the same condition but without glyphosate. The natural soil incubation included both biotic and abiotic degradations. Identical experiment run with autoclaved water and soil served as abiotic degradation. At selected time points, 5 g subsamples were collected into 50 mL centrifuge tubes and stored at -20 ºC until further analysis. All experiments were run in duplicate under the same condition.

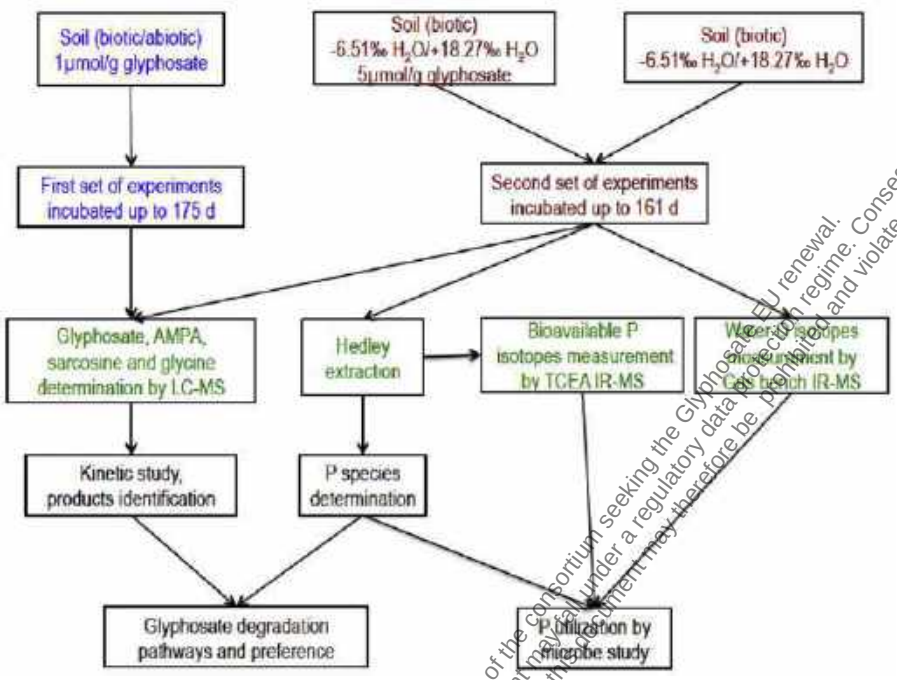
In order to identify P distribution and bioavailability during glyphosate degradation, the second set of experiments was performed in two ^{18}O-labeled waters ($\delta^{18}O_{H2O}$ = -6.51 and +18.27 ‰). To collect sufficient P for isotope analyses, 5 µmol/g unlabeled glyphosate was spiked into 300 g soil, and incubated with 600 mL ^{18}O-labeled water at 20 ºC in the dark for 161 d. The spiked glyphosate concentration is much higher than application dose in agriculture (about 1 kg/ha), but is required to obtain reliable phosphate isotopic analyses. The experimental containers were tightly capped to avoid any water evaporation that compromises the water oxygen isotopes. The containers were shaken every day for ~15 min to homogenize the system and then briefly ventilated to replenish ambient oxygen and to preserve the oxic condition. The control experiments were run under the same condition but in the absence of glyphosate. Subsampling and processing followed a similar procedure as described above.

Extraction and analyses of glyphosate, AMPA, glycine, and sarcosine
The extraction of glyphosate, AMPA, glycine, and sarcosine was based on the published method. Briefly, 1 g lyophilized soil samples degradation experiments were extracted with 5 mL 0.6 M KOH for 1 h by shaking at 140 rpm, then centrifuged at 2755 x g for 30 min. One mL of supernatant was removed and neutralized by HCl and then 0.12 mL of borate buffer (pH = 9) and 0.12 mL FMOC-Cl (12 g/L) were added and shaken for 1 min on a vortex mixer. After an overnight reaction at room temperature, the mixture was filtered with a 0.45 µm syringe filter for LC-MS analysis.

Glyphosate, AMPA, glycine, and sarcosine standards were prepared to develop the separation method by using an Acclaim 120, C18 column (2.1 x 250 mm) under a gradient eluent program. After testing and running several programs, the optimized gradient was identified to be effective with a mixture of two mobile phases with a flow rate of 0.35 mL/min with (A) acetonitrile and (B) 5 mmol/L HAc/NH_4Ac: 0-6 min, 20-40 % A, 80-60 % B; 6-9 min, 40-75 % A, 60-25 % B; 9-10.2 min, 75-100 % A, 65-0 % B; 10.2-12 min, 100 % A, 0 % B; 12-12.1 min, 100-20 % A, 0-80 % B; 12.1-14 min, 20 % A, 80 % B.

The chromatographic separation for each sample required 14 min.

Figure 7.1.1.1-2: Flowchart outlining glyphosate degradation experiments in the water-soil system



Glyphosate and its degradation products were identified and quantified by a Waters single quadrupole LC-MS equipped with PDA and SQ detector. The optimized MS parameters are as follows: ESI positive mode, capillary voltage 3 kV, cone voltage 40 V, desolvation temperature 200 ºC, desolvation gas flow 650 L/hr, and full mass scan from 100 to 500 m/z. The unlabeled glyphosate and labeled sarcosine were quantified with labeled glyphosate and unlabeled sarcosine as internal standards. Similarly, labeled glycine was quantified by labeled glycine as an external standard to avoid any interference from glycine already present in soil. AMPA was determined by the soil spiked external standards. Labeled glyphosate degradation samples were analyzed with a high resolution mass spectrometry-Q Extractive Orbitrap Mass Spectrometry (Thermo, Germany) at the University of Delaware. Orbitrap MS data were acquired under the positive mode with scan range from 100 to 1000 m/z. Glycine formation during labeled glyphosate degradation were determined by external standard prepared by spiking labeled glycine in soil to avoid the interference of soil original glycine.

The extraction and derivatization methods for glyphosate, AMPA, glycine, and sarcosine were validated by spiking known amounts of these compounds in soil. The recovery ranged from 85 to 107 % for glyphosate, 79-93 % for AMPA, 74-88 % for glycine, and 80-97 % for sarcosine with RSD below 20 %, which is considered satisfactory. The limit of quantification (LOQ) for glyphosate and AMPA is 10 nmol/g soil and for glycine and sarcosine is 50 nmol/g in single quadrupole LC-MS, while it was largely improved by using Orbitrap (0.5 nmol/g).

Distribution of P derived from glyphosate into soil P pools

To differentiate and quantify the distribution of glyphosate-derived P in soil, samples from both control and glyphosate spiked soils (from the second set of experiments) were analyzed. A 0.3 g lyophilized soil was weighed and extracted with 30 mL DI water for 2 h using the modified [redacted] (1982) sequential extraction method ([redacted] *et al.*, 1984). The supernatant was collected as H_2O extractable P_i (most labile P_i), and residual soil was extracted with 30 mL of 0.5 M $NaHCO_3$ for 16 h to collect labile and weakly adsorbed P_i. Inorganic P from those two pools represents microbially available P_i. The soil was further extracted for 16 h first with 30 mL of 0.1 M NaOH and then with 1 M HCl to obtain the NaOH extractable

P_i (strongly sorbed P, fixed by Fe and Al oxides) and HCl extractable P_i (strongly fixed Ca-P), respectively. The concentration of P_i in each pool was measured by using the phosphomolybdate blue method. The residual P in the soils after the completion of sequential extraction was quantified using ICP-MS.

Measurement of oxygen isotope ratios
Soil samples from control and glyphosate spiked (5 µmol/g) experiments with two ^{18}O-labeled waters were centrifuged first to extract waters to measure water oxygen isotopes ($\delta\,^{18}O_W$) by CO_2 equilibration method. The measurement was done in a Finnigan GasBench II coupled with an isotope ratio mass spectrometer (IRMS; Thermo, Darmstadt, Germany) in the Environmental Biogeochemistry Laboratory at the University of Delaware.

To understand the P bioavailability, the H_2O- and $NaHCO_3$- extracted P_i pools were combined and processed for the measurement of phosphate oxygen isotope ratios ($\delta\,^{18}O_P$). Five grams of lyophilized soil samples from the second set of degradation experiments were processed following the [redacted] (2018) method to purify and finally convert P_i into silver phosphate. The O-isotope ratios were measured by a thermochemolysis/elemental analyzer (TC/EA) couples with IRMS. All isotopes from samples and standards were run at least in triplicate.

The measured $\delta\,^{18}O_P$ values of P_i were calibrated against two silver phosphate standards (YR 1aR-2 and YR 3-2, with the $\delta\,^{18}O_P$ values of -5.49 and +33.63 ‰, respectively). Similarly, the $\delta\,^{18}O_W$ values of porewater were calibrated with two USGS water standards ($\delta\,^{18}O_W$ values of -1.97 and -9.25 ‰, respectively). All isotope values are reported in per mil (‰) relative to the Vienna Standard Mean Ocean Water (VSMOW).

Results and Discussion
Degradation kinetics of glyphosate and its metabolites
The typical chromatography spectra of glyphosate, AMPA, sarcosine, and glycine are shown in Figure 7.1.1.1-3. Based on the LC-MS results, the concentrations of the compounds were calculated and are shown in Figure 7.1.1.1-4. Glyphosate gradually degraded over time and the extent of degradation reached >80 % by 35 d of incubation but traces of residual glyphosate were still detected until 175 d.

AMPA, the major metabolite of glyphosate, appeared after several days, accumulated during incubation, and reached its maximum concentration at 35 and 56 d in the experiment with 1 µmol/g and 5 µmol/g glyphosate, respectively. Afterwards, its degradation dominated over accumulation. Neither the degradation of glyphosate nor the formation of AMPA was observed in the sterilized soil incubation (abiotic only experiment), indicating microorganisms play a crucial role in degrading glyphosate in soils.

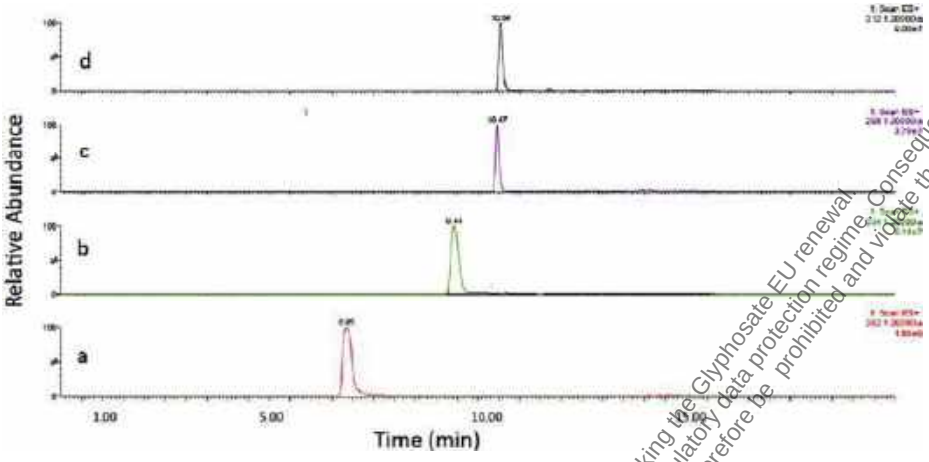
The degradation of glyphosate with time is often described according to first-order kinetics:

$$\ln(C/C_0) = -kt \quad (1)$$

$$t_{1/2} = \ln 2/k \quad (2)$$

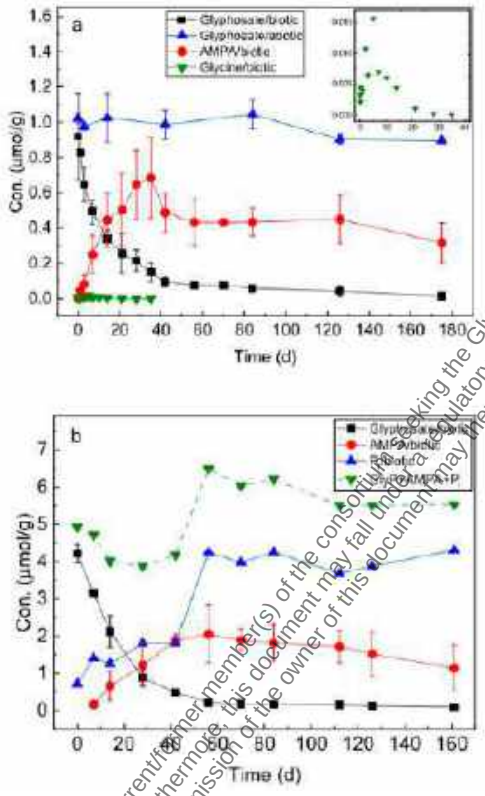
where C_0 is the initial concentration, C is the concentration at time t, and k is the degradation rate constant. The maximum accumulated concentration of AMPA is used as its initial concentration since more than 80 % of glyphosate was degraded at the time. The results show that both glyphosate and AMPA degradation follow first order kinetics with a strong correlation coefficient ($R^2 > 0.85$). The calculated half-lives of glyphosate under two sets of experiments are 28.9 and 31.5 d, respectively, consistent with the published results. A calculation based on the maximum amount of AMPA accumulated in the soil shows that the AMPA accounts for 48-68 % of the products from glyphosate degradation. It shows much longer half-lives (138.6 and 173.3 d), which highlights the high risk because of its toxicity and persistence in the environment.

Figure 7.1.1.1-3: Typical spectrum of glyphosate, AMPA, glycine and sarcosine analyzed by LC-MS (soil spiked with 1 µmol/g standards). a) glyphosate, b) AMPA, c) glycine, and d) sarcosine



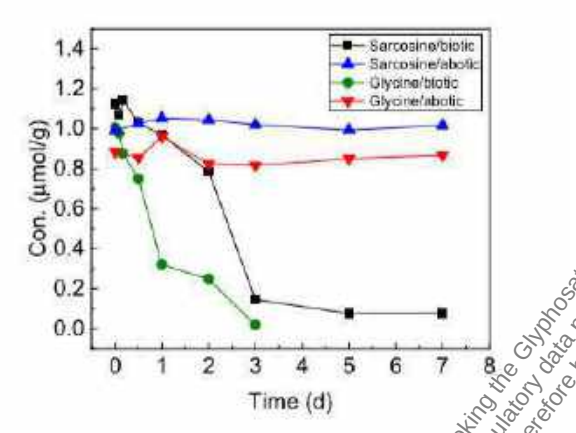
Glycine is a common amino acid and commonly present in soil and other environment. The isotope labeled glyphosate provides the reliability of detection because the labeled element is present in glycine as well. Labeled glycine appeared only after few days, accumulated, and reached the highest concentration after 5 days and then decreased but was still detectable after 35 days incubation (Figure 7.1.1.1-4a).

Figure 7.1.1.1-4: Kinetics of glyphosate biotic (natural soil) and abiotic (sterilized soil) degradation and its products. a) incubated with 1 µmol/g glyphosate, and b) incubated with 5 µmol/g glyphosate. Please note that the natural soil incubation includes both biotic and abiotic components of degradation



The concentration of labeled glycine is low, probably due to glycine derived from glyphosate was readily incorporated into microbial biomass soon after it formed. Results from a separate labeled glycine incubation experiment showed a rapid decline of soil-spiked glycine (1 µmol/g) with half-life of 0.89 days (Figure 7.1.1.1-5). Abiotic experiment showed no significant decline in glycine concentration in sterilized soil, validating methodology as well as indicating that soil microorganisms play a major role in glycine transformation. A recent study of labeled glyphosate reported the distribution of ^{13}C and ^{15}N into several amino acids including glycine, which our results corroborate. These findings, together, confirm that glyphosate derived glycine in the experiments should have rapidly utilized and metabolized by soil microorganisms.

Figure 7.1.1.1-5: Biotic (natural soil) and abiotic (sterilized soil) degradation of glycine and sarcosine in soil with spiked concentration of 1 µmol/g of each. Please note that the natural soil incubation includes both biotic and abiotic components of degradation



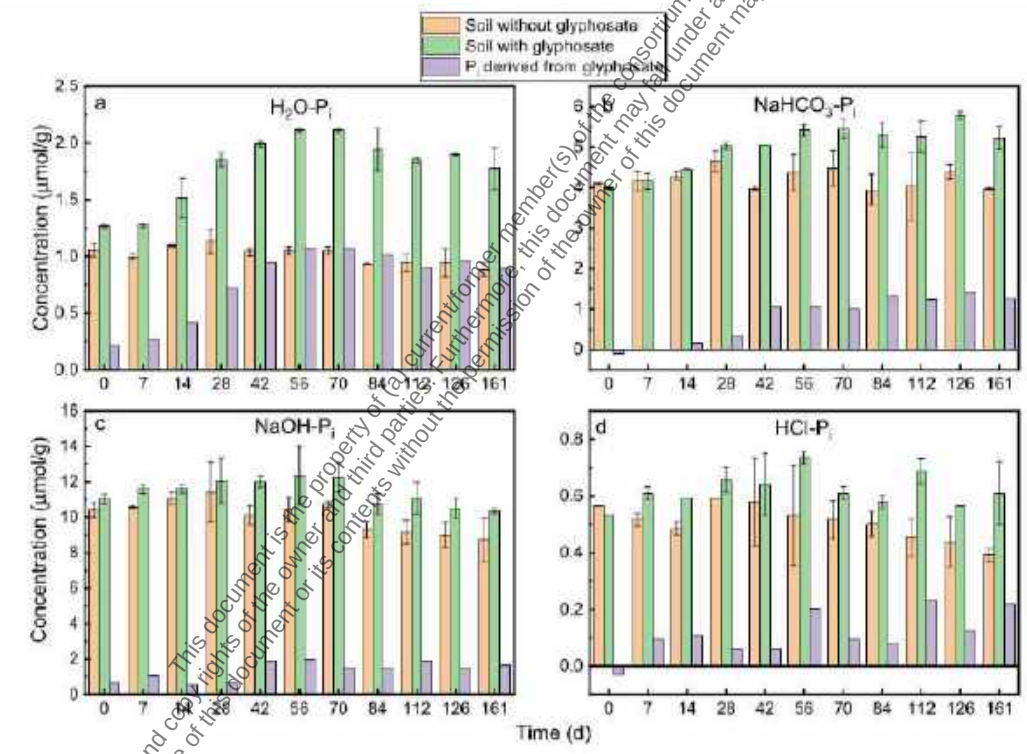
Sarcosine is a commonly recognized precursor to glycine during glyphosate degradation primarily on pure cultures that include bacteria isolated from soils, but rarely from the natural or simulated environments. In this study, sarcosine was not detected in any soil treatments including labeled glyphosate and high glyphosate (5 µmol/g) incubations. There might be three possibilities for the observed results: inefficient extraction from soil, fast oxidation of sarcosine, or presence below the detection limits of the analytical method. However, the recovery test performed by artificially spiking sarcosine in the same soil revealed that the method used could efficiently extract and accurately quantify sarcosine (yield 80-97 %). The individual incubation experiment showed that sarcosine could be degraded fast in the biotic experiment (with half-life of 0.99 days) but no significant decline in sterilized soil, which indicates degradation possible only by soil microorganisms. These lines of evidences suggest analytical method is not the reason, particularly since the high resolution Obitrap MS (detection limit of 0.5 nmol/g of sarcosine and glycine) was used. In the labeled glyphosate degradation experiments, soil samples were collected in several time points (0, 1, 2, 4 h, …until 35 days). The analytical method used successfully monitored the glycine formation and accumulation under extremely low concentration. If sarcosine was actually formed as a precursor to glycine, it should have detected by Obitrap MS since both sarcosine and glycine have similar half-lives. In a recent study, sarcosine was not detected in the abiotic degradation of glyphosate catalyzed by Mn minerals. These authors used advanced analytical methods including NMR, HPLC, and density functional theory (DFT) based electronic structure calculations and concluded that sarcosine was not a necessary intermediate product. Overall, the reliable extraction and analytical methods and intensive time point sampling verified that sarcosine was not formed during glyphosate degradation by soil microorganisms in this study.

Distribution of glyphosate-derived phosphorous in soil

Concentrations of four soil P_i pools in the control and glyphosate-spiked soils during the second set of incubations are shown in Figure 7.1.1.1-6. The experiments performed in two ^{18}O-labeled waters are considered duplicates because the difference in water oxygen isotopes does not impact the kinetics and extent of glyphosate degradation. Clearly, the control soil without glyphosate already contains high P_i and concentrations of P_i in different pools vary. It is noticeable, however, that the concentrations of P_i in these four pools remained essentially constant during the long-term incubation, with H_2O-P_i (1.01 ± 0.08 µmol/g), $NaHCO_3$-P_i (4.21 ± 0.23 µmol/g), NaOH-P_i (10.08 ± 0.91 µmol/g), and HCl-P_i (0.52 ± 0.06 µmol/g). This means that no significant transfer of P pools and organic-inorganic transformation occurred during the long-term incubation. The NaOH-P_i pool was the largest, indicating that Fe and Al minerals associated P is the major P sink in this soil, which is consistent with several other soils.

The results from the experiment in which glyphosate was spiked show that P_i derived from glyphosate transferred into different pools, resulting in an increase of corresponding pool size. The maximum concentration of H_2O-P_i was 2.11 µmol/g at 70 d of incubation. The difference between control (soil without glyphosate) and glyphosate spiked soil shows that there was 1.06 µmol/g glyphosate-derived P_i transferred into this pool. Similarly, a significant net increase of P_i was observed in $NaHCO_3$-P_i (1.40 µmol/g), NaOH-P_i (1.93 µmol/g), and HCl-P_i (0.23 µmol/g) pools, with the highest P_i concentrations measured around 56-126 d of incubation. It is interesting that the order of P pool was the same as that in the original (control) soil: NaOH-P_i >$NaHCO_3$-P_i >H_2O-P_i >HCl-P_i. Calculated P mass balance shows that the total increase in P_i among 4 pools was 4.30 µmol/g at the end of incubation, which accounts for ~86 % of spiked glyphosate (5 µmol/g). The residual P in the control and glyphosate spiked soils were similar (7.99 ± 0.69 and 7.67 ± 0.69 µmol/g, respectively), indicating that there was no significant incorporation of glyphosate-derived P in the residual P pool. It also means that the Hedley extraction could efficiently extract almost all P and account P derived from biodegradation of glyphosate.

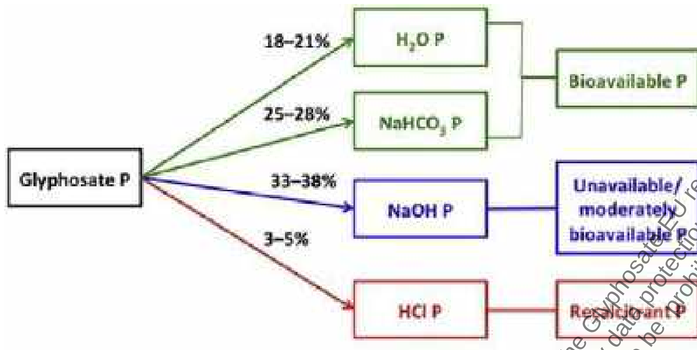
Figure 7.1.1.1-6: Concentrations of P in different pools in original soil and glyphosate incubated soil during biotic degradation. H_2O and $NaHCO_3$ extracted P pools are considered bioavailable P in soil. Soil was spiked with 5 µmol/g glyphosate. Glyphosate derived P was calculated as the different between soil with and without glyphosate



In terms of distribution P_i derived from glyphosate (Figure 7.1.1.1-7), the H_2O- and $NaHCO_3$-P_i pools, which are considered readily available P_i for uptake by microorganisms and plant roots, received almost half (44 %) of it. Meanwhile, around 33-38 % of glyphosate P transformed into the NaOH-P_i pool, an unavailable or moderately bioavailable P pool depending on the soil P conditions and plant efficiency and time. This means that this conditionally unavailable P pool might be further transported into open water systems by leaching or soil erosion and could increase the risk of polluting waters. The HCl-P_i, which is not directly utilized by plants and microorganisms and normally remains as an unavailable P pool in agricultural soil, only received 3-5 % of P derived from glyphosate. These results highlight the fact that P load derived from a large amount of glyphosate application (with estimated 130 million kg used in the U.S.)

cannot be ignored.

Figure 7.1.1.1-7: Distribution of glyphosate-derived P to different P pools during its biodegradation in the soil-water system. Soil incubated with 5 µmol/g glyphosate



Given that the P_i derived from glyphosate is steady means that it was gradually released as the degradation continues and distributed more into the bioavailable pool, and it may be a better P source for plants. Phosphorus fertilizer is the major P supply for plants with estimated 4 billion kg used in the U.S in 2014 with 50-70 % use efficiency. However, its fast P release kinetics do not match the dynamic needs of different crop growth stages well and this offset causes nutrient loss from soil to aquatic systems. Given the slow but steady P release from glyphosate degradation, it might be slightly more synchronous than commercial fertilizers, but still too fast than plant needs. Furthermore, multiple sprays of glyphosate during the crop lifetime (average of 1.6 times per crop year) support the possibility of fractionating more into bioavailable P that plants can readily take up. This demands reconsidering glyphosate not only use as a herbicide but a bonus P source to crops and should be included in estimations of crop P needs to improve the P efficiency of plant uptake as well reducing the P loss from agricultural soils.

Bioavailability of glyphosate-derived phosphorus

Once inside the cell, P_i is involved in several metabolic reactions catalyzed by enzymes including incorporation into cell biomass and ATP-ADP conversion. One of the unique enzymes is pyrophosphatase (PPase), which is highly conserved across all three domains of life, catalyzes the hydrolysis of pyrophosphate into P_i. This is a reversible reaction and leads to exchange of all four O atoms in P_i with O in ambient water and thus achieves O-isotopic equilibrium between phosphate and water. The equilibrium isotope depends on the temperature and water oxygen isotope value.

To further test the bioavailability and rate of microbial utilization of glyphosate-derived Pi, phosphate oxygen isotopes ($\delta^{18}O_P$) of P_i in the soil incubated with and without glyphosate were measured and compared with the equilibrium isotope values calculated from the temperature and oxygen isotopes of water ($\delta^{18}O_w$) in the experiments. The $\delta^{18}O_w$ values remained constant at -6.51 ± 0.30 ‰ and +18.27 ± 0.12 ‰ for two ^{18}O-labeled water experiments in the long-term incubation except at the end of the experiment (161 d), when an inadvertent evaporation resulted in slight enrichment of isotopes (-4.90 ‰ and +20.71 ‰, respectively). The expected isotopic equilibrium value ($\delta^{18}O_{P\text{-}eq}$) was calculated based on the ([redacted] 2015) equation as:

$$\delta^{18}O_{P-eq} = e^{(\frac{14.43}{T} - 0.0265)} \times (\delta^{18}O_w + 1000) - 1000 \qquad (3)$$

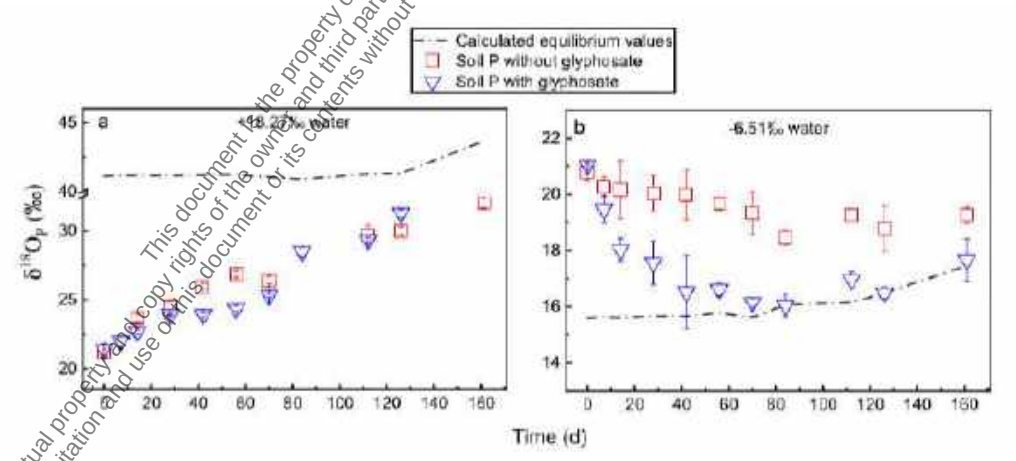
The $\delta^{18}O_{P\text{-}eq}$ values in the experiments incubated with -6.51 ‰ and +18.27 ‰ water are +15.83 ± 0.31 ‰ and +41.16 ± 0.12 ‰ (Figure 7.1.1.1-8), respectively, and remained constant during the incubation period (except at 161 days, in which water mass was not conserved). The starting isotope values of extracted P_i were consistent in all treatments: 20.77 ± 0.26 ‰, 21.02 ± 0.10 ‰, 21.38 ± 0.42 ‰ and 21.21 ± 0.16 ‰ in

two controls (soil without glyphosate) and two glyphosate spiked experiments with -6.51 ‰ and +18.27 ‰ ^{18}O-labeled waters, respectively. It means that there are no different O sources or contaminants that might have impacted isotope values during the incubation period, besides the degradation of glyphosate.

The measured $\delta^{18}O_P$ values in the bioavailable P in ^{18}O spiked (+18.27 ‰) water became gradually heavier (Figure 7.1.1.1-8a), shifting towards the equilibrium values (+41.16 ‰) and reached 32.04 ‰ at the end of incubation. This result reveals the rapid uptake of the available P by soil microorganisms and the release of cycled P back to the soil. At the early stage, $\delta^{18}O_P$ values of P_i in the soil spiked with glyphosate were consistent with those in original control experiments. However, they became lighter after 14 d and remained 1.2-2.5 ‰ lighter for a long period. This is due to the contribution from a much lighter isotope value of P_i (~4-9 ‰) derived from glyphosate. The newly derived P_i from glyphosate degradation mixed with soil P_i pool and turned them into isotopic lighter and away from the equilibrium value (around +41.2 ‰). This result is consistent with P_i distribution that P_i was heavily released from glyphosate from 14 days to 84 days (Figure 7.1.1.1-6) and preserving isotope record of the lighter glyphosate derived P_i in the system (see Figure 7.1.1.1-6). However, the difference in isotope values between those two treatments gradually narrowed and eventually erased at 161 days, indicating that the soil microorganisms were efficient to uptake and cycle almost all of bioavailable P in the soil both from originally present soil and from glyphosate derived P_i.

The isotope trend in the experiments performed in -6.51 ‰ water (Figure 7.1.1.1-8b) is comparable to heavy water, but with a minor difference. For example, the $\delta^{18}O_P$ values in glyphosate spiked soil became much lighter and reached the equilibrium value sooner than those from control soil (without glyphosate). The reason is that the P_i derived from glyphosate carries much lighter $\delta^{18}O_P$ values (as explained above), which brings the isotope values close to equilibrium (which is lighter: +15.83 ± 0.31 ‰, due to the lighter water oxygen isotopes). The gap between the two treatments was 0.8 ‰, and then increased to 2.3 ‰ due to the large contribution of lighter isotopes of glyphosate derived P_i, but with the enhancement of microbe turnover, it decreased again but still 1.6 ‰ off at the end of the incubation.

Figure 7.1.1.1-8: Changes in phosphate oxygen isotopes during glyphosate biodegradation in the water-soil system. The calculated equilibrium values assumes all P is completely recycled by microorganisms. The closer the isotope values toward the equilibrium values, the higher the extent of P cycling



The observed results explained above provide several new insights on degradation of glyphosate and its metabolites and recycling of glyphosate derived-P and together have several implications on the fate and impact of glyphosate in soils.

First, it proves that the isotope signature of glyphosate degradation can be detected in the experiments

mimicking environmental systems. Second, it indicates that the degradation of glyphosate is faster than the microbial uptake and turn-over of P, so that the unique signature could be measured at the early to middle stage of the reaction.

Third, if the $\delta^{18}O_P$ values of the P derived from organic compounds are farther/closer to the equilibrium range compared to those present in-situ, they could easily shift/overprint bulk isotope value (due to mixing), leading to the inaccurate estimation of the biological activities.

Microbial turnover of P in the soil-water system

To evaluate the extent of P taken up and recycled by soil microorganisms, the P turnover was calculated from the starting $\delta^{18}O_P$ values ($\delta^{18}O_{P\text{-}t0}$) at 0 hours, measured values at time t ($\delta^{18}O_{P\text{-}t}$) and the equilibrium values ($\delta^{18}O_{P\text{-}eq}$):

$$\%P\ \ turnover = \frac{(\delta^{18}O_{p-t} - \delta^{18}O_{p-to})}{(\delta^{18}O_{p-eq} - \delta^{18}O_{p-t})} \times 100 \qquad (4)$$

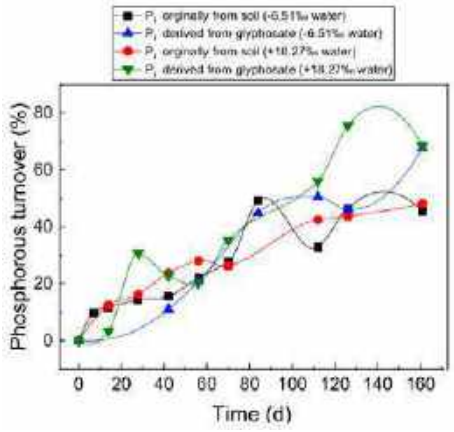
As the equation shows, the closer the values of $\delta^{18}O_{P\text{-}t0}$ to $\delta^{18}O_{P\text{-}eq}$, the higher the microbial turnover efficiency. The results show that P_i in the control experiment was rapidly exchanged by soil microorganisms and driven closer to the equilibrium values, with the turnover efficiency of 22-28 % at 56 days and 45-48 ‰ at 161 days in two ^{18}O-labeled waters (Figure 7.1.1.1-9). As expected, the efficiency of P turnover was similar irrespective of the starting isotopic values of ^{18}O-labeled water (-6.51 ‰ or +18.27 ‰).

In the glyphosate spiked experiments, the $\delta^{18}O_P$ value at time t ($\delta^{18}O_{P\text{-}t/spike}$) is the sum of glyphosate derived P_i ($\delta^{18}O_{P\text{-}t/gly}$) and the original P_i from control soil ($\delta^{18}O_{P\text{-}t/con}$), which can be calculated from a simple mass balance equation as follows:

$$\delta^{18}O_{p-t/spike} = x\delta^{18}O_{p-t/gly} + (1-X)\delta^{18}O_{p-t/con} \qquad (5)$$

where x is the fraction of P_i derived from glyphosate in the spiked samples. We calculated the starting isotope values of glyphosate derived P_i in two ^{18}O-labeled water systems at 0 hours using previous results, which are +6.92 ‰ and ±12.14 ‰ in -6.51 ‰ and +18.27 ‰ waters, respectively. Based on the starting values of glyphosate-derived P_i, its microbial turnover was calculated using equation (4). As shown in Figure 8, the trend of P turnover in the soils receiving glyphosate-derived P_i was similar to that of control soil (without glyphosate), but the recycling efficiency was higher (67-75 ‰). Overall, phosphate oxygen isotopes allowed discrimination of sources and variable recycling efficiency of soil P vs glyphosate-derived P_i.

Figure 7.1.1.1-9: Microbial turnover efficiency of soil P and glyphosate-derived P



Glyphosate degradation pathways in soil
To understand the degradation pathways and specific preferences in the soil system studied, the released P_i extractable from four pools were combined together. The total P mass from glyphosate source was also calculated by adding glyphosate, AMPA, and released P_i and are shown in Figure 7.1.1.1-4b. The released P_i steadily increased and reached the peak concentration around 56 days. AMPA remained at the accumulation stage and started to degraded at that time only when more than 80 % of glyphosate was already degraded. There was slight decrease in total P (from original concentration of 4.92 µmol/g) at the early stage of degradation, and then remained almost constant during the incubation period. Consider the efficient extraction of the glyphosate-derived P_i, it implies that there might be some other non-detected P speciation during the early stage of glyphosate degradation besides glyphosate, AMPA, and inorganic P. A potential P compound could be methylphosphonic acid, which can be generated synchronously if glycine forms directly from glyphosate. Based on the data generated in this study and foregoing assumptions and published results, revised pathways and temporal preference of glyphosate degradation in the soil-water system is proposed and shown in Figure 7.1.1.1-10. Under the action of soil microorganisms, at the early stage of degradation, glyphosate is cleaved at C(3)-N position to form glycine and methyl-phosphonic acid, the latter one is further degraded to form P_i, which accumulates in the system. Another bond cleavage occurs at C(2)-N position and form AMPA and glyoxylic acid. AMPA accumulates at the late stage of degradation. No sarcosine was generated in the soil-water system in this study, so it is not the required intermediate metabolite to form glycine.

Conclusion
In this study, we studied degradation glyphosate and its metabolites and successfully utilized phosphate oxygen isotopes to confirm the biological availability of glyphosate-derived P in the simulated soil-water system. The broader conclusions derived from this study and the implications thereof are as follows:

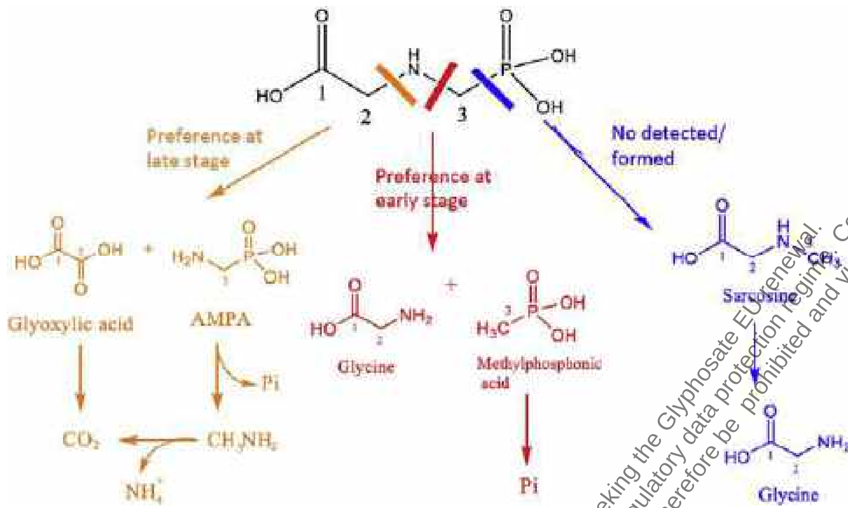
1) A satisfactory method of extraction and separation of glyphosate and its major metabolites in soil was developed, which could be used to identify the fate of glyphosate in a variety of environments. The absence of degradation in sterilized soil showed the soil microorganisms play the essential role on the degradation of glyphosate. Temporal presence of glycine and AMPA varied as well as their microbial uptake and degradation. AMPA was found to be 3-6 times resistant than glyphosate against degradation, which brings a higher concern to the safety of environment.

2) The distribution of glyphosate-derived P_i in a soil was investigated. About half of the glyphosate-derived P_i transferred into the readily bioavailable P pool. A slow but steady release of P_i from the degradation of glyphosate could mean that its supply could be slightly more synchronous with plant P demand during plant growth especially because it is applied more than one time during a crop cycle. This means that a higher proportion of glyphosate-derived P, than P from commercial fertilizers which release P all at once, could be taken up by plants.

3) Glyphosate-derived P_i has a distinct isotopic signature and can aid in identification of its source. The natural environment, however, is complex and could pose additional challenges, most likely due to the low content of glyphosate and inappropriate sampling time could miss to detect significant offset of isotope values. This is because isotope signature could be erased or overprinted due to biological cycling of glyphosate-derived P.

4) ^{18}O-labeling in water and application of phosphate oxygen isotope method allowed explicit understanding of microbial uptake and extent of biological turnover of glyphosate derived-P. The microbial turnover of original P in soil and glyphosate-derived P was comparable, but it was found that the microorganisms were more efficient to utilize and recycle glyphosate-derived P. The research tool developed could be further used to investigate the extent of microbial activities in soils and other natural environments.

Figure 7.1.1.1-10: (Bio)degradation pathways of glyphosate and preference of degradation in the water-soil system used in this study



3. Assessment and conclusion

Assessment and conclusion by applicant:
The article deals with aerobic soil degradation with non-labelled and stable-labelled glyphosate. AMPA formation was confirmed to occur as metabolite. The article focuses mainly on the fate of the phosphorous moiety to investigate for potential transformation products of glyphosate origin. In general, the methods and results are described, but there is a lack of details reported to allow for the evaluation of all potential deviations from OECD 307. For example, soil properties are not reported, exact soil water content during incubation is unclear, the size/mass of soil samples incubated is not clearly stated (1 g soil was used for extraction). Further, procedures of work-up including procedural recoveries for glyphosate and AMPA are presented in figures, but not in detail (tabulated values). DT_{50} values according to SFO were calculated for glyphosate and AMPA (based on max. concentration) but no details on quality of fits and statistics are provided. No new transformation products were reported. The article is therefore considered as reliable with restrictions.

Assessment and conclusion by RMS:

CA 7.1.1.2 Anaerobic degradation

An overview on existing studies investigating the route of degradation in anaerobic soil is given in Table 7.1.1.2-1 below.

The degradation of glyphosate under anaerobic conditions of the laboratory was investigated in one soil in one study considered as valid to address the data point ([redacted] 2003, CA 7.1.1.2/003).

Following establishment of anaerobic conditions, degradation of glyphosate was found to slow down while the pattern of degradates remained identical to that under aerobic conditions. Mineralisation was negligible and formation of non-extractable residues (NER) was found to increase by 6 to 10 % AR during the anaerobic incubation phase compared to the start. Aminomethylphosphonic acid (AMPA) was the only major degradation product observed at a maximum of 30.2 % AR after 84 days of anaerobic incubation.

The results of [redacted] 2003, CA 7.1.1.2/003) were kinetically evaluated according to the current FOCUS kinetic guidance ([redacted] 2020, CA 7.1.2.1.3/001).

In addition, the following two existing studies on anaerobic degradation of glyphosate or glyphosate-trimesium are considered as supportive information ([redacted] 2004, CA 7.1.1.2/002 and [redacted] 2000, CA 7.1.1.2/004). For evaluation and further assessment, only the results for the glyphosate (PMG) anion are considered. The results from supportive studies generally confirm those of the study regarded as valid.

In the invalid study by [redacted] (2004, CA 7.1.1.2/001), an unknown component (Peak P3) was observed at more than 10 % AR. Though attempts were made for identification, it was not finally possible to identify and thus to assign the peak to any potential degradate of glyphosate. In view of the overall non-compliance of study and its outcome with the current guidelines, the information is considered to not have an impact on the risk assessment.

Table 7.1.1.2-1: Studies on anaerobic soil degradation with glyphosate (route)

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.1.2/001	[redacted], 2004	Anaerobic soil degradation	Glyphosate	Invalid	
CA 7.1.1.2/002	[redacted] 2004	Anaerobic soil degradation	Glyphosate	Supportive	
CA 7.1.1.2/003	[redacted] 2003	Anaerobic soil degradation	Glyphosate	Valid	Updated kinetic evaluation in [redacted] 2020, CA 7.1.2.1.3/001
CA 7.1.1.2/004	[redacted], 2000	Anaerobic soil degradation	Glyphosate	Supportive	
CA 7.1.1.2/005	[redacted] 1987	Anaerobic soil degradation	Glyphosate-trimesium	Invalid	
CA 7.1.1.2/006	[redacted] 1972	Anaerobic soil degradation	Glyphosate	Invalid	

Anaerobic soil degradation studies with glyphosate as test item

1. Information on the study

Data point:	CA 7.1.1.2/001
Report author	[redacted]
Report year	2004
Report title	[14C]-Glyphosate: Anaerobic Soil Metabolism (Rate and Route of Degradation in a Sandy Loam Soil)
Report No	SNN/05
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From OECD 307: - Test substance directly applied to water phase after adaptation to anaerobic conditions for 50 days - discrepancies between chromatograph labelling and characterisation of radioactivity in soil extracts
Previous evaluation	Yes, accepted in RAR as supplementary (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The degradation of [^{14}C] -glyphosate was investigated in a sandy loam soil under anaerobic conditions of the laboratory in the dark at 20 ± 2 °C for 120 days. Anaerobic conditions were achieved by flooding the soil with Milli-Q grade water to a depth of 3 cm over the soil surface and purging of the samples with nitrogen.

The test was performed in a flow-through system with the test system connected to two traps, one with aqueous potassium hydroxide solution and one with ethanolamine/2-ethoxyethanol, both to collect carbon dioxide and an ethyl digol trap to collect volatile organic compounds. The nominal application rate was 4.8 µg/g soil (dry weight), corresponding to a field use rate of 3.6 kg a.s./ha.

Duplicate samples from each system were processed and analysed at 0, 1, 3, 7, 14, 30, 59, 91 and 120 days after treatment (DAT). The traps for volatile radioactivity were assayed at each sampling interval, otherwise exchanged at weekly intervals during the first month and 10 days interval thereafter. Soil microbiological activity was determined at the time of application of the test substance and after 120 days of incubation.

Mass balances (mean values) ranged from 90.5 % to 98 % of applied radioactivity (% AR) except day 14 where the recovery was 88.4 % AR. Volatiles formed as CO_2 as indicated by its finding in potassium hydroxide traps, reached a maximum of 25.7 % at 120 DAT. Other volatile radioactivity did not exceed 0.2 % AR in the course of the test. Radioactivity in water decreased from 97.2 % AR at time of application to 5.5 % AR after 120 days of incubation. The radioactivity extractable from soil increased from 0.8 % AR to 62.9 AR (14 DAT) and subsequently slightly decreased to 54.6 % AR at 120 DAT. Non-extractable residues (NER) increased from <0.1 % AR at 0 DAT to 11.4 % AR at 60 DAT to slightly decrease to 10.6 % AR at 120 DAT.

In water, the amount of glyphosate steadily decreased from 0 DAT to 120 DAT from 93.2 to 3.6 % AR. In parallel, the amount of soil-extracted glyphosate increased from 0 DAT to 30 DAT from 0.1 to 34.9 % AR and subsequently decreased to 1.7 % AR at 120 DAT. The metabolite AMPA was found predominantly in soil extracts where it reached a maximum amount of 44.2 % AR at 120 DAT. In water, AMPA was found with maximum 5.3 % AR at 91 DAT. In the soil extracts, an unknown peak (P3) was observed at levels above 10 % AR (max. 14.9 % AR at 91 DAT). In water, amounts of P3 were ≤2.5 % AR. For the current submission, further attempts were made for identification (see statement below). No other compounds were detected above 5 % AR at any time. In soil extracts, up to 18.2 % of radioactivity could not be assigned to a designated peak in the chromatogram. In water, unassigned radioactivity was ≤2.1 % AR

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Radiolabelled Test Material:
Identification: glyphosate, phosphonomethyl-2-^{14}C
Lot No.: 102K9424
Specific activity: 164.28 MBq/mg (4.44 µCi/mg)
Radiochemical purity: 97.0 %

Non-radiolabelled Test Material
Identification: glyphosate
Lot No.: 90K37441
Chemical purity: 96 %

2. Soil:
Soil was sieved to ≤2 mm. The soil was received immediately before testing and was air dried before sieving. Characteristics of the test soil are presented in the table below.

Table 7.1.1.2-2: Characteristics of test soil

Parameter	Results
Soil	Manningtree A
Country	UK
Textural Class (MAFF)	Sandy loam
Sand (%)	66
Silt (%)	26
Clay (%)	8
pH (medium not indicated)	6.5
Organic carbon (%)	1.0
Cation exchange capacity (meq/100 g)	7.9
Maximum Water Holding Capacity (% m/m)	36.4
Water Holding Capacity at 1/3 bar (% m/m)	18.2
Bulk Density (disturbed) (g/cm^3)	1.4
Microbial biomass (mg C/100 g): Before application (0 DAT) Study end (120 DAT)	 16.55 18.49

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of cylindrical glass vessels of 250 mL capacity filled with soil flooded with purified water to a depth of 3 cm. Each vessel was equipped with separate glass-flow system in a series as follows: pre-test system was a Dreschel bottle with sintered stem for uniform gas dispersion containing water to humidify gas flow. This was connected to a glass tube in the test vessel bringing the gas flow just below the water surface. Behind the test vessel an empty bottle was connected to prevent transfer of trapping solutions to the test vessel followed by 3 trapping bottles containing (a) ethyl digol for trapping organic volatiles, (b) 1 M aqueous potassium hydroxide solution with phenolphthalein indicator for trapping CO_2, and (c) ethanolamine/2-ethoxyethanol (1/3, v/v as backup CO_2 trap).

50 g of sieved soil (dry weight equivalents, ca. 52 g wet weight) and 70 mL of purified water were added to each test vessel. To establish anaerobic conditions, the flooded samples were purged with a stream of moist oxygen free nitrogen. Anaerobic conditions were monitored by regular measurement of the redox potential.

The test systems were acclimated for 50 days at test conditions (20 °C) prior to application of test item.

A test solution of [^{14}C]-glyphosate with a concentration of 6.96 mg/mL (38.67 mg diluted in 5.56 mL destilled water) was prepared. Aliquots of 100 µL diluted to 25 mL with distilled water were analysed by LSC. 200 µL of the [^{14}C]-glyphosate solution was applied to each test system. Dose checks confirmed that each test vessel received 0.21 mg [^{14}C]-glyphosate. Based on dose checks, the actual application rate was 4.8 mg/kg soil, corresponding to a field use rate of 3600 g a.s./ha. After application the test vessels (except 0 DAT) were closed with trap attachments.

Test systems were incubated under anaerobic conditions in the dark at 20 ± 2 °C for 120 days in maximum.

2. Sampling

Duplicate test systems were processed and analysed 0, 1, 3, 7, 14, 30, 59, 91 and 120 days after treatment (DAT). All samples were processed on the day of sampling. Trapping media were analyzed and replaced at each sampling interval, then at weekly intervals during the first month and about 10-day intervals thereafter.

3. Analytical procedures

At each sampling interval, the soil and water phase were separated by decanting the water from the test vessel. The total volume of the water layer and concentration of radioactivity in the water was measured. The water was then stored at -15° C until chromatographic analysis.

Soil samples were extracted five times: three times with 0.5 M ammonium hydroxide and 2 times with 1 M hydrochloric acid. Extracts and soil were separated by centrifugation. The volume of each extract was measured and aliquots were analysed by LSC.

Water samples and soil extracts were further analysed by TLC and HPLC.

Radiolabelled components on thin-layer chromatograms were detected and quantified using prelayed cellulose TLC plates, layer thickness 0.25 mm. The developing system was Butanol:Water:Acetic acid with 6:5:2 v/v.

The Radio-HPLC isocratic method used was a Hamilton PRP-X400 cation exchange column run with an aqueous mobile phase at pH 1.9 (5mM potassium phosphate). No limit of quantification (LOQ) or limit of detection (LOD) are given but lowest reported values are 0.1 % AR.

Test item and metabolites were identified by comparison with reference items, however test items are not reported.

The amount of volatiles and non-extractable residues was determined by LSC and combustion/LSC, respectively.

The presence of CO_2 in the potassium hydroxide traps was confirmed by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

For characterisation of unextractable radioactivity selected samples of extracted soils containing ≥10 % AR were further extracted with 0.5M NaOH solution for 18-24 hours on a rotary shaker at ambient temperature. After centrifugation, the aqueous layer was decanted. The soil debris was rinsed with further 0.5 M NaOH and these solutions combined with the initial 0.5 M NaOH extract for determination of radioactivity by LSC. After air drying, the radioactivity in the soil debris (humin fraction) was determined by combustion/LSC.

The 0.5M NaOH extract was adjusted to pH 1 with concentrated HCl and stored at room temperature for 18-24 hours. After centrifugation, the precipitate was washed with 1M HCl, the solution was combined with the pH 1 extract (fulvic acid fraction) and analyzed with LSC. The radioactivity of the precipitate (humic acid fraction) was measured by combustion/LSC.

The additional approach with an exaggerated application rate is not reported in this summary as no results on the characterisation of radioactivity is given in the report which was the main purpose for this additional setup.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]-glyphosate and metabolites under anaerobic conditions in a sandy loam soil are summarised for soil and water extracts in Table 7.1.1.2-3 and Table 7.1.1.2-4

Table 7.1.1.2-3: Recovery of radioactivity in water and soil incubated under anaerobic conditions (expressed as percent of applied radioactivity) following the application of [^{14}C]-glyphosate

		DAT								
Fraction	**Replicate**	**0**	**1**	**3**	**7**	**14**	**30**	**59**	**91**	**120**
Water	A	97.7	62.6	35.6	32.5	19.6	10.2	7.1	6.1	4.8
	B	96.7	63.9	44.8	26.8	14.4	11.4	9.1	9.6	6.1
	Mean	*97.2*	*63.3*	*40.2*	*29.7*	*17.0*	*10.8*	*8.1*	*7.9*	*5.5*
Carbon Dioxide	A	<0.1	0.4	0.3	0.3	5.2	10.0	14.3	27.8	31.1
	B	<0.1	0.5	0.3	0.3	1.1	11.9	19.0	9.2	20.3
	Mean	*<0.1*	*0.5*	*0.3*	*0.3*	*3.2*	*11.0*	*16.7*	*18.5*	*25.7*
Volatile organic compounds	A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	<0.1	<0.1	<0.1	<0.1	0.3	<0.1	<0.1	<0.1	<0.1
	Mean	*<0.1*	*<0.1*	*<0.1*	*<0.1*	*0.2*	*<0.1*	*<0.1*	*<0.1*	*<0.1*
Total extractable residues	A	0.6	29.4	51.6	50.6	58.7	61.1	58.4	52.4	45.6
	B	0.9	27.2	42.0	62.3	67.0	58.7	52.0	66.8	63.5
	Mean	*0.8*	*28.3*	*46.8*	*56.5*	*62.9*	*59.9*	*55.2*	*59.6*	*54.6*
Non-extractable Residues	A	<0.1	2.3	3.9	6.7	5.0	9.5	11.3	8.5	9.9
	B	<0.1	1.1	5.0	6.5	5.7	8.2	11.5	8.0	11.3
	Mean	*<0.1*	*1.7*	*4.5*	*6.6*	*5.4*	*8.9*	*11.4*	*8.3*	*10.6*
Mass balance	A	98.3	94.7	91.4	90.1	88.5	90.8	91.1	94.8	91.4
	B	97.6	92.7	92.1	95.9	88.2	90.2	91.6	93.6	101.2
	Mean	*98.0*	*93.7*	*91.8*	*93.0*	*88.4*	*90.5*	*91.4*	*94.2*	*96.3*

DAT: days after treatment

Table 7.1.1.2-4: Characterisation of radioactivity in water and soil extracts incubated under anaerobic conditions following treatment with [^{14}C]-glyphosate (expressed as percent of applied radioactivity), HPLC analysis

Water										
		DAT								
Compound	**Replicate**	**0**	**1**	**3**	**7**	**14**	**30**	**59**	**91**	**120**
Glyphosate	A	93.1	59.4	34.3	21.2	10.6	6.7	0.1	0.3	2.7
	B	93.3	59.9	42.4	25.4	12.5	4.4	1.0	0.5	4.5
	Mean	*93.2*	*59.7*	*38.4*	*23.3*	*11.6*	*5.6*	*0.6*	*0.4*	*3.6*
AMPA (P2)	A	1.8	1.3	0.6	5.3	5.1	1.4	5.1	3.1	1.2
	B	1.8	1.7	0.9	0.7	0.7	2.7	3.6	7.5	1.0
	Mean	*1.8*	*1.5*	*0.8*	*3.0*	*2.9*	*2.1*	*4.4*	*5.3*	*1.1*
P3 (15 min)	A	nd	nd	nd	4.5	2.5	1.5	1.3	0.9	0.6
	B	nd	nd	nd	0.3	0.5	3.4	3.0	1.1	nd
	Mean	-	-	-	*2.4*	*1.5*	*2.5*	*2.2*	*1.0*	*0.6*
Others[1]	A	2.7	1.9	0.7	1.5	1.3	0.6	0.5	1.8	0.4
	B	1.5	2.3	1.1	0.4	0.7	0.8	1.6	0.5	0.6
	Mean	*2.1*	*2.1*	*0.9*	*1.0*	*1.0*	*0.7*	*1.1*	*1.2*	*0.5*
Soil										
		DAT								
Compound	**Replicate**	**0**	**1**	**3**	**7**	**14**	**30**	**59**	**91**	**120**
Glyphosate	A	0.1	4.0	25.8	11.1	18.7	34.9	nm	11.6	nm
	B	0.1	17.4	16.9	29.8	19.5	nm	15.3	15.4	1.7
	Mean	*0.1*	*10.7*	*21.4*	*20.5*	*19.1*	*34.9*	*15.3*	*13.5*	*1.7*
AMPA (P2)	A	0.5	20.3	8.6	26.8	18.7	18.7	nm	15.0	nm
	B	0.6	5.6	9.1	19.4	19.5	nm	18.1	25.7	44.2
	Mean	*0.6*	*13.0*	*8.9*	*23.1*	*19.1*	*18.7*	*18.1*	*20.4*	*44.2*
P3 (15 min)	A	nd	nd	12.9	3.5	2.8	nd	nm	20.4	nm
	B	nd	nd	7.3	4.7	10.3	nm	11.0	9.4	9.9
	Mean	-	-	*10.1*	*4.1*	*6.6*	-	*11.0*	*14.9*	*9.9*
Others[1]	A	<0.1	5.1	4.3	9.2	18.7	7.4	7.6	5.4	nm
	B	0.2	4.2	8.7	8.5	17.7	nm	nm	16.4	7.7
	Mean	*0.2*	*4.7*	*6.5*	*8.9*	*18.2*	*7.4*	*7.6*	*10.9*	*7.7*

DAT: days after treatment
nd: not detected
nm: not measured
[1] Regions of radioactivity which cannot be assigned to a designated peak

B. MASS BALANCE

Mass balances ranged from 90.5 % to 98.0 % of applied radioactivity except day 14 where the recovery was 88.4 % AR.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 120 DAT from 97.2 to 5.5 % AR.

In parallel, soil extractable residues increased until 14 DAT from 0.8 and 62.9 % AR and subsequently slightly decreased to 54.6 % AR at 120 DAT.

The amount of non-extractable residues (NER) increased from 0 DAT to 59 DAT from <0.1 to 11.4 % AR and subsequently slightly decreased to 10.6 % AR at 120 DAT.

Fractionation of non-extractable residues into fulvic acid, humic acid and humin fractions in a representative soil sample resulted in ca. 65 % fulvic acid, 2 % AR humic acid and 30 % AR humins.

D. VOLATILE RADIOACTIVITY
Formation of $^{14}CO_2$ increased steadily during the experimental period. Maximum amounts of carbon dioxide reached at study end (120 DAT) were 25.7 % AR. Organic volatiles determined were ≤0.2 % AR.

E. TRANSFORMATION OF THE TEST ITEM
In water, the amount of glyphosate steadily decreased from 0 DAT to 120 DAT from 93.2 to 3.6 % AR. Decrease from water was paralleled by an increase of glyphosate extracteable from soil from 0 DAT to 30 DAT from 0.1 to 34.9 % AR and subsequently decrease to 1.7 % AR at 120 DAT.

The metabolite AMPA was found predominantly in soil extracts where it reached a maximum amount of 44.2 % AR at 120 DAT. In water, AMPA was found with maximum 5.3 % AR at 91 DAT.

In the soil extracts, an unknown peak (P3) was observed at levels above 10 % AR (max. 14.9 % AR at 91 DAT). In water, amounts of P3 were ≤2.5 % AR. For the current submission, further attempts were made for identification (see statement below).

No other compounds were detected above 5 % AR at any time.

In soil extracts, up to 18.2 % AR could not be assigned to a distinct peak in chromatographic analysis. In water, unassigned radioactivity was ≤2.1 % AR

F. KINETICS
New kinetic calculations based on recent guidance were not provided due to the supporting character of the study.

III. CONCLUSIONS

Glyphosate was rapidly degraded in an anaerobic water/soil system. Glyphosate degraded to AMPA which was then mineralised to carbon dioxide.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides information on the degradation behavior of glyphosate in soil under strict anaerobic conditions in the laboratory. Such application to strictly anaerobic conditions (50 days) is not in agreement with the current guideline. Further considerations on identification of the unknown compound P3 is given below as well as examples for discrepancies in peak identification.
The study is considered as invalid.

Assessment and conclusion by RMS:

Expert statement to [redacted] (2004, CA 7.1.1.2/001): [^{14}C]-Glyphosate: Anaerobic Soil Metabolism (Rate and Route of Degradation in a Sandy Loam Soil)

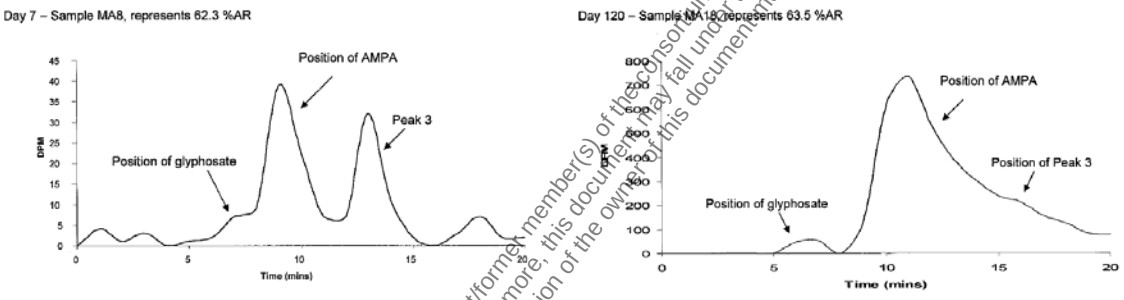
This statement compiles additional information on the finding of unknown component "P3" in the study occurring at a maximum of 14.9 % AR after 91 days of incubation.

The Certificate of Analysis in the report identifies the test item as the single radiolabelled compound glyphosate-(phosphonomethyl-^{14}C) which is the monosodium salt of the acid active. The radio-HPLC isocratic method used (Hamilton PRP-X400-poly (styrene-divinyl-benzene) sulfonate cation-exchange column) has an aqueous mobile phase at pH 1.9 which is specifically used for glyphosate. The strong cation

exchange column separates glyphosate and AMPA according to the overall positive charge of these molecules. The order of elution is based upon the ionic form of the molecule under the specific acidic pH conditions and the more positive a molecule, the longer the retention time. Hence, glyphosate elutes first, followed by AMPA. Both, glyphosate and AMPA, are present as zwitterions at pH 1.9.

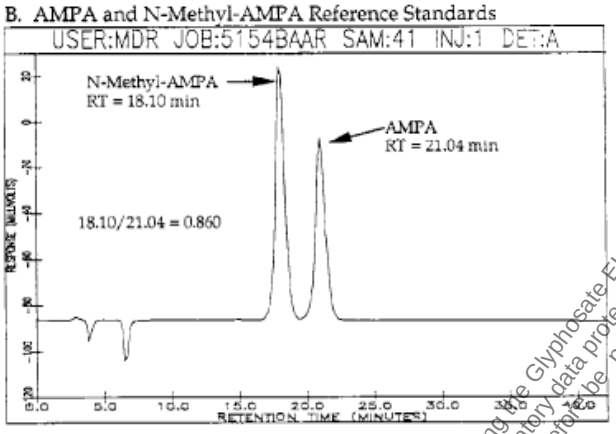
The unknown radio-peak "P3" could supposedly be the amino acid sarcosine or N-methyl-AMPA based on the position of the ^{14}C-label in the glyphosate test material (glyphosate-phosphonomethyl-2-C^{14}), where N-methyl-AMPA would be a zwitterion as well at pH 1.9, and sarcosine would be a cation at this pH. This could potentially mean that N-methyl-AMPA would co-elute with AMPA whereas sarcosine would definitely elute after AMPA as indicated in the chromatograph (however in left figure probably glyphosate 9-11 min, AMPA at 13 min and Peak 3 at 18 min if comparing to %AR reported for 7 DAT in results table). Peak identification is not clear in all available chromatographs of soil extracts. Positions in graph of 7 DAT do not agree with the findings in Table 7.1.1.2-4 in summary above. Similary, the peak identification in soil at 120 DAT seems rather speculative. With the peak at 18 min being supposedly "P3" on 7 DAT, this peak would be less prominent than indicated by the values in the table. The elution time of the indicated peak is later than elution times of glyphosate or AMPA.

Figure 7.1.1.2-1: Representative HPLC radio chromatogram following analysis of soil extract (Figure 4 from [redacted] 2004 Anaerobic Soil Metabolism study)



In order to have some confirmation on the identity of the potential degradation product of glyphosate, verification was seeked in similarly conducted analyses and found in a soybean metabolism study on glyphosate where N-methyl AMPA was identified ([redacted] *et al.*, 1994, CA 6.2.1/022). The chromatogram in Figure 7.1.1.2-2 (Figure 73B of the report), shows reference standards of AMPA and N-methyl AMPA under cation exchange chromatography conditions in phosphate buffer at pH 2.0 and shows N-methyl AMPA eluting earlier than AMPA.

Figure 7.1.1.2-2: Comparison of HPLC retention times of AmPA and N-methyl AMPA (CX HPLC/Refractive Index Detection Chromatogram; Figure 73B from [redacted] *et al.*, 1994, CA 6.2.1/022)



The amino acid sarcosine would be another option based on the position of the ^{14}C-label. However, sarcosine has not been found in GLP soil degradation studies and was rarely found in literature and if only in highly specific conditions, e.g. the presence of certain bacterial strains or the absence of phosphate. It might be, that in anaerobic conditions given specific circumstances, the C-P lyase pathway would be triggered to provide phosphate and this was the case in [redacted] (2004, CA 7.1.1.2/001). It would be unclear why sarcosine was not faster degraded to glycine, but this degradation step might be slowed down in anaerobic conditions. It would also remain unclear why sarcosine was never found in other anaerobic studies with similar conditions (SETAC 1995 protocol).

The tentative identification shows that peak "P3" was most likely not N-methyl-AMPA (due to the elution time). No further identification was possible. Overall, the available chromatographs on soil extracts are difficult to read and the labelling in the graphs show discrepancies to the characterisation of the single components of glyphosate, AMPA and "P3" in % AR. The study is considered as invalid to address the data point due to study design and issues in residue identification. The findings would therefore not be relevant for the current submission.

1. Information on the study

Data point:	CA 7.1.1.2/002
Report author	[redacted]
Report year	2003
Report title	Route and Rate of Anaerobic Soil Degradation of Glyphosate According to SETAC, Part 1, 1.2 (March 1995)
Report No	IF-02/00005224
Document No	
Guidelines followed in study	SETAC, Part 1, 1.2 (March 1995)
Deviations from current test guideline	From OECD 307: - Application of test substance to water layer after establishment of anaerobic conditions (no aerobic incubation phase prior flooding)
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

Glyphosate was tested in anaerobic soil according to SETAC Guideline, Part I, 1.2 March 1995). The study was performed in the laboratory using a mixture of radioactive glyphosate (radiopurity > 9 %) and unlabelled glyphosate, both analytical grades. The degradation of glyphosate was investigated under anaerobic conditions at 20°C in a flooded soil (Hofheim), classified as silt loam (USDA textural class). The test concentration of 5.8 mg/kg dry soil corresponding to a field rate of 4.32 kg a.s./ha assuming homogeneous penetration of the test .item in the top 5 cm of the soil and a soil density of 1.5 g/cm³. The flooded soil system was acclimatised under a dynamic atmosphere of nitrogen gas to maintain anaerobic conditions. Sampling intervals were at zero time, after 6 hours, 1 d, 7 d, 14 d, 32 d, 60 d, 90 d, and 120 days of incubation.

Processed water and soil extracts were assayed separately by liquid scintillation counting and radio-chromatography using TLC- and HPLC-chromatographic systems. Total recoveries of radioactivity ranged from 93.7 % to 101.0 % AR throughout the 120 days of incubation. Approximately 20 % AR were evolved as radioactive carbon dioxide within the scope of the experiment. The identification of $^{14}CO_2$ was performed by precipitation of $BaCO_3$ using barium chloride. Radioactivity in the water phases declined steadily to approximately 6 % AR at day 120. Corresponding levels in the soils yielded for approximately 70 % AR on day 120.

The non-extractable radioactivity in the soils accounted for 34.4 to 37.8 % AR on day 120. Most of the residual radioactivity (24.4 to 29.9 % AR) was found to be bound to the humin fraction of the soil after 120 days of anaerobic incubation and is not expected to be bioavailable.

Glyphosate degraded rapidly in the water phase of the test system and decreased to approximately 40 % AR and 5 % AR (equivalent to 2.32 and 0.29 mg/kg) at the day 1 and day 60, respectively. In the processed soil extractable radioactivity glyphosphate was found after 6 hrs with an average value of approximately 21 % AR and increased to approximately 43 % AR at day 32. At experimental end (120 days) glyphosate accounted for approximately 13 % AR (equivalent to 0.75 mg/kg). In the processed water and soil extracts unknown metabolites reached maximum levels < 5 % AR (equivalent to 0.29 mg/kg parent equivalents). The major metabolite of glyphosate AMPA was identified by TLC- and HPLC chromatographic analysis using radioactive known reference item. AMPA was identified in the water specimens and predominantly in the soil phase of the test system reaching a maximum < 5 % AR (0.29 mg/kg parent equivalents) in the water phase. In the processed soil extractable radioactivity AMPA remained at a plateau value of approximately 20 % AR by day 60 to day 120. Glyphosate was converted in both anaerobic compartments of the test system into unknown degradates of low concentration (max. 2.3 % AR in soil). The anaerobic degradation of glyphosate was reflected by the formation of AMPA, residual residues and the formation of $^{14}CO_2$.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled test material

Identification: [^{14}C]-glyphosate
Lot No.: Amersham Pharmacia CFQ 12960/BE9180
Specific activity: 57 mCi/mg
Radiochemical purity: 98.1 %
Chemical purity: not provided

Non-radiolabelled test compound

Identification: Glyphosate
Lot No.: sigma Aldrich 1025x
Chemical purity: 99 %

2. Soil:

Soils was sieved to ≤ 2 mm. The soil was received and stored in a sealed transport container at ambient temperature. Characteristics of the test soil are presented in the table below.

Table 7.1.1.2-5: Characteristics of test soil

Parameter	Results
Soil	Hofheim
Country	Germany
Textural Class (USDA)	Silt loam
Sand (50 µm – 2 mm) (%)	29.9
Silt (2 µm – 50µmm) (%)	52.3
Clay (< 2 µm) (%)	17.8
pH (water)	6.06
pH ($CaCl_2$)	5.10
Organic carbon (%)	1.24
Organic matter (%)	2.14
Cation exchange capacity (meq/100 g)	13.5 meq/100g dry soil
Maximum Water Holding Capacity (%)	43.0
Bulk Density (disturbed) (g/cm^3)	1119 g/L
Microbial biomass after sampling (initial value) (mg C/100g dry soil)	24
Microbial biomass at study end (day 120) (mg C/100g dry soil)	3

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

Soil equivalent to 100 g dry weight (moistened to approximately 40 % maximum water holding capacity) were bottled into 1000 mL glass vessels. Bottled soil was flooded by addition of reagent water (water column height ca. 2 cm) and maintained under a dynamic atmosphere of nitrogen gas, in the dark, at 20 °C. Nitrogen gas leaving the system was passed sequentially through a series of traps to collect any carbon dioxide and organic volatiles produced. The study was carried out with duplicate specimens at each

sampling point. Metabolism vessels and trapping system were connected via tubing. On a weekly basis the nitrogen gas stream of approximately 10-15 rnL/min was measured. Flooded soil was acclimatized under a dynamic atmosphere of nitrogen gas. A pre-incubation phase of approximately 2 months was needed to reach an anaerobic equilibrium of the test matrix based on measurable variables (redox potential of water and sediment oxygen concentration of water, and pH-value of water and soil).

The stability of [^{14}C]-glyphosate in the application solution was confirmed by LSC and radio-chromatography before and after application. Reserved test matrix for the determination of aged microbial activity of anaerobic soils was treated at 5.8 mg/kg dry soil using unlabelled glyphosate. .

Each individual test vessel was treated with the application solution to give glyphosate concentrations of 5.8 mg/kg. Aliquots of glyphosate application solution were added using a 250 µl syringe. The test item was applied in reagent water onto the surface of the water phase.

If the water level dropped more than 10 % below 2 cm equivalent to 180 mL (in weight equivalents), oxygen free reagent water was added until the desired water level was obtained. The redox potential of water and soil, oxygen concentration of water and pH value of water and soils were determined at each sampling interval or at about 14 day intervals using specimens for the determination of aged microbial activity of soils, and specimens taken for analysis.

2. Sampling

Duplicated specimen were taken at the following sampling times: zero time, 6 hours, and 1, 7, 14, 32, 60, 90 and 120 days. Aliquots from the volatile traps were radio-assayed at each sampling time (excluding zero time) or at about 14 day intervals, whichever came first.

3. Analytical procedures

After removal of the water phase from the test system by decantation, water and soil were assayed separately for their radiocarbon concentration and their radiocarbon composition.

The soils were transferred quantitatively into 750 mL vessels and extracted several times by shaking with 100 mL of 1 M Nik-solution. The extraction solvent was separated from the soil by centrifugation (10 minutes at 4500 rpm). The sequential extractability of radioactivity of each individual extract as well as the combined extraction solutions on a per specimen basis was radio-assayed by liquid scintillation counting (LSC). The final extraction step resulted in < 5 % of the applied radioactivity (% AR). The combined extraction solutions were adjusted to pH 2 by the addition of HCL and again centrifuged. There was no loss radioactivity during acidification of specimens.

Specimen extracts were subjected to further concentration using freeze drying if necessary, and specimen residues were reconstituted with reagent water. The volume of the specimen, extract concentrate was measured and subjected to LSC for confirmation that there was no loss of radioactivity during specimen concentration. Processed specimen extracts were subjected to radio-chromatography (HPLC and TLC). Each chromatographic analysis was performed in duplicate.

After exhaustive soil extraction the residual radioactivity in soils was assayed by combustion. Remaining soil was stored at room temperature in tightly closed storage containers.

The extracted soils of the 120 day samplings (air dried-and ground) were subjected to further characterisation of soil radioactivity which remained bound to the humic and fulvic acids and the humin fraction.

After separation from the soil phase, the volume of the water phase was measured and aliquots analysed by LSC. A concentration of the water phases was performed by freeze drying, if needed. specimen residues were reconstituted with reagent water. The volume of the specimen extract concentrate was measured and subjected to LSC for confirmation that there was no loss of radioactivity during specimen concentration. Processed specimen extracts were subjected to radio-chromatography (HPLC and TLC) in duplicate.

Radioactivity in solution was determined by liquid scintillation counting (LSC) in triplicate per specimen.

Thin Layer Chromatography (TLC) was performed on pre-coated plates of lonex-25 SA-Na. One dimensional thin layer chromatography was used for the separation of specimen extract aliquots. The TLC plates were developed under chamber saturation conditions with a general target development distance of approximately 16 cm.

HPLC was based on a glyphosate cation-exchange column by Pickering. After HPLC separation of specimen aliquots, radioactive signals were detected by means of a radioactivity monitor/UV-photometer. The resulting peaks observed by the radioactive monitor were taken and quantified in relation to the summed radiochemical signals of the run time of interest (% area). Radioactive signals were quantified and characterised by comparing their retention time with the retention times of the pure reference items.

The identification of CO_2 was performed by precipitation $BaCO_3$ using barium chloride.

II. RESULTS AND DISCUSSION

A. DATA

Table 7.1.1.2-6: Recovery of radioactivity in water and soil under anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

Fraction	Replicate	DAT								
		0	0.25	1	7	14	32	60	90	120
Carbon dioxide	A	n.p.	0.1	0.2	0.6	1.1	6.0	15.3	19.1	20.1
	B	n.p.	0.1	0.2	0.6	1.1	6.0	15.3	19.1	20.1
	Mean	n.p.	0.1	0.2	0.6	1.1	6.0	15.3	19.1	20.1
Volatile organic compounds	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total water	A	96.5	65.3	42.7	36.5	24.0	8.5	5.5	3.5	5.8
	B	96.4	62.2	44.5	38.1	17.7	7.0	6.9	4.7	5.7
	Mean	96.5	63.8	43.6	37.3	20.9	7.8	6.2	4.1	5.8
Total extractables soil	A	1.9	25.4	41.1	40.4	51.6	51.8	50.2	45.1	35.8
	B	1.8	28.2	40.7	37.5	54.7	53.4	49.5	42.8	32.7
	Mean	1.9	26.8	40.9	39.0	53.2	52.6	49.9	44.0	34.3
Non-extractable Residues	A	0.5	3.4	9.7	19.8	20.1	32.2	24.3	31.7	34.4
	B	0.6	7.4	8.7	21.8	27.0	29.9	29.3	31.5	37.8
	Mean	0.6	5.4	9.2	20.8	23.6	31.1	26.8	31.6	36.1
Mass balance	A	98.9	94.2	93.7	97.3	96.8	98.5	95.3	99.4	96.1
	B	98.8	97.9	94.1	98.0	100.5	96.3	101.0	98.1	96.3
	Mean	98.9	96.1	93.9	97.7	98.7	97.4	98.2	98.8	96.2

DAT: days after treatment
nd: not detected
np: not performed

Table 7.1.1.2-7: Characterisation of radioactivity in water and soil extracts under anaerobic conditions following application of [14C]-glyphosate (expressed as percent of applied radioactivity)

Compound		Replicate	DAT								
			0	0.25	1	7	14	32	60	90	120
Glyphosate	Water	A	92.9	59.4	41.3	34.0	20.9	7.5	4.8	1.5	2.1
		B	93.0	58.3	42.2	36.0	15.8	5.8	6.2	2.4	2.3
		Mean	93.0	58.9	41.8	35.0	18.4	6.7	5.5	2.0	2.2
	Soil	A	n.p.	4.7	7.4	8.1	8.2	5.8	20.6	19.5	19.5
		B	n.p.	6.2	7.5	8.5	8.3	9.5	17.4	19.1	17.1
		Mean	n.p.	5.5	7.5	8.3	8.3	7.7	19.0	19.3	18.3
AMPA	Water	A	2.7	3.0	0.8	1.5	1.4	0.4	0.8	1.2	3.2
		B	2.2	2.3	1.1	1.2	1.3	0.6	0.8	1.4	3.0
		Mean	2.5	2.7	1.0	1.4	1.4	0.5	0.8	1.3	3.1
	Soil	A	n.p.	4.7	7.4	8.1	8.2	5.8	20.6	19.5	19.5
		B	n.p.	6.2	7.5	8.5	8.3	9.5	17.4	19.1	17.1
		Mean	n.p.	5.5	7.5	8.3	8.3	7.7	19.0	19.3	18.3
Largest unknown	Water	A	1.0	1.5	0.6	1.0	0.7	0.5	nd	0.7	0.6
		B	1.3	1.1	0.7	1.0	0.4	0.6	nd	0.9	0.5
		Mean	1.2	1.3	0.7	1.0	0.6	0.6	nd	0.8	0.6
	Soil	A	n.p.	nd	nd	nd	1.1	1.6	nd	nd	2.4
		B	n.p.	nd	nd	nd	1.3	1.1	nd	nd	2.0
		Mean	n.p	nd	nd	nd	1.2	1.4	nd	nd	2.2
All unknowns	Water	A	1.0	3.1	0.8	1.0	1.8	0.8	nd	0.9	0.6
		B	1.3	1.6	1.3	1.0	0.9	0.6	nd	1.1	0.5
		Mean	1.2	2.4	1.1	1.0	1.4	0.7	nd	1.0	0.6
	Soil	A	n.p.	nd	nd	nd	1.8	2.6	nd	nd	3.1
		B	n.p.	nd	nd	nd	2.1	1.7	nd	nd	2.8
		Mean	n.p	nd	nd	nd	2.0	2.2	nd	nd	3.0

Table 7.1.1.2-8: Characterisation of radioactivity in flooded soil (sum of water and soil extracts) under anaerobic conditions following application of [14C]-glyphosate (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	7	14	32	60	90	120
Glyphosate	A	92.9	80.2	75.0	66.4	62.5	51.0	34.4	27.1	15.5
	B	93.0	80.3	75.4	65.0	60.2	48.1	38.3	26.2	15.3
	Mean	93.0	80.3	75.2	65.7	61.4	50.6	36.4	26.7	15.4
AMPA	A	2.7	7.7	8.2	9.6	9.6	6.2	21.4	20.7	22.7
	B	2.2	8.5	8.6	9.7	9.6	10.1	18.2	20.5	20.1
	Mean	2.5	8.1	8.4	9.7	9.6	8.2	19.8	20.6	21.4
Largest unknown	A	1.0	1.5	0.6	1.0	1.8	2.1	nd	0.7	3.0
	B	1.3	1.1	0.7	1.0	1.7	1.7	nd	0.9	2.5
	Mean	1.2	1.3	0.7	1.0	1.8	1.9	nd	0.8	2.8
All unknowns	A	1.0	3.1	0.8	1.0	3.6	3.4	nd	0.9	3.7
	B	1.3	1.6	1.3	1.0	3.0	2.3	nd	1.1	3.3
	Mean	1.2	2.4	1.1	1.0	3.3	2.9	nd	1.0	3.5

Table 7.1.1.2-9: Fractionation of day 120 post extracted soil (in percent of applied radioactivity)

Experiment	Fulvic acid	Humic acid	Humin
120 DAT A 20 °C	6.3	3.8	24.4
120 DAT B 20 °C	5.2	2.6	29.9

B. MASS BALANCE
The mass balance range for the individual sampling times (0, 6 hrs, 1, 7, 14, 32, 60, 90, and 120 days) was 93.7 to 101.0 % AR.

Radioactivity disappeared very fast from the treated water phases. At zero time 96.4 and 96.5 % AR were found in the soil surface water. After 1 day of incubation approximately 40 % AR were detected in the water phases. At experimental end (120 day's) the remaining radioactivity in the water phases was 5.8 % AR.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
The extractable radioactivity in the soil increased over time, reaching a plateau value of approximately 50 % AR by day 14 to 60. At experimental end the soil extractable radioactivity was reduced to 32.7 and 35.8 % AR. Respectively.

The non-extractable radioactivity in the soils increased over time. The residual radioactivity in the soil by day 120 was 34.4 and 37.8 % AR.

D. VOLATILE RADIOACTIVITY
After 120 days of anaerobic incubation the amounts of CO_2 evolved accounted for 20.1 % AR maximum. Liberated volatile organics were below 0.1 % of the applied radioactivity at experimental end (120 days).

E. TRANSFORMATION OF THE TEST ITEM
Glyphosate was present at zero time in the assayed water phases with an average value of approximately 93 % AR. It is level decreased rapidly to approximately 40 % and 5 % AR (equivalent to 2.32 and 0.29 mg/kg) at the 1 day and 60 day sampling times, respectively. Glyphosate was found at the 6 hour time in the processed soil extractable radioactivity with an average value of approximately 21 % AR. It is level increased to approximately 43 % AR in the processed soil extractable radioactivity of day 32. At experimental end (120 days) glyphosate accounted for approximately 13 % AR in the processed soil extractable radioactivity.

In the processed water and soil extracts unknown metabolites reached maximum levels less than 5 % AR (maximum: 2.4 % AR in the soil and 3.0 % AR in the total test system) of the applied radioactivity. The major metabolite of glyphosate, namely AMPA (aminomethylphosphonic acid), was identified by HPLC- and TLC- chromatographic analysis using a known reference item. AMPA was identified in the soil and water specimens of the test system and reached a maximum level of below 5 % AR in the water phases. In the processed soil extractable radioactivity AMPA underwent a plateau value of approximately 20 % AR by day 60 to 120. Further, glyphosate was converted in both anaerobic compartments of the test system into unknown degradates of low concentration (3.0 % AR maximum fraction in the total test system equivalent to 0.17 mg/kg parent equivalents).

F. KINETICS
New kinetic calculations based on recent guidance were not provided due to the supporting character of the study.

III. CONCLUSIONS

Glyphosate degraded rapidly in the water phase of the test svstem.

The major degradation products of glyphosate produced under anaerobic conditions were AMPA and carbon dioxide. AMPA was found in the water specimens and predominantly in the soil phases of the test system.

AMPA reached a maximum level of below 5 % AR in the water phase in the processed soil extractable radioactivity AMPA underwent a plateau value of approximately 20 % of the applied radioactivity (% AR) to the test system. The evolved CO_2 accounted for 20 % AR at experimental end. Unknown degradation products of low concentration (3.0 % AR maximum fraction in the total test system equivalent to 0.17 mg/kg parent equivalents) were formed in the flooded soil system.

The appearance of AMPA, the formation of bound residues, and the formation of carbon dioxide reflect the anaerobic degradation pathway of glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides information on the degradation behavior of glyphosate in soil under established thus strict anaerobic conditions. Such application to strictly anaerobic conditions (50 days) is not in agreement with the current guideline.
Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.2/003
Report author	[redacted]
Report year	2003
Report title	The degradation of [14C]-Glyphosate in soil under anaerobic conditions
Report No	22581
Document No	
Guidelines followed in study	OECD 307
Deviations from current test guideline	From OECD 307: none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The route and rate of [^{14}C]-glyphosate degradation at a nominal concentration of 5 mg/kg soil under anaerobic conditions following an aerobic ageing period was investigated in a UK sandy loam soil.

Samples were incubated in the dark at 20 °C for 10 days (pre-determined aerobic half-life) under aerobic conditions and were then flooded by the addition of water and incubated for an additional 120 days under a nitrogen atmosphere.

At various intervals up to 120 days post-flooding, duplicate samples were removed and the radioactive distribution determined.

The mean overall recovery of applied radioactivity from the aerobic phase ranged from 104 % to 109 % AR (applied radioactivity). The mean recovery during the anaerobic phase ranged from 97 % to 105 % AR.

Glyphosate was degraded rapidly during the aerobic period of 10 days. At zero time (aerobic), glyphosate accounted for 102.8 % AR. Following 10 days of aerobic incubation, glyphosate accounted for 55.0 % of the applied radioactivity. The only significant degradation products detected were AMPA (19.2 % AR, mean of replicates) and $^{14}CO_2$ (12 % of AR).

Upon initiation of anaerobic conditions, the rate of degradation was observed to slow down significantly. During the anaerobic phase, levels of glyphosate declined from 57.7 % of the applied radioactivity 1 h after flooding to 39.1 % after 120 days. During the anaerobic phase, liberation of $^{14}CO_2$ was significantly reduced when compared to aerobic ageing, and AMPA was the only significant metabolite generated reaching a maximum of 30.2 % of applied radioactivity after 84 days of anaerobic incubation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate (N-(phosphonomethyl) glycine)
Lot No.: 2213-04.3
Specific activity: 38.79 µCi/mg or 6.56 mCi/mmol
Radiochemical purity: Radiochemical purity 97.28 % (HPLC)

2. Soil:

The soil was collected from the upper 20 cm layer of a grassland site by removing surface vegetation and collecting the top soil. Soil was sieved (2 mm) prior to use on the study. The sandy loam soil was supplied by Landlook, Midlands, UK from a site with no pesticide or organic fertiliser treatments for at least five years prior to collection. Characteristics of the test soils are presented in the table below.

Table 7.1.1.2-10: Characteristics of test soil

Parameter	
Soil	Landlook,
Country	UK
Textural Class (USDA)	Sandy loam
Sand (50 µm – 2 mm) (%)	69.43
Silt (2 µm – 50µmm) (%)	18.85
Clay (< 2 µm) (%)	11.72
pH (KCl)	5.9

Table 7.1.1.2-10: Characteristics of test soil

Organic carbon (%)	1.8
Cation exchange capacity (meq/100 g)	15.7
Water Holding Capacity at 1/3 bar (%)	16.0
Microbial biomass (mg C/100g)	32.4

B. STUDY DESIGN

1. Experimental conditions

A total of 22 soil samples (ca. 50 g oven dry equivalent) were prepared. The moisture content of each soil sample was adjusted to ca 50 % maximum water holding capacity and maintained at this level throughout the aerobic phase of the study. Soil samples were pre-incubated under aerobic conditions at 20 ± 2 C for 8 days prior to application for an acclimation period.

The dosing solution was prepared by combining an aliquot of non-labelled glyphosate standard with an aliquot of [^{14}C]-glyphosate test substance dissolved in water. The resulting [^{14}C]-glyphosate treatment solution which had a specific activity of 29.96 µCi/mg was used for dosing. Treatment solution was applied drop-wise to the surface of the soil with the radioactive application of 7.82 µCi, equivalent to an application rate of 5.22 mg/kg (dry weight equivalent).

Following test material application, the samples were re-connected to the continuous air flow system and incubated under aerobic conditions at 20 ± 2 C in the dark for a period of 10 days after application of glyphosate (pre-determined aerobic half-life). The gas mixture leaving each flask was passed through four traps, the first one acting as a safety trap, the second one contained ethanediol to trap non-specific organic volatiles and the final 2 traps contained 2 M sodium hydroxide to trap liberated $^{14}CO_2$.

Following removal of day 10 aerobic incubates, all remaining soil samples were then flooded by the addition of approximately 100 mL milli-Q water to give a depth of 1-3 cm. A stream of moist nitrogen was then introduced to the test system. The samples were maintained under anaerobic conditions at 20 ± 2 C in the dark for a period of 120 days post-flooding. Two additional samples were prepared for the in situ redox measurements during the anaerobic phase. The measured redox potential indicates that the test system achieved anaerobic conditions 14 days post flooding.

2. Sampling

During the aerobic phase (10 days), duplicate soil samples were taken for analysis at zero time (immediately post-application) and at the pre-determined aerobic soil half-life (10 days after application). Duplicate soil samples were removed and analysed at 1 h, and 3, 7, 14, 28, 56, 84 and 120 days post-flooding. Trapping solutions were removed and analysed at the time of removal of the respective incubation flasks.

3. Analytical procedures

For the aerobic phase of the study, soil samples were transferred into centrifuge bottles and extracted three times with 0.5 M ammonium hydroxide (100 mL) using an end-over-end shaker for a period of approximately one hour. After shaking, the extract was separated from the residue by centrifugation (2200 g, 30 min) and the radioactivity in the supernatant was determined by liquid scintillation counting. The quantitative distribution of radiolabelled components in the combined soil extracts was determined using ion exchange HPLC.

For the anaerobic phase of the study, the surface water was separated from soils by decanting. The remaining soils were processed in the same way as aerobic soil sample. Surface waters containing >5 % of applied radioactivity were also subjected to chromatographic analysis (HPLC and TLC). Following extraction, the radioactivity remaining in the post-extracted soil was determined by combustion analysis.

The distribution of radioactivity in organic matter in selected post-extracted soil samples was determined. Each sample was extracted by shaking in 0.5 M sodium hydroxide (ca 100 mL) for about one hour. The extracts were separated by centrifugation from soil residues and the radioactive content of the soil (humin) was determined by combustion analysis. The extract was adjusted to ca pH 1 using concentrated hydrochloric acid, to precipitate the humic acid fraction. The extract was centrifuged and the supernatant containing the fulvic acid fraction, was removed and aliquots were submitted for liquid scintillation counting. Radioactivity associated with the humic acid fraction was quantified by dissolving directly in scintillation fluid.

Prior to chromatographic analysis for each individual soil sample, an aliquot of each extract was combined. All combined soil extracts and surface water samples containing >5 % of the applied radioactivity were analysed using HPLC.

For TLC analyses, aliquots of selected sample extracts and surface waters were applied to Polygram Ionex-25 SA-Na TLC plates which were subsequently developed in 0.01 M potassium dihydrogen phosphate (adjusted to ca pH 2 with concentrated phosphoric acid): methanol (9:1, v/v). Non-radiolabelled glyphosate and AMPA prepared in Milli-Q grade water were chromatographed at each sample. Following chromatography, the areas of radioactivity present on TLC plates were quantified using a Molecular Dynamics phosphor imager or a Fuji FLA5000 phosphor imager. Standards were visualised using Ninhydrin spray reagent.

For combustion analyses, cellulose powder and Combustaid® (ca 100 µL) were added to triplicate portions of air-dried soil residues (ca 0.3 g) prior to combustion in oxygen using a Packard Sample Oxidiser, Model 307. The combusted products were absorbed in Carbo-Sorb®, mixed with Permafluor®E+ and the radioactivity determined by liquid scintillation counting.

All extract aliquots, surface water aliquots, trap solution aliquots and apparatus wash aliquots were added directly to scintillates and submitted for liquid scintillation counting. All radioassays were performed in duplicate. Radioactivity was quantified using a liquid scintillation analyser (Packard 1600TR or Packard 2100TR), with automatic quench correction by external standard-channels ratio. A limit of reliable determination of 30 dpm above background has been instituted in these laboratories.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts are summarised in Table 7.1.1.2-11 to Table 7.1.1.2-14.

Table 7.1.1.2-11: Recovery radioactivity from water and soil under aerobic followed by anaerobic conditions following application of [^{14}C]-glyphosate

Time point	Study phase	Sample	% of Applied Radioactivity						
			Water	Soil extract	$^{14}CO_2$[1]	Volatiles[2]	Non-extractable residue	Apparatus wash	Mass Balance
Zero time	Aerobic phase	Rep A	NS	107.42	NS	NS	2.33	NS	109.75
		Rep B	NS	106.08	NS	NS	2.45	NS	108.53
		Mean	-	106.75	-	-	2.39	-	109.14
Day 10		Rep A	NS	75.59	12.37	0.01	15.11	0.01[3]	103.09
		Rep B	NS	76.66	12.50	ND	14.78	0.01[3]	103.95
		Mean	-	76.13	12.44	0.01	14.95	0.01[3]	103.52
1 h	Anaerobic phase	Rep A	0.49	76.96	12.49	ND	12.65	0.05	102.64
		Rep B	0.49	78.18	12.46	ND	13.21	0.02[3]	104.36
		Mean	0.49	77.57	12.48	-	12.93	0.04[3]	103.50
Day 3		Rep A	1.20	72.76	13.13	0.01	16.38	0.01[3]	103.49
		Rep B	1.47	74.61	12.97	0.01	16.73	0.01[3]	105.80
		Mean	1.34	73.69	13.05	0.01	16.56	0.01[3]	104.65
Day 7		Rep A	0.97	71.36	12.14	ND	17.68	0.02[3]	102.17
		Rep B	1.17	70.46	1.64[4]	ND	17.88	0.01[3]	91.16
		Mean	1.07	70.91	6.89	-	17.78	0.02[3]	96.67
Day 14		Rep A	1.82	67.30	13.08	ND	20.22	0.01[3]	102.43
		Rep B	2.06	66.94	13.32	0.01	21.05	0.01[3]	103.39
		Mean	1.94	67.12	13.20	0.01	20.64	0.01[3]	102.91
Day 28		Rep A	5.90	65.25	13.40	0.01	20.57	0.01[3]	105.14
		Rep B	4.36	67.61	13.44	0.01	20.41	ND	105.83
		Mean	5.13	66.43	13.42	0.01	20.49	0.01[3]	105.49
Day 56		Rep A	5.41	62.15	12.18	0.01	24.74	0.01[3]	104.50
		Rep B	5.48	63.06	1.73[4]	0.01	24.52	0.01[3]	94.81
		Mean	5.45	62.61	6.96	0.01	24.63	0.01[3]	99.66
Day 84		Rep A	5.72	62.19	12.52	0.52	22.02	0.03[3]	103.00
		Rep B	6.37	61.73	13.01	0.27	22.53	0.01[3]	103.92
		Mean	6.05	61.96	12.77	0.40	22.28	0.02[3]	103.46
Day 120		Rep A	6.31	61.55	11.89	0.28	22.54	ND	102.57
		Rep B	6.78	60.36	13.13	0.29	22.49	0.01[3]	103.06
		Mean	6.55	60.96	12.51	0.29	22.52	0.01[3]	102.82

[1] trapped with 2M sodium hydroxide
[2] non-specific organic volatiles: trapped with ethanediol
[3] results calculated from data less than 30 dpm above background
[4] low recoveries of $^{14}CO_2$ are probably caused by a leak in the flow-through apparatus
NS = no sample, ND = not detected

Table 7.1.1.2-12: Characterization of radioactivity in soil extracts under aerobic followed by anaerobic conditions following application of [^{14}C]-glyphosate

Time point	Study phase	Sample	% of applied radioactivity – Soil extracts – Glyphosate	AMPA	Unknown B	Unknown C
Zero time	Aerobic phase	Rep A	104.41	3.01	ND	ND
		Rep B	101.20	4.88	ND	ND
Day 10		Rep A	55.85	18.92	0.82	ND
		Rep B	54.05	19.38	ND	3.23
1 h	Anaerobic phase	Rep A	57.50	19.46	ND	ND
		Rep B	56.99	21.19	ND	ND
Day 3		Rep A	53.35	19.41	ND	ND
		Rep B	54.85	19.76	ND	ND
Day 7		Rep A	53.08	18.28	ND	ND
		Rep B	51.84	18.62	ND	ND
Day 14		Rep A	46.38	20.92	ND	ND
		Rep B	46.44	20.49	ND	ND
Day 28		Rep A	39.06	26.19	ND	ND
		Rep B	42.37	25.24	ND	ND
Day 56		Rep A	31.59	30.56	ND	ND
		Rep B	40.52	22.54	ND	ND
Day 84		Rep A	32.85	29.34	ND	ND
		Rep B	31.74	29.99	ND	ND
Day 120		Rep A	31.62	29.93	ND	ND
		Rep B	33.40	26.96	ND	ND

NS = no sample, ND = not detected, NP = not profiled as < 5 % applied radioactivity in sample

Table 7.1.1.2-13: Characterization of radioactivity in water under aerobic followed by anaerobic conditions after application of [^{14}C]-glyphosate

Time point	Study phase	Sample	% of applied radioactivity – Water – Glyphosate	AMPA	Unknown B	Unknown C
Zero time	Aerobic phase	Rep A	NS	NS	NS	NS
		Rep B	NS	NS	NS	NS
Day 10		Rep A	NS	NS	NS	NS
		Rep B	NS	NS	NS	NS
1 h	Anaerobic phase	Rep A	NP	NP	NP	NP
		Rep B	NP	NP	NP	NP
Day 3		Rep A	NP	NP	NP	NP
		Rep B	NP	NP	NP	NP
Day 7		Rep A	NP	NP	NP	NP
		Rep B	NP	NP	NP	NP
Day 14		Rep A	NP	NP	NP	NP
		Rep B	NP	NP	NP	NP
Day 28		Rep A	5.90	ND	ND	ND
		Rep B	NP	NP	NP	NP
Day 56		Rep A	5.41	ND	ND	ND
		Rep B	5.48	ND	ND	ND
Day 84		Rep A	5.46	0.26	ND	ND
		Rep B	5.47	0.90	ND	ND
Day 120		Rep A	6.31	ND	ND	ND
		Rep B	6.78	ND	ND	ND

NS = no sample, ND = not detected, NP = not profiled as <5 % applied radioactivity in sample

Table 7.1.1.2-14: Characterization of radioactivity in water/soil system under aerobic followed by anaerobic conditions following application of [^{14}C]-glyphosate

Time point	Study phase	Sample	% of applied radioactivity			
			Total			
			glyphosate	AMPA	Unknown B	Unknown C
Zero time	Aerobic phase	Rep A	104.41	3.01	ND	ND
		Rep B	101.20	4.88	ND	ND
Day 10		Rep A	55.85	18.92	0.82	ND
		Rep B	54.05	19.38	ND	3.23
1 h	Anaerobic phase	Rep A	57.99[1]	19.46	ND	ND
		Rep B	57.48[1]	21.19	ND	ND
Day 3		Rep A	54.55[1]	19.41	ND	ND
		Rep B	56.32[1]	19.76	ND	ND
Day 7		Rep A	54.05[1]	18.28	ND	ND
		Rep B	53.01[1]	18.62	ND	ND
Day 14		Rep A	48.20[1]	20.92	ND	ND
		Rep B	48.50[1]	20.49	ND	ND
Day 28		Rep A	44.96	26.19	ND	ND
		Rep B	46.73[1]	25.24	ND	ND
Day 56		Rep A	37.00	30.56	ND	ND
		Rep B	46.00	22.54	ND	ND
Day 84		Rep A	38.31	29.60	ND	ND
		Rep B	37.21	30.89	ND	ND
Day 120		Rep A	37.93	29.93	ND	ND
		Rep B	40.18	26.96	ND	ND

NS = no sample, ND = not detected, NP = not profiled as <5 % applied radioactivity in sample
[1] Radioactivity in surface water for these samples accounted for <5 % applied activity. It was assumed to be glyphosate and was included in total.

B. MASS BALANCE
The mean total recoveries from the sand loam soil incubated for 10 days, during the aerobic phase of the study were in a range of ca. 104 % to 109 % AR (mean of replicates).

The mean recoveries under anaerobic conditions incubated subsequently for up to 120 days were in the range of ca. 97 to ca. 105 % AR.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
Extractable radioactivity accounted for 107 % AR at zero time (aerobic phase), declining to 63 % AR after 56 days post flooding (anaerobic phase), remaining relatively constant.

Radioactivity associated with surface water following flooding was observed to increase slowly from <1 % applied after 1 hour to 5 % after 28 days. At day 120 levels of radioactivity associated with the surface water accounted for 7 % AR.

Non-extractable residue increased from a minimum of 2 % AR at zero time to a maximum of 25 % after 56 days. At day 120, levels recovered as non-extractable residues accounted for 23 % AR (mean of two replicates).

D. VOLATILE RADIOACTIVITY
$^{14}CO_2$ was observed during the aerobic phase of the study and accounted for 12 % of applied radioactivity in the majority of study samples prior to flooding. Production of $^{14}CO_2$ after initiation of anaerobic conditions decreased to levels of less than 2 % of applied radioactivity for the remainder of incubation. Radioactivity associated to ethanediol trap (non-specific volatiles) and apparatus washings accounted for <0.1 % of applied radioactivity.

E. TRANSFORMATION OF THE TEST ITEM

At zero time (aerobic), levels of glyphosate in the water/soil system were quantified and subsequently declined to 55.0 % (mean of two replicates) after 10 days of aerobic incubation. Upon initiation of anaerobic conditions (1-hour post-flooding), levels of glyphosate accounted for 57.7 % applied radioactivity and decreased to 39.1 % after 120 days of incubation under anaerobic conditions.

The only significant degradation product detected was AMPA. At zero time AMPA accounted for 3.9 % applied radioactivity, increasing to a maximum of 30.2 % after 84 days and subsequently declining to 28.4 % after 120 days of incubation (all values representing mean of two replicates).

No other compounds were detected above 5 % AR at any time.

F. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in Anagu (2020, CA 7.1.2.1.3/001).

III. CONCLUSIONS

Glyphosate was degraded rapidly during the aerobic period of the study. The only significant metabolites detected were AMPA and $^{14}CO_2$. Following initiation of anaerobic conditions, the rate of degradation was observed to slow down significantly. During the anaerobic phase, liberation of $^{14}CO_2$ was significantly reduced when compared to the aerobic ageing period, and AMPA was the only significant metabolite.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study adequately describes the degradation behavior of glyphosate in soil under anaerobic conditions of the laboratory. No deficiencies or deviations occurred. The study wasused for subsequent kinetic evaluation following latest EU guidance.
The study is considered valid to adress the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.2/004
Report author	[redacted]
Report year	2000
Report title	The degradation of [^{14}C]-Glyphosate in soil under anaerobic conditions
Report No	18201
Document No	
Guidelines followed in study	SETAC (1995)
Deviations from current test guideline	From OECD 307: - Application of test substance to samples following establishment of strict anaerobic conditions (no aerobic incubation phase prior flooding)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The objective of this study was to investigate the rate and route of degradation of radiolabeled glyphosate in a flooded sandy loam soil under anaerobic conditions at a nominal temperature of 20 °C. The study was conducted using procedures outlined in SETAC Document "Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides (March 1995)".

Samples of sandy loam soil (50 g dry weight equivalent) were dispensed into incubation flasks and flooded with Milli-Q grade water to a depth of ca. 3 cm over the soil surface. To establish anaerobic conditions, the flooded soil samples were purged with a stream of moist, oxygen-free nitrogen. The establishment of anaerobic conditions was monitored regularly by measuring the redox potential of the soil in selected samples. Following a pre-incubation period of 39 days under anaerobic conditions, samples of flooded soil were treated with [^{14}C]-glyphosate at a rate of 5 mg/kg.

The samples were incubated in the dark at a nominal temperature of 20 °C for up to 120 days under anaerobic conditions. Ethanediol and ethanolamine were used to collect non-specific volatiles and $^{14}CO_2$, respectively. At intervals throughout the 120-day incubation period, duplicate samples were removed for analysis of total radioactivity. The trap reagents were collected and replenished when samples were removed for analysis or at 4 weekly intervals (whichever was first). At each sampling interval the surface water was separated from the soil and analysed by liquid scintillation counting. The radioactivity present in the water was analysed by HPLC and TLC. The soil was extracted with 0.5 M ammonium hydroxide and the nature of radioactivity extracted from the soil was investigated by HPLC and TLC analysis.

The mean recovery of applied radioactivity from flooded sandy loam soil up to and including 120 DAT ranged from 95 % to 102 %. The total levels of radioactivity extracted from the soil increased from 8 % at zero time to 59 % at 14 DAT and remained around this level for the remainder of the incubation period. As the total levels of extractable radioactivity increased with time, a concomitant decrease in the levels of radioactivity present in the surface water resulted. At zero time, 93 % of applied radioactivity was associated with the surface water and levels decreased to 18 % by 14 DAT. Beyond 14 DAT, levels of radioactivity in the surface water declined more slowly, accounting for 10 % AR at study termination. Radioactivity associated with the non-extractable residue increased from 2 % AR at zero time to 20 % AR

at 14 DAT and remained around this level for the remainder of the incubation period. Radioactivity recovered as $^{14}C0_2$ and non-specific-volatiles was very low (<1 %).

Following extraction, the organic matter from single replicates from 90 DAT and 120 DAT was fractionated into humin, humic acid and fulvic acid. Radioactivity associated with the humin, fulvic acid and humic acid accounted for up to 9, 10 and 5 %, respectively.

HPLC analysis of the surface water and soil extracts indicated that the principal component detected co-chromatographed with glyphosate. At zero time, levels of glyphosate in the test system accounted for 95 % of applied radioactivity. As the incubation progressed, levels of parent compound declined, accounting for 68 % at study termination. In addition to parent compound, low levels of the degradation products AMPA (aminomethylphosphonic acid) and HMPA (Hydroxymethyl phosphonic acid) were detected in samples at intervals throughout the study, accounting for up to 8 and 1 % of applied radioactivity, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled test material

Identification: [^{14}C]-glyphosate
Lot No.: C-2417.2
Specific activity: 12.35 µCi/mg)
Radiochemical purity: 99.2 %; 100.0 % (HPLC) 97.6 % (TLC)

Non-radiolabelled test compound

Identification: Glyphosate
Lot No.: GLP-9606-7189-A
Chemical purity: 99.92 % (impurities weight: 0.078 %: 0.034 % Iminobis, 0.024 % MAMPA, 0.02 % AMPA)

2. Soil:

A freshly collected sample of sandy loam was used. The soil was collected from the upper 20 cm layer of a grassland site by removing surface vegetation and bagging the top soil immediately below. Characterisation data is presented in the table below. Soil was sieved (2 mm) prior to use on the study and its moisture content determined.

Table 7.1.1.2-15: Characteristics of test soil

Parameter	
Soil	PT 200
Country	not indicated
Textural Class (USDA)	Loamy sand
Sand (50 µm – 2 mm) (%)	67.2
Silt (2 µm – 50µmm) (%)	16.1
Clay (< 2 µm) (%)	16.7
pH (KCl)	5.8
Organic carbon (%)	1.7
Organic matter (%)	2.9
Cation exchange capacity (meq/100 g)	18.2
Water Holding Capacity at 0 bar (%)	65.3

Table 7.1.1.2-15: Characteristics of test soil

Water Holding Capacity at 1/3 bar (%)	19.0
Microbial biomass prior to study initiation (mg C/100 g oven dry soil equivalent)	67

B. STUDY DESIGN

1. Experimental conditions

Samples of soil (20, including 4 for contingency purposes; ca 50 g oven dry equivalent) were weighed into previously silanised Erlenmeyer flasks (250 mL capacity). Milli-Q grade water was added to each flask to create a layer of water (ca 3 cm depth; ca 135 g) over the soil. The depth of water of 3 cm was maintained for the duration of the study. Additionally, 2 units were prepared in Erlenmeyer flasks fitted with side-arm attachments. A platinum combination redox electrode was placed in each side-arm to allow in situ redox measurements at the base of the soil during the incubation period. A stream of moist, O_2-free nitrogen, at a flow rate of ca 5-15 mL min^{-1}, was passed over the surface of each sample. The gas mixture leaving each flask was passed through a series of 3 traps. The first trap was a safety trap to prevent back flow, the second contained ethanediol to trap non-specific ^{14}C-organic volatiles and the third trap contained ethanolamine to trap liberated $^{14}CO_2$. Air leaving each incubation unit were combined and passed over a copper II oxide catalyst at ca 800°C to oxidise any radioactivity to $^{14}CO_2$ (which was subsequently trapped in ethanolamine). Connections between traps and incubation flasks were made using a combination of glass connectors and PVC tubing.

The flooded soils were pre-incubated under an atmosphere of nitrogen for 39 days in the dark at a nominal temperature of 20°C. During the pre-incubation period the redox potential of the two control units was measured and the establishment of anaerobic conditions was confirmed when a redox potential of less than 200 mV was obtained.

Separate stock solutions of [^{14}C]-glyphosate and non-radiolabelled glyphosate were prepared in Milli-Q grade water and aliquots of each stock, containing 4.98 mg of [^{14}C]-glyphosate and 7.62 mg of non-radiolabelled glyphosate respectively, transferred to a volumetric flask and filled up to 5 mL with Milli-Q grade water Test solution (100 µl), containing 0.252 mg of glyphosate was applied dropwise to the surface of the water in each incubation flask. The application rate was 5.04 mg per kg soil (oven dry equiv.). The radioactive application to each sample was determined as 7.41 µCi. Following test material application, the samples were re-connected to the continuous gas flow system. The samples were then incubated in the dark at a nominal temperature of 20°C for up to 120 days.

2. Sampling

Duplicate incubates were sampled immediately following application of test solution, 3, 7, 14, 30, 60, 90 and 120 days. At each sampling interval, the redox potential of the soil and pH of the surface water were recorded.

Traps were sampled and replenished at regular intervals throughout the incubation period. Trapping solutions associated with the catalytic converter were stored at ambient temperature and not analysed further.

3. Analytical procedures

Surface waters were separated from soils by careful decanting. The soil residues were transferred into separate Nalgene® centrifuge bottles and extracted with 0.5 M ammonium hydroxide (3 x ca 100 mL; ca 1 h) and end-over-end shaking. After shaking, the extract was separated from the residue by centrifugation (ca 3500 r.p.m.; ca 30 min) and the amount of radioactivity in the supernatant determined by liquid scintillation counting. Surface water and soil extracts were subjected to HPLC and TLC analyses.

Following extraction, the radioactivity remaining in the soil was determined by combustion analysis in order to quantify residual radioactive content. The organic matter in selected extracted residues (Flask 21, 90 DAT and Flask 23, 120 DAT) was then fractionated. Each sample was extracted with 0.5 M sodium hydroxide (2 x ca 20 mL) by shaking (ca 30 min) and sonication (ca 5 min). The extracts were separated from the residue by centrifugation (ca 1000 r.p.m.; ca 15 min) and the radioactive content of the soil (humin) was determined by combustion analysis. The pH of the combined sodium hydroxide extracts was adjusted to ca 1 using concentrated hydrochloric acid and stirring, to precipitate out the humic acid fraction. The sample was then centrifuged and the supernatant, which contained the fulvic acid fraction, removed. The humic acid fraction was quantified by oxidation of the precipitate.

Following decanting, aliquots of each surface water were submitted for liquid scintillation counting followed by HPLC and TLC analyses. Following trap sampling, aliquots of each solution were submitted for liquid scintillation counting. After removal of samples from the flasks, the flasks were soaked in acetone to remove any residual radioactivity. Aliquots of each apparatus wash were submitted for liquid scintillation counting.

Radiolabelled glyphosate and its degradation products extracted from soil and present in the surface water were characterised and quantified by HPLC with TLC as confirmatory method. For each individual sample, an aliquot (ca 10 % by volume) of each of its extracts was combined. For HPLC analysis, the pH of a sub-sample of each combined extract and surface water was adjusted to ca 2-3 using concentrated phosphoric acid, prior to chromatographic analysis. Quantification of radioactivity was determined by collecting fractions of HPLC column eluate (1 min intervals) and submitting these for liquid scintillation counting. Reference substances (glyphosate, AMPA, MAMPA and HMPA) were used to determine the order of elution and standard retention times.

Further preparation of samples for TLC analysis was required to optimise chromatographic resolution. To an aliquot of each pH adjusted combined extract sample, 0.1 M EDTA (50 pl) was added and the solution sonicated prior to chromatographic analysis. For the surface water samples, an aliquot of the original sample was mixed with 0.5 M ammonium hydroxide, the pH adjusted to ca 2-3 using concentrated phosphoric acid and the sample centrifuged. 0.1 M EDTA was added to a sub-sample of the supernatant and the sample sonicated prior to chromatographic analysis. For TLC analysis aliquots of each sample extract and surface water were applied to a Polygram lonex-25 SA-Na TLC plate (Macherey-Nagei, Germany) which was then developed in 0.015 M potassium dihydrogen phosphate (adjusted to ca pH 2.4 with concentrated phosphoric acid): methanol (9:1, v/v). Following chromatography, the areas of radioactivity present on TLC plates were quantified using a Molecular Dynamics phosphor imager. Standards were visualised using ninhydrin spray reagent. The limit of quantification for determination of radioactivity is 30 d.p m. above the background (not given). No detailed information on the limit of detection (LOD) and limit of quantification (LOQ) is provided.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]-glyphosate and metabolites in soil extracts are summarised in Table 7.1.1.2-16 to Table 7.1.1.2-19 for the sandy loam soil.

Table 7.1.1.2-16: Recovery of radioactivity of [^{14}C]-glyphosate applied to sandy loam under anaerobic conditions (expressed as percent of applied radioactivity)

Sampling Interval	Flask Number	Percentage of Applied Radioactivity Recovered as:						
		Water	Soil Extracts	^{14}C-Organic Volatiles	$^{14}CO_2$	Non-extractable Residue	Apparatus Wash	Total
0 DAT	8	92.59	8.04	NS	NS	1.63	ND	102.27
	9	93.83	7.35	NS	NS	1.37	ND	102.56
	Mean	93.21	7.70	-	-	1.50	-	102.41
3 DAT	10	45.40	40.00	ND	0.07	9.95	0.04[1]	95.45
	11	49.34	39.08	ND	0.05	8.76	0.02[1]	97.25
	Mean	47.37	39.54	—	0.06	9.35	0.03	96.35
7 DAT	12	31.71	50.69	ND	0.12	12.27	0.05[1]	94.83
	13	33.31	50.62	ND	0.08	13.37	0.02[1]	97.40
	Mean	32.51	50.66	-	0.10	12.82	0.03	96.12
14 DAT	14	16.42	57.02	ND	0.21	18.37	ND	92.03
	15	19.30	61.42	ND	0.20	21.54	0.01[1]	102.47
	Mean	17.86	59.22	-	0.20	19.96	0.01	97.25
30 DAT	16	14.98	60.57	0.02	0.66	18.64	0.02[1]	94.89
	17	15.33	61.56	0.02	0.71	18.23	0.02[1]	95.86
	Mean	15.15	61.06	0.02	0.68	18.44	0.02	95.38
60 DAT	18	12.74	66.29	0.02	0.74	20.56	0.02[1]	100.37
	19	12.01	70.46	0.21	0.57	20.16	0.02[1]	103.43
	Mean	12.38	68.37	0.11	0.66	20.36	0.02	101.90
90 DAT	20	10.91	62.88	0.22	0.93	22.76	0.02[1]	97.71
	21	9.98	64.04	0.02	0.77	25.48	0.02[1]	100.30
	Mean	10.44	63.46	0.12	0.85	24.12	0.02	99.00
120 DAT	22	10.53	64.80	0.04	0.79	20.87	0.01[1]	97.05
	23	10.40	64.79	0.02	0.95	20.89	0.01[1]	97.07
	Mean	10.46	64.79	0.03	0.87	20.88	0.01	97.06

NS = No sample
ND = Not detected
[1]= Results calculated from data less than 30 d.p.m. above background

Table 7.1.1.2-17: Characterisation of radioactivity in water following application of [^{14}C]-glyphosate under anaerobic conditions (expressed as percent of applied radioactivity) with HPLC

Sampling Interval	Flask Number	Component as a percentage of applied radioactivity		
		Glyphosate	AMPA	HMPA
0 DAT	8	87.04	5.00	0.54
	9	88.41	4.76	0.68
	Mean	87.73	4.88	0.61
3 DAT	10	40.91	4.49	ND
	11	47.04	2.30	ND
	Mean	43.98	3.40	-
7 DAT	12	30.55	1.16	ND
	13	31.75	1.56	ND
	Mean	31.15	1.36	-
14 DAT	14	15.65	0.77	ND
	15	18.36	0.53	0.41
	Mean	17.01	0.65	0.21
30 DAT	16	14.98	ND	ND
	17	14.88	0.45	ND
	Mean	14.93	0.23	-
60 DAT	18	12.74	ND	ND
	19	11.78	0.23	ND
	Mean	12.26	0.12	-
90 DAT	20	10.91	ND	ND
	21	9.98	ND	ND
	Mean	10.45	-	-
120 DAT	22	10.30	0.23	ND
	23	10.12	0.28	ND
	Mean	10.21	0.26	-

NS = No sample
ND = Not detected

Table 7.1.1.2-18: Characterisation of radioactivity in soil extract following application of [^{14}C]-glyphosate under anaerobic conditions (expressed as percent of applied radioactivity) with HPLC

Sampling Interval	Flask Number	Component as a Percentage of Applied Radioactivity	
		Glyphosate	AMPA
0 DAT	8	7.28	0.76
	9	6.62	0.73
	Mean	6.95	0.75
3 DAT	10	34.42	5.58
	11	34.51	4.57
	Mean	34.47	5.08
7 DAT	12	43.72	6.97
	13	44.65	5.97
	Mean	44.19	6.47
14 DAT	14	50.13	6.89
	15	55.14	6.28
	Mean	52.04	6.59
30 DAT	16	52.90	7.67
	17	53.74	7.82
	Mean	53.32	7.75
60 DAT	18	59.02	7.27
	19	62.34	8.12
	Mean	60.68	7.70
90 DAT	20	56.06	6.82
	21	57.47	6.57
	Mean	56.77	6.70
120 DAT	22	57.31	7.49
	23	58.09	6.70
	Mean	57.70	7.10

NS = No sample
ND = Not detected

Table 7.1.1.2-19: Characterisation of radioactivity in soil/water system following application of [^{14}C]-glyphosate under anaerobic conditions (expressed as percent of applied radioactivity) with HPLC

Sampling interval	Flask Number	Component as a percentage of applied radioactivity		
		Glyphosate	AMPA	HMPA
0 DAT	8	94.32	5.76	0.54
	9	95.03	5.49	0.68
	Mean	94.68	5.63	0.61
3 DAT	10	75.33	10.07	ND
	11	81.55	6.87	ND
	Mean	78.44	8.47	—
7 DAT	12	74 27	8.13	ND
	13	76.40	7.53	ND
	Mean	75.34	7.83	-
14 DAT	14	65.78	7.66	ND
	15	73.50	6.81	0.41
	Mean	69.64	7.24	0.21
30 DAT	16	67.88	7.67	ND
	17	68.62	8.27	ND
	Mean	66.25	7.97	-
60 DAT	18	71.76	7.27	ND
	19	74.12	8.35	ND
	Mean	72.94	7.81	-
90 DAT	20	66.97	6.82	ND
	21	67.45	6.57	ND
	Mean	67.21	6.70	-
120 DAT	22	67.61	7.72	ND
	23	68.21	6.98	ND
	Mean	67.91	7.35	-

ND = Not detected

B. MASS BALANCE

The mean recovery of applied radioactivity from flooded soil up to and including 120 DAT ranged from 95 % to 102 %.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The total levels of radioactivity extracted from the soil increased from 8 % at zero time to 59 % at 14 DAT and remained around this level for the remainder of the incubation period.

As the total levels of extractable radioactivity increased with time, a concomitant decrease in the levels of radioactivity present in the surface water resulted. At zero time, 93 % of applied radioactivity was associated with the surface water and levels decreased to 18 % by 14 DAT. Beyond 14 DAT, levels of radioactivity in the surface water declined more slowly, accounting for 10 % at study termination.

Radioactivity associated with the non-extractable residue increased form 2 % at zero time to 20 % at 14 DAT and remained around this level for the remainder of the incubation period. Following extraction, the organic matter from single replicates from 90 DAT and 120 DAT was fractionated into humin, humic acid and fulvic acid Radioactivity associated with the humin, fulvic acid and humic acid accounted for up to 9, 10 and 5 %, respectively.

D. VOLATILE RADIOACTIVITY
Radioactivity recovered as $^{14}CO_2$ as non-specific ^{14}C-volatiles and as washings in the apparatus was very low (<1 %).

E. TRANSFORMATION OF THE TEST ITEM
At zero time, levels of glyphosate in the total flooded test system accounted for 95 % of applied radioactivity. As the incubation progressed, levels of parent compound declined, accounting for 68 % at study termination. In addition to parent compound, low levels of AMPA and HMPA were detected in samples at intervals throughout the study, accounting for up to 8 and 1 % of applied radioactivity, respectively.

F. KINETICS
New kinetic calculations based on recent guidance were not provided due to the supporting character of the study.

III. CONCLUSIONS

In conclusion, following incubation in a flooded sandy loam soil, glyphosate disappeared quickly from the aqueous phase of the test system into the soil. Glyphosate slowly degraded to AMPA under anaerobic conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides information on the degradation behavior of glyphosate in soil under established strict anaerobic conditions. Such application to strictly anaerobic conditions (50 days) is not in agreement with the current guideline.
Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.2/005
Report author	[redacted]
Report year	1987
Report title	SC-0224: Anaerobic soil metabolism study: fate of the carboxymethyaminomethylphosphonic acid moiety
Report No	PMS-217
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From OECD 307: - Soil samples either aerated with pure oxygen (aerobic incubation) or flushed with nitrogen (anaerobic incubation) under positive pressure. - Duration of study only 66 days instead of 120 days (degradation not >90 % at test end) - Two sampling dates for aerobic and anaerobic phase, respectively, first sample in anaerobic conditions after 30 days - No confirmatory method used

	- Mass balance based on recovery of 0 DAT - Total recovery below 80 % AR for sample of 66 DAT - No determination of biomass
Previous evaluation	Yes, accepted in RAR as supplementary (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary
[^{14}C]-glyphosate trimesium (SC-0224) radiolabelled in the carboxymethylaminomethyl-phosphonate (CMAMP) moiety was surface applied at a rate of 30 ppm to a moist loam soil and incubated in biometer flasks under aerobic conditions. The study was conducted with an initial aerobic aging period equivalent to one half-life (3 days) followed by 63 days of incubation under flooded anaerobic conditions. At selected timepoints, analyses were run for trapped extractable ^{14}C by extraction with 0.5 M ammonium hydroxide, bound ^{14}C and ^{14}C present in floodwater.

[^{14}C]-glyphosate trimesium was metabolised extensively; nearly 45 % of the applied radiocarbon was trapped as CO_2 during the 66 day study. Soil-bound ^{14}C was the only significant component remaining in soil after 3 days, TLC analyses showed that parent and aminomethylphosphonic acid (AMPA), a primary metabolite, were the only ^{14}C components of the ammonium hydroxide extracts. Floodwater represented less than 3 % of the ^{14}C applied to the soil and was composed mostly of parent glyphosate.

The overall distribution of ^{14}C recovered from [^{14}C]-glyphosate trimesium treated soils in the range of 85 % AR to 100 % AR using the ^{14}C recovery from 0 time soil as the basis for dosage determination. The recovery of ^{14}C from 0 time represented 93.0 % AR of the theoretical applied.

Over the 66 day duration of the study, 43 % AR of the applied ^{14}C was recovered from NaOH traps, confirmed to be $^{14}CO_2$ by precipitation as barium salts (> 98 % AR of the trapped ^{14}C). No ^{14}C was retained by the polyurethane foam traps.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: [^{14}C-CMAMP] SC-0224 (trimethylsulfonium carboxymethyl)
Lot No.: WRC-7615-29-01
Specific activity: 30 µCi/mg
Radiochemical purity: 98.1 % (after purification)

2. Soil:
Soil was sieved to ≤ 2 mm. Moisture content of the air-dried soil was determined to be 1.97 g H_2O/100 g based on weight loss from 4-5 g samples of soil -heated for 10 min in a microwave oven.

Table 7.1.1.2-20: Characteristics of test soils

Parameter	Results
Soil	Sorrento
Country	IT
Textural Class (USDA)	Sandy loam
Sand (50 µm – 2 mm) (%)	53.2
Silt (2 µm – 50µmm) (%)	34.4
Clay (< 2 µm) (%)	12.4
pH (water)	6.9
Organic carbon (%)[1]	1.28
Organic matter (%)	2.2
Cation exchange capacity (meq/100 g)	17.6
Half saturation [2]	21 %
Bulk Density (disturbed) (g/cm^3)	1.43
Soil moisture adjusted to 75 % field capacity	42g water/100g soil

[1] Calculated from organic matter according to OC = OM x 0.58
[2] Not given if volume metric or gravimetric value

B. STUDY DESIGN

1. Experimental conditions

200 g of air-dried soil were placed into each of 10 one-L biometer flasks. Using a volumetric pipet 10 mL of the soil treatment solution were slowly and uniformly applied to the surface of the soil in each biometer flask. Three additional flasks, two containing 200 g untreated soil and one containing no soil, were set up as controls.Soil moisture was adjusted to 75 % of field capacity (field capacity – 42 g water / l00 g soil) by adding 47.8 mL water to the soil or each glyphosate trimesium treated flask and 57.8 mL to the soils of the two control flasks. Two trapped flasks were set aside for immediate analysis as 0 time samples. The remaining flask was then fitted with a polyurethane foam plug in the flask bridge. The sidearm of each flask was then filled with 1.0 M sodium hydroxide. All flasks were placed in an environmental chamber maintained at 23 °C and in total darkness for the duration of the study. The flasks were maintained initially under aerobic conditions, all being connected to a gas distribution line of oxygen. Pressure was maintained under pressure by connecting the oxygen line to a “U” tube containing mineral oil.

After three days of incubation, anaerobic conditions were achieved by flooding each soil vessel with water (200 mL) and substituting nitrogen for oxygen in the gas suppling system.

2. Sampling

Duplicate test systems were processed and analysed 0, 3, 33 and 66 days after treatment (DAT). NaOH solutions were collected and replaced with fresh solution at each soil sampling interval.

3. Analytical procedures

Each aerobic soil was transferred into 250 mL polypropylene centrifuge bottles and extracted with 1.0 M ammonium hydroxide (two times, approximately 150 mL each extraction). Each extraction step was conducted by hand shaking followed by separation of soil and extract by centrifugation at 10000 x G).Each extract was decanted and immediately neutralized to pH 7 with HCL to prevent base hydrolysis of [^{14}C]glyphosate trimesium to AMPA and radio-assayed by LSC. Each anaerobically incubated soil plus flood water was transferred equally into two 250 mL polypropylene centrifuge bottles and centrifuged. The flood water was decanted and radioassayed. The soils were extracted with ammonium hydroxide.

The ammonium, hydroxide soil extracts and the flood water, were reduced to dryness using high-vacuum rotary evaporation, and the residues were re-dissolved in 10 mL water for analysis.

Aliquots of the NaOH traps and ethyl acetate extracts were radio-assayed. The occurrence of $^{14}CO_2$ in the alkali traps was determined by $BaCl_2$ precipitation. $BaCl_2$ was added to aliquots of composited trap solutions represent in the collection intervals 0 to 68 days. The NaOH trap samples were analysed for ^{14}C both before and after $BaCl_2$ treatment by counting 0.1 mL aliquots.

The soil extracts and floodwaters were purified by cation exchange micro-column chromatography prior to metabolite characterisation via TLC. The purification step was needed to remove soil cations which were shown to interfere with the movement of glyphosate trimesium on the cation-exchange TLC plates used in this study. Column chromatography was performed using Dowex G 50W-X8 resin (hydrogen form, 200-400 mesh; Bio-Rad, Richmond, CA). The column was then rinsed with purified water added dropwise until the pH of the eluted water reached 7.0. Each soil extract/floodwater was then applied to the column and eluted with 5 mL purified water. Fractions were collected (200~400 µL each and radioassayed using one-µL aliquots counted by LSC. The column was washed with 1.0N HC1 (5 mL) then rinsed with water prior to application of the next sample. The ^{14}C in each sample emerged approximately between 3 mL and 3.5 mL total elution volume. Fractions containing this peak were analysed by TLC.

II. RESULTS AND DISCUSSION

A. DATA

Over the 66 day duration of the study, 43 % AR of the applied ^{14}C was recovered from NaOH traps, confirmed to be $^{14}CO_2$ by precipitation as barium salts (> 98 % AR of the trapped ^{14}C). No ^{14}C was retained by the polyurethane foam traps.

The bound ^{14}C decreased from 33 % AR at 0 time to 24 % AR by the end of the study at 66 days. Floodwater contained 2-3 % AR.

Radioactive mass balance and distribution of [^{14}C]-glyphosate trimesium and metabolites in soil extracts are summarised in Table 7.1.1.2-21 to Table 7.1.1.2-23 for the respective soils.

Table 7.1.1.2-21: Distribution of radioactivity under aerobic and anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity[1])

Compound	Replicate	DAT			
		0	3	33	66
		Aerobic conditions		Anaerobic conditions	
Extractable	Mean	66.70	37.78	15.82	16.02
Bound	Mean	33.30	29.82	30.00	23.59
CO_2	Mean	-	23.86	39.53	43.21
Floodwater	Mean	-	-	2.72	2.45
Total mass balance	Mean	100.00	91.47	88.06	85.27

DAT: days after treatment
[1] Recoveries based on recovery of 0 DAT

Table 7.1.1.2-22: Characterisation of radioactivity in soil extracts under aerobic and anaerobic conditions following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

TLC[1] AREA IDENTITY	Aerobic conditions		Anaerobic conditions	
	0 DAT	3 DAT	33 DAT	66 DAT
AMPA	0.33	0.13	0.50	NS
CMAMP	65.81	37.34	15.26	15.97
"Area D"[1]	0.56	0.31	0.06	0.05
ORIGIN	NS	NS	NS	NS
TOTAL	66.70	37.78	15.82	16.02

[1] Corresponds to the section of the TLC plate directly below glyphosate (presumably a tailing effect of CMAMP).
NS not significant

Table 7.1.1.2-23: Characterisation of radioactivity in water following application of [^{14}C]-glyphosate (expressed as percent of applied radioactivity)

TLC[3] AREA IDENTITY	33 DAT	66 DAT
	%	%
"Area A"[1]	0.02	NS
AMPA	0.24	0.52
CMAMP	1.73	1.38
"Area D"[2]	0.40	0.24
ORIGIN	0.33	0.31
TOTAL	2.72	2.45

[1] Corresponds to the least polar section of TLC plate
[2] Corresponds to the section of the TLC plate directly below CMAMP (presumably a tailing effect of CMAMP).
NS not significant

B. MASS BALANCE

The overall distribution of ^{14}C recovered from [^{14}C]-glyphosate trimesium treated soils in the range of 85 % AR to 100 % AR using the ^{14}C recovery from 0 time soil as the basis for dosage determination. The recovery of ^{14}C from 0 time represented 93.0 % AR of the theoretical applied.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The ammonium hydroxide-soluble ^{14}C fraction proved to be short-lived, declining from an initial level of about 67 % AR at 0 time to approximately 38 % AR at 3 days and by 16 % AR after 66 days.

D. VOLATILE RADIOACTIVITY

43 % AR of the applied ^{14}C was recovered from NaOH traps at the last study day and confirmed to be $^{14}CO_2$ by precipitation as barium salts (> 98 % AR of the trapped ^{14}C). No ^{14}C was retained by the polyurethane foam traps.

E. TRANSFORMATION OF THE TEST ITEM

Results of the TLC characterisation of the soil extracts show that unchanged [^{14}C]-glyphosate was the single major component of the soil extractable fraction and the metabolite AMPA occurred as a minor component. At all sampling intervals [^{14}C]-glyphosate accounted for between 96-99 % AR. The determined half-life of [^{14}C]-glyphosate was approximately 3 days.

TLC analysis of floodwater showed that the ^{14}C fraction consisted of mainly unchanged [^{14}C]-glyphosate (about 69 % AR). The remaining floodwater ^{14}C was in the form of the metabolite AMPA (9 % at DAA 33, 21 % AR at DAA 66.) and unresolved material more polar than [^{14}C]-glyphosate trimesium (below 1 % AR).

F. KINETICS

In view of the low number of datapoints and since supporting information, a kinetic evaluation according to current guidance was not performed.

III. CONCLUSIONS

This study has shown that [^{14}C]-glyphosate trimesium is very rapidly and extensively metabolised in anaerobic soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study provides information on the degradation behavior of glyphosate trimesium in soil under anaerobic conditions. The study is considered to have supportive character based on the low number of sampling points. Beyond the fact that this low number does not allow for the conclusion on trends in degradation from the route perspective, the study is not kinetically evaluable to derive degradation rates. The study is therefore considered as invalid to contribute adequately to the degradation behavior of glyphosate residues in soil under anaerobic conditions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.2/006
Report author	[redacted]
Report year	1972
Report title	The degradation and metabolism of MON-0573 in soil
Report No	269
Document No	
Guidelines followed in study	U.S. Department of Agriculture (ARS, Pesticides Regulation Division): Pesticide Registration (PR) Notice 70-15 "Guidelines For Studies to Determine the Impact of Pesticides on the Environment." June 23, 1970
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: aerobic/anaerobic soil metabolism, degradation in water Test item: [^{14}C] glyphosate, phosphonomethyl-label (97 % radiochemical purity), 1-glycine label (96 % radiochemical purity), 2-glycine label (99 % radiochemical purity) Test soils (soil type): Ray (silt loam), Drummer (silty clay loam), Lintonia (sandy loam), Norfolk (sandy loam) pH: 6.5, 7.0, 6.0, 5.7 (method not stated) Organic matter: 1.0 %, 6 %, 1 %, 1 %

	The total study included various tests including aerobic and anaerobic degradation (samples water-logged) in non-sterile and sterilized soil (soil Ray only). Tests with exaggerated application rates performed for identification of metabolites (soil Ray). This summary focuses on the results of aerobic degradation tests. Application rate: 109 to 126 mg/kg for the different labels, 1000 mg/kg for metabolite identification with test substance applied to water phase, i.e. not applied directly to soil Test design: 5 g soil suspended in 100 mL water, continuously agitated by shaking; 100 g soil and 1000 mL for large scale tests Volatiles trapping: CO_2: ascarite trap Organic volatiles: no trapping Incubation: 30 °C, continuous shaking, soil flooded/suspended Sampling: 0, 1, 3, 7, 14, 21, 28 days after treatment (DAT) for soil Ray, 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 66, 77, 84, 91, 105 and 112 DAT for soil Norfolk, 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 66, 77 and 84 DAT for soil Drummer, 0, 1, 3, 7, 14, 21, 28 and 35 DAT for soil Lintonia, single samples collected per soil and sampling interval Workup: taking of an aliquot of the soil-water suspension, centrifugation, washing of soil with water, lyophilisation of soil, threefold extraction with 0.5 N aqueous NH_4OH solution at ambient temperature Determination of radioactivity: Extracts: LSC NER: combustion/LSC Volatiles: ascarite treated with HCl, trapping in 0.25 N NaOH, LSC Identification of radioactive residues: TLC/radiodetection co-chromatography with reference items, ^{1}H and ^{31}P-NMR
Short description of results:	Recovery of radioactivity: 68.7 – 109.8 % AR for all glyphosate labels and soils at the day of experiment termination Mineralization: 46.8 to 55.3 % AR for soil Ray, 5.8 to 9.3 % AR for soil Norfolk, 34.7 to 41.4 % AR for soil Drummer, 14.3 % AR for soil Lintonia (for all soils at termination) Other volatiles: not measured Extractable radioactivity: 2.7 to 22.9 % AR at 28 DAT for soil Ray, 65.4 to 81.8 % AR at 112 DAT for soil Norfolk, 12.0 to 19.6 % AR at 84 DAT for soil Drummer, 18.3 % AR at 35 DAT for soil Lintonia Non-extractable radioactivity: 8.5 to 40.3 % AR at 28 DAT for soil Ray, 4.6 to 13.5 % AR at 112 DAT for soil Norfolk, 16.7 to 33.9 % AR at 84 DAT for soil Drummer, 2.6 % AR at 35 DAT for soil Lintonia Transformation of test item (TLC analysis): Glyphosate: 0.2 to 7.4 % AR at 14 DAT and not detected at 28 DAT for soil Ray, 45.6 to 80.1 % AR at 14 DAT and 0.8 to 16.3 % AR at 112 DAT for soil Norfolk, 12.5 to 25.5 % AR at 14 DAT and 7.6 to 15.7 % AR at 84 DAT for soil Drummer, 69.5 % AR at 14 DAT and 59.5 % AR at 35 DAT for soil Lintonia

	AMPA:	8.5 % AR at 14 DAT and 4.4 % AR at 28 DAT for soil Ray; 0.5 % AR at 14 DAT and 1.7 % AR at 28 DAT for soil Norfolk, 1.8 % AR at 14 DAT, 8.4 % R at 56 DAT and 8.3 % AR at 84 DAT for soil Drummer, 6.9 % AR at 14 DAT and 6.6 % AR at 35 DAT for soil Lintonia (phosphonomethyl-label only for all soils)
	No unknown metabolites were observed at >5 % AR.	
Reasons why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: - mixed aerobic/anaerobic design strongly beyond actual standards and guidelines in soil degradation testing, i.e. soil suspended in aqueous solution during incubation and application of the test substance - work-up of aliquots only instead of complete soil samples - closed system without air exchange - incubation at 30 °C - soil history, sampling and storage not reported	
Category study in AIR 5 dossier (L docs)	Category 3b	

CA 7.1.1.3 Soil photolysis

The molar decadic absorption coefficient (ε) of glyphosate is <10 L mol^{-1} cm^{-1} at wavelengths >295 nm (see Document M-CA, Section 2.4). Therefore, direct photolysis is not expected to significantly contribute to degradation of glyphosate in soil. Thus, experimental studies on soil photolysis are formally not required. For completeness, the studies previously evaluated are presented below including an updated kinetic evaluation.

The information available on soil photolysis is summarised in Table 7.1.1.3-1 below.

The photodegradation of glyphosate on soil surfaces was investigated in one soil in the course of one study considered as valid ([redacted], 1993, CA 7.1.1.3/003). Additionally, two studies are considered as supportive (Esser, 1996, CA 7.1.1.3/002 and [redacted], 1989, CA 7.1.1.3/004).

Investigations into soil photolysis under the conditions of the laboratory confirm that degradation of glyphosate in soil is not significantly affected by irradiation. Mineralisation and formation of non-extractable residues (NER) was moderate with a maximum amount of 14.6 % AR of CO_2 after 30 days and maximum amount of 19.4 % AR of NER after 14 days. In dark controls, formation of CO_2 was lower while NER formation was comparable (max. CO_2 of 5.4 % AR, max. NER of 17.4 % AR).

No particular photolytic transformation products were observed at levels above 5 % AR. Aminomethylphosphonic acid (AMPA) was the only degradation product observed at a maximum of 8.2 % AR (7 DAT) in irradiated samples compared to 6.1 % AR (3 DAT) in dark controls.

The results of the soil photolysis study with glyphosate were kinetically evaluated according to the current EU FOCUS kinetic guidance ([redacted], 2020, CA 7.1.1.3/001). The calculated DT_{50} and DT_{90} of glyphosate are 69.8 and 482 days, respectively, with hence longer DT_{50} values than those calculated forlaboratory aerobic soil degradation studies. For AMPA, no reliable half-life could be derived.

The results of the supportive studies show a similar degradation behaviour of glyphosate residues when being compared to the study considered as valid. This applies for irradiated samples as well as for dark controls.

Overall, it is concluded that photodegradation on soil surfaces does not contribute significantly to the overall elimination of glyphosate residues from the soil environment.

Within the search for peer reviewed scientific literature documented as LRR (2010-2019), no article was identified that would provide information relevant to this data point.

Table 7.1.1.3-1: Studies on soil photolysis with glyphosate

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.1.3/001	[redacted] 2020	Kinetic evaluation	Glyphosate	Valid	
CA 7.1.1.3/002	[redacted] 1996	Soil photolysis	Glyphosate	Supportive	
CA 7.1.1.3/003	[redacted] 1993	Soil photolysis	Glyphosate	Valid	Updated kinetic evaluation in [redacted] 2020
CA 7.1.1.3/004	[redacted] 1989	Soil photolysis	Glyphosate	Supportive	
CA 7.1.1.3/005	[redacted] 1983	Soil photolysis	Glyphosate-trimesium	Invalid	
CA 7.1.1.3/006	[redacted] 1978	Soil photolysis	Glyphosate	Invalid	
CA 7.1.1.3/007	[redacted] 1972	Soil photolysis	Glyphosate	Invalid	

Table 7.1.1.3-2: Summary of soil photolysis degradation parameters for glyphosate

Study	Soil type	pH[1]	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calculation
[redacted] 1993, CA 7.1.1.3/003	Les Evouettes II Loam / silt loam	6.1	22 °C dry soil	69.8 / 482	1.2	HS

[1] Medium not stated

1. Information on the study

Data point:	CA 7.1.1.3/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from a soil photolysis study
Report No	112148-007
Document No	
Guidelines followed in study	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: None
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities	No, not applicable for this study type
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
A kinetic evaluation of a soil photolysis laboratory study by van [redacted] (1993; CA 7.1.1.3/003) was performed in order to derive the trigger (persistence) endpoints for glyphosate and its major soil metabolite AMPA. The evaluation was conducted according to FOCUS kinetics guidance (2006, 2014) using the fitting software CAKE.

Residue data were taken from the soil photolysis study and adjusted according to FOCUS kinetics, where necessary.

For glyphosate, estimated trigger DT_{50} and DT_{90} are 69.8 and 482 days (HS model), respectively. For AMPA, no reliable endpoints could be derived.

I. MATERIALS AND METHODS

1. Data pre-processing
In this assessment, the irradiated experiment was evaluated. The metabolite AMPA was included in the evaluation.

The standard procedures recommended by FOCUS (2006, 2014) were followed for all residues to adjust the experimental data for the kinetic modelling.

The initial amounts of glyphosate were set to the value of the material balance at day 0, thus assigning all radioactivity observed at day 0 to the parent compound and assuming that no degradation processes have yet taken place. Accordingly, the initial amounts of the metabolites were set to 0 in the pathway fits.
Processed residue data for kinetic evaluation are presented in the following table.

Table 7.1.1.3-3: Processed residue data of glyphosate and its metabolite AMPA

Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	102.4[1]	0[2]
3	75.7	7.4
7	65.3	8.2
14	64.8	5.2
21	60.3	7.4
30	60.5	6.5

[1] Set to material balance
[2] Amounts of metabolite set to 0 at day 0

2. Kinetic models and analysis

Kinetic models
Four kinetic degradation models were considered to describe the degradation behaviour of the compounds in soil: single first-order (SFO), first-order multi-compartment (FOMC = Gustafson and Holden model), double-first-order-in-parallel (DFOP) and Hockey-stick (HS) (FOCUS; 2006, 2014).

Optimisation
The kinetic analysis was conducted using the software CAKE v3.3 (CAKE, 2016).

The data were directly fitted with the complete dataset and unconstrained initial concentration (M_0) for the substance. Iteratively Reweighted Least Square (IRLS) was used as the solver, as implemented in Cake. Optimisations were carried out for the initial soil residue (M_0), degradation model parameters k, α, β, g or t_b, depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually, based on the observed degradation pattern and preliminary model runs. By default, the initial amount of metabolite was fixed to 0. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

Criteria for selection of the appropriate kinetic model
Evaluation of model fit
The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration vs. time plots and residual plots) and statistically (Chi-square (χ^2) test). The visual inspection focused on the residuals which should not be distributed systematically around the zero line, but randomly. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered
- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered

A statistical measure of the quality of a fit is given by the χ^2-test which considers the deviations between observed and calculated values relative to the uncertainty of the measurements. The model with the smallest error percentage was defined as the most appropriate, because it described the measured data in the most robust way.

In general, it is recommended that if the χ^2 error is <15 %, then the model has adequately reflected the measured data. However, this value should only be considered as guidance and not an absolute cut-off criterion. Depending on the complexity of the curve fitting for multiple components and the scattering of the experimental data, also fits with higher χ^2 error values may be acceptable if overall the measured data are well described by the fitted curve.

Significance of parameters
A single-sided t-test was performed to evaluate whether the optimised parameters were significantly different from zero at a chosen significance level of 5 %. In case of metabolite data, a significance level of 10 % or higher may still be acceptable due to the inherent variability that often occurs in these types of data. This is particularly relevant for the degradation rate constants (k) of the SFO, DFOP and HS kinetic models. For the FOMC kinetic model, only the significance of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The CAKE software also reports a confidence interval on the optimised parameter estimates. The confidence interval should be relatively tight and not contain 0 to be considered statistically robust.

II. RESULTS AND DISCUSSION

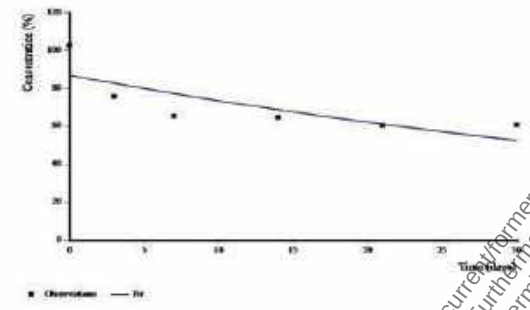
Table 7.1.1.3-4: Kinetic models and goodness-of-fit statistics of parent-only fits

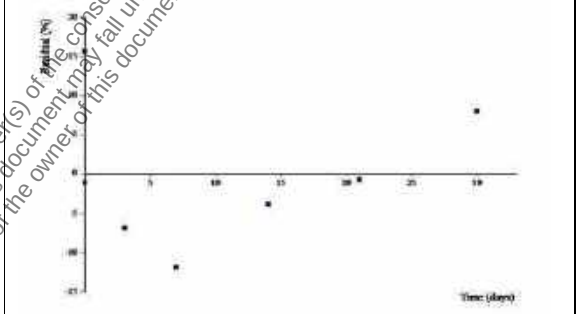
Kinetic model	Visual assess-ment	M_0	Kinetic parameter	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	86.8	k: 0.0168	10.3	k: 0.035	k: -0.002	k: 0.036	41.4	137
FOMC	Good	102.4	α: 0.0991 β: 0.1185	2.0	-[1]	β: -0.240	β: 0.477	129	>1000
DFOP	Good	102.4	k_1: 0.4257 k_2: 0.0030 g: 0.3585	1.4	k_1: 0.021 k_2: 0.149	k_1: 0.033 k_2: -0.006	k_1: 0.818 k_2: 0.012	83.5	623
HS	Good	102.4	k_1: 0.1007 k_2: 0.0039 t_b: 4.342	1.2	k_1: 0.003 k_2: 0.055	k_1: 0.065 k_2: -0.002	k_1: 0.136 k_2: 0.010	69.8	482

For the derivation of trigger endpoints, the kinetic evaluation was started by comparing SFO and biphasic models for the parent substance glyphosate. The SFO model showed visually poor results with systematic deviations and comparatively high scatter. As 10 % of the initially measured concentration was not reached within the study period and the DT_{90} was higher than the experimental period, FOMC is not a preferred fit for endpoint derivation. The DFOP and HS model show visually good fits with lowest χ^2 errors, more favourable for the HS fit, which reveals also the most significant t-test of the biphasic models.

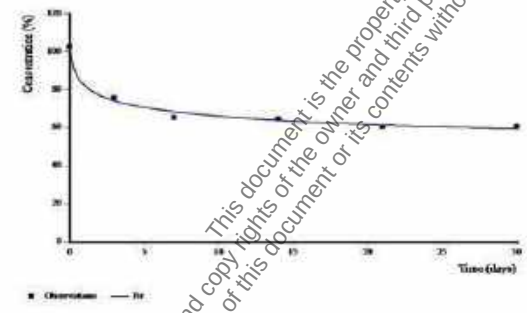
Conclusion: HS to be used in pathway fit for trigger endpoints

SFO





FOMC



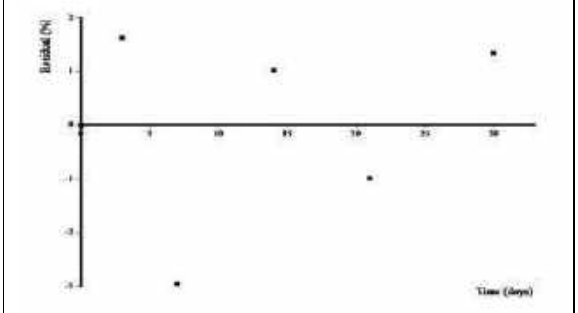
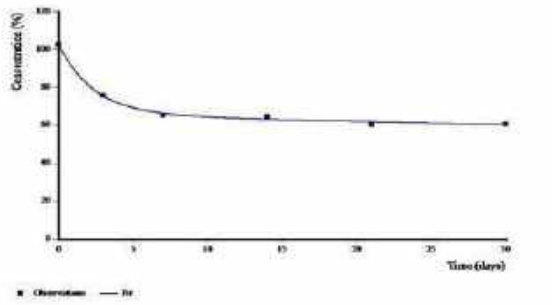
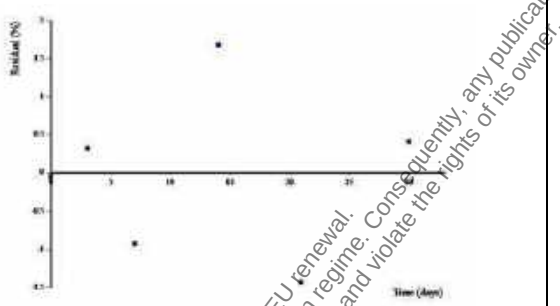
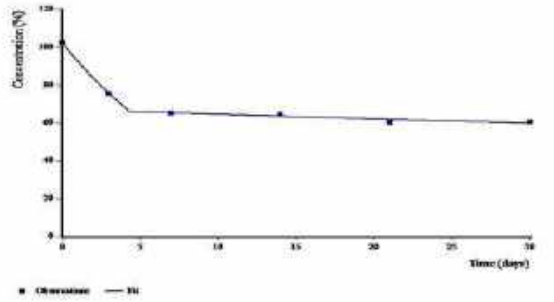
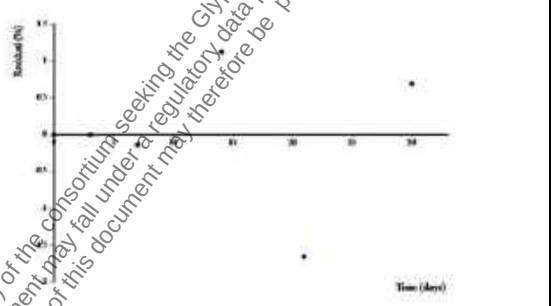


Table 7.1.1.3-4: Kinetic models and goodness-of-fit statistics of parent-only fits

DFOP	
	
HS	
	

[1] t-test not relevant for kinetic parameter β

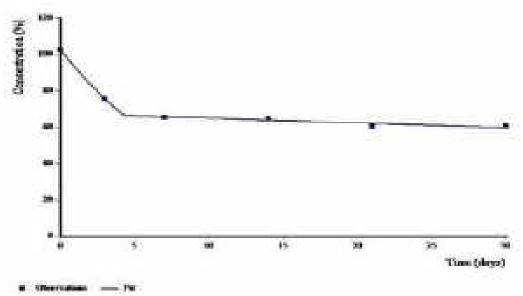
Table 7.1.1.3-5: Kinetic models and goodness-of-fit statistics of pathway fit

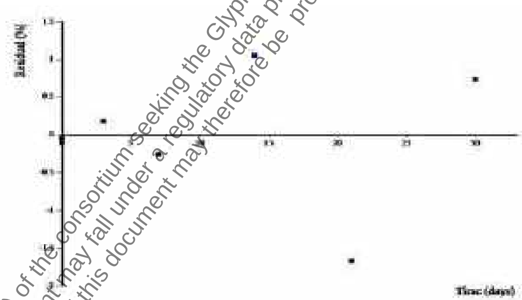
Kinetic model	Visual assess-ment	M_0	Kinetic parameter	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate HS	Good	102.4	k_1: 0.1017 k_2: 0.0040 tb: 4.282	1.2	k_1:<0.001 k_2: 0.012	k_1: 0.0828 k_2:-0.0008	k_1: 0.12 k_2:0.007	68.3	468	-
AMPA: SFO	Good	0.0	k: 0.0221	13.1	k: 0.0818	k: -0.0127	k: 0.057	31.3	104	0.244 (±0.046)

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. However, the degradation rate of AMPA is not significantly different from zero; therefore, no reliable endpoints can be derived.

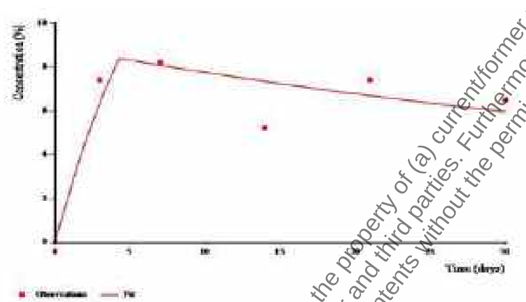
Conclusion: Parent-only HS fit to be used for deriving trigger endpoints for glyphosate
No trigger endpoints can be derived for AMPA

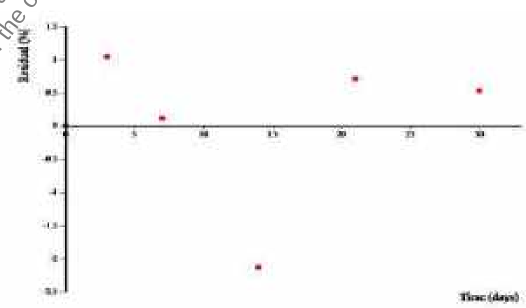
Glyphosate: HS





AMPA: SFO





Summary of trigger endpoints

For glyphosate, estimated trigger DT_{50} and DT_{90} are 69.8 and 482 days (HS model), respectively. For AMPA, no reliable endpoints could be derived.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The kinetic evaluation was performed according to the current guidances without any deviations. Thus, the study and the endpoints provided are considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.3/002
Report author	[redacted]
Report year	1996
Report title	[P-Methylene-14C]Glyphosate acid: Photodegradation in/on soil by natural sunlight
Report No	547W-1
Document No	
Guidelines followed in study	U.S. EPA 161-3
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - material balance 80.0 to 97.6 % AR - tests were not conducted with an artificial irradiation source, but samples exposed to natural sunlight of 250-700 nm range - temperature was 25°C
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

[^{14}C]glyphosate ([P-Methylene-^{14}C]Glyphosate Acid, [^{14}C]PMG) was added in an aqueous solution to a thin layer of a sandy loam soil (organic carbon 0.46 %, pH 8.3) in quartz containers. The application rate was 10.19 ± 0.60 µg/g corresponding to 11 kg/ha (10 lb/acre). The moisture of the soil in containers was adjusted to 75 % of water holding capacity at 1/3 bar just after dosing. Containers were sealed with Teflon septa and placed outdoors in a temperature controlled water bath for exposure to natural sunlight for 30 days. The average temperatures of the light exposed and dark control soil samples for the study period were 24.91 ± 0.03 and 24.80 ± 0.04 °C.

Volatiles were trapped intermittently and at each sampling time except for time 0, using one ethylene glycol to trap organic volatiles and two with 10 % NaOH to collect carbon dioxide.

Duplicate samples from each system were processed and analysed at 0, 2, 6, 12, 20 and 30 days after treatment (DAT). Traps with ethylene glycol and 10 % NaOH were sampled at all these occasions except 0 DAT. Intermittent trapping of the headspace was performed once a week starting approximately one week after dosing.

Material balances ranged from 84.0 to 95.6 % of applied radioactivity (% AR) (single values, n = 12) for light exposed samples and from 80.0 to 97.6 % AR for dark control samples (single values, n = 10).

[^{14}C]glyphosate degraded rapidly to $^{14}CO_2$, witch maximum values of 32.9 % AR and 36.7 % AR at 12 DAT in the light exposed and dark control samples, respectively. At study end at 30 DAT, 29.5 and 30.1 % AR were detected as $^{14}CO_2$ in light exposed and dark control samples, respectively, due to significant losses of $^{14}CO_2$. Considering unaccounted ^{14}C as CO_2, more CO_2 was formed in the dark-control than in the light-exposed samples (53.1 % AR and 34.2 % AR (each mean of two replicates) in dark control

and light exposed samples, respectively). Radiocarbon found in the light exposed and dark control ethylene glycol traps reached a maximum of 1.9 and 5.8 % AR, respectively, and was not further characterized.

The amount of radioactivity extractable from soil decreased from 0 DAT to 30 DAT from 92.0 % AR to 26.1 % AR (mean of two replicates) in light exposed samples and from 92.0 % AR to 29.0 % AR in dark control samples (mean of two replicates).

Bound ^{14}C-residues were always <10 % AR for the dark control samples. However, up to 33.6 % AR (mean of two replicates) was bound in light exposed samples at 30 DAT. Additional extractions with 0.1 M NaOH showed that 3.6, 6.3 and 23.7 % AR was associated with the humic acid, fulvic acid and humin fractions, respectively.

The amount of [^{14}C]glyphosate in soil extracts decreased from 0 DAT to 30 DAT from 89.1 % AR to 3.2 and 3.6 % AR (mean of two replicates) in light exposed and dark control soil samples, respectively.

The major degradate detected in light exposed and dark control soil extracts after 30 days was AMPA. AMPA reached a maximum of 28.4 and 28.0 % AR (mean of replicates) respectively, in light exposed and dark control soil samples at 20 DAT, and represented 19.8 and 24.3 % AR (mean of replicates) for light exposed and dark control soil extracts, respectively, at 30 DAT. An unidentified degradate, designated “Degradate 1”, reached maximum values of 3.4 and 1.5 % AR, respectively, in light exposed and dark control soil samples at 20 DAT. With the exception of AMPA, no other degradates above 5 % AR were detected.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [P-Methylene - ^{14}C]Glyphosate Acid ([^{14}C]PMG)
Lot No.: WRC Ref. 15617-06-02
Specific activity: 42.7 mCi/mmole
Radiochemical purity: 97.3 %
Chemical purity: not indicated

2. Soil:

Upon arrival at the testing facility, the sandy loam soil used in the study was sieved to ≤2 mm. The soil was maintained at approximately 8 °C in an incubator until experimental start of the study. Characteristics of the test soil are presented in the table below.

Table 7.1.1.3-6: Characteristics of test soil

Parameter	Results
Soil	Visalia (KOFO1A)
Country	CA, USA
Textural Class (USDA)	Sandy loam
Sand (50 µm – 2 mm) (%)	71.2
Silt (2 µm – 50µmm) (%)	20.0
Clay (< 2 µm) (%)	8.8
pH	8.3 [2]
Organic carbon (%) [1]	0.46
Organic matter (%)	0.80
Cation exchange capacity (meq/100 g)	8.14

Table 7.1.1.3-6: Characteristics of test soil

Maximum Water Holding Capacity (%)	n.i.
Water Holding Capacity at 0 bar (%)	n.i.
Water Holding Capacity at 0.33 bar (%)	11.92
Water Holding Capacity at 15 bar (%)	4.18
Bulk Density (disturbed) (g/cm^3)	1.46
Microbial biomass [3] [Colony forming units (CFU)/g soil] - Total aerobic bacteria - Total actinomycetes - Total fungi	 5.050 x 10^6 2.050 x 10^6 0.009 x 10^6

DAT = days after treatment, USDA: United States Department for Agriculture, n.i.: not indicated
[1] Calculated from organic matter according to OC = OM x 0.58
[2] Buffer medium not indicated
[3] Tested within a week of experimental start date

B. STUDY DESIGN

1. Experimental conditions

The test system consisted of thin soil layers placed in specially designed and temperature controlled round chambers (50 mm diameter, 20 mm height) made of quartz for light exposed samples and borosilicate glass covered with aluminium foil to prevent irradiation for dark control samples. Six extra soil containers were prepared. Duplicate containers of both light exposed and dark control samples were sealed with a screw cap fitted with a Teflon septum. The sample containers were submerged in a bath containing deionized water at an approximate 30° angle with respect to the horizon to maximize irradiation during periods of strong sunlight intensity. The water was circulated using a Lauda ™ Constant Temperature Circulator and maintained at approximately 25.0 ± 1.0 °C. Two small submersible pumps were placed in the bath to prevent local temperature differences. The temperature was acquired each 10 seconds using Type T thermocouples. Three thermocouples were used, one was placed in the water bath and one each placed inside and attached to the bottom of the irradiated and dark containers before adding the soil slurry.

Volatiles from each individual container were trapped by inserting a needle with tubing attached to a series of traps connected to a water aspirator pump (no flow through system). The traps consisted of one ethylene glycol trap (50 mL) to collect organic volatiles and two 10 % NaOH traps to account for carbon dioxide. Samples were weighed following each intermittent trapping to assure that moisture content was maintained at 75 % of soil water holding capacity at 1/3 bar. After intermittent trapping the punctured septa were replaced by new ones, and the sealed containers placed back into the water bath. Since some radiocarbon recoveries were low and large amounts of $^{14}CO_2$ were produced, additional trapping experiments were conducted at day 20 and 30 samplings after purging and trapping the headspace gases. Acidic phosphate buffer (5 mL of ~ pH 2.0) was injected through each septum, the containers were connected to the trapping system, and the mixtures vortexed to release $^{14}CO_2$ adsorbed to the moist soil.

The equivalent of 3.1 g of dry soil was weighed into each sample container. Deionized water (3 mL) was added to each dish to form slurries; slurries were allowed to dry and form thin soil layers (1-2 mm) on the bottom of the containers.

The dosing solution was prepared by adding aqueous [^{14}C]glyphosate stock solution (0.238 mL, 870 µg) to 2.562 mL of deionized water. Aliquots (100 µL) of the dosing solution were applied as evenly as possible to each of the previously prepared soil containers by using a glass syringe. Deionized water (177 µL) was then added to achieve 75 % water holding capacity at 1/3 bar. Aliquots of the dosing solution taken prior to, during and after the application process were radio assayed by LSC to determine the applied radiocarbon. The final concentration of test substance in the soil was 10.19 µg/g corresponding to 11 kg/ha (10 lb/acre).

Test systems were incubated for 30 days at 75 % of the maximum water holding capacity at 1/3 bar. Cloud cover data were compiled. The exposure phase was carried out in Richmond, CA at latitude 38° N, longitude 122° W, between October 18 and November 17, 1995. Sunlight intensity and cumulative energy (250 – 700 nm range) were measured and recorded at 20 minute intervals throughout the study using an International Light Radiometer. The mean total light energy was 7.02 W min/cm^2, with the cumulative light energy of 217.6 W min/cm^2.

2. Sampling

Duplicate test systems were sampled 0, 2, 6, 12, 20 and 30 days after treatment (DAT). Traps with ethylene glycol and 10 % NaOH were sampled at all these occasions except 0 DAT. Intermittent trapping of the headspace was performed once a week starting approximately one week after dosing. Trapping solutions and soil extracts were analysed by LSC on the day of collection. Extracts were analysed by HPLC within 24 hours of sampling, with the exception of 2 DAT samples, which were analysed after three days. All samples were frozen when not in use.

3. Analytical procedures

At each sampling time the soils were transferred from the containers into pre-weighed Teflon centrifuge tubes (50 mL) by rinsing the containers with 1 M aqueous KH_2P0_4 (15 mL) adjusted to ~ pH 2.0 with concentrated H_3PO_4. The mixture was shaken for ten minutes with a Wrist Action Shaker. After centrifugation (5,000 rpm, 10 minutes) the supernatant was separated from the residue, and the residue extracted once more with the extraction solvent (total of 15 mL) in the same manner as the first extraction. The supernatants were combined, the volumes recorded, and aliquots (3 x 1 mL) radio assayed by Liquid Scintillation Counting (LSC). For HPLC analyses, subsamples of each replicate sample were filtered and aliquots of the filtrates were co-injected with solutions of mixed analytical reference standards glyphosate and AMPA.

The limit of detection (LOD) for individual degradates in the HPLC radio chromatograms were determined by the dpm injected and the liquid scintillation counting detection limit. As an example a limit of 0.3 % AR is given for a background of 30 dpm and a sample size of 10,000 dpm injected of a matrix containing 5 ppm.

[^{14}C]glyphosate and its metabolites were analysed by HPLC of soil extract aliquots. Structural assignment was based on co-elution of ^{14}C-peaks with reference substances by HPLC and confirmed by one-dimensional TLC co-migration of ^{14}C-spots with reference substances.

Bound ^{14}C-residues present at ≥ 10 % AR were further characterized in selected samples (30 DAT replicate A and B light exposed extracted soil). Humic and fulvic acids residue were determined by extracting samples twice with 0.1 M NaOH (15 mL) by shaking for 24 hours under nitrogen using a wrist action shaker. After centrifugation the combined extract was acidified to pH 1 by adding a few drops of 6N HCl and humic acid allowed to precipitate overnight in an ice bath. The humic acid fraction (pellet) was separated from the fulvic acid fraction (supernatant) by centrifugation (2,000 rpm for 5 min). The volume of total supernatant was determined and aliquots (3 x 500 µl) taken for radioassay by LSC. The pellet (humic acid fraction) was redissolved in a minimal volume of 0.1M NaOH solution and the radiocarbon quantified by LSC of aliquots (3 x 200 µL).

The amount of volatiles was determined by LSC. The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents of 6 DAT samples. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in soil extracts are summarised in Table 7.1.1.3-7 to Table 7.1.1.3-10. Fractionation of non-extractable residues into fulvic acid, humic acid in humin fractions is presented in Table 7.1.1.3-11.

Table 7.1.1.3-7: Mass balance of [^{14}C]glyphosate in/on light-exposed soil

Days after application	% applied			
	^{14}C in soil extract	^{14}C non-extracted in residue soil	^{14}C as total volatiles	Total recovery
0 d Rep A	91.6	3.0	n.d.	94.7
0 d Rep B	92.4	3.1	n.d.	95.6
2 d Rep A	70.9	6.3	12.2	89.4
2 d Rep B	65.3	6.2	18.8	90.3
6 d Rep A	49.8	6.4	25.7	81.8
6 d Rep B	48.4	7.2	30.6	86.2
12 d Rep A	40.7	13.0	37.8	91.5
12 d Rep B	40.1	14.8	29.1	84.0
20 d Rep A	36.2	16.2	37.4	89.8
20 d Rep B	41.8	19.0	28.5	89.4
30 d Rep A	25.3	36.1	30.3	91.7
30 d Rep B	26.8	31.0	31.5	89.2

n.d. = not determined

Table 7.1.1.3-8: Degradation of [^{14}C]glyphosate in/on dark control soil

Days after application	% applied			
	^{14}C in soil extract	^{14}C non-extracted in residue soil	^{14}C as total volatiles	Total recovery
0 d Rep A	91.6	3.0	n.d.	94.7
0 d Rep B	92.4	3.1	n.d.	95.6
2 d Rep A	70.4	6.1	16.5	93.0
2 d Rep B	73.2	6.4	18.0	97.6
6 d Rep A	51.5	6.2	32.8	90.6
6 d Rep B	43.7	6.6	36.4	86.7
12 d Rep A	37.9	6.1	40.2	84.1
12 d Rep B	40.1	6.7	37.4	84.1
20 d Rep A	36.3	6.1	24.4	66.8 [1]
20 d Rep B	36.3	6.5	43.5	86.3
30 d Rep A	28.8	7.4	28.2	64.4 [1]
30 d Rep B	29.2	7.2	43.6	80.0

n.d. = not determined
[1] Significant losses of $^{14}CO_2$. These numbers were not considered for range of mass balance.

Table 7.1.1.3-9: Distribution of [^{14}C]glyphosate and its degradates in extracts of light exposed samples

Days after application	% applied				
	^{14}C in soil extract	Glyphosate	AMPA	Degradate 1	Others
0 d Rep A	91.6	88.69	2.49	0.21	0.21
0 d Rep B	92.4	89.60	1.89	0.17	0.74
2 d Rep A	70.9	57.82	12.58	0.00	0.50
2 d Rep B	65.3	50.99	13.67	0.64	0.00
6 d Rep A	49.8	26.34	22.08	1.38	0.00
6 d Rep B	48.4	26.62	20.69	1.02	0.08
12 d Rep A	40.7	10.85	26.82	3.02	0.00
12 d Rep B	40.1	10.48	27.54	2.07	0.00
20 d Rep A	36.2	6.36	26.60	3.15	0.09
20 d Rep B	41.8	8.05	30.10	3.65	0.00
30 d Rep A	25.3	3.41	18.61	2.57	0.71
30 d Rep B	26.8	2.91	21.06	2.83	0.00

Table 7.1.1.3-10: Distribution of [^{14}C]glyphosate and its degradates in extracts of dark control samples

Days after application	% applied				
	^{14}C in soil extract	Glyphosate	AMPA	Degradate 1	Others
0 d Rep A	91.6	88.69	2.49	0.21	0.21
0 d Rep B	92.4	89.60	1.89	0.17	0.74
2 d Rep A	70.4	57.17	12.07	0.61	0.56
2 d Rep B	73.2	60.93	11.83	0.34	0.09
6 d Rep A	51.5	30.16	20.85	0.00	0.49
6 d Rep B	43.7	19.51	23.47	0.72	0.00
12 d Rep A	37.9	10.32	26.36	1.22	0.00
12 d Rep B	40.1	13.64	25.46	0.99	0.00
20 d Rep A	36.3	7.57	27.36	1.38	0.00
20 d Rep B	36.3	5.92	28.70	1.60	0.09
30 d Rep A	28.8	2.78	25.00	1.02	0.00
30 d Rep B	29.2	4.36	23.61	1.24	0.00

Table 7.1.1.3-11: Fractionation of 30 DAT post extracted soil

Days after application	% applied		
	Fulvic acid	Humic acid	Humin
30 d Rep A	6.6	4.3	25.2
30 d Rep B	6.0	2.9	22.1
Average	6.3	3.6	23.7

B. MASS BALANCE

Material balances ranged from 84.0 to 95.6 % of applied radioactivity (% AR) (single values, n = 12) for light exposed samples and from 80.0 to 97.6 % AR for dark control samples (single values, n = 10). Since the amounts of extracted and bound radiocarbon were usually consistent between replicates, losses of radiocarbon that occurred after 2 DAT were attributed to the rapid and steady formation of large amounts of $^{14}CO_2$. This caused some leakage from the headspace of the sample containers resulting in lower

recoveries in some replicates. Intermittent purging of the headspace at 7 DAT intervals helped to mitigate the losses, but did not completely solve the problem in the dark-control samples.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from soil decreased from 0 DAT to 30 DAT from 92.0 % AR to 26.1 % AR (mean of two replicates) in light exposed samples and from 92.0 % AR to 29.0 % AR in dark control samples (mean of two replicates).

The amount of non-extractable residues (NER) increased from 0 DAT to 30 DAT from 3.1 % AR (mean of two replicates) to 33.6 % AR (mean of two replicates) in light exposed samples and to 7.3 % AR (mean of two replicates) in dark control samples. Light exposed extracted soils from 30 DAT (replicates A and B) were therefore selected for additional extraction using 01.M NaOH for characterization of bound ^{14}C-residues. Only 3.6 and 6.3 % of applied dose (average of replicates) were associated with the humic and fulvic acid fractions, respectively.

D. VOLATILE RADIOACTIVITY

Total volatiles trapped at the end of the test period amounted to 30.9 % AR and 35.9 % AR in irradiated and darck control samples (both values mean of two replicates). [^{14}C]-PMG degraded rapidly to $^{14}CO_2$, with maximum values of 32.9 % AR and 36.7 % AR (each mean of two replicates) at 12 DAT in the light exposed and dark control samples, respectively. At study end at 30 DAT, 29.5 and 30.1 % AR were detected as $^{14}CO_2$ (each mean of two replicates) in light exposed and dark control samples, respectively, due to significant losses of $^{14}CO_2$. Considering unaccounted ^{14}C as CO_2, more CO_2 was formed in the dark-control than in the light-exposed samples (53.1 % AR and 34.2 % AR (each mean of two replicates) in dark control and light exposed samples, respectively). Radiocarbon found in the light exposed and dark control ethylene glycol traps reached a maximum of 1.9 and 5.8 % AR (each mean of two replicates) at 20 DAT and 30 DAT, respectively, and was not further characterized. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

[^{14}C]glyphosate rapidly degraded in the light exposed and dark control soil samples and represented only 3.2 and 3.6 % AR (mean of two replicates), respectively, at study end at 30 DAT0. The major degradate detected in light exposed and dark control soil extracts after 30 days was AMPA. AMPA reached a maximum of 28.4 and 28.0 % AR (mean of replicates) respectively, in light exposed and dark control soil samples at 20 DAT0, and represented 19.8 and 24.3 % AR (mean of replicates) for light exposed and dark control soil extracts, respectively, at 30 DAT. An unidentified degradate, designated “Degradate 1”, reached maximum values of 3.4 and 1.5 % AR, respectively, in light exposed and dark control soil samples, respectively, at 20 DAT. With the exception of AMPA no other degradates above 5 % AR were detected. ^{14}C as total volatiles reached a maximum of 33.5 and 38.8 % AR (each mean of two replicates) in light exposed and dark control soil samples at 12 DAT, respectively.

F. KINETICS

New kinetic calculations based on more recent guidance will not be provided as this study is only delivering supplemental information. The half-life of glyphosate was calculated to be 6.5 days ($R^2 = 0.940$) for the light exposed and 6.6 days ($R^2 = 0.922$) for the dark control samples, using pseudo first order kinetics.

III. CONCLUSIONS

A study of the photodegradation of [P-Methylene-^{14}C]Glyphosate Acid ([^{14}C]PMG) in natural sunlight on sandy loam soil was conducted for 30 days at about 25 °C. Dark control samples were maintained concurrently to account for non photolytic degradation processes.

Radiocarbon recoveries ranged from 84.0 to 95.6 % of applied radioactivity (% AR) (single values, n = 12) for light exposed samples and from 80.0 to 97.6 % AR for dark control samples (single values, n = 10). Small losses of radiocarbon occurred throughout the study, due to the rapid and steady formation of $^{14}CO_2$.

Up to 32.9 % AR and 36.7 % AR in the light exposed and dark control NaOH traps was recovered as $^{14}CO_2$ at 12 DAT. Glyphosate rapidly degraded in both, light exposed and dark control, representing only 3.2 and 3.6 % AR (mean of two replicates), respectively, at study end at 30 DAT.

The major product detected in light exposed and dark control soil extracts was AMPA, which reached a maximum of 28.4 and 28.0 % AR (mean of replicates) respectively, in light exposed and dark control soil samples at 20 DAT, and represented 19.8 and 24.3 % AR (mean of replicates) for light exposed and dark control soil extracts, respectively, at 30 DAT. An unidentified degradate, designated "Degradate 1", reached maximum values of 3.4 and 1.5 % AR, respectively, in light exposed and dark control soil samples, respectively, at 20 DAT. No degradates other than AMPA were detected at ≥10 % AR. A pattern of steady increase of the major terminal metabolite $CO_2$and the rise and slight decline of the metabolite AMPA was clearly established.

The only significant difference between light exposed and dark control samples was increased post extraction soil residues in irradiated samples. The unextracted radiocarbon in the dark control soil reached 7.3 % AR at 30 DAT while amounts in light exposed soil reached 33.6 % AR at 30 DAT. Additional extractions with 01.M NaOH showed that 3.6, 6.3 and 23.7 % AR was associated with the humic acid, fulvic acid and humin fractions, respectively.

Exposure of glyphosate treated soil to light had no effect on the degradation rate of glyphosate or extractable residues found.

The results of this study indicate that photolysis in/on soil is not likely to be a significant route of dissipation for glyphosate compared to rapid microbial degradation in soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The photodegradation of [^{14}C]glyphosate on soil surfaces under natural sunlight was examined for 30 days using a field application rate of 11 kg/ha (10 lb/acre) soil. Mass balances ranged from 80.0 to 97.6 % AR.
As natural sunlight was used for the experiment instead of preferred artificial light, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.3/003
Report author	[redacted]
Report year	1993
Report title	Photodegradation study of ^{14}C-Glyphosate on soil
Report No	315764
Document No	
Guidelines followed in study	U.S. EPA 161-3
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - only single sample data is available
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The objectives of the present study were to determine the rate of photolysis of glyphosate and its degradation products in one silt loam soil (organic carbon 1.4 %, pH 6.1), prepared as thin-layers on glass-plates. Therefore, [^{14}C]glyphosate was applied to the soil at a dose level of 8.45 mg/kg soil corresponding to 3.6 kg a.i./ha and exposed to an artificial light source using a 12 hours light/dark cycle during 30 days.

The test was performed in flow-through systems connected to one NaOH trap to collect carbon dioxide and one ethylene glycol trap to collect volatile organic compounds.

Soil samples from each system were processed and analysed at 0, 3, 7, 14, 21 and 30 days (dark controls: 31 DAT). $^{14}CO_2$ and ^{14}C-volatiles were measured at each sampling interval except for 0 DAT.

Total recoveries of radioactivity amounted, on average, to 104.4 ± 1.9 % AR and 106.6 ± 1.0 % AR in irradiated and dark control samples, respectively, ranging from 102.4 % AR to 107.2 % and from 105.5 to 107.7 %, respectively.

In irradiated soil samples, extracted radioactivity decreased from 94.9 % (0 DAT) to 73.3 % AR (30 DAT). Non-extractable radioactivity was detected in amounts ranging from 7.5 % (0 DAT) to 19.4 % AR (14 DAT), and decreasing then to 15.5 % AR at study end (30 DAT). Cumulative $^{14}CO_2$ levels steadily increased from 4.3 % AR (3 DAT) to 14.6 % AR (30 DAT). No volatiles (<0.05 % AR) were trapped by means of ethylene glycol.

In the control samples incubated in the dark, extracted radioactivity decreased from 88.6 % (3 DAT) to 83.9 % AR (31 DAT) throughout the entire incubation period. Non-extractable radioactivity was measured in amounts ranging from 13.0 % (3 DAT) to *ca.* 17.4 % AR from 21 DAT onwards. Small amounts of $^{14}CO_2$, ranging from 3.9 % (3 DAT) to 5.4 % AR (31 DAT), but no volatiles (≥0.05 % AR) were trapped.

In irradiated soil samples, the amount of parent compound decreased from 94.9 % AR to 60.5 % AR (30 DAT). Besides glyphosate three radioactive fractions (M2, M3, M4) occurred. Radioactive fraction M2

(range 5.2 % - 8.2 % AR (7 DAT)) was proven to be identical to aminomethylphosphonic acid, also designated AMPA. Radioactive fractions M3 (2.5 – 5.0 % AR) and M4 (2.7 % - 3.9 % AR) were tentatively identified to be (N-methyl-N-phosphono-methyl)-glycine and hydroxymethylphosphonic acid, respectively.

In the soil samples incubated in the dark, except for small amounts of AMPA (2.9 - 6.1 % AR (3 DAT)), only parent compound was found, ranging from 83.8 % to 79.6 % AR.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate
Lot No.: CFA.745 C6
Specific activity: 12.3 MBq/mg (333 mCi/g)
Radiochemical purity: > 99.3 %
Chemical purity: not indicated

2. Soil:

The selected soil was air-dried and sieved to ≤2 mm. Before the start of the experiment, the untreated soil was stored in concrete cylinders in the open. Characteristics of the test soil are presented in the table below.

Table 7.1.1.3-12: Characteristics of test soil

Parameter	Results
Soil	Les Evouettes II
Country	Switzerland
Textural Class (USDA)	Loam / silt loam
Sand (50 µm – 2 mm) (%)	38.0
Silt (2 µm – 50µmm) (%)	50.7
Clay (< 2 µm) (%)	11.3
pH [2]	6.1
Organic carbon (%)	1.40
Organic matter (%) [1]	2.41
Cation exchange capacity (meq/100 g)	15.5
Maximum Water Holding Capacity (%)	55.3
Field capacity (%)	40.2
40 % MWC (g/100 g soil)	22.1
Bulk Density (disturbed) (g/cm^3)	0.856
Microbial biomass / Total late counts	
At the start of the experiments	2.2 x 10^5 / g soil
At 30 DAT of incubation (illuminated plate)	1.4 x 10^5 / g soil
At 31 DAT of incubation (dark control plate)	0.6 x 10^5 / g soil

DAT = days after treatment, USDA: United States Department for Agriculture
[1] Calculated from organic carbon according to OM = OC / 0.58
[2] Buffer medium not indicated

B. STUDY DESIGN

1. Experimental conditions

For illumination, the soil thin-layer plates were placed in a metal-chamber with a matt-black interior covered with a quartz plate. The metal-chamber beneath the photolysis apparatus was cooled by means of a waterbath, allowing maintenance of constant temperature. The light source was a Hanau Suntest CPS apparatus equipped with a xenon burner 1.1 kW and a UV filter system simulating natural sunlight. Radiation intensity was measured at regular time intervals and on average the light intensity was 93 Klux. The temperature was continuously monitored and remained constant (22 ± 1 °C) except for the transition period. The system was continuously ventilated with air by means of a membrane pump. The air in the metal-chamber was saturated by placing moistened filter paper against the walls. Additionally, the incoming air was moistened by bubbling through a flask containing saturated $NaHSO_4$. The outcoming air was passed through a CO_2-trapping system (NaOH) and through an ethylene glycol trap.

Dark soil samples were placed in an all-glass chamber under exclusion of light and incubated in an air-conditioned room at a temperature of 22 ± 2 °C. Air was ventilated by means of a membrane pump and trapped as described for the illuminated set-up.

100 g of sieved soil was mixed with 75 mL of bidistilled water. After homogenization (4 minutes) the soil thin-layer plates were prepared by applying the slurry to the surface of 16 clean, pre-weighed glass-plates (5 × 10 cm) using a TLC-plate coater adjusted to a layer-thickness of about 1.0 mm.

Based on a target dose of 8.4 mg/kg soil (3.6 kg a.s./ha, dry weight based), the average soil weight per soil plate (2.472 g) and a target application volume of 1.5 mL, an aliquot of 1010 µl (346.4 µg) stock solution was made up to 25 mL with bidistilled water. The application solution proved to contain 13.7 µg/mL of [^{14}C]glyphosate. Based on the concentration of test item in the application solution and the average soil weight per treatment are, 1.52 mL containing 20.8 µg [^{14}C]glyphosate were applied to each plate.

Test systems were incubated for 30 days using a 12 hours light/dark cycle.

2. Sampling

After incubation, samples were weighed, left at room temperature for about 2 hours and re-weighed to get information on the moisture content of the incubated soil. No difference in moisture content between illuminated and dark control soil plates was found.

The soil was sampled at time intervals of 0, 3, 7, 14, 21 and 30 days (dark controls: 31 DAT instead of 30 DAT). The soil was stored at -20 °C until analyses. $^{14}CO_2$ and ^{14}C-volatiles were measured at each sampling interval except for 0 DAT for both illuminated and dark samples.

For test on vitality of microbial biomass, one sample (about 5 g soil) at 0 DAT and two samples (about 5 g each) at 30 DAT (illuminated soil) and 31 DAT (dark control) was collected.

3. Analytical procedures

The air-dried soil samples (about 2-3 g) were extracted 3 times with 0.5 M NH_3 (about 4-6 mL/g soil) by shaking for 30 minutes at room temperature. For time intervals 30 DAT/31 DAT, additional extractions with H_2O and 0.1N HCl were performed. An exhaustive extraction with refluxing methanol/0.5M NH_3 (8+2, v/v) at 70 °C was performed for time intervals 21 DAT and 30 DAT/31 DAT; these additional extractions were performed to show that extraction of radioactivity was complete. After each extraction, samples were centrifuged for 10 minutes at 1900 g, the supernatant decanted and filtered through a filter paper. The radioactivity in each extract was determined by Liquid Scintillation Counting (LSC). The NH_3-extracts were combined and directly analysed by TLC. Thereafter, extracts were stored at -20 °C until HPLC. Remaining soil was air-dried, homogenized and the non-extracted radioactivity determined by combustion of aliquots (about 200-500 mg) and LSC.

The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC/radiodetection method were not reported.

The amount of volatiles was determined by LSC. The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in soil extracts are summarised in Table 7.1.1.3-13 to Table 7.1.1.3-17.

Table 7.1.1.3-13: Mass balance for [^{14}C]glyphosate in irradiated samples (expressed as percent of applied radioactivity)

Compound	Sampling intervals (days)					
	0	3	7	14	21	30
Extracted						
Room temperature						
1. 0.5M NH_3	74.6	65.0	60.2	54.9	53.2	43.3
2. 0.5M NH_3	16.1	18.9	17.0	17.6	16.2	19.4
3. 0.5M NH_3	4.2	5.7	5.2	5.9	5.3	6.1
- H_2O	n.d.	n.d.	n.d.	n.d.	n.d.	2.2
- 0.1N HCl	n.d.	n.d.	n.d.	n.d.	n.d.	1.3
Subtotal	94.9	89.6	82.4	78.4	74.7	72.3
Reflux at 70 °C - MeOH/0.5M NH_3 (8+2, v/v)	n.d.	n.d.	n.d.	n.d.	0.3	1.0
Subtotal	94.9	89.6	82.4	78.4	75.0	73.3
Non-extracted	7.5	13.3	14.6	19.4	17.3	15.5
Cumulative volatiles						
- NaOH trapped	n.d.	4.3	6.3	8.6	11.3	14.6
- Ethylene glycol trapped	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
Total	**102.4**	**107.2**	**103.3**	**106.4**	**103.6**	**103.4**
Total mean ± SD	104.4 ± 1.9					

n.d. = not determined, SD = Standard deviation

Table 7.1.1.3-14: Mass balance for [^{14}C]glyphosate in dark control samples (expressed as percent of applied radioactivity)

Compound	Sampling intervals (days)					
	0	3	7	14	21	31
Extracted						
Room temperature						
1. 0.5M NH_3	74.6	67.0	63.5	61.5	60.5	52.2
2. 0.5M NH_3	16.1	16.4	18.7	18.7	18.0	21.2
3. 0.5M NH_3	4.2	5.2	5.5	5.6	6.0	6.4
- H_2O	n.d.	n.d.	n.d.	n.d.	n.d.	2.4
- 0.1N HCl	n.d.	n.d.	n.d.	n.d.	n.d.	1.2
Subtotal	94.9	88.6	87.7	85.8	84.5	83.3
Reflux at 70 °C - MeOH/0.5M NH_3 (8+2, v/v)	n.d.	n.d.	n.d.	n.d.	0.4	0.6
Subtotal	94.9	88.6	87.7	85.8	84.9	83.9

Table 7.1.1.3-14: Mass balance for [^{14}C]glyphosate in dark control samples (expressed as percent of applied radioactivity)

Compound	Sampling intervals (days)					
	0	3	7	14	21	31
Non-extracted	7.5	13.0	15.0	15.5	17.4	16.5
Cumulative volatiles						
- NaOH trapped						
- Ethylene glycol trapped	n.d. n.d.	3.9 <0.05	5.0 <0.05	5.1 <0.05	5.2 <0.05	5.4 <0.05
Total	**102.4**	**105.5**	**107.7**	**106.4**	**107.5**	**105.8**
Total mean ± SD	**106.6 ± 1.0**					

n.d. = not determined, SD = Standard deviation

Table 7.1.1.3-15: Characterisation of extractables following treatment with [^{14}C]glyphosate in irradiated samples (expressed as percent of applied radioactivity)

Compound	Sampling interval (Days)					
	0	**3**	**7**	**14**	**21**	**30**
Glyphosate	94.9	75.7	65.3	64.8	60.3	60.5
AMPA (M2)	n.d.	7.4	8.2	5.2	7.4	6.5
M3 [1]	n.d.	3.6	5.0	4.8	4.3	2.5
M4 [2]	n.d.	2.9	3.9	3.6	2.7	2.8
Total	**94.9**	**89.6**	**82.4**	**78.4**	**74.7**	**72.3**

n.d. = not detected
[1] Tentatively identified as (N-methyl-N-phosphono-methyl)-glycine
[2] Tentatively identified as hydroxymethylphosphonic acid

Table 7.1.1.3-16: Characterisation of extractables following treatment with [^{14}C]glyphosate in dark control samples (expressed as percent of applied radioactivity)

Metabolite Code	Sampling interval (Days)					
	0	**3**	**7**	**14**	**21**	**31** [1]
Glyphosate	94.9	82.5	83.8	82.9	80.8	79.6
AMPA	n.d.	6.1	3.9	2.9	3.7	3.7
Total	**94.9**	**88.6**	**87.7**	**85.8**	**84.5**	**83.3**

n.d. = not detected

Table 7.1.1.3-17: Amount of [^{14}C]glyphosate in irradiated soil samples after correction for the degradation in the dark (expressed as percent of applied radioactivity initially applied to each plate)

	Sampling interval (Days)					
	0	**3**	**7**	**14**	**21**	**30/31** [1]
I	94.9	75.7	65.3	64.8	60.3	60.5
II	94.9	82.5	83.8	82.9	80.8	79.6
III	94.9	88.1	76.4	76.8	74.4	75.8

I: Amount of 14C-Glyphosate in irradiated samples
II: Amount of 14C-Glyphosate in dark controls
III: Amount of 14C-Glyphosate in irradiated samples after correction for its degradation in the dark (III = 94.9 % - (II - I))
[1] Irradiated: 30 days; dark controls: 31 days

B. MASS BALANCE
Total recovery of radioactivity ranged from 102.4 % AR to 107.2 % and from 105.5 to 107.7 % AR in irradiated and dark control samples, respectively.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
Extracted radioactivity decreased from 94.9 to 73.3 % AR and from 88.6 to 83.9 % AR in irradiated and dark control samples, respectively.

Non-extracted radioactivity was 7.5 % AR at 0 DAT and was at similar levels in irradiated and dark control samples from DAT 3 to study end. NER increased to 19.4 % AR (14 DAT) and to 17.4 % AR (21 DAT) in irradiated and dark control samples, respectively, and decreased then to 15.5 and 16.5 % AR at study end (30 DAT in irradiated, 31 DAT in dark control samples).

D. VOLATILE RADIOACTIVITY
In irradiated samples high amounts of $^{14}CO_2$ were evolved, increasing from 4.3 % AR (3 DAT) to 14.6 % AR at 30 DAT (cumulative levels), while the cumulative levels of $^{14}CO_2$ were similar during 31 days of incubation in the dark, ranging from 3.9 (3 DAT) to 5.4 % AR (31 DAT). Organic volatiles determined were <0.05 % AR for irradiated and dark control samples at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

The occurrence of higher amounts of $^{14}CO_2$ in irradiated samples as compared to the dark controls indicated that glyphosate could be mineralized by the process of photolysis.

E. TRANSFORMATION OF THE TEST ITEM
In irradiated samples, the amount of glyphosate decreased from 94.9 % AR to 60.5 % AR (30 DAT). From 3 DAT on, besides glyphosate three radioactive fractions, M2 (AMPA), M3 and M4 were detected, with M3 and M4 tentatively identified as (N-methyl-N-phosphono-methyl)-glycine) and hydroxymethylphosphonic acid, respectively. AMPA was detected with a maximum amount of 8.2 % AR at 7 DAT, with similar amounts at other sampling times, ranging from 5.2 (14 DAT) to 7.4 % AR (3 DAT/21 DAT). The amount of radioactive fraction M3 increased from 3 DAT (3.6 % AR) to 5.0 % AR at 7 DAT and, thereafter, decreased to 2.5 % AR at 30 DAT. Radioactive fraction M4 had similar levels of radioactivity from 3 DAT to 30 DAT, ranging from 2.7 % (21 DAT) to 3.9 % AR (7 DAT). No other metabolites were detected above 5 % AR at any time.

In dark control samples, the amount of glyphosate decreased from 94.9 % AR to 79.6 % AR (31 DAT). Except for 0 DAT, one radioactive fraction (AMPA) was detected throughout the incubation interval, with a maximum amount of 6.1 % AR at 3 DAT. Thereafter, the amounts were somewhat smaller, ranging from 3.9 % (7 DAT) to2.9 % AR (14 DAT).

A different metabolite pattern was found after irradiation as compared to the dark control during 30 days if incubation. Radioactive fraction M2 occurred in both, irradiated and dark control samples. Therefore, radioactive fractions M3 and M4 were specific photolytic products of [^{14}C]glyphosate on soil.

F. KINETICS
New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. The new evaluation is reported under Porschewski (2020, CA 7.1.1.3/0001.

III. CONCLUSIONS

The present data indicated that the degradation of [^{14}C]glyphosate on soil under irradiation conditions simulating natural sunlight (light/dark cycle: 12 hours) proceeded faster than in the dark.

After TLC-analyses of the extracted radioactivity from irradiated soil plates, mainly parent compound (M1) was found at all time intervals. With increasing irradiation time, one major (M2) and two minor radioactive fractions (M3 and M4) were detected. Radioactive fraction M2 was proven to be identical to aminomethylphosphonic acid (Ref. B), also designated AMPA. Radioactive fractions M3 and M4 were tentatively identified to be (N-methyl-N-phosphono-methyl)-glycine (Ref. D) and hydroxymethylphosphonic acid (Ref. E), respectively.

In conclusion, taking into account the specific occurrence of $^{14}CO_2$ in the irradiated samples as compared to the dark controls, the present data showed that glyphosate could be slowly mineralized by the process of photolysis.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The photodegradation of [^{14}C]glyphosate on soil surfaces was examined using an artificial light source at an application rate of 3.6 kg/ha soil. Mass balances ranged from 102.4 to 107.7 % of applied radioactivity (% AR).
The study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.3/004
Report author	[redacted]
Report year	1989
Report title	Photodegradation of [^{14}C]Glyphosate in/on soil by natural sunlight
Report No	153W
Document No	
Guidelines followed in study	U.S. EPA 161-3
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - tests were not conducted with an artificial irradiation source, but samples exposed to natural sunlight - no constant temperature
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The photoreactions of the herbicide [^{14}C]glyphosate (N-phosphonomethylglycine), under natural sunlight have been examined on a sandy loam soil surface (organic carbon 0.9 %, pH 7.6 (H_2O)). Uniform soil layers

on the bottom of petri dishes were placed in temperature controlled chambers for 31 days. The average temperature of soil surface during the study was 22.6 ± 0.2 °C and 21.9 ± 0.2 °C for light exposed and dark control samples, respectively.

The test was performed in flow-through systems connected to three traps, one containing ethylene glycol and two containing 10 % NaOH solution for collection of volatile organic compounds and carbon dioxide, respectively.

The study was conducted at a typical field application rate of 4.48 kg/ha soil (4.0 lb/acre).

Duplicate samples from each system were processed and analysed at 0, 3, 7, 11, 20 and 31 days after treatment (DAT). The trapping solution were collected for analysis and replaced with fresh solution at the same sampling times to determine the amount of volatile organic compounds and carbon dioxide.

Mass balances ranged from 96.3 to 111.2 % of applied radioactivity (% AR). Recoveries averaged 89.5 ± 4.0 % (average ± SD, n = 12) and 89.3 ± 5.9 % (average ± SD, n = 10) for light exposed and dark control samples, respectively.

Maximum amounts of carbon dioxide reached at study end (31 DAT) were 4.0 % AR in irradiated soil and 6.6 % AR in dark control. Organic volatiles determined were ≤ 0.5 % AR for both irradiated and dark control samples at all sampling points.

The amount of radioactivity extractable from soil decreased from 0 DAT to 20 DAT from 103.1/106.5 to 87.6/85.6 % AR in irradiated soil and from 102.5/93.2 to 84.8/83.2 % AR in dark control soil followed by a slight increase to 91.5/90.7 and 87.5/85.0 % in irradiated and dark control samples, respectively, at 31 DAT.

The amount of non-extractable residues (NER) increased from 0 DAT to 31 DAT from 1.9/3.6 to 11.5/14.8 % AR in irradiated soil. Similar amount were detected in dark control samples (from 2.8/3.2 at 0 DAT to 10.5/13.2 % AR at 31 DAT).

The amount of glyphosate in irradiated soil samples decreased from 101.6/104.8 % AR (0 DAT) to 78.9/77.2 % AR (31 DAT). Similar results were found for dark control samples (78.6/74.3 % AR at 31 DAT).

Besides carbon dioxide, one major metabolite was detected, both in irradiated and dark control samples. AMPA was detected with a maximum amount of 13.0 % AR (mean of two replicates) at 31 DAT; amounts in dark control samples are somewhat lower with 9.8 % AR (mean of two replicates) at 31 DAT. No other metabolites were detected above 0.7 % AR at any time.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate (N-phosphonomethylglycine)
Lot No.:	not indicated
Specific activity:	8.08 mCi/mmol
Radiochemical purity:	98.9 %
Chemical purity:	not indicated

2. Soil:

Soil was sieved to ≤2 mm. The soil was received and stored in a freezer (-20 °C) prior to use. Characteristics of the test soil are presented in the table below.

Table 7.1.1.3-18: Characteristics of test soil

Parameter	Results
Soil	KP8 Composite
Country	Kentucky, USA
Textural Class (USDA)	Sandy loam
Sand (50 µm – 2 mm) (%)	74
Silt (2 µm – 50µmm) (%)	16
Clay (< 2 µm) (%)	10
pH (water)	7.6
pH ($CaCl_2$)	n.i.
Organic carbon (%) [1]	0.9
Organic matter (%)	1.6
Cation exchange capacity (mgq/100 gm)	6

[1] Calculated from organic matter according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

The test was performed in flow-through systems connected to three traps, one containing ethylene glycol and two containing 10 % NaOH solution for collection of volatile organic compounds and carbon dioxide, respectively. The test system consisted of thin layers of soil in petri dishes placed in temperature controlled stainless steel chambers. Aliquots of the soil (3.1 g) were weighed into 50 mm petri dishes. Distilled water (3 mm) was added to each dish and the slurries were allowed to dry, forming a uniform layer on the bottom of the petri dishes. At dosing, the soil surface (19.6 cm^{-2} per petri dish) was treated with aliquots (200 µl) of a glyphosate stock solution (4.725 mg [^{14}C]glyphosate plus 21.28 mg unlabelled glyphosate) in a circular pattern. The volume of water in the dosing solution was calculated to provide 75 % of the soil water holding capacity. The amount of glyphosate applied to each petri dish was equivalent to an application rate of 4.48 kg glyphosate/ha (4.0 lb/acre).

After dosing, petri dishes were placed in temperature controlled stainless steel chambers: one set was covered with dark material to prevent exposure of dark control samples to light, the other with quartz glass plates for the light exposed samples. Sample chambers were exposed to natural sunlight at 37.45° N ad longitude 122.26° W (Richmond, California) from February 24 through March 27, 1989 corresponding to 31 days of incubation. Sunlight intensity and cumulative sunlight energy were measured and recorded at 10 minute intervals throughout the study.

Each chamber was equipped with a coolant (Prestone antifreeze:water (1:1)); temperature was continuously monitored at 10 minute intervals using thermocouples attached to the soil surface in both irradiated and dark conditions. The temperature range was 15.6 to 30.7 °C in the light exposed samples, and 15.8 to 28.5 °C in the dark control samples.

Humidified air was drawn through each sample chamber and then consecutively through three traps, one with ethylene glycol and the other two with 10 % NaOH solution for trapping of volatile organic compounds and carbon dioxide, respectively.

2. Sampling

Duplicate test systems were processed and analysed 0, 3, 7, 11, 20 and 31 days after treatment (DAT). All soil samples were processed on the day of sampling. Trapping solutions were collected for analysis and replaced with fresh solutions at the same sampling times.

3. Analytical procedures

At each sampling interval, soil samples were extracted twice with 0.5 N KOH (1x 20 mL, 1 x 15 mL) by vortexing and subsequent centrifugation. Extracts were combined and the total volume recorded; then, aliquots (3 x 0.5 mL) were analysed for radioactivity by liquid scintillation counting (LSC). Extracted soil samples were dried and aliquots (2 x 500 mg) analysed for unextracted radiocarbon by combustion followed by LSC. Glyphosate and its potential degradates were identified by HPLC. Identities of degradates were confirmed by TLC.

Soil samples in which > 9 % AR remained bound after extraction with 0.5 N KOH as determined by combustion, were re-extracted to reduce the radiocarbon level in soil. Aliquots of the soil samples (0.25 g) were shaken on a wrist action shaker for one hour with 0.03 M Na_2EDTA (20 mL). Radiocarbon was measured and selected extracts were analysed by HPLC.

The limit of detection (LOD) and limit of quantification (LOQ) for both chromatographic methods (HPLC, TLC) were 0.5 % AR and 0.1 % AR, respectively. LOD and LOQ for the radiodetection method were not reported. [^{14}C]Carbon dioxide was trapped in sodium hydroxide solutions. Its presence was confirmed by precipitation with barium chloride.

II. RESULTS AND DISCUSSION

A. DATA

The radioactive mass balance is summarised in Table 7.1.1.3-19. The distribution of glyphosate and metabolites in soil extracts are summarised in Table 7.1.1.3-20.

Table 7.1.1.3-19: Mass balance for [^{14}C]glyphosate in irradiated and dark control samples (expressed as percent of applied radioactivity)

Sample description/ Replicate	Extractable	Unextracted [^{14}C] in soil			Volatiles		Total [2]
		Original unextracted	Extracted with EDTA	Residual unextracted	NaOH	Ethylene glycol	
Day 0							
Irradiated (1)	103.1	1.9	- [1]	1.9	< LOQ	< LOQ	105.0
Irradiated (2)	106.5	3.6	- [1]	3.6	< LOQ	< LOQ	110.2
Dark Control (1)	102.5	2.8	- [1]	2.8	< LOQ	< LOQ	105.2
Dark Control (2)	93.2	3.2	- [1]	3.2	< LOQ	< LOQ	96.3
Day 3							
Irradiated (1)	94.2	9.6	4.9	4.7	1.3	0.06	105.1
Irradiated (2)	96.9	9.7	4.9	4.8	1.3	0.06	107.9
Dark Control (1)	96.9	10.1	4.8	5.3	4.1	0.01	111.2
Dark Control (2)	96.1	8.6	- [1]	8.6	4.1	0.01	108.9
Day 7							
Irradiated (1)	98.2	10.4	4.2	6.2	1.7	0.15	110.4
Irradiated (2)	95.1	9.9	5.3	4.6	1.7	0.15	106.8
Dark Control (1)	91.6	6.4	- [1]	6.4	4.8	0.02	102.8
Dark Control (2)	89.2	9.6	3.7	5.9	4.8	0.02	103.5
Day 11							
Irradiated (1)	95.6	6.8	- [1]	6.8	1.9	0.24	104.5
Irradiated (2)	96.6	5.8	- [1]	5.8	1.9	0.24	104.6
Dark Control (1)	93.1	7.4	- [1]	7.4	5.1	0.05	105.6
Dark Control (2)	93.6	5.1	- [1]	5.1	5.1	0.05	103.9
Day 20							
Irradiated (1)	87.6	10.1	9.4	0.7	2.3	0.34	100.4
Irradiated (2)	85.6	13.8	6.4	7.4	2.3	0.34	102.1
Dark Control (1)	84.8	11.8	5.8	6.0	5.8	0.08	102.5
Dark Control (2)	83.2	10.3	5.7	4.6	5.8	0.08	99.4

Table 7.1.1.3-19: Mass balance for [^{14}C]glyphosate in irradiated and dark control samples (expressed as percent of applied radioactivity)

Sample description/ Replicate	Extractable	Unextracted [^{14}C] in soil			Volatiles		Total[2]
		Original unextracted	Extracted with EDTA	Residual unextracted	NaOH	Ethylene glycol	
Day 31							
Irradiated (1)	91.5	11.5	10.4	1.1	4.0	0.5	107.4
Irradiated (2)	90.7	14.8	5.1	9.7	4.0	0.5	110.0
Dark Control (1)	87.5	10.5	6.5	4.0	6.6	0.09	104.7
Dark Control (2)	85.0	13.2	5.1	8.1	6.6	0.09	104.9

[1] Soil samples were not re-extracted as <9 %AR remained bound after extraction with 0.5N KOH
[2] There may be slight discrepancies due to rounding errors

Table 7.1.1.3-20: Characterisation of extractables following treatment with [^{14}C]glyphosate in irradiated and dark control samples (expressed as percent of applied radioactivity)

Sample description/ Replicate	Glyphosate	AMPA	Unknowns
Day 0			
Irradiated (1)	101.6	1.5	0.0
Irradiated (2)	104.8	1.6	0.1
Dark Control (1)	100.8	1.6	0.0
Dark Control (2)	91.7	1.4	0.0
Day 3			
Irradiated (1)	85.6	8.3	0.2
Irradiated (2)	88.3	8.4	0.3
Dark Control (1)	90.1	6.8	0.1
Dark Control (2)	89.9	6.2	0.1
Day 7			
Irradiated (1)	88.9	9.3	0.2
Irradiated (2)	85.6	9.5	0.2
Dark Control (1)	84.3	7.3	0.0
Dark Control (2)	81.9	7.2	0.1
Day 11			
Irradiated (1)	85.9	9.7	0.2
Irradiated (2)	85.9	10.7	0.2
Dark Control (1)	83.7	9.3	0.1
Dark Control (2)	85.3	8.3	0.1
Day 20			
Irradiated (1)	76.8	10.8	0.3
Irradiated (2)	75.1	10.5	0.3
Dark Control (1)	76.2	8.7	0.1
Dark Control (2)	74.7	8.5	0.1
Day 31			
Irradiated (1)	78.9	12.6	0.5
Irradiated (2)	77.2	13.3	0.7
Dark Control (1)	78.6	8.8	0.1
Dark Control (2)	74.3	10.7	0.1

B. MASS BALANCE

Radiocarbon recoveries averaged 105.1 % based on nominal applied radioactivity (% AR). Single values ranged from 96.3 to 111.2 % (% AR). Recoveries averaged 89.5 ± 4.0 % (average ± SD, n = 12) and 89.3 ± 5.9 % (average ± SD, n = 10) for light exposed and dark control samples, respectively.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from soil decreased from 0 DAT to 20 DAT from 103.1/106.5 to 87.6/85.6 % AR in irradiated soil and from 102.5/93.2 to 84.8/83.2 % AR in dark control soil followed by a slight increase at 31 DAT to 91.5/90.7 and 87.5/85.0 % in irradiated and dark control samples, respectively.

Non-extractable ^{14}C increased over the study period in both light exposed and dark control samples, from 0 DAT to 31 DAT from 1.9/3.6 to 11.5/14.8 % AR in irradiated soil and with similar amounts in dark control samples (from 2.8/3.2 at 0 DAT to 10.5/13.2 % AR at 31 DAT). Soil samples in which >9 % AR remained bound following extraction with 0.5N KOH were re-extracted with 0.03N Na_2EDTA. HPLC analysis of a representative light exposed extract indicated that the bound material was glyphosate and AMPA. Although the low [^{14}C] concentration and high Na_2EDTA concentration in the dark control extracts precluded HPLC analysis, it is highly probable that the extracted radiocarbon was likewise comprised of glyphosate and AMPA.

D. VOLATILE RADIOACTIVITY

Formation of $^{14}CO_2$ increased during the experimental period. Maximum amounts of carbon dioxide reached at study end (31 DAT) were 4.0 % AR in irradiated soil and 6.6 % AR in dark control. No radiocarbon was detected in the ethylene glycol traps at levels >0.5 % AR. $^{14}CO_2$ evolved during the study was quantitated as sodium carbonate and its identity confirmed by precipitation with barium chloride.

E. TRANSFORMATION OF THE TEST ITEM

The major degradation product observed in both light and dark samples was the AMPA derivative of glyphosate. Formation of carbon dioxide was observed in both light exposed and dark control samples with slightly higher yields in the latter. AMPA appears to generally form in somewhat greater amounts in the light (31 DAT: maximum amounts of 12.6/13.3 % AR and 8.8/10.7 % AR in light exposed and dark control samples, respectively). However, the combined amounts of both degradation products (AMPA and CO_2) are essentially constant between irradiated and dark control samples. No other metabolites were detected above 0.7 % AR at any time.

F. KINETICS

New kinetic calculations based on more recent guidance will not be provided as this study is only delivering supplemental information. DT_{50} values for glyphosate, based upon a linear extrapolation to the first order model, were 90.2 days (R = 0.82) in sunlight and 96.3 days (R = 0.86) in the dark.

III. CONCLUSIONS

The photodegradation of [^{14}C]glyphosate on soil surfaces to its AMPA derivative is not a photochemically accelerated process. A significant difference in degradation rates for light exposed and dark control samples was not obtained.

The results of this study support that the degradation of glyphosate to AMPA on soil is microbially induced.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The photodegradation of [^{14}C]glyphosate on soil surfaces was examined at an application rate of 4.48 kg/ha soil under natural sunlight. Mass balances ranged from 96.3 to 111.2 % of applied radioactivity (% AR).

As natural sunlight was used for the experiment instead of preferred artificial light, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.3/005
Report author	[redacted]
Report year	1983
Report title	The photodegradation of SC-0224 applied to soil
Report No	PMS-137
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - test duration not clear - samples exposed during day (192 hours) and frozen over night - tests were not conducted with an artificial irradiation source, but samples exposed to natural sunlight
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

Thin layers of Felton loamy sand soil (organic carbon 1.5 %, pH 5.4) were treated with a solution of unlabbelled SC-0224 (trimethylsulfonium carboxymethylaminomethylphosphonate) at a rate of 30 mg/kg soil and subsequently illuminated with sunlight for a net illumination time of 192 h to determine photodecomposition rates.

Petri plates containing the soil supplemented with SC-0224 were placed in an outdoor area during daytime with dark control samples loosely wrapped in aluminium foil. Each evening, all samples were covered and frozen until the following exposure day.

Quadruplicate samples were collected after 0, 6, 12, 18, 36, 48, 96, 144 and 192 hours of incubation, i.e. the time where samples were frozen during night-time was excluded.

Two duplicate samples per sampling events were used for analytical determination of the trimethylsulfonium (TMS) cation; residues of glyphosate (carboxymethylaminomethylphosphonate anion, CMAMP) and AMPA were analysed in the other two samples. Photodegradation of glyphosate was biphasic: a fast phase lasting 60 h where 34 % of the applied anion was lost; and a slow phase lasting to the end of the study (192 h, total), resulting in another 6 % loss of the anion. No photodegradation of trimesium occurred, although there was an instantaneous loss of about 35 % of the total trimesium applied to the soil, probably due to chemical hydrolysis. Subsequently, trimesium recovery, with two unduplicated exceptions, ranged between 55 and 69 %.

One major metabolite was detected in illuminated samples. AMPA is photolysed from glyphosate with about 15 % loss of total recovery after 192 h illumination. The amount of AMPA increased during the study period towards a maximum amount of 24.3 % (molar basis) at study end (192 h). There was no corresponding formation of AMPA in the dark controls.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Analytical SC-0224, trimethylsulfonium carboxymethylaminomethylphosphonate, consisting of 57.04 % SC-0224 and 40.4 % water
Lot No.: WRC-7466-14-01
Chemical purity: 95.7 % on an anhydrous basis

2. Soil:

A Felton loam sand soil was selected as test soil and sieved to 500 µ (0.5 mm). Characteristics of the test soil are presented in the table below.

Table 7.1.1.3-21: Characteristics of test soil

Parameter	Results
Soil	Felton
Country	Not indicated
Textural Class	Loamy Sand
Sand (1.0 – 2.0 mm µm – 2 mm) (%)	0.1
Course sand (0.5 – 1.0 mm)	16.0
Medium fine and very fine sand (0.05 – 0.5 mm)	72.0
Silt (0.002 mm – 0.05 mm) (%)	6.8
Clay (< 0.002 mm) (%)	5.1
pH [2]	5.4
Organic carbon (%) [1]	1.5
Organic matter (%)	2.6
Cation exchange capacity (meq/100 g)	10.9

[1] Calculated from organic matter according to OC = OM x 0.58
[2] Medium not stated

B. STUDY DESIGN

1. Experimental conditions

5 g of the selected soil was weighed into 9 cm (diameter) pyrex petri plates. The addition of 3.0 - 3.5 mL deionized water helped to spread the soil into a thin, even layer in each plate. The soil in uncovered plates dried overnight. Due to its sandy nature, the dry soil did not adhere well to the plate and was thus dampened by spraying it with a small amount of water. Subsequently, the soil was treated with 1 mL of the SC-0224 solution (non-labelled test item), sprayed on the soil holding a DVilbiss sprayer 5.1 - 7.6 cm above the plate. To ensure that the entire dose reached the soil, each application was rinsed through the sprayer with 0.5 mL water, also sprayer onto the soil.

The application solution of SC-0224 contained 2.61 x 10^{-2} g analytical SC-0224 (100 mL H_2O)$^{-1}$ and accordingly 1 mL of the solution sprayed on the surface of a thin layer of 5 g soil in a petri plate resulted in a concentration of 30 mg SCC-0224/kg soil.

Following treatment, all samples were covered with a box and allowed to partially dry overnight. Subsequently, the samples to be illuminated were set uncovered on a bench in an outdoor area exposed to sunlight. Dark controls were grouped according to total exposure time and each group was loosely wrapped in aluminium foil. Temperature and light meter readings were taken throughout the day; thermometers were located both inside and outside the soil. Each evening all samples were covered and frozen (- 20 °C) until

the following exposure day. Dark controls were handled expediently to minimize exposure to light. No temperature and moisture control was taking place, the graphical temperature plot shows variances of 20 to 40 °C.

Soil in petri plates were incubated under outdoor conditions for 192 hours of exposure in the daytime.

2. Sampling
Quadruplicate samples were collected after 0, 6, 12, 18, 36, 48, 96, 144 and 192 hours of incubation, i.e. the time where samples were frozen during night-time was excluded. Sampling of quadruplicates allowed the separate analysis of glyphosate anion and trimesium (TMS) cation, each in duplicates. Following treatment, samples from 0 hours were immediately frozen until analysis.

3. Analytical procedures
A separate extraction and analysis of glyphosate anion and trimesium cation was conducted.

The anion was extracted with 0.5 M NH_4OH, filtered, concentrated to dryness and derivatized with 9-fluorenylmethyl chloroformate. Samples were analysed using HPLC with a strong anion exchange column and a variable wavelength fluorescence detector. The solvent system was 0.02 M borate buffer (pH 9.0, flow rate 2 mL min^{-1}). This analysis procedure detected both glyphosate and its photolyte, aminomethylphosphonate (AMPA). The theoretical maximum concentration of AMPA that could form (13.6 ppm (13.6 mg/L)) was used to calculate the percent of AMPA found. Samples were not analysed for other photolytes. The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC method were 0.01 and 0.1 mg/L, respectively.

The cation was extracted from the soil with water and treated with 20 % sucrose and 3.6 % aqueous NaOH at 100 °C to dealkylace the trimesium to form dimethylsulfide (DMS). The DMS was trapped in toluene and analysed by gas chromatography and detected by flame photometry in a sulfur-specific mode. The LOD was approximately 0.2 mg/L.

II. RESULTS AND DISCUSSION

A. DATA
The distribution of glyphosate and its metabolite AMPA as well as the DMS cation in soil extracts is summarised in Table 7.1.1.3-22.

Table 7.1.1.3-22: Characterisation of extractables following treatment with SC-0224 in illuminated and dark control samples (expressed as % of nominal amount of glyphosate applied, all values are means of two replicates if not indicated otherwise)

Illumination length (h)	Glyphosate		AMPA		Glyphosate + AMPA (Illuminated)	TMS	
	Illuminated	Dark	Illuminated	Dark		Illuminated	Dark
0	98.8	93.2	4.8	<0.7 [1,2]	103.6	69.9	64.0
6	92.8	93.2	4.1	1.5	96.9	63.5	61.2
12	92.0	93.2 [1]	5.2	<0.7 [1,2]	97.2	67.2	65.1
18	83.1	85.3	6.3	<0.7 [1,2]	89.4	66.7	62.4
36	74.9	91.6	9.6	<0.7 [1,2]	84.5	64.5	69.4
48	75.9	91.1	12.5	<0.7 [1,2]	88.4	62.9	54.8
96	64.3	87.0 [1]	16.2 [1]	8.1 [1]	80.5	77.4 [1]	82.8 [1]
144	62.6	89.4	19.9 [1]	<0.7 [1,2]	82.5	50.5 [1]	47.3 [1]
192	59.7	84.1	24.3 [1]	<0.7 [1,2]	84.0	63.5	56.5

[1] Only one of the duplicates was analysed
[2] AMPA concentration was less than the LOQ of 0.1 mg/L (i.e. 0.7 % of the total possible theoretical concentration)

B. DEGRADATION OF THE TEST ITEM

In illuminated samples, the sum of the % glyphosate anion and % AMPA constituted recoveries between 80.3 to 103.6 % of the nominally applied glyphosate (mean of two replicates). Material balance cannot be determined. Over the course of the study, glyphosate decreased from initially 98.8 % of nominally applied to 59.7 % after 192 h of irradiation. The amount of AMPA (molar base, relative to glyphosate nominally applied) increased during the study period towards a maximum amount of 24.3 % at study end (192 h) in irradiated samples. Recovery of glyphosate in dark control samples declined gradually throughout the experiment, from 93.2 % to 84.1 % of the glyphosate applied. There was no corresponding formation of AMPA in the dark controls.

Trimesium recovery generally was between 55 and 69 % except for two samples (96 h and 144 h) in both the illuminated and dark control samples where duplicates were not analysed. Throughout the study, the resulting recoveries in dark controls and illuminated samples parallel each other with no apparent overall decrease in concentration. This indicates that no photodegradation of TMS occurred throughout the study period. Recovery of trimesium from 0 DAT samples was between 64 and 70 % for dark control and illuminated samples, respectively, and recovery of trimesium from the soil fortified just prior to analysis resulted in recoveries between 62.5 – 72 % of that added. Apparently an instantaneous, chemical breakdown of trimesium occurs in soil, described also in other experiments in soil and aquatic/sediment systems. In summary, approximately 35 % of trimesium was lost instantly from both illuminated and dark control samples. Subsequently, recovery was stable indicating no photodegradation on trimesium.

C. KINETICS

New kinetic calculations based on more recent guidance will not be provided as this study is not considered valid to describe the photolytic behaviour of glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The photodegradation of unlabelled glyphosate (applied as analytical trimethylsulfonium carboxymethylaminomethylphosphonate SC-0224) on soil surfaces was examined at a concentration of 300 mg SC-0224/kg soil. The duration of the study was 192 hours, discontinued each evening when all samples were covered and frozen until the following exposure day.
Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.3/006
Report author	[redacted]
Report year	1978
Report title	Photodegradation and anaerobic aquatic metabolism of Glyphosate, N-Phosphono-Methylglycine
Report No	MSL-0598
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - study duration of 3 days - temperature on soil surface 54°C - important basic data not available (e.g. LOD/LOQ, amount of soil used)
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The photodegradation of [^{14}C]glyphosate ([^{14}C]PMG) applied to the surface of one soil (organic carbon 0.7 %, pH 8.1) was investigated under simulated sunlight for 72 hours of irradiation.

Sections (8 x 8 cm) of TLC plates covered with thin layers of soil were treated with 717 µg of a mixture of 5 µg of [^{14}C]glyphosate and 712 µg of unlabelled glyphosate which is equivalent to 4.5 kg/ha (4 lbs/A). Dark control samples were covered with aluminium foil.

The prepared sections were exposed to artificial sunlight for 0, 24 and 72 hours.

The total recovery of ^{14}C-activity extracted from soils was 100.2, 102.7, 102.7 and 105.7 % for the zero time control, the 72 h dark control, the 24 h irradiated soil and the 72 h exposed soil, respectively.

The degradation of [^{14}C]glyphosate in soil extracts was relatively slow, with 4.6/6.6 % degradation after 24 hours and 5.2/8.3 % degradation after 72 hours of irradiation as determined by TLC/HPLC analysis, respectively. Degradation of glyphosate was lower in dark control samples, with 2.2 and 2.3 % degradation according to TLC and HPLC results, respectively.

Besides photolysis on soil surfaces, the study examined also the anaerobic aquatic metabolism and the photodegradation of glyphosate in natural water. However, this summary only refers to the photodegradation of glyphosate on soil surfaces.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate (PMG)
Lot No.: not indicated
Specific activity: 10.12 mC/mM
Radiochemical purity: 98 – 99 % (TLC)

2. Soil:

Soils were sieved to ca. 0.6 mm. Characteristics of the test soils are presented in the table below.

Table 7.1.1.3-23: Characteristics of test soil

Parameter	Results
Soil	Ray silt loam
Country	not indicated
Textural Class	Silt loam
Sand (%)	4.6
Silt (%)	84.2
Clay (%)	10.0
pH [2]	8.1
Organic carbon (%) [1]	0.7
Organic matter (%)	1.2

[1] Calculated from organic matter according to OC = OM x 0.58
[2] Medium not stated

B. STUDY DESIGN

1. Experimental conditions

Sieved soil was slurried with water to prepare test plates (20 x 20 cm). The plates were divided into four sections and trimmed so that each section was 8 x 8 cm. The plates were exposed to a 275-watt GE sunlamp for 72 hours in order to eliminate microbial degradation of glyphosate.

Each section of the plate was treated with 717 µg of a mixture of 5 µg of [^{14}C]glyphosate and 712 µg of unlabelled glyphosate. This treatment is equivalent to 4.5 kg/ha (4 lb/acre). Following treatment, sections were exposed to artificial sunlight. An additional section was treated, covered with aluminium foil and placed under the sunlamp to serve as control.

The lamp was placed 15 cm above the soil surface so that the greatest intensity of light, 1500 watts/m-2, as determined by a Radiometer, was at the centre of the plate and the intensity of the plate at the extreme corners was equal.

2. Sampling

The prepared sections were exposed to artificial sunlight for 0, 24 and 72 hours.

3. Analytical procedures

After the appropriate exposure period, the soil from the exposed and control sections was scraped from the plate and extracted two times with 0.5 N NH_4OH. Radioactivity was quantified by LSC and degradation of glyphosate analysed by HPLC and TLC. One section of the plate was treated and extracted immediately to determine the recovery of ^{14}C-activity at zero time.

The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC/TLC//LSC were not reported.

II. RESULTS AND DISCUSSION

A. DATA

Data on the degradation of [^{14}C]glyphosate in soil extracts are summaries in Table 7.1.1.3-24.

Table 7.1.1.3-24: Degradation over time following treatment with [^{14}C]glyphosate in light exposed and dark control samples (expressed as % of initial amount)

Exposure	TLC	HPLC
Dark control (72 h)	2.2	2.3
Irradiated (24 h)	4.6	6.6
Irradiated for (72 h)	5.2	8.3

B. MASS BALANCE

The total recovery of ^{14}C-activity extracted from soils was 100.2, 102.7, 102.7 and 105.7 % for the zero time control, the 72 h control, the 24 h irradiated soil and the 72 h exposed soil, respectively. In view of these recoveries it is evident that there was no loss of ^{14}C-activity indicating that glyphosate is not volatilized from the dry soil surface.

C. EXTRACTABLE RESIDUES

The degradation of [^{14}C]glyphosate in soil extracts was relatively slow, with 4.6/6.6 % degradation after 24 hours and 5.2/8.3 % degradation after 72 hours of irradiation determined by TLC/HPLC analyses, respectively. Degradation of glyphosate was lower in dark control samples, with 2.2 and 2.3 % degradation based on TLC and HPLC results, respectively.

E. KINETICS

In view of the low number of data points, a kinetic assessment is not feasible.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The photodegradation of [^{14}C]glyphosate on soil surfaces was examined under simulated sunlight at a rate of 4.5 kg/ha soil. The study duration was only 3 days. The temperature on the soil surface was 54 °C. Mass balances ranged from 100.2 to 105.7 % AR. Limited information on the soil given in the study report.

Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.1.3/007
Report author	[redacted]
Report year	1972
Report title	MON-0573, Residue and metabolism. Part 2: The photolysis, run-off, and leaching of MON-0573 on or in soil
Report No	258
Document No	
Guidelines followed in study	US PR Notice 70-15 (1970)
Deviations from current test guideline	From SETAC 1995 – Procedures for assessing the environmental fate and ecotoxicity of pesticides: - study duration of 48 hours - temperature on soil surface 30-31°C - application rate unclear - important basic data not available (e.g. amount of soil used, light intensity). - tests were conducted with an artificial irradiation source, but samples exposed to UV light of 100-380 nm range.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The photodegradation of [^{14}C]MON-0573 (glyphosate) on soil surfaces was examined in three soils (organic carbon 0.6 – 3.5 %; pH 5.7 – 7.0) using an artificial light source for 48 hours.

TLC plates were covered with three soil layers of 2 cm width and 0.75 mm thickness. Each of the bands was spotted with 10 µL of the stock solution, containing each 1,050,000 dpm corresponding to 10 µg [^{14}C]glyphosate. Separate sheets of aluminium foil were used to cover the left and middle 2 cm bands of glyphosate on the three soil TLC´s. Plates were placed under a black light fluorescent fixture so that the exposed 2 cm bands were ca. 2.5 cm directly below one of the fluorescent tubes. The black light utilised was a 91 cm fluorescent fixture equipped with three 40 watt GE-F4OBL fluorescent tubes.

A recording thermometer with a probe next to the soil TLC´s indicated the temperature ranged from 30 to 31 °C during UV exposure.

The 2 cm band of glyphosate were exposed to UV light for 0, 24 and 48 hours. Plates were developed twice with water and evaluated after each development.

No significant degradation products were detected that were moved from the origin after two developments of the soil TLC´s with water.

Besides photolysis on soil surfaces, the study examined also run-off from and leaching of glyphosate through soil. However, this summary only refers to the photodegradation of glyphosate on soil surfaces.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]MON-0573
Lot No.: not indicated
Specific activity: 8.06 mc/mMol (analysis prior to experimental start)
Radiochemical purity: 96.0 % (analysis prior to experimental start)
Chemical purity: not indicated

2. Soil:

All soils were sieved to ca. 0.6 mm. Characteristics of the test soils are presented in the table below.

Table 7.1.1.3-25: Characteristics of test soils

Parameter	Results		
Soil	Ray	Drummer	Norfolk
Country			
Textural Class	Silt loam	Silty clay loam	Sandy loam
Sand (%)	6.0	2.0	86.0
Silt (%)	83.2	55.4	11.0
Clay (%)	9.6	36.8	2.3
pH [2]	6.5	7.0	5.7
Organic carbon (%) [1]	0.6	3.5	0.6
Organic matter (%)	1.0	6.0	1.0

[1] Calculated from organic matter according to OC = OM x 0.58
[2] Buffer medium not indicated

B. STUDY DESIGN

1. Experimental conditions

Sieved soil (400, 300 and 300 g for soils Norfolk, Ray and Drummer, respectively) and water (107, 135 and 176 mL for soils Norfolk, Ray and Drummer, respectively) were mixed and formed a slurry on TLC plates of 0.75 mm thickness. Following spreading onto TLC plates by using a Shandon spreader, the soil slurry was allowed to dry overnight.

A stock solution was prepared by dissolving 46.75 mg of [^{14}C]glyphosate in 46.75 mL of 0.1 M NH_4HCO_3. Each of the three 2 cm bands located on the origin (3 cm from the bottom) was spotted with 10 µL of the stock solution, containing each 1,050,000 dpm corresponding to 10 µg [^{14}C]glyphosate. Separate sheets of aluminium foil were used to cover the left and middle 2 cm bands of glyphosate on the three soil TLC´s.

All three plates were then placed under a black light fluorescent fixture so that the exposed 2 cm bands were ca. 2.5 cm directly below one of the fluorescent tubes. The black light utilised was a 91 cm fluorescent fixture equipped with three 40 watt GE-F4OBL fluorescent tubes. After a 24 hours exposure to UV light, the aluminium foil was removed from the middle band and an additional 24 hours UV exposure was carried out. As a result, the three 2 cm bands were exposed to UV light for 0, 24 and 48 hours, respectively.

A recording thermometer with a probe next to the soil TLC´s indicated the temperature ranged from 30 – 31 °C during UV exposure.

2. Sampling

The 2 cm band of soil treated with glyphosate were exposed to UV light for 0, 24 and 48 hours.

3. Analytical procedures
Following development of soil TLC plates with water in a horizontal chromatography chamber, the soil TLC´s were allowed to dry horizontally overnight before evaluation with a Beta Camera. After evaluation of the first development, the soil TLC´s were developed with water a second time as before.

II. RESULTS AND DISCUSSION

A. TRANSFORMATION OF THE TEST ITEM
After UV exposure of [^{14}C]-glyphosate on soil for 48 hours there were no significant degradation products that were moved from the origin after two developments of the soil TLC´s with water.

B. KINETICS
In view of the low number of data points, a kinetic assessment is not feasible.

III. CONCLUSIONS
Irradiation of three soil TLC plates for 48 hours failed to give any soil mobile decomposition products. Photolysis is not considered to be a major cause of breakdown of glyphosate on soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The photodegradation of [^{14}C]-glyphosate on soil surfaces was examined using an artificial light source according to a pertinent guideline at the time of conduct. In view of current guidelines there are several deviations. The application rate is not clear. The study duration was only 48 hours. The temperature on the soil surface ranged from 30 to 31 °C. Limited information on the soil, preparation of soil layers, amount of soil used, light intensity and application procedure given in the study report.
Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

CA 7.1.2 Rate of Degradation in Soil

The rate of degradation of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in soil under aerobic conditions was investigated in various studies under laboratory and field conditions.

The results of the studies were kinetically evaluated according to the current EU guidances (FOCUS, 2006, 2014) to derive degradation rates for glyphosate and AMPA for comparison with trigger values and as endpoints for input in modelling.

For glyphosate, the DT_{50}-values derived for comparison with EU triggers (persistence endpoints) under laboratory conditions at 20 to 25 °C range from 0.6 to 60.2 days (Table 7.1.2-1). For the DT_{90} the range is from 9.7 to >1000 days. At lower temperatures (8 – 10 °C) degradation was lower compared to tests performed at 20 °C with the same soils.

Normalised (20°C, pF2) modelling endpoints in terms of the DT_{50} in soil range from 2.1 to 126.2 days (Table 7.1.2-1).

Under field conditions, the DT_{50}-values serving for comparison with trigger values range from 2.1 to 147 days for glyphosate. The corresponding DT_{90}–values range from 35.3 to >1000 days (Table 7.1.2-3). Normalised DT_{50}-values to serve as modelling endpoints range from 12.7 to 182 days (Table 7.1.2-4).

For metabolite AMPA, the trigger DT_{50} and DT_{90} of laboratory studies, range from 29.4 to 1040~~497~~ days and from 97.7 to 3450~~>1000~~ days, respectively (Table 7.1.2-2). Normalised modelling endpoints range from 14.2 to 1040~~497~~ days (Table 7.1.2-2).

Under field conditions, the trigger DT_{50} and DT_{90} for AMPA range from 65.0 to 634 days and from 216 to >1000 days, respectively (Table 7.1.2-6). Normalised modelling endpoints range from 90.7to 471 days (Table 7.1.2-7).

An evaluation based on the "EFSA $DegT_{50}$ Endpoint Selector" suggests that normalised DT_{50} values from laboratory and field studies do not differ significantly for glyphosate and AMPA (see Figure 7.1.2-1 and Figure 7.1.2-2). Consequently, for modelling purposes, DT_{50} values from laboratory and field studies were considered as one dataset for each, glyphosate and AMPA (see MCP Section 9).

Normalised DT_{50} values of glyphosate were assessed on pH dependency (Table 7.1.2-8 and Figure 7.1.2-3) and indicated a significant correlation for the laboratory studies. For the field studies, no such significance in correlation was observed while the correlation became significant for the combined data set of laboratory and field studies. Therefore, for modelling purposes, pH dependency of the DT_{50} was assumed for glyphosate (see MCP Section 9).

The assessment of pH dependency of the normalised DT_{50} of AMPA (see Table 7.1.2-9 and Figure 7.1.2-4) showed ~~did not show~~ a significant correlation for the laboratory studies, but showed no significant correlation for the field studies and the combined data set of laboratory and field studies. ~~Similar overall findings are expected following inclusion of the preliminary findings in Simmonds (2020, CA 7.1.2.1.2/002) once the study is finalised.~~

Table 7.1.2-1: Summary of aerobic degradation rates in soil for glyphosate - laboratory studies: trigger and modelling endpoints

Study	Soil type	pH ($CaCl_2$)	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	DT_{50} (d) 20 °C pF 2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted], 1993: Les Evouettes, CA 7.1.1.1/006	Silt loam	-	6.1[2]	20 / 40	9.7 / 184	6.5	DFOP	26.0[3]	6.5	DFOP
[redacted] 1995: Arrow, CA 7.1.1.1/005	Sandy loam	5.9	6.5[3]	20 / 40	37.8 / >1000	2.3	FOMC	126.2[4]	3.6	DFOP
[redacted] 1996: Soil B, CA 7.1.1.1/003	Sandy loam	-	6.7	25 / 75[6]	1.1 / 21.3	7.0	FOMC	6.9[3]	7.0	FOMC
[redacted] 1996: Speyer 2.1, CA 7.1.1.1/004	Sand	5.9	6.5[3]	20 / 45	8.3 / 51.3	2.5	DFOP	15.5[3]	2.5	DFOP
[redacted] 1996: Speyer 2.2 CA 7.1.1.1/004	Loamy sand	5.6	6.2[3]	20 / 45	18.1 / 162	5.9	DFOP	64.2[4]	5.9	DFOP
[redacted], 1996: Speyer 2.3, CA 7.1.1.1/004	Loamy sand	6.4	6.9[3]	20 / 45	2.7 / 13.0	7.5	DFOP	2.8	8.9	SFO
[redacted] 1996: Speyer 2.3 CA 7.1.1.1/004	*Loamy sand*	6.4	6.9[3]	*10 / 45*	*7.9 / 50.9*	*2.4*	*DFOP*	*5.9[3]*	*2.4*	*DFOP*
[redacted] 2010: Gartenacker, CA 7.1.1.1/001	Loam	-	7.1	20 / 50	8.1 / 55.4	3.1	DFOP	9.2[3]	3.1	DFOP
[redacted] 1992: Speyer 2.1, dose group A, CA 7.1.2.1.1/005	Sand	-	6.9	20 / 40	4.5 / 68.9	5.6	DFOP	20.8[3]	5.6	DFOP
[redacted] 1992: Speyer 2.1, dose group B, CA 7.1.2.1.1/005	*Sand*	-	*6.9*	*20 / 20*	*3.2 / 76.7*	*2.8*	*DFOP*	*15.2[4]*	*2.8*	*DFOP*
[redacted] 1992: Speyer 2.1, dose group C. CA 7.1.2.1.1/005	*Sand*	-	*6.9*	*8 / 40*	*41.6 / 201*	*2.3*	*HS*	*22.0[5]*	*2.3*	*HS*
[redacted] 1992: Speyer 2.1, dose group D (sterile), CA 7.1.2.1.1/005	*Sand*	-	*6.3*	*20 / 40*	*15.8 / 134*	*4.4*	*DFOP*	*50.6[5]*	*4.4*	*DFOP*

Table 7.1.2-1: Summary of aerobic degradation rates in soil for glyphosate - laboratory studies: trigger and modelling endpoints

Study	Soil type	pH ($CaCl_2$)	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	DT_{50} (d) 20 °C pF 2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] 1992: [redacted] 2.1, dose group E (lower dose rate), CA 7.1.2.1.1/005	Sand	-	6.9	20 / 40	7.0 / 73.2	6.2	DFOP	22.0[4]	6.2	DFOP
[redacted] 1992: Beedon manor, dose group F, CA 7.1.2.1.1/005	Clay loam	-	7.8	20 / 40	0.6 / 9.7	2.6	DFOP	2.6[4]	2.6	DFOP
[redacted], 1993: Speyer 2.1, CA 7.1.2.1.1/003	Sand	-	6.1[2]	20 / 40	10.8 / 84.0	3.3	DFOP	13.9[4]	3.3	DFOP
[redacted], 1993: Speyer 2.2, CA 7.1.2.1.1/003	Sand	-	6.0[2]	20 / 40	6.3 / 157	7.8	HS	47.0[5]	7.8	HS
[redacted], 1993: Speyer 2.3, CA 7.1.2.1.1/003	Loamy sand	-	6.9[2]	20 / 40	5.8 / 22.2	2.5	DFOP	3.5[4]	2.5	DFOP
[redacted] 2010: Drusenheim, CA 7.1.2.1.1/002	Loam	-	7.4	20 / 50	2.2 / 14.4	5.0	FOMC	2.1[4]	5.0	FOMC
[redacted] 2010: Pappelacker, CA 7.1.2.1.1/002	Loamy sand	-	7.0	20 / 50	3.6 / 37.6	5.5	DFOP	6.4[4]	5.5	DFOP
[redacted] 2010: 18-Acres, CA 7.1.2.1.1/002	Sandy clay loam	-	5.7	20 / 50	60.2 / >1000	2.0	FOMC	98.7[5]	2.9	DFOP
Geometric mean of soil Speyer 2.3 (n = 2; [redacted] 1996):								4.1		
Geometric mean of soil Speyer 2.1 (n = 4), [redacted] 1992):								19.8		
pH-dependence								Yes		

Italic font experiments were conducted under non-standard conditions, i.e. lower temperature, lower soil moisture or sterile soil. Trigger endpoints are not considered for further evaluation. For modelling endpoints, results from sterile soil were excluded from further assessment while results from experiments with lower temperature or soil moisture were normalised to standard conditions.

[1] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
[2] Medium not reported, H_2O assumed
[3] Calculated with German Input Decision Tool v. 3.3
[4] Calculated as DT_{50}/3.32 as 10 % of initially measured concentration reached within experimental period
[5] Calculated as ln(2)/k_2 as 10 % of initially measured concentration not reached within experimental period
[6] 75 % of water holding capacity at 1/3 bar

Table 7.1.2-2: Summary of aerobic degradation rates in soil for AMPA - laboratory studies: trigger and modelling endpoints

Study	Soil type	pH ($CaCl_2$)	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	ff	DT_{50} (d) 20 °C pF 2/10 kPa[1]	St. (χ^2)	Method of calc.
[redacted] 1993: Les Evouettes, CA 7.1.1.1/006	Silt loam	-	6.1[2]	20 / 40	424 / >1000	15.4	DFOP-SFO	0.346	199	15.4	DFOP-SFO
[redacted] 1995: Arrow, CA 7.1.1.1/005	Sandy loam	5.9	6.5	20 / 40	n.a.[5]	-	-	n.a.[3]	n.a.[3]	-	-
[redacted] 1996: Soil B, CA 7.1.1.1/003	Sandy loam	-	6.7	25 / 75[5]	99.4 / 330	8.9	FOMC-SFO	0.264	106	8.9	FOMC-SFO
[redacted], 1996: Speyer 2.1, CA 7.1.1.1/004	Sand	5.9	6.5	20 / 45	n.a.[5]	-	-	n.a.[3]	n.a.[3]	-	-
[redacted] 1996: Speyer 2.2 CA 7.1.1.1/004	Loamy sand	5.6	6.2	20 / 45	497 / >1000	8.8	DFOP-SFO	0.548	497	8.8	DFOP-SFO
[redacted] 1996: Speyer 2.3, CA 7.1.1.1/004	Loamy sand	6.4	6.9[3]	20 / 45	41.4 / 137	15.8	DFOP-SFO	0.424	43.1	18.2	SFO-SFO
[redacted] 1996: Speyer 2.3 CA 7.1.1.1/004	*Loamy sand*	6.4	6.9[3]	*10 / 45*	*129 / 429*	*8.2*	*DFOP-SFO*	*0.454*	*50.0*	*8.2*	*DFOP-SFO*
[redacted] 2010: Gartenacker, CA 7.1.1.1/001	Loam	-	7.1	20 / 50	119 / 396	8.2	DFOP-SFO	0.183	65.7	8.2	DFOP-SFO
[redacted] 1992: Speyer 2.1, dose group A, CA 7.1.2.1.1/005	Sand	-	6.9	20 / 40	n.a.[5]	-	-	n.a.[3]	n.a.[3]	-	-
[redacted] 1992: Speyer 2.1, dose group B, CA 7.1.2.1.1/005	*Sand*	-	*6.9*	*20 / 20*	*n.a.[5]*	-	-	*n.a.[3]*	*n.a.[3]*	-	-
[redacted] 1992: Speyer 2.1, dose group C. CA 7.1.2.1.1/005	*Sand*	-	*6.9*	*8 / 40*	*n.a.[5]*	-	-	*n.a.[3]*	*n.a.[3]*	-	-

Table 7.1.2-2: Summary of aerobic degradation rates in soil for AMPA - laboratory studies: trigger and modelling endpoints

Study	Soil type	pH ($CaCl_2$)	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	ff	DT_{50} (d) 20 °C pF 2/10 kPa[1]	St. (χ^2)	Method of calc.
[redacted] *1992: Speyer 2.1, dose group D (sterile), CA 7.1.2.1.1/005*	*Sand*	-	*6.3*	*20 / 40*	*n.a.*[5]	-	-	*n.a.*[3]	*n.a.*[3]	-	-
[redacted] 1992: Speyer 2.1, dose group E (lower dose rate), CA 7.1.2.1.1/005	Sand	-	6.9	20 / 40	283 / 940	6.4	DFOP-SFO	0.393	283	6.4	DFOP-SFO
[redacted] 1992: Beedon manor, dose group F, CA 7.1.2.1.1/005	Clay loam	-	7.8	20 / 40	67.3 / 224	16.4	DFOP-SFO	0.149	59.0	16.4	DFOP-SFO
[redacted] 1993: Speyer 2.1, CA 7.1.2.1.1/003	Sand	-	6.1[2]	20 / 40	86.5 / 288	13.7	DFOP-SFO	0.687	47.7	13.7	DFOP-SFO
[redacted] 1993: Speyer 2.2, CA 7.1.2.1.1/003	Sand	-	6.0[2]	20 / 40	110 / 365	8.9	HS-SFO	0.683	76.0	8.9	HS-SFO
[redacted] 1993: Speyer 2.3, CA 7.1.2.1.1/003	Loamy sand	-	6.9[2]	20 / 40	85.0 / 282	8.8	DFOP-SFO	0.336	44.8	8.8	DFOP-SFO
[redacted] 2010: Drusenheim, CA 7.1.2.1.1/002	Loam	-	7.4	20 / 50	29.4 / 97.7	3.8	FOMC-SFO	0.285	14.2	3.8	FOMC-SFO
[redacted] 2010: Pappelacker, CA 7.1.2.1.1/002	Loamy sand	-	7.0	20 / 50	90.9 / 302	6.2	DFOP-SFO	0.192	51.4	6.2	DFOP-SFO
[redacted] 2010: 18-Acres, CA 7.1.2.1.1/002	Sandy clay loam	-	5.7	20 / 50	n.a.[5]	-	-	n.a.[3]	n.a.[3]	-	-
[redacted] 2020: 18-Acres, CA 7.1.2.1.2/002	Sandy clay loam	5.3	5.5	20 / pF 2	1040 / 3450	3 04	SFO[4]	-	1040	3.04	SFO[4]
[redacted] 2020: Brierlow, CA 7.1.2.1.2/002	Silt loam	5.4	5.7	20 / pF 2	1000 / 1000[6]	-	SFO[4,6]	-	1000[6]	-	SFO[4,6]

Table 7.1.2-2: Summary of aerobic degradation rates in soil for AMPA - laboratory studies: trigger and modelling endpoints

Study	Soil type	pH ($CaCl_2$)	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	ff	DT_{50} (d) 20 °C pF 2/10 kPa[1]	St. (χ^2)	Method of calc.
[redacted] 2017: Warsop, CA 7.1.2.1.2/003	Loamy sand	3.9	4.6	20 / 45	326 / >1000	1.25	SFO[4]	-	326	1.25	SFO[4]
Arithmetic mean of soil Speyer 2.3 (n = 2, [redacted] 1996):								0.439	-		
Geometric mean of soil Speyer 2.3 (n = 2, [redacted] 1996):								-	46.4		
pH dependence									No		

Italic font experiments were conducted under non-standard conditions, i.e. lower temperature, lower soil moisture or sterile soil. Trigger endpoints are not considered for further evaluation. For modelling endpoints, results from sterile soil were excluded from further assessment while results from experiments with lower temperature or soil moisture were normalised to standard conditions.

[1] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7

[2] Medium not reported, H_2O assumed

[3] No reliable endpoints were derived for AMPA as there was no real decline phase visible until test termination

[4] Metabolite applied

[5] 75 % of water holding capacity at 1/3 bar

[6] Default value as no reliable endpoints were derived as no significant degradation was observed

Table 7.1.2-3: Summary of aerobic degradation rates in soil for glyphosate - field studies: trigger endpoints

Study	Soil type (USDA)	Location (country or USA state)	pH (KCl)	pH (H_2O)	Depth (cm)	DT_{50} (d) actual	DT_{90}(d) actual	St. (χ^2)	Method of calculation
[redacted] 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany		6.4[1]	0 - 30	40.7	187	6.6	DFOP
[redacted] 1992, CA 7.1.2.2.1/013	Sandy loam (bare soil)	Klein-Zecher, Germany	-	7.0[1]	0 - 30	29.1	364	12.7	DFOP
[redacted] 1992, CA 7.1.2.2.1/013	Loam (bare soil)	Unzhurst, Germany	-	6.7[1]	0 - 30	27.0	126	8.5	DFOP
[redacted] 1992, CA 7.1.2.2.1/013	Silt loam (bare soil)	Rohrbach, Germany	-	8.5[1]	0 - 30	24.4	81.0	16.0	SFO
[redacted] 1992, CA 7.1.2.2.1/013	Clay loam (bare soil)	Herrngiersdorf, Germany	-	8.0[1]	0 - 30	33.7	112	10.6	SFO
[redacted], 1992, CA 7.1.2.2.1/013	Silt loam (bare soil)	Wang-Inzkofen, Germany	-	7.2[1]	0 - 30	15.8	180	9.2	FOMC
[redacted] 1992 CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1	7.5[2]	0 - 30	6.1	118	5.0	DFOP
[redacted], 1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33	7.8[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0	6.6[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73	5.6[2]	0 - 30	5.8	201	9.4	DFOP
[redacted] 1992, CA 7.1.2.2.1/014	Silty clay (bare soil)	Ontario, Canada	-	7.9[1]	0 - 30	2.4	49.2	15.9	FOMC
[redacted] 1993, CA 7.1.2.2.1/006	Clay loam (bare soil)	Arizona, USA	-	8.0[1]	0 - 121.9	-[3]	-[3]	-	-

Table 7.1.2-3: Summary of aerobic degradation rates in soil for glyphosate - field studies: trigger endpoints

Study	Soil type (USDA)	Location (country or USA state)	pH (KCl)	pH (H_2O)	Depth (cm)	DT_{50} (d) actual	DT_{90}(d) actual	St. (χ^2)	Method of calculation
[redacted] 1993, CA 7.1.2.2.1/006	Loamy sand (bare soil)	California, USA	-	6.3[1]	0 - 121.9	13.5	101	12.7	FOMC
[redacted], 1993, CA 7.1.2.2.1/006	Silty clay loam (bare soil)	Iowa, USA	-	6.0[1]	0 - 121.9	147	>1000	14.6	FOMC
[redacted], 1993, CA 7.1.2.2.1/006	Loam (bare soil)	Minnesota, USA	-	6.5[1]	0 - 121.9	-[3]	-[3]	-	-
[redacted], 1993, CA 7.1.2.2.1/006	Sandy clay loam (bare soil)	New York, USA	-	5.8[1]	0 - 121.9	-[3]	-[3]	-	-
[redacted], 1993, CA 7.1.2.2.1/006	Loam (bare soil)	Ohio, USA	-	7.8[1]	0 - 121.9	2.1	62.8	13.5	DFOP
[redacted] 1993, CA 7.1.2.2.1/005	Loamy sand (bare soil)	Ontario, Canada	-	6.8[1]	0 - 45	9.5	53.9	16.4	DFOP
[redacted]1989, CA 7.1.2.2.1/016	Sandy loam (bare soil)	California, USA	-	7.1[1]	0 - 121.9	5.4	35.3	5.1	FOMC

[1] Medium not reported, H_2O assumed
[2] Calculated with German Input Decision Tool v. 3.3
[3] No reliable endpoint could be determined

Table 7.1.2-4: Summary of aerobic degradation rates in soil for glyphosate - field studies: modelling endpoints

Study	Soil type (USDA)	Location (country or USA state)	pH (KCl)	pH (H_2O)	Depth (cm)	DT_{50} (d) Norm[1]	St. (χ^2)	Method of calculation
[redacted]1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	-	6.4[2]	0 - 30	23.0	12.9	SFO
[redacted]1992, CA 7.1.2.2.1/013	Sandy loam (bare soil)	Klein-Zecher, Germany	-	7.0[2]	0 - 30	27.9	13.1	SFO
[redacted] 1992, CA 7.1.2.2.1/013	Loam (bare soil)	Unzhurst, Germany	-	6.7[2]	0 - 30	25.9	13.4	SFO
[redacted]1992, CA 7.1.2.2.1/013	Silt loam (bare soil)	Rohrbach, Germany	-	8.5[2]	0 - 30	12.7	1.9	SFO
[redacted]1992, CA 7.1.2.2.1/013	Clay loam (bare soil)	Herrngiersdorf, Germany	-	8.0[2]	0 - 30	21.5	11.4	SFO
[redacted]1992, CA 7.1.2.2.1/013	Silt loam (bare soil)	Wang-Inzkofen, Germany	-	7.2[2]	0 - 30	26.4	11.9	SFO

Table 7.1.2-4: Summary of aerobic degradation rates in soil for glyphosate - field studies: modelling endpoints

Study	Soil type (USDA)	Location (country or USA state)	pH (KCl)	pH (H_2O)	Depth (cm)	DT_{50} (d) Norm[1]	St. (χ^2)	Method of calculation
[redacted] 1992 CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1	7.5[3]	0 - 30	51.0[4]	6.8	HS
[redacted] 1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33	7.8[3]	0 - 30	-[5]	-	-
[redacted] 1992, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0	6.6[3]	0 - 30	-[5]	-	-
[redacted] 1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73	5.6[3]	0 - 30	46.0[4]	6.8	HS
[redacted] 1992, CA 7.1.2.2.1/014	Silty clay (bare soil)	Ontario, Canada	-	7.9[2]	0 - 30	-[5]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Clay loam (bare soil)	Arizona, USA	-	8.0[2]	0 - 121.9	-[5]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Loamy sand (bare soil)	California, USA	-	6.3[2]	0 - 121.9	32.6	22.0	SFO
[redacted] 1993, CA 7.1.2.2.1/006	Silty clay loam (bare soil)	Iowa, USA	-	6.0[2]	0 - 121.9	182	15.9	SFO
[redacted] 1993, CA 7.1.2.2.1/006	Loam (bare soil)	Minnesota, USA	-	6.5[2]	0 - 121.9	-[5]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Sandy clay loam (bare soil)	New York, USA	-	5.8[2]	0 - 121.9	-[5]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Loam (bare soil)	Ohio, USA	-	7.8[2]	0 - 121.9	-[5]	-	-
[redacted] 1993, CA 7.1.2.2.1/005	Loamy sand (bare soil)	Ontario, Canada	-	6.8[2]	0 - 45	-[5]	-	-
[redacted] 1989, CA 7.1.2.2.1/016	Sandy loam (bare soil)	California, USA	-	7.1[2]	0 - 121.9	-[5]	-	-
pH dependence						No		

[1] $DegT50_{matrix}$ according to EFSA (2014) and FOCUS (2006, 2014)
[2] Medium not reported, H_2O assumed
[3] Calculated with German Input Decision Tool v. 3.3
[4] Calculated from the slow phase: $\ln(2)/k_2$
[5] No reliable endpoint could be determined

Table 7.1.2-5: Summary of maximum occurrence of AMPA in field study trials – adapted relative to glyphosate residues recovered at day 0

Study	Soil type (USDA)	Location (country or USA state)	pH (H_2O)	AMPA (%)	DAT
[redacted] 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[2]	36.2	91
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[2]	41.5	201
	Loam (bare soil)	Unzhurst, Germany	6.7[2]	25.8	90
	Silt loam (bare soil)	Rohrbach, Germany	8.5[2]	41.3	56
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[2]	28.0	28
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[2]	43.8	29
[redacted] 1992, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[1]	27.5	194
[redacted], 1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[1]	37.9	62
[redacted] /010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[1]	26.3	61
[redacted] 1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[1]	48.8	271
[redacted] 1992, CA 7.1.2.2.1/014	Silty clay (bare soil)	Ontario, Canada	7.9[2]	28.4	14
[redacted], 1993, CA 7.1.2.2.1/006	Clay loam (bare soil)	Arizona, USA	8.0[2]	49.5	21
	Loamy sand (bare soil)	California, USA	6.3[2]	34.3	456
	Silty clay loam (bare soil)	Iowa, USA	6.0[2]	45.2	458
	Loam (bare soil)	Minnesota, USA	6.5[2]	**63.0**	95
	Sandy clay loam (bare soil)	New York, USA	5.8[2]	30.8	90
	Loam (bare soil)	Ohio, USA	7.8[2]	45.5	21
[redacted] 1993, CA 7.1.2.2.1/005	Loamy sand (bare soil)	Ontario, Canada	6.8[2]	32.7	86
[redacted] 1989, CA 7.1.2.2.1/016	Sandy loam (bare soil)	California, USA	7.1[2]	20.2	31

[1] Measured in KCl
[2] Method of pH determination not reported, H_2O assumed

Table 7.1.2-6: Summary of aerobic degradation rates in soil for AMPA - field studies: trigger endpoints

Study	Soil type (USDA)	Location (country or USA state)	pH (KCl)	pH (H_2O)	Depth (cm)	DT_{50} (d) actual	DT_{90} (d) actual	St. (χ^2)	Method of calculation
[redacted] 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	-	6.4[1]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/013	Sandy loam (bare soil)	Klein-Zecher, Germany	-	7.0[1]	0 - 30	521	>1000	13.9	DFOP-SFO
[redacted] 1992, CA 7.1.2.2.1/013	Loam (bare soil)	Unzhurst, Germany	-	6.7[1]	0 - 30	634	>1000	11.9	DFOP-SFO
[redacted] 1992, CA 7.1.2.2.1/013	Silt loam (bare soil)	Rohrbach, Germany	-	8.5[1]	0 - 30	255	847	15.5	SFO-SFO
[redacted] 1992, CA 7.1.2.2.1/013	Clay loam (bare soil)	Herrngiersdorf, Germany	-	8.0[1]	0 - 30	288	958	11.0	SFO[4]
[redacted] 1992, CA 7.1.2.2.1/013	Silt loam (bare soil)	Wang-Inzkofen, Germany	-	7.2[1]	0 - 30	273	907	15.8	FOMC-SFO
[redacted] 1992 CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1	7.5[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33	7.8[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0	6.6[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73	5.6[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/014	Silty clay (bare soil)	Ontario, Canada	-	7.9[1]	0 - 30	155	514	16.5	FOMC-SFO
[redacted] 1993, CA 7.1.2.2.1/006	Clay loam (bare soil)	Arizona, USA	-	8.0[1]	0 - 121.9	97.6	630	15.3	DFOP[4]
[redacted] 1993, CA 7.1.2.2.1/006	Loamy sand (bare soil)	California, USA	-	6.3[1]	0 - 121.9	-[3]	-[3]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Silty clay loam (bare soil)	Iowa, USA	-	6.0[1]	0 - 121.9	-[3]	-[3]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Loam (bare soil)	Minnesota, USA	-	6.5[1]	0 - 121.9	302	>1000	10.3	SFO[4]
[redacted] 1993, CA 7.1.2.2.1/006	Sandy clay loam (bare soil)	New York, USA	-	5.8[1]	0 - 121.9	-[3]	-[3]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Loam (bare soil)	Ohio, USA	-	7.8[1]	0 - 121.9	65.0	216	17.5	DFOP-SFO
[redacted] 1993, CA 7.1.2.2.1/005	Loamy sand (bare soil)	Ontario, Canada	-	6.8[1]	0 - 45	-[3]	-[3]	-	-
[redacted] 1989, CA 7.1.2.2.1/016	Sandy loam (bare soil)	California, USA	-	7.1[1]	0 - 121.9	111	370	15.4	FOMC-SFO

[1] Medium not reported, H_2O assumed
[2] Calculated with German Input Decision Tool v. 3.3
[3] No reliable endpoint could be determined
[4] Decline fit

Table 7.1.2-7: Summary of aerobic degradation rates in soil for AMPA - field studies: modelling endpoints

Study	Soil type (USDA)	Location (country or USA state)	pH (KCl)	pH (H_2O)	Depth (cm)	DT_{50} (d) Norm[1]	ff	St. (χ^2)	Method of calculation
[redacted]1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	-	6.4[2]	0 - 30	-[4]	-[4]	-	-
[redacted]1992, CA 7.1.2.2.1/013	Sandy loam (bare soil)	Klein-Zecher, Germany	-	7.0[2]	0 - 30	471	0.1984	9.2	SFO-SFO
[redacted]1992, CA 7.1.2.2.1/013	Loam (bare soil)	Unzhurst, Germany	-	6.7[2]	0 - 30	238	0.3192	8.9	SFO-SFO
[redacted]1992, CA 7.1.2.2.1/013	Silt loam (bare soil)	Rohrbach, Germany	-	8.5[2]	0 - 30	119	0.2399	1.2	SFO-SFO
[redacted]1992, CA 7.1.2.2.1/013	Clay loam (bare soil)	Herrngiersdorf, Germany	-	8.0[2]	0 - 30	90.7	0.2508	7.8	SFO-SFO
[redacted] 1992, CA 7.1.2.2.1/013	Silt loam (bare soil)	Wang-Inzkofen, Germany	-	7.2[2]	0 - 30	142	0.2308	7.2	SFO-SFO
[redacted]1992 CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1	7.5[3]	0 - 30	-[4]	-[4]	-	-
[redacted]1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33	7.8[3]	0 - 30	-[4]	-[4]	-	-
[redacted] 1992, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0	6.6[3]	0 - 30	-[4]	-[4]	-	-
[redacted]1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73	5.6[3]	0 - 30	-[4]	-[4]	-	-
[redacted] 1992, CA 7.1.2.2.1/014	Silty clay (bare soil)	Ontario, Canada	-	7.9[2]	0 - 30	-[4]	-[4]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Clay loam (bare soil)	Arizona, USA	-	8.0[2]	0 - 121.9	303	-	21.1	SFO[5]
[redacted]1993, CA 7.1.2.2.1/006	Loamy sand (bare soil)	California, USA	-	6.3[2]	0 - 121.9	-[4]	-[4]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Silty clay loam (bare soil)	Iowa, USA	-	6.0[2]	0 - 121.9	-[4]	-[4]	-	-
[redacted]1993, CA 7.1.2.2.1/006	Loam (bare soil)	Minnesota, USA	-	6.5[2]	0 - 121.9	-[4]	-[4]	-	-
[redacted]1993, CA 7.1.2.2.1/006	Sandy clay loam (bare soil)	New York, USA	-	5.8[2]	0 - 121.9	-[4]	-[4]	-	-
[redacted] 1993, CA 7.1.2.2.1/006	Loam (bare soil)	Ohio, USA	-	7.8[2]	0 - 121.9	-[4]	-[4]	-	-
[redacted] [redacted]1993, CA 7.1.2.2.1/005	Loamy sand (bare soil)	Ontario, Canada	-	6.8[2]	0 - 45	-[4]	-[4]	-	-
[redacted]1989, CA 7.1.2.2.1/016	Sandy loam (bare soil)	California, USA	-	7.1[2]	0 - 121.9	-[4]	-[4]	-	-
pH dependence						No			

[1] $DegT50_{matrix}$ according to EFSA (2014)
[2] Medium not reported, H_2O assumed
[3] Calculated with German Input Decision Tool v. 3.3
[4] No reliable endpoint could be determined
[5] Decline fit

Evaluation of modelling endpoints derived from laboratory and field degradation studies with "EFSA DegT50 Endpoint Selector"

Figure 7.1.2-1: EFSA DegT$_{50}$ Endpoint Selector for glyphosate

Calculations — Laboratory studies

Cont. No. i	DegT50 values A x_i	logarithmic DegT50 values D $l_i = \ln(x_i)$ =	deviation from mean μ G $d_i=(l_i-\mu_{lab})$ =	squared deviation from mean μ H d_i^2 =
1	x_1 = 26	l_1 = 3 258	d_1 = 0 630	d_1^2 = 0 3964
2	x_2 = 126 2	l_2 = 4 838	d_2 = 2 209	d_2^2 = 4 8815
3	x_3 = 6 9	l_3 = 1 932	d_3 = -0 697	d_3^2 = 0 4857
4	x_4 = 15 5	l_4 = 2 741	d_4 = 0 112	d_4^2 = 0 0126
5	x_5 = 64 2	l_5 = 4 162	d_5 = 1 534	d_5^2 = 2 3518
6	x_6 = 4 1	l_6 = 1 411	d_6 = -1 217	d_6^2 = 1 4822
7	x_7 = 9 2	l_7 = 2 219	d_7 = -0 409	d_7^2 = 0 1675
8	x_8 = 19 8	l_8 = 2 986	d_8 = 0 357	d_8^2 = 0 1276
9	x_9 = 2 6	l_9 = 0 956	d_9 = -1 673	d_9^2 = 2 7987
10	x_{10} = 13 9	l_{10} = 2 632	d_{10} = 0 003	d_{10}^2 = 0 0000
11	x_{11} = 47	l_{11} = 3 850	d_{11} = 1 222	d_{11}^2 = 1 4925
12	x_{12} = 3 5	l_{12} = 1 253	d_{12} = -1 376	d_{12}^2 = 1 8925
13	x_{13} = 2 1	l_{13} = 0 742	d_{13} = -1 887	d_{13}^2 = 3 5590
14	x_{14} = 6 4	l_{14} = 1 856	d_{14} = -0 772	d_{14}^2 = 0 5962
15	x_{15} = 98 7	l_{15} = 4 592	d_{15} = 1 964	d_{15}^2 = 3 8558

Number of studies	B N=	15		
Degrees of freedom	C df_{lab} = N-	14		
Sum over all studiess	E L= $\Sigma_i l_i$ =	39 427	I D^2= $\Sigma_i d_i^2$ =	24 1002
Mean of logarithmic values	F μ_{lab}= L/N =	2 628		
Variance of logarithmic values			J σ_{lab}^2= D^2/df_{lab} =	1 7214
Standard deviation of logarithmic values			σ_{lab}=	1 3120

Calculations — Field studies

Cont. No. j	DegT50 values K z_j	logarithmic DegT50 values N $k_j = \ln(x_j)$ =	deviation from mean μ Q $c_j=(l_j-\mu_{fld})$ =	squared deviation from mean μ R c_j^2 =
1	x_1 = 23 0	k_1 = 3 135	c_1 = -0 370	c_1^2 = 0 1365
2	x_2 = 27 9	k_2 = 3 329	c_2 = -0 176	c_2^2 = 0 0311
3	x_3 = 25 9	k_3 = 3 254	c_3 = -0 251	c_3^2 = 0 0629
4	x_4 = 12 7	k_4 = 2 542	c_4 = -0 963	c_4^2 = 0 9282
5	x_5 = 21 5	k_5 = 3 068	c_5 = -0 437	c_5^2 = 0 1909
6	x_6 = 26 4	k_6 = 3 273	c_6 = -0 232	c_6^2 = 0 0537
7	x_7 = 51	k_7 = 3 932	c_7 = 0 427	c_7^2 = 0 1822
8	x_8 = 46	k_8 = 3 829	c_8 = 0 324	c_8^2 = 0 1047
9	x_9 = 32 6	k_9 = 3 484	c_9 = -0 021	c_9^2 = 0 0004
10	x_{10} = 182	k_{10} = 5 204	c_{10} = 1 699	c_{10}^2 = 2 8866

Number of studies	L M=	10		
Degrees of freedom	M df_{fld} = M-	9		
Sum over all studiess	O K= $\Sigma_j k_j$ =	35 050	S C^2= $\Sigma_j c_j^2$ =	4 5772
Mean of logarithmic values	P μ_{fld}= K/M =	3 505		
Variance of logarithmic values			T σ_{fld}^2= C^2/df_{fld} =	0 5086
Standard deviation of logarithmic values			σ_{fld}=	0 7131

Comparison between laboratory and field studies

Sum of degrees of freedom			U **df** = df_{lab} + df_{fld} =	23
Sum of reciprocal sample sizes	V **h** = (1/N)+(1/M) =	0 1667		
Difference between means	W **A** = $\mu_{lab} - \mu_{fld}$ =	-0 877		
Sum of squared deviations			X **B** = $D^2 + C^2$ =	28 6774
Combined variance of logarithmic values			Y σ^2 = B/df =	1 2468
Standard deviation of the difference between the means			Z s = $\sqrt{h \cdot \sigma^2}$ =	0 4559
Statistic of Student's t-test			AA **t** = A/s =	-1 9229
Significance level of the test α (as given in the procedure)			AB **α** =	25%
Upper 1-α quantile of t-distribution with df degrees of freedom			AC $t_{df;1-\alpha}$ =	0 6853

AD Is Student's t-statistic **t** larger than the t-quantile $t_{df;1-\alpha}$?

	→ Test confirms that field studies show shorter DegT50 than laboratory studies	NO	→ Observations do not contradict the hypothesis that field studies show equal DegT50 than laboratory studies

Figure 7.1.2-2: EFSA $DegT_{50}$ Endpoint Selector for AMPA

Calculations	Laboratory studies							
	Cont. No. i	DegT50 values A x_i		logarithmic DegT50 values D $l_i = \ln(x_i)$=		deviation from mean μ G $d_i=(l_i-\mu_{lab})$=		squared deviation from mean μ H d_i^2=
	1	x_1=	199	l_1=	5.293	d_1=	0.810	d_1^2= 0.6554
	2	x_2=	106	l_2=	4.663	d_2=	0.180	d_2^2= 0.0323
	3	x_3=	497	l_3=	6.209	d_3=	1.725	d_3^2= 2.9752
	4	x_4=	46.4	l_4=	3.837	d_4=	-0.646	d_4^2= 0.4179
	5	x_5=	65.7	l_5=	4.185	d_5=	-0.299	d_5^2= 0.0892
	6	x_6=	283	l_6=	5.645	d_6=	1.162	d_6^2= 1.3496
	7	x_7=	59	l_7=	4.078	d_7=	-0.406	d_7^2= 0.1650
	8	x_8=	47.7	l_8=	3.865	d_8=	-0.619	d_8^2= 0.3829
	9	x_9	76	l_9=	4.331	d_9=	-0.153	d_9^2= 0.0234
	10	x_{10}=	44.8	l_{10}=	3.802	d_{10}=	-0.682	d_{10}^2= 0.4645
	11	x_{11}=	14.2	l_{11}=	2.653	d_{11}=	-1.830	d_{11}^2= 3.3507
	12	x_{12}=	51.4	l_{12}=	3.940	d_{12}=	-0.544	d_{12}^2= 0.2960
	13	x_{13}=	326	l_{13}=	5.787	d_{13}=	1.303	d_{13}^2= 1.6983
Number of studies		B N=	13					
Degrees of freedom		C df_{lab} = N-	12					
Sum over all studiess				E L= $\Sigma_i l_i$ =	58.288	I $D^2 = \Sigma_i d_i^2$ =		11.9002
Mean of logarithmic values				F μ_{lab}= L/N =	4.484			
Variance of logarithmic values						J σ_{lab}^2= D^2/df_{lab} =		0.9917
Standard deviation of logarithmic values						σ_{lab}=		0.9958

Calculations	Field studies							
	Cont. No. j	DegT50 values K z_j		logarithmic DegT50 values N $k_j = \ln(z_j)$=		deviation from mean μ Q $c_j=(l_j-\mu_{lab})$=		squared deviation from mean μ R c_j^2=
	1	x_1=	471	k_1=	6.155	c_1=	0.891	c_1^2= 0.7938
	2	x_2=	238	k_2=	5.472	c_2=	0.208	c_2^2= 0.0434
	3	x_3=	119	k_3=	4.779	c_3=	-0.485	c_3^2= 0.2350
	4	x_4=	90.7	k_4=	4.508	c_4=	-0.756	c_4^2= 0.5720
	5	x_5=	142	k_5=	4.956	c_5=	-0.308	c_5^2= 0.0949
	6	x_6=	303	k_6=	5.715	c_6=	0.450	c_6^2= 0.2024
Number of studies		L M=	6					
Degrees of freedom		M df_{fld} = M-	5					
Sum over all studiess				O K= $\Sigma_j k_j$ =	31.583	S $C^2 = \Sigma_j c_j^2$ =		1.9415
Mean of logarithmic values				P μ_{fld}= K/M =	5.264			
Variance of logarithmic values						T σ_{fld}^2= C^2/df_{fld} =		0.3883
Standard deviation of logarithmic values						σ_{fld}=		0.6231

Comparison between laboratory and field studies					
Sum of degrees of freedom			U df= df_{lab} + df_{fld} =		17
Sum of reciprocal sample sizes	V h = (1/N)+(1/M)=	0.2436			
Difference	W A = μ_{lab} - μ_{fld} =	-0.780			
Sum of squared deviations			X B = $D^2 + C^2$ =		13.8428
Combined variance of logarithmic values			Y σ^2 = B/df =		0.8142
Standard deviation of the difference between the means			Z s = $\sqrt{h \cdot \sigma^2}$ =		0.4453
Statistic of Student's t-test			AA t = A/s=		-1.7518
Significance level of the test α (as given in the procedure)			AB α =		25%
Upper 1−α quantile of t-distribution with df degrees of freedom			AC $t_{df;1-\alpha}$ =		0.6892
AD Is Student's t-statistic t larger than the t-quantile $t_{df;1-\alpha}$?					
	→ Test confirms that field studies show shorter DegT50 than laboratory studies	NO	→ Observations do not contradict the hypothesis that field studies show equal DegT50 than laboratory studies		

Calculations	Laboratory studies								
	Cont. No.	DegT50 values		logarithmic DegT50 values		deviation from mean μ		squared deviation from mean μ	
	i	A x_i		D $l_i = \ln(x_i)$=		G $d_i=(l_i-\mu_{lab})$=		H d_i^2=	
	1	x_1=	199	l_1=	5.293	d_1=	0.484	d_1^2=	0.2340
	2	x_2=	106	l_2=	4.663	d_2=	-0.146	d_2^2=	0.0213
	3	x_3=	497	l_3=	6.209	d_3=	1.399	d_3^2=	1.9578
	4	x_4=	46.4	l_4=	3.837	d_4=	-0.972	d_4^2=	0.9453
	5	x_5=	65.7	l_5=	4.185	d_5=	-0.624	d_5^2=	0.3899
	6	x_6=	283	l_6=	5.645	d_6=	0.836	d_6^2=	0.6987
	7	x_7=	59	l_7=	4.078	d_7=	-0.732	d_7^2=	0.5358
	8	x_8=	47.7	l_8=	3.865	d_8=	-0.945	d_8^2=	0.8923
	9	x_9=	76	l_9=	4.331	d_9=	-0.479	d_9^2=	0.2293
	10	x_{10}=	44.8	l_{10}=	3.802	d_{10}=	-1.007	d_{10}^2=	1.0147
	11	x_{11}=	14.2	l_{11}=	2.653	d_{11}=	-2.156	d_{11}^2=	4.6496
	12	x_{12}=	51.4	l_{12}=	3.940	d_{12}=	-0.870	d_{12}^2=	0.7567
	13	x_{13}=	326	l_{13}=	5.787	d_{13}=	0.977	d_{13}^2=	0.9552
	14	x_{14}=	1040	l_{14}=	6.947	d_{14}=	2.137	d_{14}^2=	4.5685
	15	x_{15}=	1000	l_{15}=	6.908	d_{15}=	2.098	d_{15}^2=	4.4025
Number of studies		B N=	15						
Degrees of freedom		C $df_{lab}=N-$	14						
Sum over all studies				E $L=\Sigma_i l_i$ =	82.143	I $D^2=\Sigma_i d_i^2$ =			22.2514
Mean of logarithmic values				F $\mu_{lab}=L/N$ =	4.610				
Variance of logarithmic values						J $\sigma_{lab}^2=D^2/df_{lab}$ =			1.5894
Standard deviation of logarithmic values								σ_{lab}=	1.2607

Calculations	Field studies								
	Cont. No.	DegT50 values		logarithmic DegT50 values		deviation from mean μ		squared deviation from mean μ	
	j	K z_j		N $k_j=\ln(z_j)$=		Q $c_j=(l_j-\mu_{dt})$=		R c_j^2=	
	1	x_1=	471	k_1=	6.155	c_1=	0.891	c_1^2=	0.7938
	2	x_2=	258	k_2=	5.472	c_2=	0.208	c_2^2=	0.0434
	3	x_3=	119	k_3=	4.779	c_3=	-0.485	c_3^2=	0.2350
	4	x_4=	90.7	k_4=	4.508	c_4=	-0.756	c_4^2=	0.5720
	5	x_5=	142	k_5=	4.956	c_5=	-0.308	c_5^2=	0.0949
	6	x_6=	305	k_6=	5.714	c_6=	0.450	c_6^2=	0.2024
Number of studies		L M=	6						
Degrees of freedom		M $df_{dt}=M-$	5						
Sum over all studies				O $K=\Sigma_j k_j$ =	31.583	S $C^2=\Sigma_j c_j^2$ =			1.9415
Mean of logarithmic values				P $\mu_{dt}=K/M$ =	5.264				
Variance of logarithmic values						T $\sigma_{dt}^2=C^2/df_{dt}$ =			0.3883
Standard deviation of logarithmic values								σ_{dt}=	0.6231

Comparison between laboratory and field studies					
Sum of degrees of freedom				U $df=df_{lab}+df_{dt}$ =	19
Sum of reciprocal sample sizes	V $h=(1/N)+(1/M)$ =	0.2333			
Difference	W $A=\mu_{lab}-\mu_{dt}$ =	-0.654			
Sum of squared deviations				X $B=D^2+C^2$ =	24.1930
Combined variance of logarithmic values				Y $\sigma^2=B/df$ =	1.2733
Standard deviation of the difference between the means				Z $s=\sqrt{h\cdot\sigma^2}$ =	0.5451
Statistic of Student's t-test				AA $t=A/s$=	-0.8330
Significance level of the test α (as given in the procedure)				AB α =	25%
Upper $2\cdot\alpha$ quantile of t-distribution with df degrees of freedom				AC $tdf_{1-\alpha}$ =	0.6876
AD Is Student's t-statistic t larger than the t-quantile $t_{df;1-\alpha}$?					
→ Test confirms that field studies show shorter DegT50 than laboratory studies		NO	→ Observations do not contradict the hypothesis that field studies show equal DegT50 than laboratory studies		

Assessment of pH dependency of normalised DT_{50} of glyphosate in soil

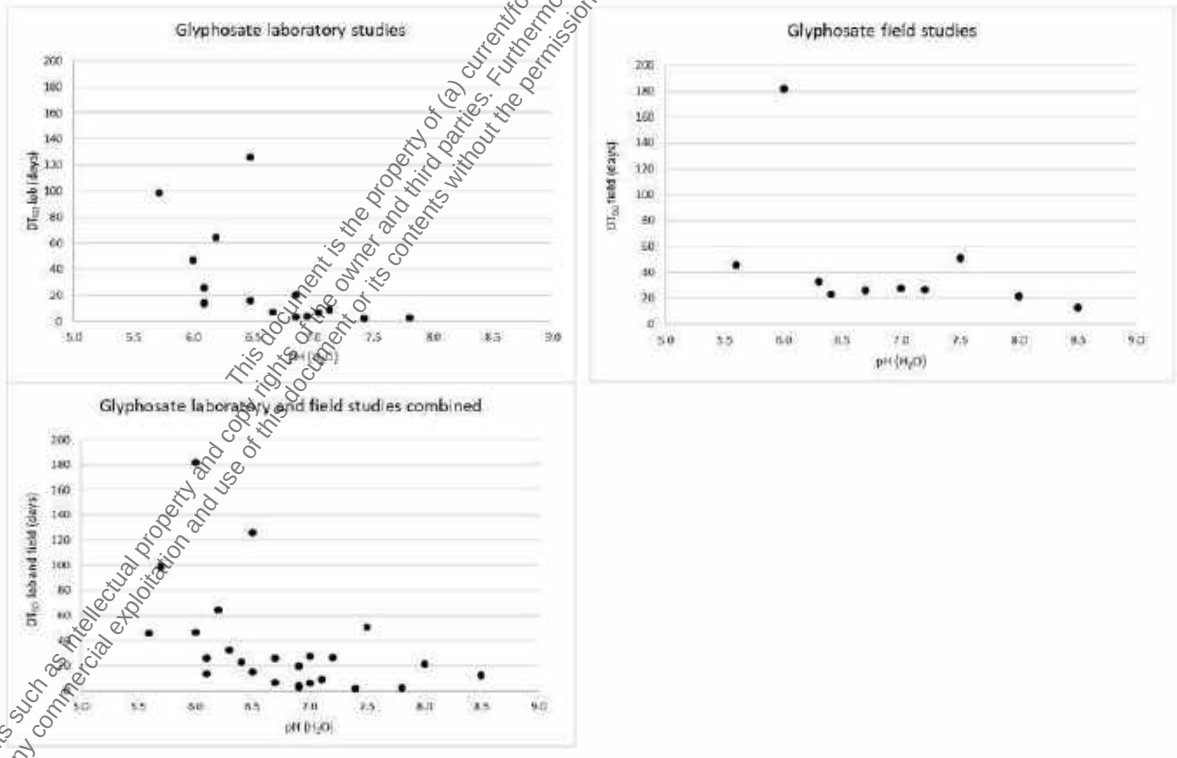
The pH dependency of the normalised DT_{50} of glyphosate in soil was assessed using the German Input Decision tool 3.3 (*Holdt, G. et al., 2012: Recommendations for simulation calculation to predict environmental concentrations for active substances of plant protection products and their metabolites in groundwater (PECgw) in the National Authorisation procedure in Germany*). If pH values measured in H_2O were not available, they were converted from available measured pH values. The results of laboratory and field studies were evaluated separately. The assessment shows a significant correlation between the pH values and the normalised DT_{50} for glyphosate derived from laboratory soil degradation studies. No significant correlation between the pH values and the normalised DT_{50} for glyphosate derived from field studies was shown. For the combined data from laboratory and field studies, there is a significant correlation between the pH values and the normalised DT_{50} for glyphosate.

Table 7.1.2-8: Correlation parameters for DT_{50} values and pH values for glyphosate derived from laboratory and field studies

Compound	Study type	Kendall tau (stringency of the correlation)	p (level of significance)
Glyphosate	Laboratory studies	-0.599	0.002
Glyphosate	Field studies	-0.467	0.074
Glyphosate	Laboratory and field studies combined	-0.399	0.006

p values <0.05 are highlighted in grey, a correlation between the data points of the investigated factors exists

Figure 7.1.2-3: Correlation between pH and DT_{50} values of glyphosate derived from laboratory and field studies



Assessment of pH dependency of aerobic degradation of AMPA in soil

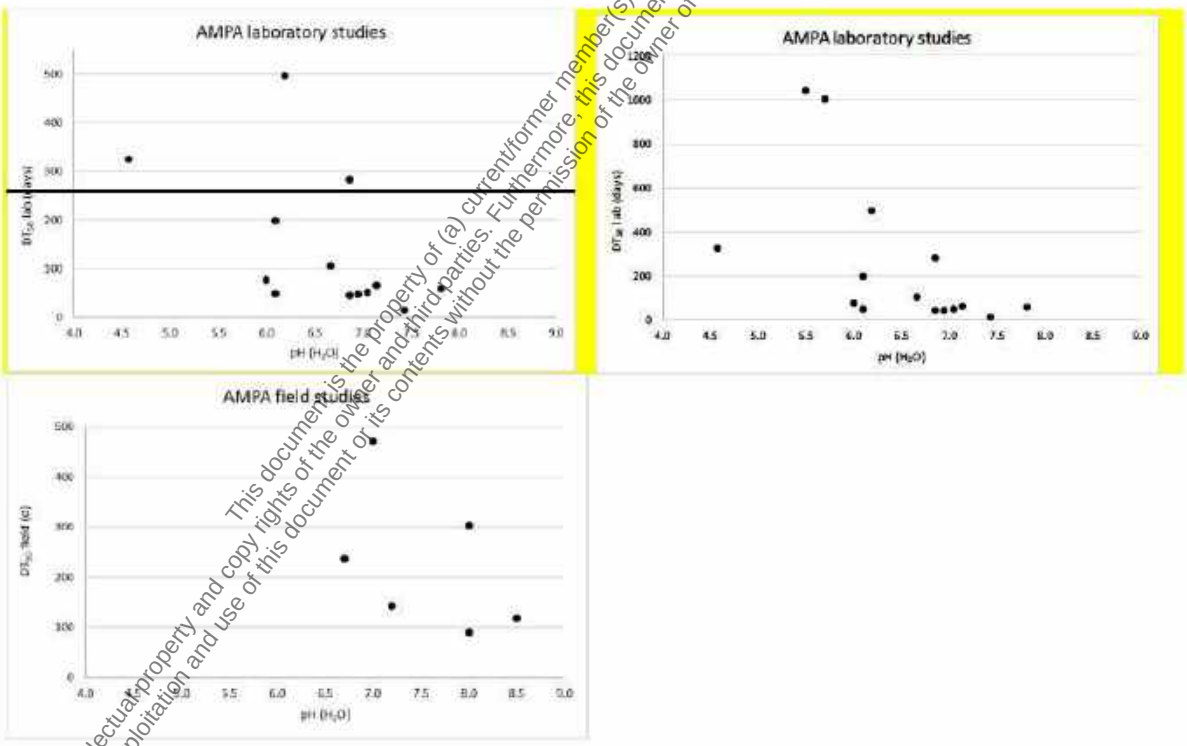
The pH dependency of the the normalised DT_{50} of AMPA in soil was assessed using the German Input-Decision Tool 3.3 (Holdt *et al.*, 2012). If pH values measured in H_2O were not available, they were converted from measured pH values. The results of laboratory and field studies were evaluated separately. The assessment ~~did not show~~ showed a significant correlation between the pH values and the normalised DT_{50} for AMPA for data derived from laboratory studies. For field studies and combined laboratory and field studies, no significant correlation between pH values and the normalised DT_{50} for AMPA was found.

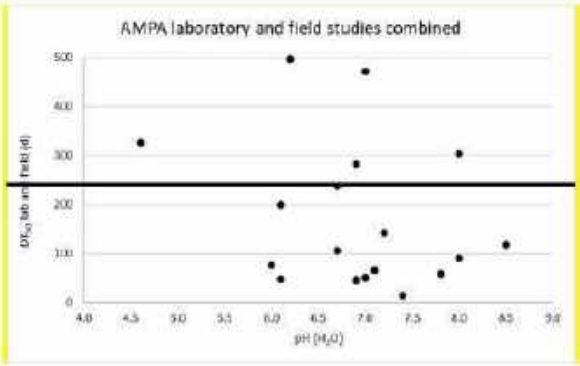
Table 7.1.2-9: Correlation parameters for DT_{50} values and pH values for AMPA

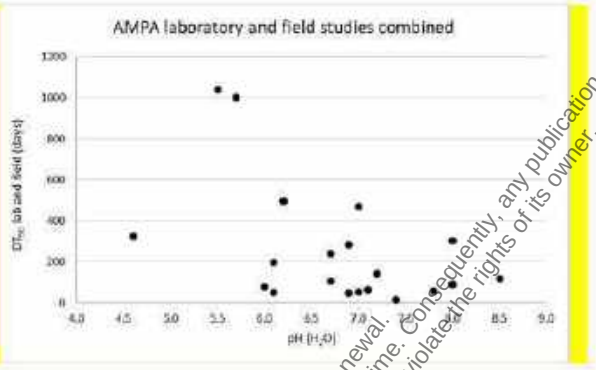
Compound	Study type	Kendall tau (stringency of the correlation)	p (level of significance)
AMPA	laboratory studies	~~-0.364~~ -0.490	~~0.098~~ 0.013
AMPA	field studies	-0.414	0.339
AMPA	laboratory and field studies combined	~~-0.096~~ -0.247	~~0.598~~ 0.130

p values <0.05 are highlighted in grey, a correlation between the data points of the investigated factors exists
AMPA: aminomethylphosphonic acid

Figure 7.1.2-4: Correlation between pH and DegT$_{50}$ values of AMPA derived from laboratory and field studies







CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

The rate of degradation of glyphosate in soil under aerobic conditions was investigated in the course of eight studies with glyphosate or glyphosate-trimesium which are considered valid to address the data point ([redacted] 2010, CA 7.1.1.1/001, [redacted] 1996, CA 7.1.1.1/003, [redacted] 1996, CA 7.1.1.1/004, [redacted] 1995, CA 7.1.1.1/005, [redacted] 1993, CA 7.1.1.1/006, [redacted] 2010, CA 7.1.2.1.1/002, [redacted] 1993, CA 7.1.2.1.1/003 and its addendum [redacted] 2002, CA 7.1.2.1.1/004 and [redacted] 1992, CA 7.1.2.1.1/005). For studies performed with glyphosate-trimesium, only the results for the glyphosate (PMG) anion are considered for evaluation and further assessment. The results of the studies were evaluated according to the current kinetic guidances (FOCUS, 2006, 2014, [redacted] 2020, CA 7.1.2.1.1/001). In addition, the studies on route of degradation under aerobic conditions were included in the evaluation. The trigger DT_{50} and DT_{90} of glyphosate range from 0.6 to 60.2 days and from 9.7 to >1000 days, respectively. An overview of degradation endpoints for glyphosate under aerobic laboratory conditions is given in Table 7.1.2-1.

In the scientific literature review for glyphosate (2010-2019), eleven articles were identified to provide further information relevant to the data point. The reliability of these articles was assessed as "reliable with restrictions" as either test conditions were not in agreement with current test guideline or insufficient information was reported to allow evaluation according to the current kinetic guidance. Thus, no new endpoints were derived, and the articles are considered as supportive information.

In Zhelezova *et al.* (2017, CA 7.1.2.1.1/010) it was shown, that the degradation of glyphosate was not consistently affected by biochar available in soil. Based on a correlation between degradation rate and adsorption coefficients it was concluded that the degradation of glyphosate may be limited by availability. In Cassigneul *et al.* (2016, CA 7.1.2.1.1/011) the half-life of glyphosate increased in the presence of decomposing cover crops. This was attributed to differences in composition and availability to microorganisms. Norgaard *et al.* (2015, CA 7.1.2.1.1/012) showed in soil degradation experiments with microplates that mineralisation of glyphosate was linked to soil clay and orgnic carbon content. The article of Kanissery *et al.* (2015, CA 7.1.2.1.1/013) showed in aerobic and anaerobic degradation experiments that degradation and mineralisation of glyphosate is slower under anoxic conditions. The addition of soil phosphate was found to stimulate degradation in anoxic soils only. Based on degradation experiments with Argentinian soils from different cultivation systems, Rampoldi *et al.* (2014, CA 7.1.2.1.1/014) suggested a correlation between glyphosate mineralisation and adsorption. Further, for two of the three soils, mineralisation rates were higher when the soil originates form monoculture compared to crop rotation systems. The article of Al-Rajab *et al.* (2014, CA 7.1.2.1.1/015) showed that about 60 % of applied glyphosate were mineralised within 80 days and calculated a half-life of extractable glyphosate of 14.5 days. Nguyen *et al.* (2013, CA 7.1.2.1.1/016) focused on the relationships between soil properties and degradation of glyphosate in 21 different soils. It was shown, that glyphosate mineralization may correlate with extractable H^+ cations, Ca^{2+} and plant available potassium. In the article of Bergström *et al.* (2011, CA 7.1.2.1.1/017) degradation of glyphosate was found to correlate with adsorption. Further, it is suggested that AMPA degradation can be faster than that for glyphosate. The article from Ghafoor *et al.* (2011, CA 7.1.2.1.1/018) found that the adsorption coefficient (K_f) and soil lactase activity together explained 88 % of the variation in degradation rate of glyphosate. Investigations of Alexa *et al.* (2010, CA 7.1.2.1.1/019) showed, that the extend of (bio-)degradation of glyphosate in soil samples, collected from vine plantations, varied when inorganic supplements were added (decrease of degradation capacity) or presence of straw (accumulation of $^{14}CO_2$). The article of Al-Rajab (2010, CA 7.1.2.1.1/020) showed that degradation and mineralisation of glyphosate in three agricultural soils varied in three different soils. Based on mineralisation rates, half-lifes of 12 to 42 days were estimated.

Table 7.1.2.1.1-1: Studies on aerobic rate of degradation of glyphosate

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.2.1.1/001	[redacted] 2020	Kinetics evaluation	Glyphosate, AMPA	Valid	
CA 7.1.2.1.1/002	[redacted] 2010	Aerobic rate	Glyphosate	Valid	Updated kinetic evaluatoin in CA 7.1.2.1.1/001
CA 7.1.2.1.1/003	[redacted] 1993.	Aerobic rate	Glyphosate	Valid	Addendum: CA 7.1.2.1.1/004 Updated kinetic evaluatoin in CA 7.1.2.1.1/001
CA 7.1.2.1.1/004	[redacted] 2002	Addendum	Glyphosate	Valid	Addendum: to CA 7.1.2.1.1/003
CA 7.1.2.1.1/005	[redacted] 1992.	Aerobic rate	Glyphosate-Trimesium	Valid	Updated kinetic evaluatoin in CA 7.1.2.1.1/001
CA 7.1.2.1.1/006	[redacted] 1991	Aerobic rate	Glyphosate-Trimesium	Invalid	
CA 7.1.2.1.1/007	[redacted] 1991	Aerobic rate	Glyphosate	Invalid	
CA 7.1.2.1.1/008	[redacted] 1980	Aerobic rate	Glyphosate	Invalid	
CA 7.1.2.1.1/009	[redacted] 1972	Aerobic rate	Glyphosate	Invalid	

Table 7.1.2.1.1-2: Aerobic rate of degradation - relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.2.1.1/010	Zhelezova *et al*., 2017	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/011	Cassigneul *et al*., 2016	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/012	Norgaard *et al*., 2015	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/013	Kanissery *et al*., 2015	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/014	Rampoldi *et al*., 2014	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/015	Al-Rajab & Hakami, 2014	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/016	Nguyen *et al*., 2013	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/017	Bergstrom *et al*., 2011	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/018	Ghafoor *et al*., 2011	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/019	Alexa *et al*., 2010	Aerobic rate	Glyphosate	Reliable with restrictions	
CA 7.1.2.1.1/020	Al-Rajab & Schiavon, 2010	Aerobic rate	Glyphosate, AMPA	Reliable with restrictions	

Rate of degradation studies with glyphosate

1. Information on the study

Data point:	CA 7.1.2.1.1/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from aerobic laboratory soil degradation studies
Report No	112148-001
Document No	
Guidelines followed in study	FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp. FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not applicable for this study type
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A kinetic evaluation of eight aerobic soil degradation studies (including 20 soils / experiments) was performed in order to derive trigger (persistence) and modelling endpoints for glyphosate and its major soil metabolite AMPAAMPA. The evaluation was conducted according to FOCUS kinetics guidance (2006, 2014) using the fitting software CAKE. Residue data were directly taken from the aerobic soil degradation studies and adjusted according to FOCUS Kinetics. The soils investigated covered a broad range of soil types and pH values and were incubated in the dark at different temperatures and soil moistures.

I. MATERIALS AND METHODS

The purpose of this assessment was to conduct a kinetic modelling evaluation for glyphosate and its major soil metabolite AMPAAMPA using results from laboratory soil degradation studies. The aim of the evaluation was to derive the following endpoints:

- Trigger endpoints to be used as triggers for higher-tier environmental fate studies
- Modelling endpoints for use in calculating predicted environmental concentrations.

The degradation of glyphosate under aerobic conditions was investigated in eight laboratory studies ([redacted] 1993, CA 7.1.1.1/006; [redacted] 1995, CA 7.1.1.1/005; [redacted] 1996, CA 7.1.1.1/003; [redacted] 1996, CA 7.1.1.1/004; [redacted] 2010, CA 7.1.1.1/001 and

CA 7.1.2.1.1/002; [redacted], 1992, CA 7.1.2.1.1/005; [redacted], 1993, CA 7.1.2.1.1/003). The test soils covered a broad range of soil types and pH values. A total number of 20 soil degradation experiments under dark aerobic conditions were conducted for up to 364 days. Incubation temperatures were between 8 and 25 °C. The soils moisture content ranged between 20 and 50 % MWHC. Additionally, degradation of glyphosate was tested under sterile conditions or with reduced application rate ([redacted] 1992, CA 7.1.2.1.1/005, dose groups D and E, respectively).

1. Data pre-processing

The standard procedures recommended by FOCUS (2006, 2014) were followed to adjust the experimental data for kinetic modelling. Replicate samples were available for all of the studies except [redacted] (1996, CA 7.1.1.1/004) and [redacted] (1993, CA 7.1.2.1.1/003).

All measured data points derived from the study reports were included in the kinetic evaluation even if the material balance of single measurements dropped below the level of 90 % of the applied radioactivity as either the material balances were close to 90 % or lower material balances could be attributed to potential loss of $^{14}CO_2$ during the experiments (soil Speyer 2.1 of study [redacted], 1993, CA 7.1.2.1.1/003; soil Drusenheim of study [redacted] 2010, CA 7.1.2.1.1/002).

For experiments exceeding the recommended duration of 120 days (e.g. [redacted], 1993, CA 7.1.1.1/06; [redacted], 1995, CA 7.1.1.1/005; [redacted], 2010, CA 7.1.1.1/001) all data points were included for kinetic evaluation as microbial biomass measurements and ongoing decline of glyphosate concentrations indicated that microbial degradation still occurred.

The initial amounts of glyphosate were set to the value of the material balance at day 0, thus assigning all radioactivity observed at day 0 to the parent compound and assuming that no degradation processes have yet taken place. Accordingly, the initial amounts of the metabolites were set to 0.

It is recommended that values below the LOD should be replaced by half the LOD (FOCUS; 2006, 2014). Processed residue data are presented in the following tables.

Table 7.1.2.1.1-3: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1993, CA 7.1.1.1/006), soil Les Evouettes

Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	98.6[1]	0.0[2]
3	65.6	6.2
3	69.0	6.4
7	49.5	14.9
7	58.6	11.5
14	48.9	12.2
14	38.7	13.5
28	36.7	19.7
28	36.1	21.9
56	24.3	21.1
56	25.4	22.7
84	19.4	28.3
84	19.6	30.4
112	16.3	28.3
112	21.8	26.9
168	9.4	16.6
168	10.8	21.7
252	8.3	17.7
252	8.4	18.8
364	7.4	21.2
364	6.0	21.4

[1] Set to material balance
[2] Amounts of metabolite set to 0 at day 0

Table 7.1.2.1.1-4: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1995, CA 7.1.1.1/005), soil Arrow

Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	97.6[1]	0.0[2]
0	96.6	0.0[2]
3	87.0	3.9
3	82.2	3.1
7	74.0	6.9
7	73.9	6.6
14	64.2	10.4
14	69.5	8.3
30	54.0	14.4
30	54.6	13.7
60	41.1	22.1
60	38.4	22.3
90	32.5	27.5
90	35.5	25.4
120	28.1	28.0
120	29.0	26.6

Table 7.1.2.1.1-4: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1995, CA 7.1.1.1/005), soil Arrow

Time (d)	Glyphosate (% AR)	AMPA (% AR)
180	26.5	25.8
180	27.6	25.3

[1] Set to material balance
[2] Amounts of metabolites set to 0 at day 0

Table 7.1.2.1.1-5: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1996, CA 7.1.1.1/003), soil B

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]
0	100.3[2]	0.0[3]
0	99.2[2]	0.0[3]
1	50.5	16.7
1	50.5	15.4
3	36.6	16.6
3	36.4	16.1
7	20.0	20.8
7	19.6	20.8
14	11.3	20.5
14	13.6	19.6
30	6.3	21.4
30	5.4	20.7
63	7.8	11.5
63	2.4	16.2
90	2.1	14.5
90	2.1	13.6
121	1.9	11.9
121	2.1	14.7

[1] Soil extracts were analysed using both HPLC and TLC method. As analysis of the ammonia extracts by TLC and HPLC showed the chromatographic profiles to be very similar at each sampling interval, results of both methods were used as analytical replicates and were averaged for kinetic analysis.
[2] Set to material balance
[3] Amounts of metabolites set to 0 at day 0

Table 7.1.2.1.1-6: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1996, CA 7.1.1.1/004)

Time (d)	Glyphosate (% AR)	AMPA (% AR)
Speyer 2.1		
0	97.7[1]	0.0[2]
1	84.8	12.1
2	74.3	12.9
4	59.2	25.1
7	53.9	27.3
15	38.2	27.5
29	21.0	37.9
60	8.5	42.3
90	2.2	50.1
Speyer 2.2		
0	103.8[1]	0.0[2]
1	96.1	4.3
2	84.2	7.9
4	77.1	12.9
7	71.8	15.7
15	60.3	21.0
29	41.7	34.5
60	26.7	42.4
90	25.9	39.0
120	19.0	40.9
Speyer 2.3, 20°C		
0	99.1[1]	0.0[2]
1	76.2	13.0
2	63.9	27.0
4	34.2	25.7
7	18.4	32.0
15	13.3	25.3
29	0.05[3]	31.1
60	3.0	18.5
Speyer 2.3, 10°C		
0	99.3[1]	0.0[2]
1	87.3	8.7
2	80.0	9.2
4	62.2	19.3
7	54.9	22.1
15	35.9	25.8
29	21.7	28.7
60	7.5	34.3

[1] Set to material balance
[2] Amounts of metabolites set to 0 at day 0
[3] Value below LOD (= 0.1 %AR) set to ½ LOD

Table 7.1.2.1.1-7: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (2010, CA 7.1.1.1/001), soil Gartenacker

Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	100.1[1]	0.0[2]
0	99.2[1]	0.0[2]
3	71.1	4.3
3	69.2	4.6
6	58.1	7.0
6	56.6	7.2
10	44.4	8.2
10	43.4	8.0
20	33.3	11.0
20	29.2	13.7
34	17.6	11.5
34	18.0	12.7
55	10.5	14.9
55	9.3	14.5
90	4.5	12.1
90	4.7	12.3
112	3.0	9.9
112	3.4	10.2
132	2.3	8.8
132	2.7	7.8

[1] Set to material balance

[2] Amounts of metabolites set to 0 at day 0

Table 7.1.2.1.1-8: Processed residue data of glyphosate and its metabolite AMPA in [redacted] l (1992, CA 7.1.2.1.1/005)

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]
Speyer 2.1, dose group A (20 °C, 40 % MWHC, 4 mg/kg)		
0	99.2[2]	0.0[3]
0	99.1[2]	0.0[3]
2	53.7	7.1
2	65.0	9.4
4	54.6	15.3
4	55.0	15.3
8	46.9	18.6
8	41.8	17.4
16	35.0	25.5
16	32.0	24.2
33	21.0	29.2
33	21.2	29.8
64	13.1	33.9
64	13.3	29.7
104	7.5	29.9
104	8.0	29.7

Table 7.1.2.1.1-8: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1992, CA 7.1.2.1.1/005)

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]
Speyer 2.1, dose group B (20 °C, 20 % MWHC, 4 mg/kg)		
0	100.1[2]	0.0[3]
0	97.6[2]	0.0[3]
2	57.7	10.0
2	58.6	9.2
4	48.6	15.7
4	45.0	15.7
8	34.1	15.3
16	31.3	16.6
16	28.5	23.1
33	20.9	27.0
33	21.0	28.0
64	11.4	28.3
64	12.9	26.6
104	8.0	26.6
104	6.5	28.6
Speyer 2.1, dose group C (8 °C, 40 % MWHC, 4 mg/kg)		
0	98.9[2]	0.0[3]
0	98.3[2]	0.0[3]
2	66.7	5.6
2	77.3	5.1
4	76.5	7.7
4	68.8	6.8
8	68.9	9.9
8	69.3	8.2
16	65.7	13.7
16	61.3	14.2
33	54.3	16.5
33	59.9	18.2
64	35.6	17.8
64	37.4	19.4
104	25.1	23.8
104	28.5	22.6

Table 7.1.2.1.1-8: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1992, CA 7.1.2.1.1/005)

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]
Speyer 2.1, dose group D (20 °C, 40 % MWHC, 4 mg/kg, sterile)		
0	98.1[2]	0.0[3]
0	95.3[2]	0.0[3]
2	63.0	5.4
2	61.2	5.7
4	61.8	7.4
4	60.2	8.4
7	52.5	9.8
7	57.7	7.2
16	43.5	10.7
16	42.5	15.7
34	47.4	11.6
34	30.2	17.4
70	23.9	21.1
70	24.3	19.6
Speyer 2.1, dose group E (20 °C, 40 % MWHC, 0.4 mg/kg)		
0	101.4[2]	0.0[3]
0	103.1[2]	0.0[3]
2	71.8	10.6
2	75.8	11.4
4	75.1	18.2
4	65.4	15.0
8	46.7	16.8
8	NaN	NaN
16	33.0	24.0
16	33.4	25.0
33	23.0	27.4
33	24.1	31.0
64	12.7	30.8
64	12.9	32.1
104	7.6	33.7
104	6.9	27.3

Table 7.1.2.1.1-8: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1992, CA 7.1.2.1.1/005)

Time (d)	Glyphosate (% AR)[1]	AMPA (% AR)[1]
Beedon manor, dose group F (20 °C, 40 % MWHC, 0.4 mg/kg)		
0	96.6[2]	0.0[3]
0	89.8[2]	0.0[3]
2	22.5	7.4
2	19.5	7.1
4	17.4	10.9
4	13.9	8.9
8	10.9	13.6
8	10.4	13.5
16	5.0	12.4
16	5.2	12.3
33	2.4	12.7
33	2.2	12.2
64	0.8	6.9
64	0.7	6.9
104	0.4	3.4
104	0.7	3.5

[1] Residues are mean values of two solvent system (solvent system 1 and solvent system 5). As data in the two solvent systems are similar, mean values were calculated and used for kinetic analysis.
[2] Set to material balance
[3] Amounts of metabolites set to 0 at day 0

Table 7.1.2.1.1-9: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1993, CA 7.1.2.1.1/003)

Time (d)	Glyphosate (% AR)	AMPA (% AR)
Speyer 2.1		
0	91.1[1]	0.0[2]
7	56.0	21.7
14	38.1	41.2
28	22.6	32.6
56	9.7	40.0
84	9.7	38.7
105	8.0	23.5
Speyer 2.2		
0	97.6[1]	0.0[2]
7	41.4	42.4
14	48.8	31.4
28	33.3	33.1
56	31.3	34.6
84	19.3	33.9
105	13.5	35.4

Table 7.1.2.1.1-9: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (1993, CA 7.1.2.1.1/003)

Time (d)	Glyphosate (% AR)	AMPA (% AR)
Speyer 2.3		
0	92.3[1]	0.0[2]
7	39.4	13.6
14	19.7	25.1
28	5.5	25.1
56	4.3	18.9
84	3.0	18.5
105	2.5	12.1

[1] Set to material balance

[2] Amounts of metabolites set to 0 at day 0

Table 7.1.2.1.1-10: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (2010, CA 7.1.2.1.1/002)

Time (d)	Glyphosate (% AR)	AMPA (% AR)
Drusenheim		
0	102.2[1]	0.0[2]
0	100.9[1]	0.0[2]
1	64.9	9.6
1	66.2	7.7
3	43.5	15.0
3	44.1	15.1
8	18.3	21.2
8	18.1	21.1
14	10.2	19.7
14	10.8	18.9
27	4.9	17.5
27	3.3	15.9
48	1.6	9.5
48	1.5	9.8
70	1.1	6.2
70	0.9	6.1

Table 7.1.2.1.1-10: Processed residue data of glyphosate and its metabolite AMPA in [redacted] (2010, CA 7.1.2.1.1/002)

Time (d)	Glyphosate (% AR)	AMPA (% AR)
Pappelacker		
0	102.2[1]	0.0[2]
0	102.0[1]	0.0[2]
1	77.1	4.2
1	77.2	3.9
3	59.0	7.4
3	58.1	7.9
8	27.4	14.5
8	29.2	13.7
14	19.1	14.2
14	29.6	12.2
27	10.1	13.7
27	18.2	13.2
48	4.5	13.6
48	9.1	15.4
70	2.3	10.4
70	2.9	11.6
91	2.0	10.0
91	1.8	9.5
120	2.0	9.1
120	2.2	9.0
18-Acres		
0	101.3[1]	0.0[2]
0	99.5[1]	0.0[2]
8	73.9	3.3
8	73.9	3.4
14	69.4	3.9
14	73.1	2.9
21	65.6	6.4
21	65.3	7.2
41	55.9	9.1
41	54.4	8.5
63	47.0	11.7
63	49.3	12.0
91	44.7	13.3
91	46.7	13.2
120	42.1	14.3
120	41.3	12.1

[1] Set to material balance

[2] Amounts of metabolites set to 0 at day 0

2. Kinetic models and analysis

Kinetic models

Four kinetic degradation models were considered to describe the degradation behaviour of the compounds in soil: single first-order (SFO), first-order multi-compartment (FOMC = Gustafson and Holden model), double-first-order-in-parallel (DFOP) and Hockey-stick (HS) (FOCUS; 2006, 2014). The HS model was tested only in cases where none of the other models were able to provide a visually and statistically reliable fit.

For the parent compound, the best-fit model was accepted for deriving trigger endpoints, while the DT_{50} calculated from SFO model was preferably selected as modelling endpoint. If SFO did not provide an acceptable fit, modelling endpoints were derived from an appropriate bi-phasic model. If 10 % of the initial concentration was reached within the experimental period, the DT_{50} was back-calculated from DT_{90} as $DT_{50} = DT_{90}/3.32$. Otherwise, the DT_{50} was derived from the slow-phase degradation rate of the DFOP or HS model.

For the metabolite, pathway fits were conducted using the appropriate kinetic model for trigger and modelling endpoints for the parent determination and SFO for the metabolite.

In general, kinetic endpoints for parent and metabolite were derived from acceptable pathway fits. In cases where no reliable pathway fit could be established, kinetic endpoints for the parent were derived from the corresponding parent-only fit, and decline fits were conducted for the metabolite, starting from the maximum observed concentration. The respective day was defined as 0 days after maximum concentration, and later time points were adjusted accordingly.

Optimisation

The kinetic analyses were conducted using the software CAKE 3.3.

The data were directly fitted with the complete dataset and unconstrained initial concentration (M_0) for the parent substance. Iteratively Reweighted Least Square (IRLS) was used as the solver, as implemented in CAKE. Optimisations were carried out for the initial soil residue (M_0), degradation model parameters k, α, β, g or t_b, depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually, based on the observed degradation pattern and preliminary model runs. By default, the initial amount of metabolite was fixed to 0. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of $1 * 10^{-5}$ and 100, respectively.

If a pathway fit did not yield visually and/or statistically reliable results, the kinetic model was further optimised by fixing one or more of the model parameters to either the value derived from a reliable parent-only fit (e.g. M_0, k-rates) or to values derived from previous pathway fits with unbound parameters (e.g. ff). A stepwise fixing procedure has been applied in these cases which is further described in the results chapter for the respective pathway fits.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration vs. time plots and residual plots) and statistically (Chi-square (χ^2) test). The visual inspection focused on the residuals which should not be distributed systematically around the zero line, but randomly. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered
- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered

A statistical measure of the quality of a fit is given by the χ^2-test. The χ^2-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements. The model with the smallest error percentage was defined as the most appropriate, because it described the measured data in the most robust way.

In general, for parent compounds, it is recommended that if the χ^2 error is ≤15 %, then the model has adequately reflected the measured data. However, this value should only be considered as guidance and not an absolute cut-off criterion. The guidance is less clear for metabolites due to the complexity of the curve fitting for multiple components, and so this criterion is a little more relaxed.

Significance of parameters

A single-sided t-test was performed to evaluate whether the optimised degradation rate constants (k) of the SFO, DFOP and HS kinetic models were significantly different from zero at a chosen significance level of 5 %. For the FOMC kinetic model, only the confidence interval of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The CAKE software also reports a 95 % confidence interval on the estimated parameters. It should be relatively tight and not contain 0 to be considered statistically robust.

3. Normalisation

Modelling endpoints (DT_{50} values) derived from kinetic analyses have to be normalised to the soil moisture content at field capacity (pF2) and a temperature of 20 °C to be used in environmental fate models.

Moisture correction was carried out by multiplying the respective DT_{50} values by a moisture correction factor. A Walker exponent of 0.7 was used for the correction. The gravimetric water content during the study (θ_{act}) was calculated using the soil water characteristics that were given in the respective study reports except for [redacted] (1995, CA 7.1.1.1/005) for which the FOCUS default value of 27 g/100 g was used. For the gravimetric water content at pF2 (θ_{ref}) the default values for the relevant soil types as given by FOCUS (2000) were used.

A temperature correction was necessary for all experiments which were not conducted at 25 °C. A Q_{10} value of 2.58 was used for the correction.

A detailed overview of the moisture and temperature correction procedure can be found in the following table.

Table 7.1.2.1.1-11: Temperature and moisture correction factors for normalisation of modelling endpoints

Study	Soil type	Temperature		Moisture					Overall correction factor ($f_{overall}$) (-)
		During study[1] (°C)	Correction factor (f_{temp}) (-)	During study[1] (% of MWHC)	Gravimetric water content at MWHC[1] (g / 100 g)	Gravimetric water content during study (θ_{act})[2] (g / 100 g)	Gravimetric water content at pF2 (θ_{ref})[3] (g / 100 g)	Correction factor (f_{moist}) (-)	
[redacted] (1993, CA 7.1.1.1/006): Les Evouettes	Silt loam	20	1.00	40	22.1	8.8	26	0.47	0.47
[redacted] (1995, CA 7.1.1.1/005): Arrow	Sandy loam	20	1.00	40	27[3]	10.8	19	0.67	0.67
[redacted] /003): Soil B	Sandy loam	25	1.61	75[4]	14.25	10.7	19	0.67	1.07
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.1	Sand	20	1.00	45	31	14.0	12	1.00	1.00
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.2	Loamy sand	20	1.00	45	48	21.6	14	1.00	1.00
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	20	1.00	45	39	17.6	14	1.00	1.00
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	10	0.39	45	39	17.6	14	1.00	0.39
[redacted] (2010, CA 7.1.1.1/001): Gartenacker	Loam	20	1.00	50[4]	21.4[5]	10.7	25	0.55	0.55
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group A	Sand	20	1.00	40	32.95	13.2	12	1.00	1.00
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group B	Sand	20	1.00	20	32.95	6.6	12	0.66	0.66

Table 7.1.2.1.1-11: Temperature and moisture correction factors for normalisation of modelling endpoints

Study	Soil type	Temperature		Moisture					Overall correction factor ($f_{overall}$) (-)
		During study[1] (°C)	Correction factor (f_{temp}) (-)	During study[1] (% of MWHC)	Gravimetric water content at MWHC[1] (g / 100 g)	Gravimetric water content during study (θ_{act})[2] (g / 100 g)	Gravimetric water content at pF2 (θ_{ref})[3] (g / 100 g)	Correction factor (f_{moist}) (-)	
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group C	Sand	8	0.32	40	32.95	13.2	12	1.00	0.32
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group D (sterile)	Sand	20	1.00	40	31.31	12.5	12	1.00	1.00
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group E (lower rate)	Sand	20	1.00	40	32.95	13.2	12	1.00	1.00
[redacted] (1992, CA 7.1.2.1.1/005): Beedon manor, dose group F	Clay loam	20	1.00	40	57.94	23.2	28	0.88	0.88
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.1	Sand	20	1.00	40	12.8	5.1	12	0.55	0.55
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.2	Sand	20	1.00	40	17.7	7.1	12	0.69	0.69
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.3	Loamy sand	20	1.00	40	14	5.6	14	0.53	0.53
[redacted] (2010, CA 7.1.2.1.1/002): Drusenheim	Loam	20	1.00	50[4]	17.6[5]	8.8	25	0.48	0.48
[redacted] (2010, CA 7.1.2.1.1/002): Pappelacker	Loamy sand	20	1.00	50[4]	12.4[5]	6.2	14	0.57	0.57

Table 7.1.2.1.1-11: Temperature and moisture correction factors for normalisation of modelling endpoints

Study	Soil type	Temperature		Moisture					Overall correction factor ($f_{overall}$) (-)
		During study[1] (°C)	Correction factor (f_{temp}) (-)	During study[1] (% of MWHC)	Gravimetric water content at MWHC[1] (g / 100 g)	Gravimetric water content during study (θ_{act})[2] (g / 100 g)	Gravimetric water content at pF2 (θ_{ref})[3] (g / 100 g)	Correction factor (f_{moist}) (-)	
[redacted] (2010, CA 7.1.2.1.1/002): 18-Acres	Sandy clay loam	20	1.00	50[4]	19.7[5]	9.9	22	0.57	0.57

[1] Measured values taken from study reports
[2] Calculated: moisture during study (% MWHC) / 100 × gravimetric water content at MWHC
[3] FOCUS default value
[4] Percent of gravimetric water content at 1/3 bar
[5] Gravimetric water content at 1/3 bar, reported values

II. RESULTS AND DISCUSSION

[redacted] (1993, CA 7.1.1.1/006)

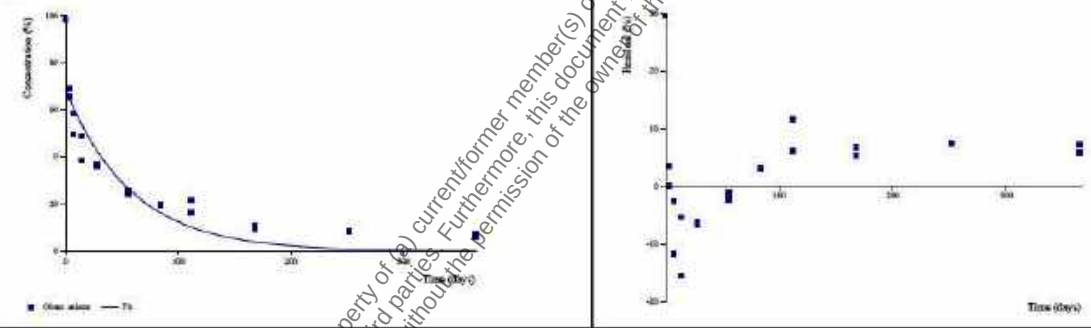
Table 7.1.2.1.1-12: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Les Evouettes of study [redacted] (1993, CA 7.1.1.1/006)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	69.0	k: 0.0171	24.9	k: <0.001	k: 0.0108	k: 0.023	40.5	135
FOMC	Good	96.5	α: 0.4936 β: 3.316	6.2	-[1]	β: 1.5133	β: 5.110	10.2	349
DFOP	Good	97.1	k_1: 0.2332 k_2: 0.0082 g: 0.5408	6.1	k_1: <0.001. k_2: <0.001	k_1: 0.1456 k_2: 0.0061	k_1: 0.321 k_2: 0.010	8.6	185
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide equally reliable and visually acceptable results but the least χ^2 error is provided by the DFOP model.

Conclusion: DFOP to be used in pathway fit for trigger endpoints.
DFOP to be used in pathway fit for modelling endpoints

SFO



FOMC

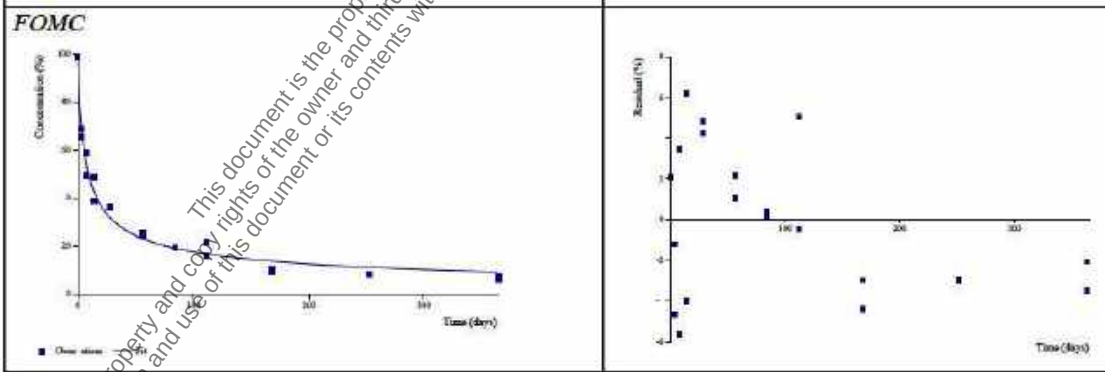
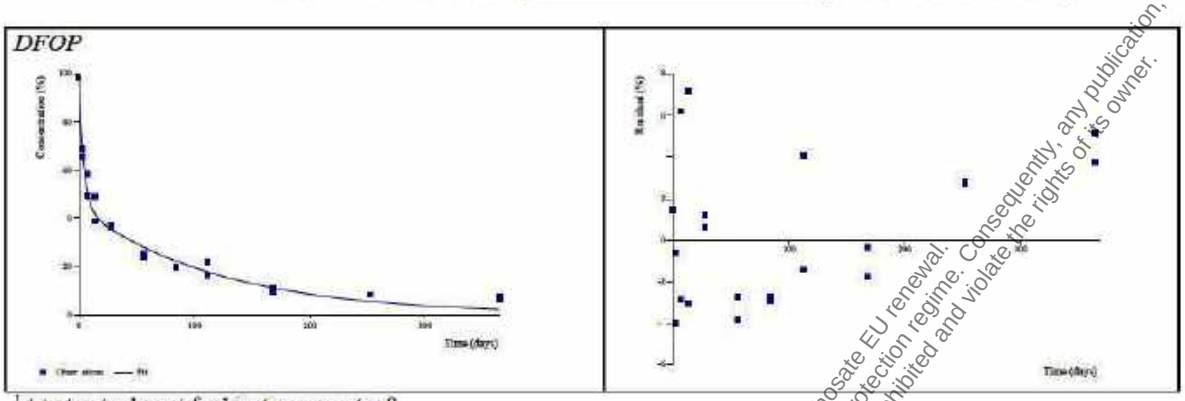


Table 7.1.2.1.1-12: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Les Evouettes of study [redacted] (1993, CA 7.1.1.1/006)

DFOP



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-13: Kinetic models and goodness-of-fit statistics of pathway fit for soil Les Evouettes of study [redacted] (1993, CA 7.1.1.1/006) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	95.2	k_1: 0.2083 k_2: 0.0083 g: 0.5355	6.5	k_1: <0.001 k_2: <0.001	k_1: 0.1346 k_2: 0.0062	k_1: 0.282 k_2: 0.01	9.7	184	-
AMPA: SFO	Accep-table	-	k: 0.0016	15.4	k: 0.002	k: 0.0005	k: 0.003	424	>1000	0.346 (±0.033)

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

Glyphosate (DFOP)

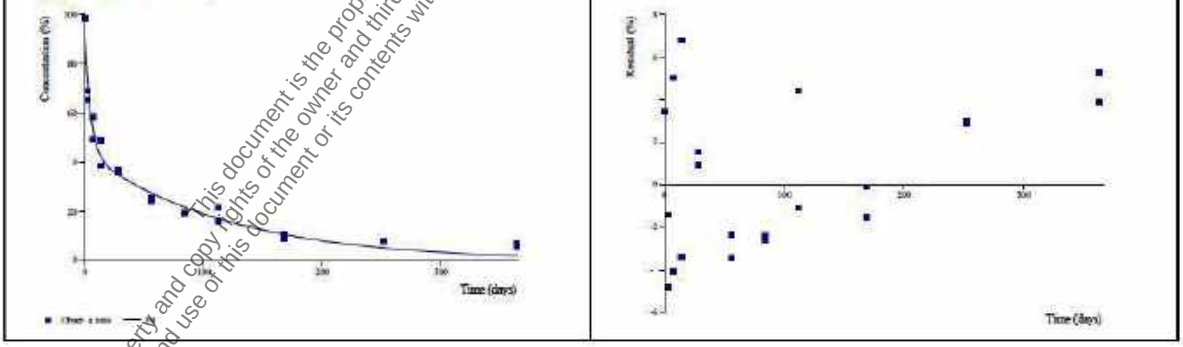
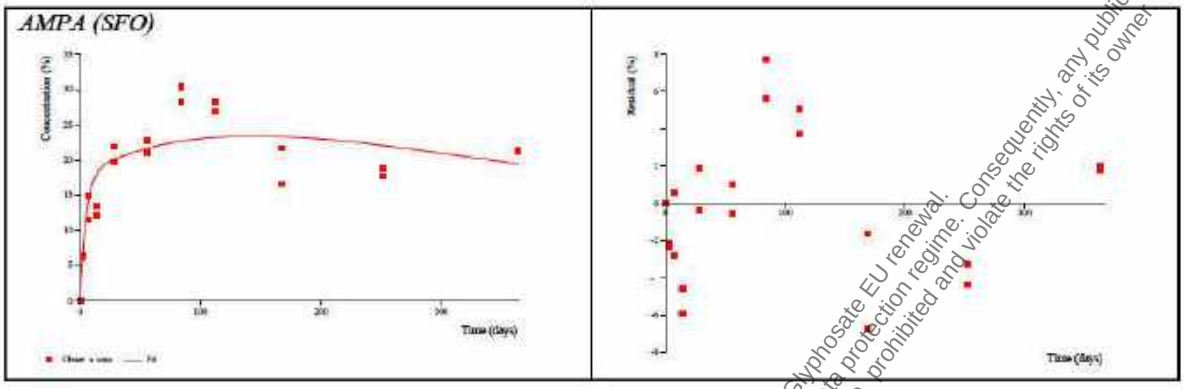


Table 7.1.2.1.1-13: Kinetic models and goodness-of-fit statistics of pathway fit for soil Les Evouettes of study [redacted] (1993, CA 7.1.1.1/006) – trigger and modelling endpoints

AMPA (SFO)



[redacted] (1995, CA 7.1.1.1/005)

Table 7.1.2.1.1-14: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Arrow of study [redacted] (1995, CA 7.1.1.1/005)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > 4 (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	84.0	k: 0.0099	10.9	k: <0.001	k: 0.0076	k: 0.012	69.8	232
FOMC	Good	96.0	α: 0.4539 β: 10.47	3.3	-[1]	β: 6.312	β: 14.63	37.8	>1000
DFOP	Good	94.3	k_1: 0.0595 k_2: 0.0097 g: 0.4852	3.6	k_1: <0.001 k_2: <0.001	k_1: 0.0345 k_2: 0.0017	k_1: 0.085 k_2: 0.006	37.4	440
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The FOMC model provides a slightly better visual fit (M_0 as well as the residues at the last two sampling dates) and the lowest χ^2 error. Thus, the FOMC model is selected as the best-fit model. As 10 % of the initial concentration was not reached within the experimental period, the DFOP model is selected for derivation of modelling endpoints.

Conclusion: FOMC to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

SFO

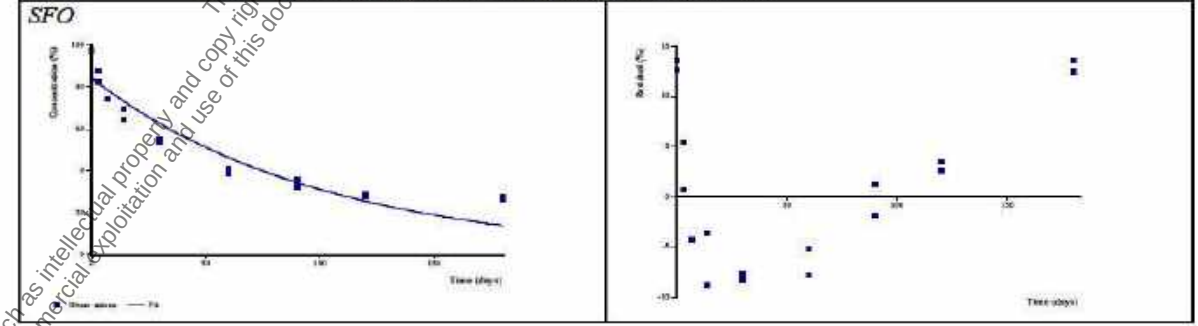
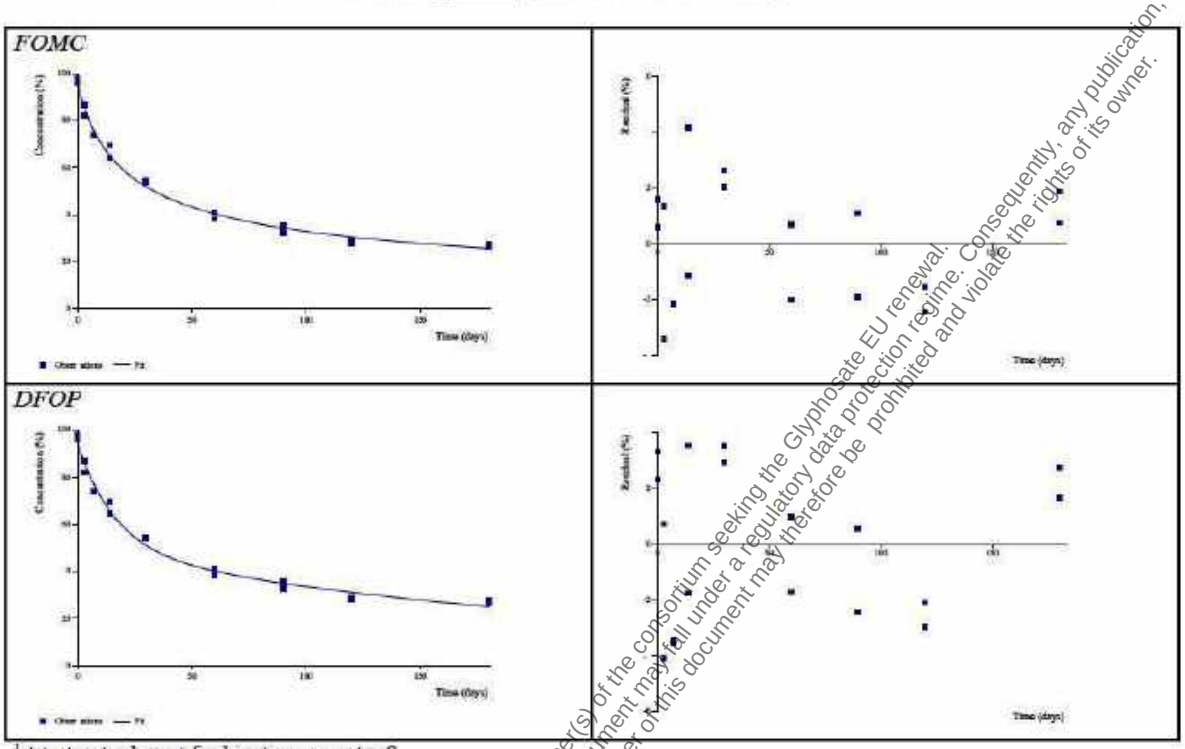


Table 7.1.2.1.1-14: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Arrow of study [redacted] (1995, CA 7.1.1.1/005)



[1] t-test not relevant for kinetic parameter β

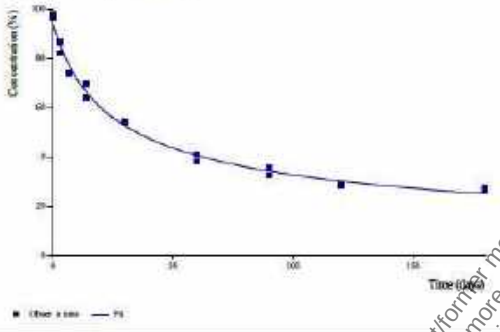
Table 7.1.2.1.1-15: Kinetic models and goodness-of-fit statistics of pathway fits for soil Arrow of study [redacted] (1995, CA 7.1.1.1/005) – trigger endpoints

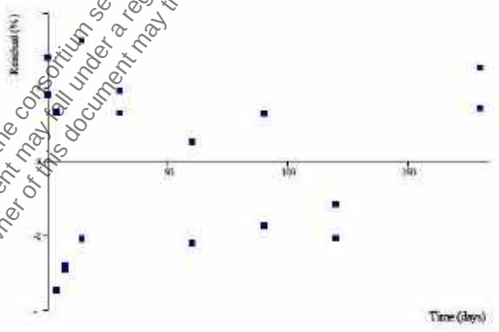
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (+ std. dev.)
Glypho-sate: FOMC	Good	94.8	α: 0.496 β: 13.22	2.5	-[1]	β: 8.158	β: 18.28	40.3	>1000	-
AMPA: SFO	Poor	-	k: 0	8.3	k: 0.5	k: -0.0013	k: 0.001	>1000	>1000	0.395 (±0.028)

The degradation of glyphosate is well described by the pathway fit. For AMPA, the degradation rate is not significantly different from zero and the visual fit is poor (the fitted curve still increases towards the end of the study while the measured residue data starts to decrease). Thus, the pathway fit is not acceptable. A decline fit for AMPA was not performed due to the limited number of data points after the peak.

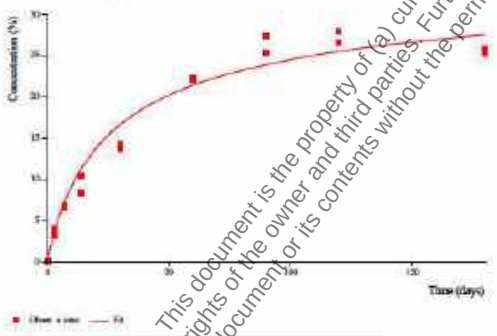
Conclusion: Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate
No trigger endpoints can be derived for AMPA

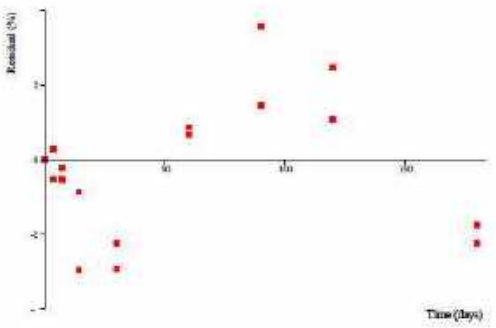
Glyphosate (FOMC)





AMPA (SFO)





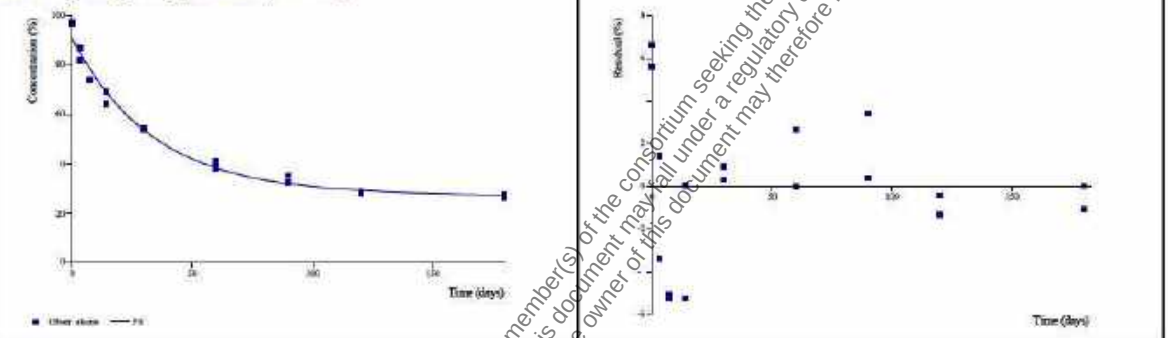
[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-16: Kinetic models and goodness-of-fit statistics of pathway fits for soil Arrow of study [redacted] (1995, CA 7.1.1.1/005) – modelling endpoints

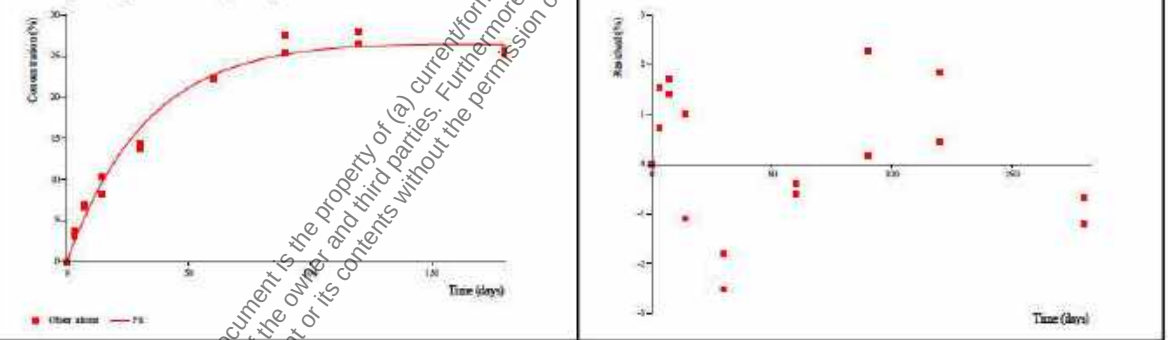
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Initial fitting										
Glypho-sate: DFOP	Poor	91.0	k_1: 0.0301 k_2: 0.0003 g: 0.6852	4.7	k_1: <0.001 k_2: 0.436	k_1: 0.0210 k_2:-0.0031	k_1: 0.039 k_2: 0.004	42.8	>1000	-
AMPA: SFO	Accep-table	-	k: 0.0003	5.9	k: 0.325	k: -0.0011	k: 0.002	>1000	>1000	0.437 (±0.033)

For the parent, the visual fit is poor (M_0 is underestimated compared to parent-only DFOP fit) and the parameter k_2 is not significantly different from zero. As the residue data of AMPA are acceptably described, the fitting was repeated with initial parameters for parent (M_0, k_1, k_2 and g) fixed to results from parent-only DFOP fit.

Initial fitting: Glyphosate (DFOP)



Initial fitting: AMPA (SFO)



Repeated fitting: initial parameters for parent fixed to results from parent-only fit

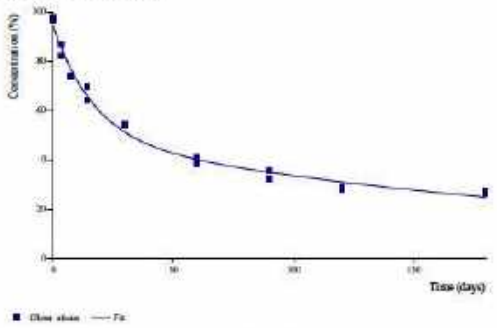
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 94.3	fixed to k_1: 0.0595 k_2: 0.0037 g: 0.4852	3.1	[1]	[1]	[1]	37.5	440	-
AMPA: SFO	Poor	-	k:<0.0001	9.3	k: 0.5	k: -0.0013	k: 0.001	>1000	>1000	0.398 (±0.023)

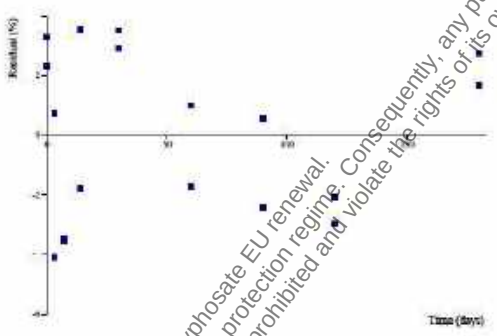
For AMPA, the degradation rate is not significantly different from zero and the visual fit is poor (the fitted curve still increases towards the end of the study while the measured residue data starts to decrease). Thus, the pathway fit is not acceptable. A decline fit for AMPA was not performed due to the limited number of data points after the peak.

Conclusion: Parent-only DFOP fit to be used for deriving modelling endpoints for glyphosate
No modelling endpoints can be derived for AMPA

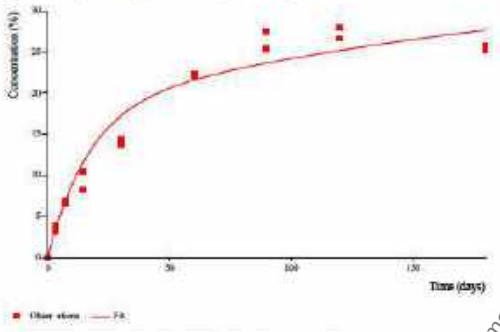
Table 7.1.2.1.1-16: Kinetic models and goodness-of-fit statistics of pathway fits for soil Arrow of study [redacted] (1995, CA 7.1.1.1/005) – modelling endpoints

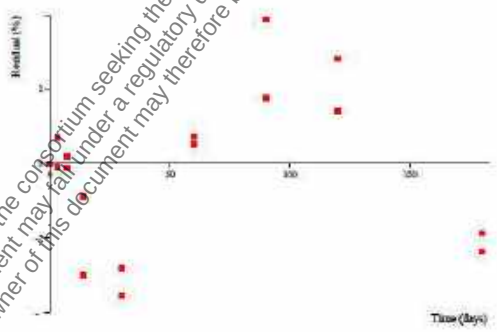
Repeated fitting: Glyphosate (DFOP) – fixed initial parent parameters





Repeated fitting: AMPA (SFO)





[1] Not determined due to fixed parameters

[redacted] (1996, CA 7.1.1.1/003)

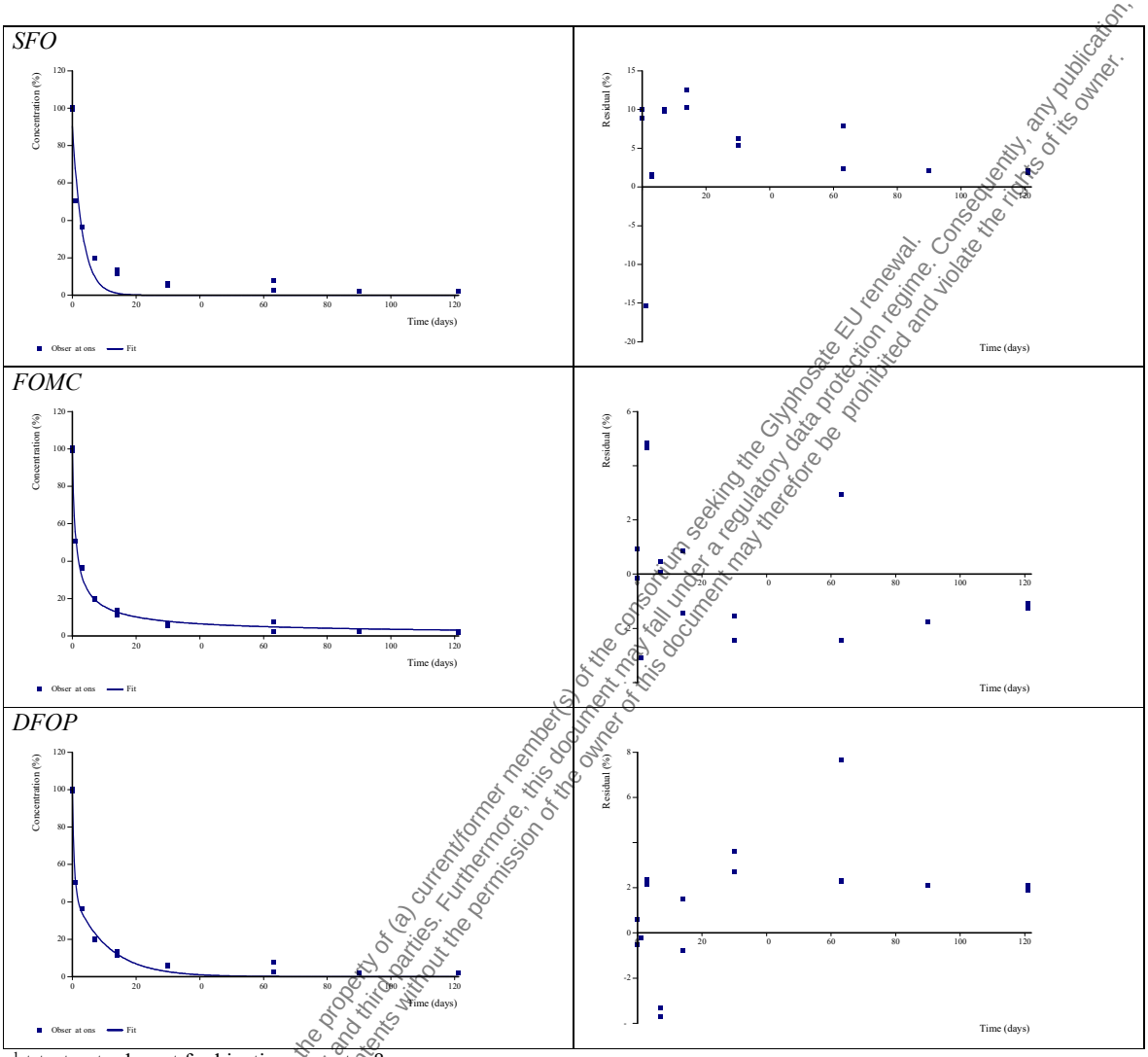
Table 7.1.2.1.1-17: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Soil B of study [redacted] (1996, CA 7.1.1.1/003)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.3	k: 0.3155	25.6	k: <0.001	k: 0.2065	k: 0.425	2.2	7.3
FOMC	Good	99.4	α: 0.6566 β: 0.6408	6.9	-[1]	β: 0.3872	β: 0.894	1.2	20.7
DFOP	Acceptable	99.7	k_1: 1.722 k_2: 0.0937 g: 0.5492	9.0	k_1: <0.001 k_2: <0.001	k_1: 0.8916 k_2: 0.0607	k_1: 2.553 k_2: 0.127	1.0	16.1
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The FOMC model provides the best visual fit (M_0 as well as the residues at the last five sampling dates) and the lowest χ^2 error. Thus, the FOMC model is selected as the best-fit model for parent-only fit. As 10 % of the initial concentration was reached within the experimental period, the FOMC model can also be used for derivation modelling endpoints.

Conclusion: FOMC to be used in pathway fit for trigger endpoints
FOMC to be used in pathway fit for modelling endpoints

Table 7.1.2.1.1-17: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Soil B of study [redacted] (1996, CA 7.1.1.1/003)



[1] t-test not relevant for kinetic parameter β

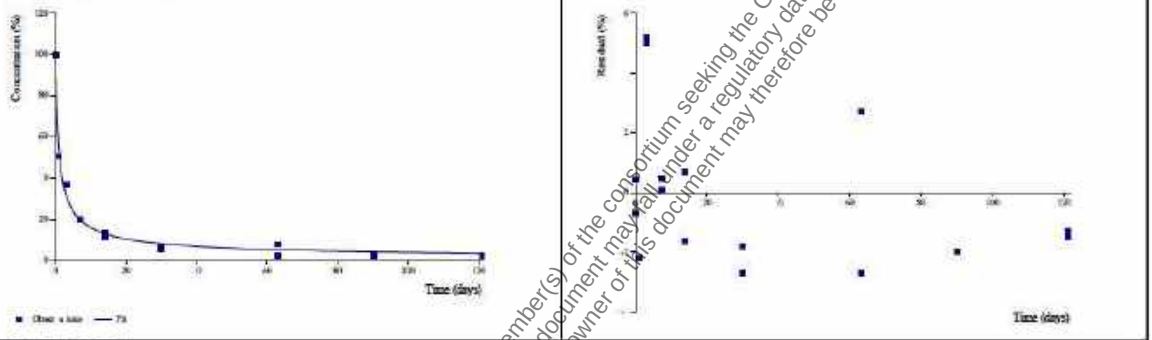
Table 7.1.2.1.1-18: Kinetic models and goodness-of-fit statistics of pathway fit for soil Soil B of study [redacted] (1996, CA 7.1.1.1/003) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (+ std. dev.)
Glypho-sate: FOMC	Good	99.9	α: 0.6314 β: 0.571	7.0	-[1]	β: 0.354	β: 0.788	1.1	21.3	-
AMPA: SFO	Accep-table	-	k: 0.007	8.9	k: <0.001	k: 0.0049	k: 0.009	99.1	330	0.264 (±0.014)

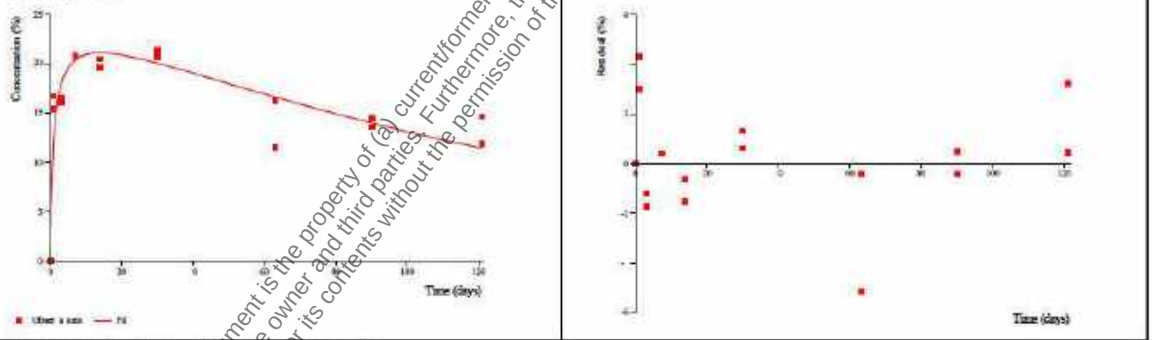
Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

Conclusion: FOMC-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

Glyphosate (FOMC)



AMPA (SFO)



[1] t-test not relevant for kinetic parameter β

[redacted] (1996, CA 7.1.1.1/004)

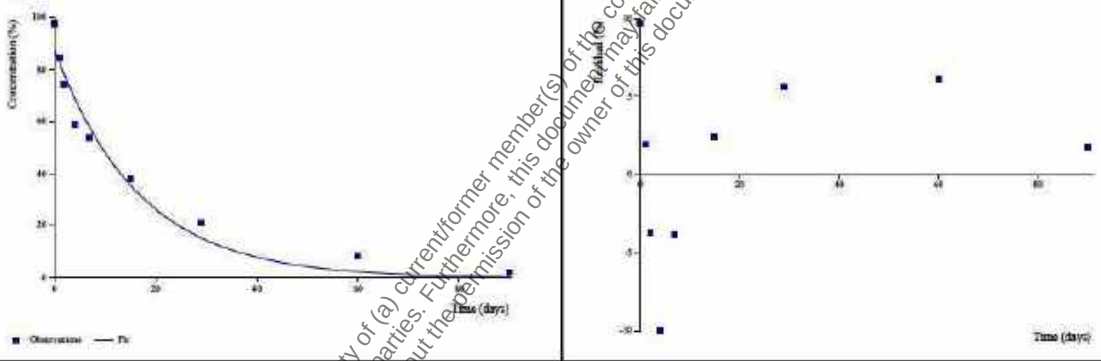
Table 7.1.2.1.1-19: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1 of study [redacted] (1996, CA 7.1.1.1/004)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	88.0	k: 0.0601	9.5	k: <0.001	k: 0.0385	k: 0.082	11.5	38.3
FOMC	Good	94.9	α: 1.13 β: 9.71	5.7	-[1]	β: -0.8596	β: 20.28	8.3	64.8
DFOP	Good	98.3	k_1: 0.474 k_2: 0.0371 g: 0.3278	2.5	k_1: 0.003 k_2: <0.001	k_1: 0.2054 k_2: 0.0288	k_1: 0.743 k_2: 0.046	8.3	51.3
HS	Not calculated								

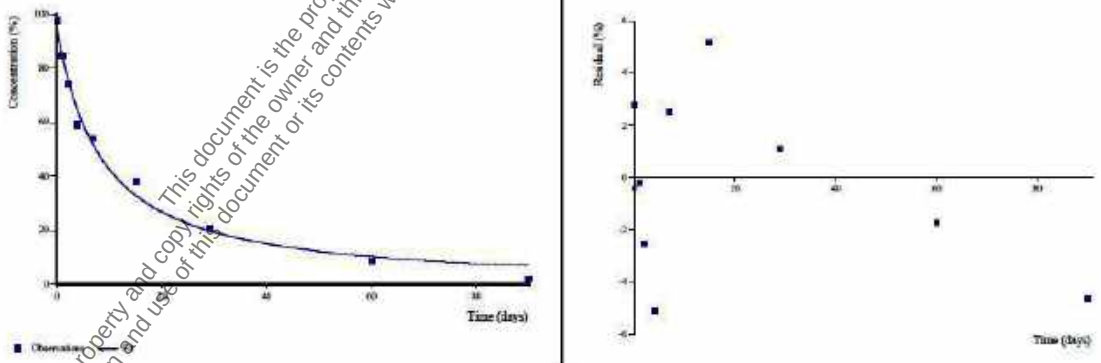
Degradation of glyphosate was best described by the FOMC and DFOP biphasic models. The DFOP model provides a better visual fit (M_0 as well as the residues at the last four sampling dates) and the lowest χ^2 error.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

SFO

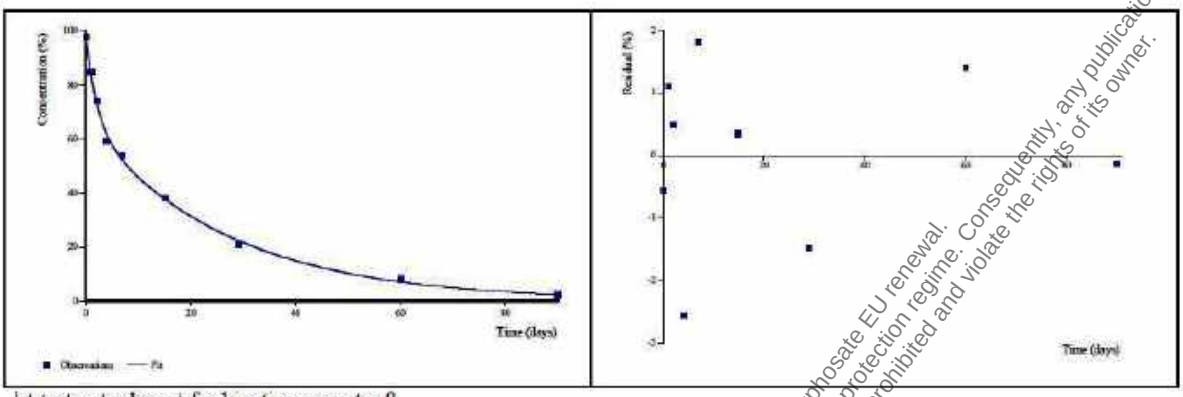


FOMC



DFOP

Table 7.1.2.1.1-19: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1 of study [redacted] (1996, CA 7.1.1.1/004)



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-20: Kinetic models and goodness-of-fit statistics of pathway fit for soil Speyer 2.1 of study [redacted] (1996, CA 7.1.1.1/004) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	98.8	k_1: 0.4904 k_2: 0.0367 g: 0.3344	2.5	k_1: <0.001 k_2: <0.001	k_1: 0.2753 k_2: 0.0300	k_1: 0.705 k_2: 0.043	8.1	51.7	-
AMPA: SFO	Accep-table	-	k: 0.0008	8.4	k: 0.327	k: -0.0032	k: 0.005	829	>1000	0.523 (±0.047)

The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable.

Conclusion: Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate
No trigger endpoints can be derived for AMPA

Glyphosate (DFOP)

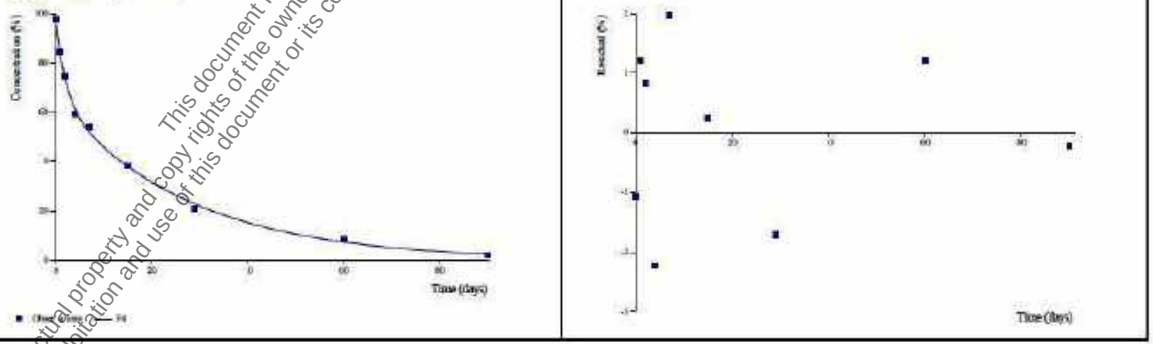


Table 7.1.2.1.1-20: Kinetic models and goodness-of-fit statistics of pathway fit for soil Speyer 2.1 of study [redacted] (1996, CA 7.1.1.1/004) – trigger and modelling endpoints

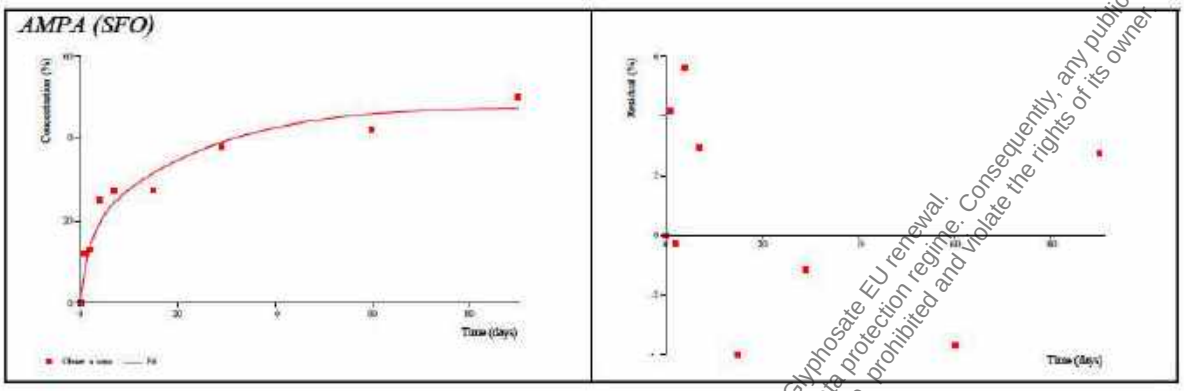


Table 7.1.2.1.1-21: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.2 of study [redacted] (1996, CA 7.1.1.1/004)

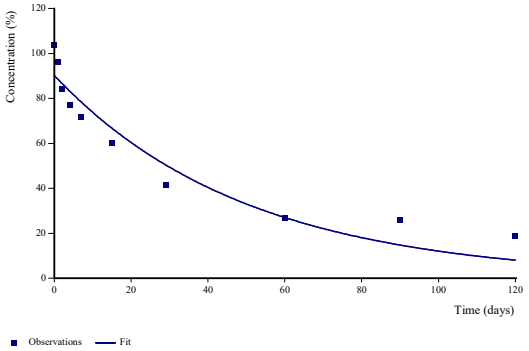
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.3	k: 0.0201	11.0	k: 0.001	k: 0.0119	k: 0.028	34.5	115
FOMC	Good	101.3	α: 0.5744 β: 7.976	4.0		β: 1.585	β: 14.37	18.7	431
DFOP	Good	100.7	k_1: 0.1338 k_2: 0.0098 g: 0.4358	5.0	k_1: 0.023 k_2: 0.005	k_1: 0.0040 k_2: 0.0034	k_1: 0.264 k_2: 0.016	19.3	176
HS	Not calculated								

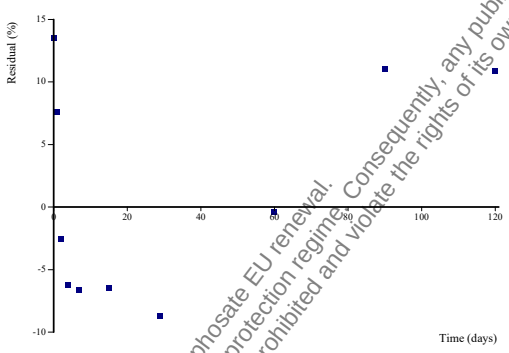
Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide similar reliable and visually acceptable results. As 10 % of the initial concentration was not reached within the experimental period, the DFOP model is selected as the best-fit model as well as for deriving modelling endpoints.

Conclusion: DFOP to be used in pathway fits for trigger endpoints
DFOP to be used in pathway fits for modelling endpoints

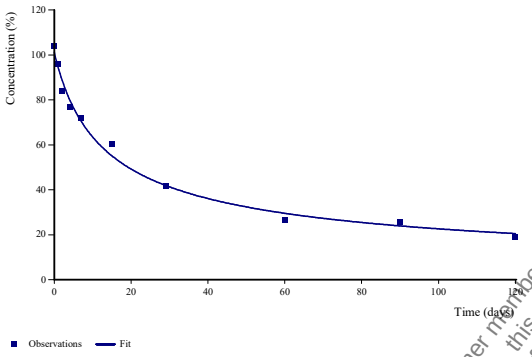
Table 7.1.2.1.1-21: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.2 of study [redacted] (1996, CA 7.1.1.1/004)

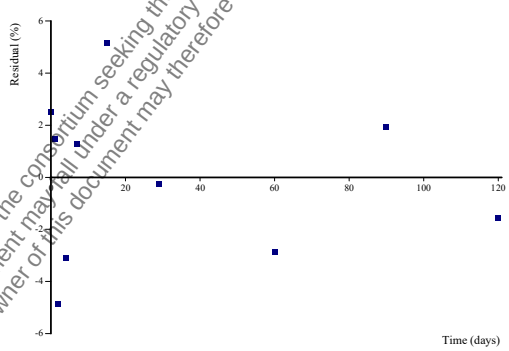
SFO



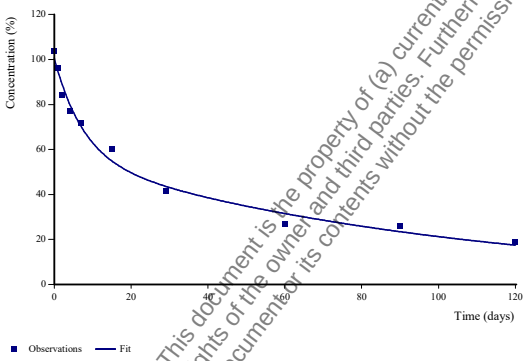


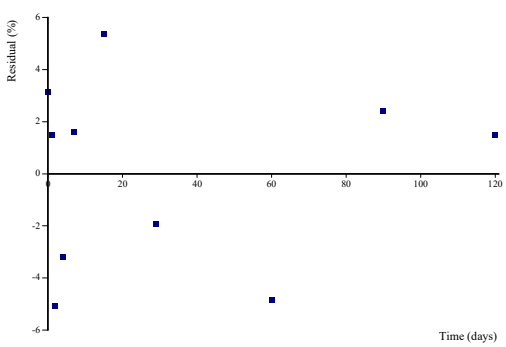
FOMC





DFOP





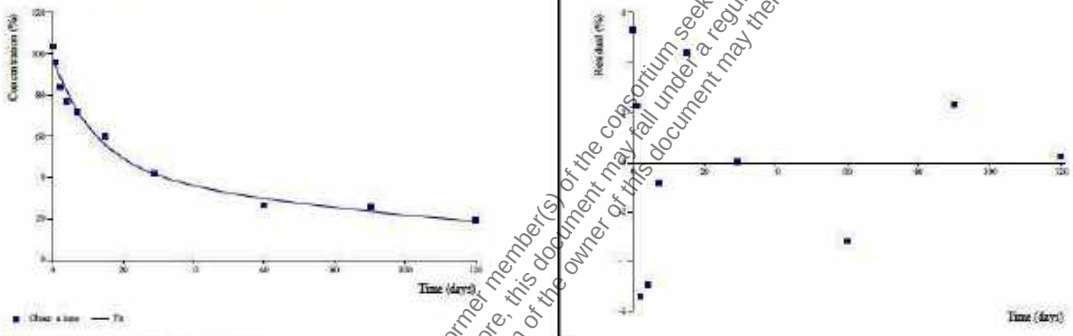
[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-22: Kinetic models and goodness-of-fit statistics of pathway fit for soil Speyer 2.2 of study [redacted] (1996, CA 7.1.1.1/004) – trigger and modelling endpoints

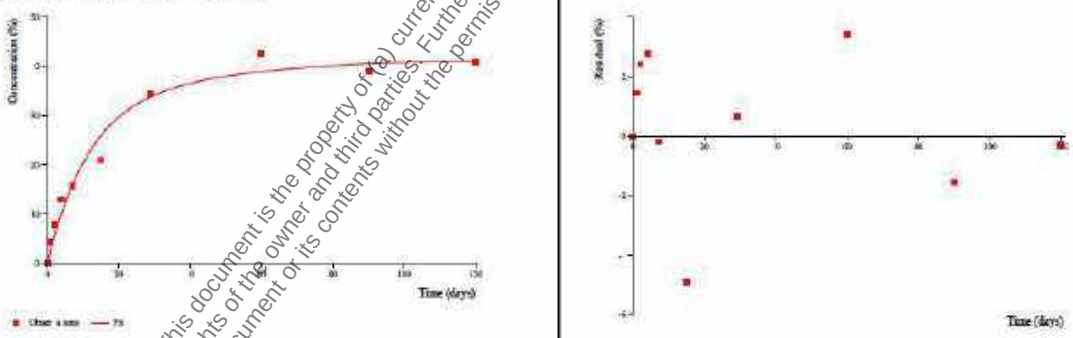
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Initial fitting										
Glypho-sate: DFOP	Accep-table	98.5	k_1: 0.0858 k_2: 0.0076 g: 0.5275	5.1	k_1: 0.001 k_2: 0.016	k_1: 0.0354 k_2: 0.0007	k_1: 0.136 k_2: 0.014	20.1	205	-
AMPA: SFO	Good	-	k: 0.0019	8.1	k: 0.122	k: -0.0015	k: 0.005	362	>1000	0.618 (±0.071)

For glyphosate the visual fit is acceptable but M_0 is underestimated compared to the measured value. As the residue data of AMPA are well described, the fitting was repeated with initial parameter M_0 for parent fixed to the measured initial concentration (103.8 %).

Initial fitting: Glyphosate (DFOP)



Initial fitting: AMPA (SFO)



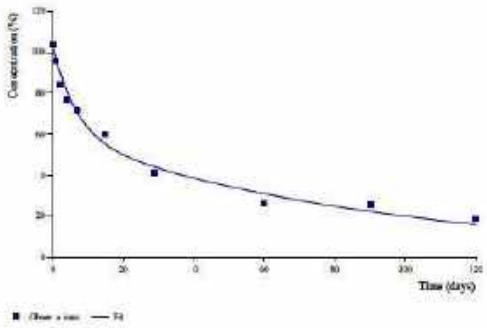
Repeated fitting 1: parent M_0 fixed to measured initial concentration

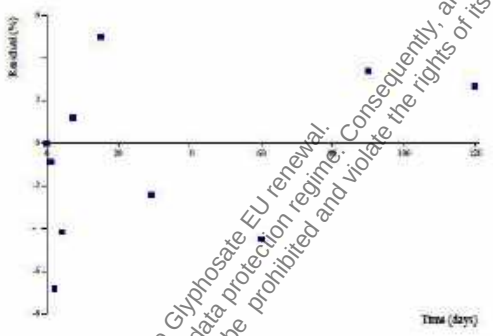
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 103.8	k_1: 0.1507 k_2: 0.0108 g: 0.4259	5.9	k_1: 0.001 k_2: <0.001	k_1: 0.0621 k_2: 0.0056	k_1: 0.239 k_2: 0.016	18.1	162	-
AMPA: SFO	Accep-table	-	k: 0.0014	9.2	k: 0.191	k: 0.0019	k: 0.005	497	>1000	0.548 (±0.052)

The visual fit of glyphosate improved. For AMPA, the parameter k is not significantly different from zero, but overall the formation of AMPA is well described and the estimated formation fraction is plausible with a low standard deviation. Therefore, the fitting was repeated again with additionally fixing ff for AMPA to the estimated value (0.548).

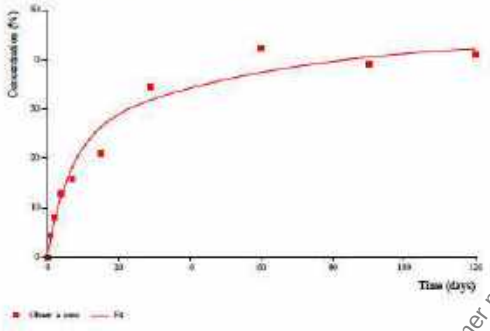
Table 7.1.2.1.1-22: Kinetic models and goodness-of-fit statistics of pathway fit for soil Speyer 2.2 of study [redacted] (1996, CA 7.1.1.1/004) – trigger and modelling endpoints

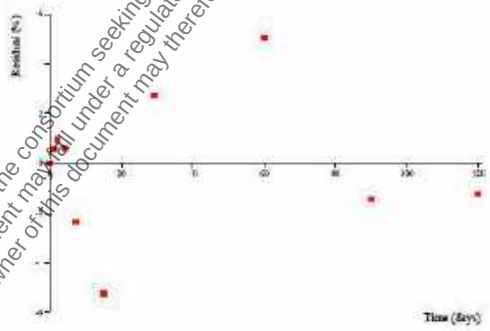
Repeated fitting 1: Glyphosate (DFOP) – fixed parent M_0





Repeated fitting 1: AMPA (SFO)





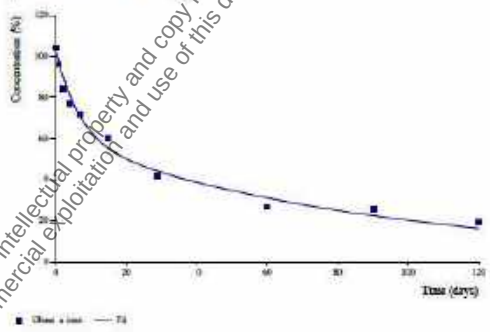
Repeated fitting 2: parent M_0 fixed to measured initial concentration, AMPA ff fixed to previously estimated value

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	Error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 103.8	k_1: 0.1507 k_2: 0.0108 g: 0.4139	5.9	k_1: <0.001 k_2: <0.001	k_1: 0.0667 k_2: 0.0060	k_1: 0.235 k_2: 0.016	18.1	162	-
AMPA: SFO	Accep-table	-	k: 0.0014	8.8	k: 0.043	k: -0.0002	k: 0.003	497	>1000	fixed to 0.548

The estimated parameters for glyphosate and AMPA are statistically reliable.

Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

Repeated fitting 2: Glyphosate (DFOP) – fixed parent M_0 and metabolite ff



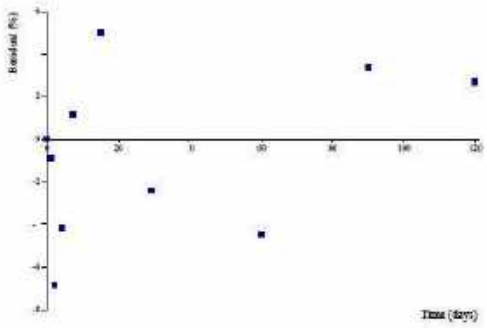
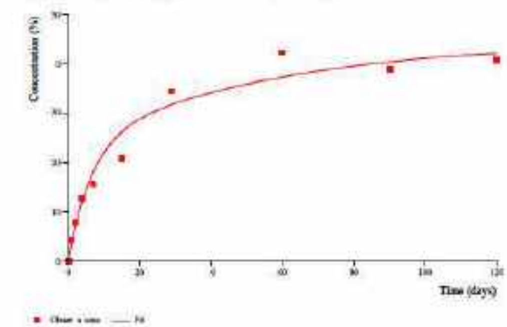


Table 7.1.2.1.1-22: Kinetic models and goodness-of-fit statistics of pathway fit for soil Speyer 2.2 of study [redacted] (1996, CA 7.1.1.1/004) – trigger and modelling endpoints

Repeated fitting 2: AMPA (SFO)



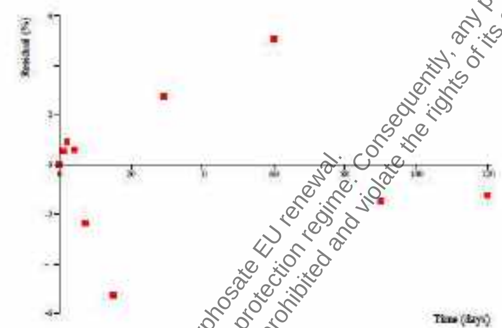


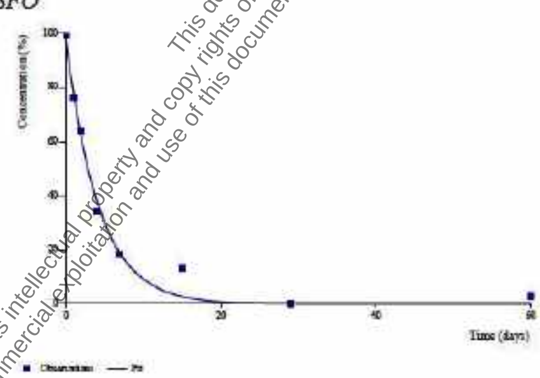
Table 7.1.2.1.1-23: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.3, 20 °C, of study [redacted] (1996, CA 7.1.1.1/004)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	98.3	k: 0.2365	8.8	k: <0.001	k: 0.1771	k: 0.296	2.9	9.7
FOMC	Good	100.3	α: 2.691 β: 9.209	7.7	-1	β: -9.483	β: 27.9	2.7	12.5
DFOP	Good	100.1	k_1: 0.3169 k_2: 0.0497 g: 0.8345	7.5	k_1: 0.016 k_2: 0.252	k_1: 0.0433 k_2:-0.1388	k_1: 0.59 k_2: 0.238	2.7	13.0
HS	Not calculated								

The SFO model provides an acceptable visual and statistically reliable fit. The FOMC and DFOP bi-phasic models improve the visual fit. The DFOP model provides the best visual fit and the lowest χ^2 error. The parameter k_2 of the DFOP model is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k_1 as indicated by a high value for parameter g (0.8345).

Conclusion: DFOP to be used in pathway fit for trigger endpoints
SFO to be used in pathway fit for modelling endpoints

SFO



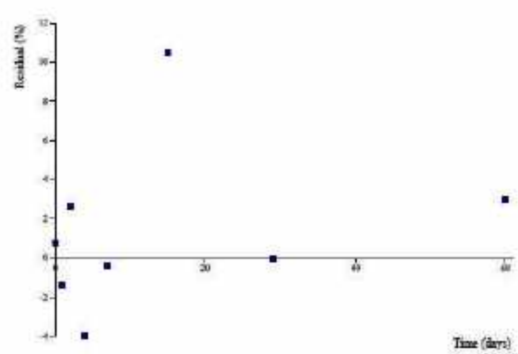
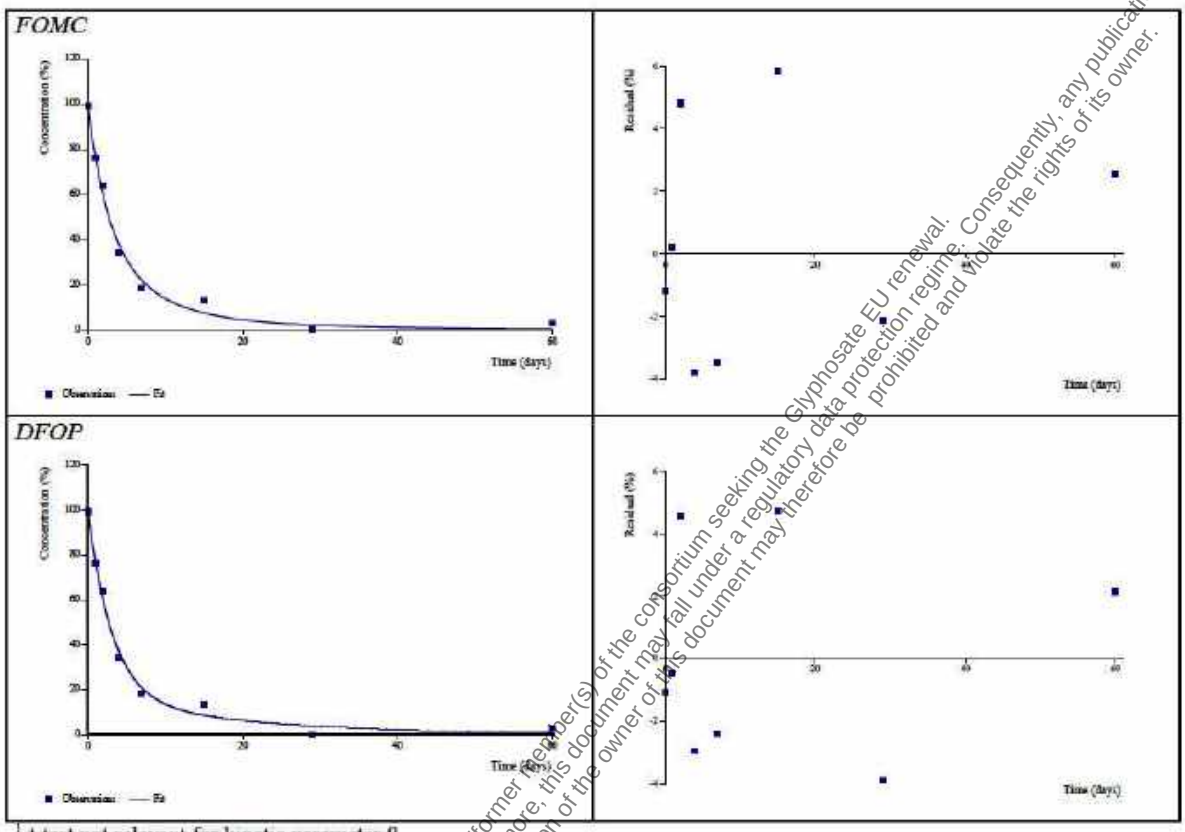


Table 7.1.2.1.1-23: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.3, 20 °C, of study [redacted] (1996, CA 7.1.1.1/004)



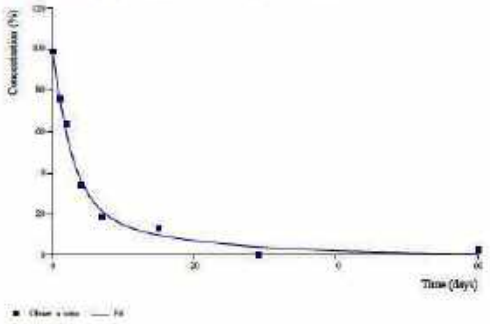
[1] t-test not relevant for kinetic parameter β

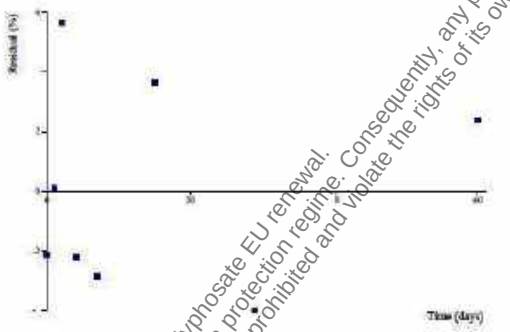
Table 7.1.2.1.1-24: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.3 (20 °C) of study [redacted] (1996, CA 7.1.1.1/004) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Initial fitting										
Glypho-sate: DFOP	Good	101.2	k_1: 0.3621 k_2: 0.0599 g: 0.7742	7.7	k_1: 0.006 k_2: 0.162	k_1: 0.1025 k_2: -0.07	k_1: 0.622 k_2: 0.19	2.6	14.3	-
AMPA: SFO	Good	-	k: 0.0166	14.8	k: 0.015	k: 0.0021	k: 0.031	41.7	138	0.434 (±0.059)
The visual fits for glyphosate and AMPA are good, but for glyphosate the parameter k_2 of is not significantly different from zero. As the residue data of AMPA are well described, the fitting was repeated with initial parameters for parent (M_0, k_1, k_2 and g) fixed to results from parent-only DFOP fit.										

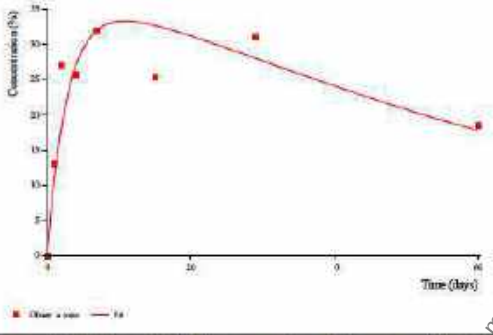
Table 7.1.2.1.1-24: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.3 (20 °C) of study [redacted] (1996, CA 7.1.1.1/004) – trigger endpoints

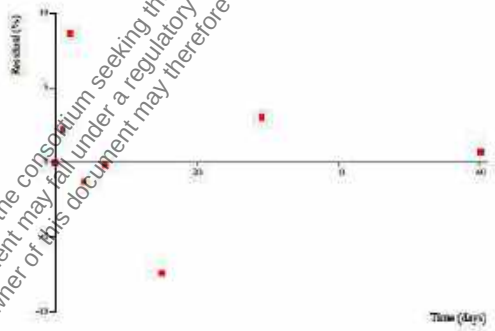
Initial fitting: Glyphosate (DFOP)





Initial fitting: AMPA (SFO)





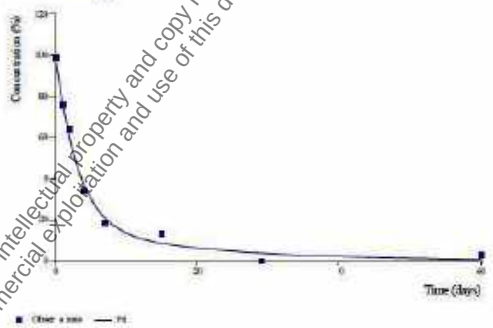
Repeated fitting: initial parameters for parent fixed to results from parent-only fit

Kinetic model	Visual assess-ment	M₀	Kinetic para-meters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT₅₀ (d)	DT₉₀ (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 100.1	fixed to k_1: 0.3169 k_2: 0.0497 g: 0.8395	2.9	-[1]	-[1]	-[1]	2.7	13.0	-
AMPA: SFO	Good	-	k: 0.0168	15.8	k: 0.007	k: 0.0040	k: 0.03	41.4	137	0.440 (±0.048)

The repeated fitting resulted in reliable degradation parameters for glyphosate and AMPA.

Conclusion: DFOP-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

Glyphosate (DFOP) – fixed input parameters from parent-only fit



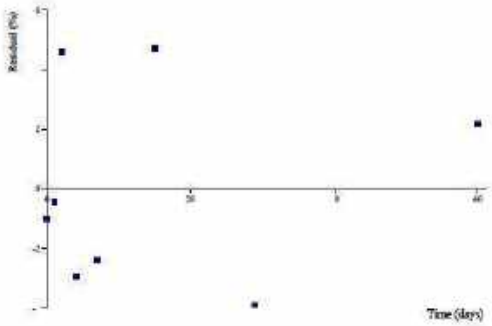
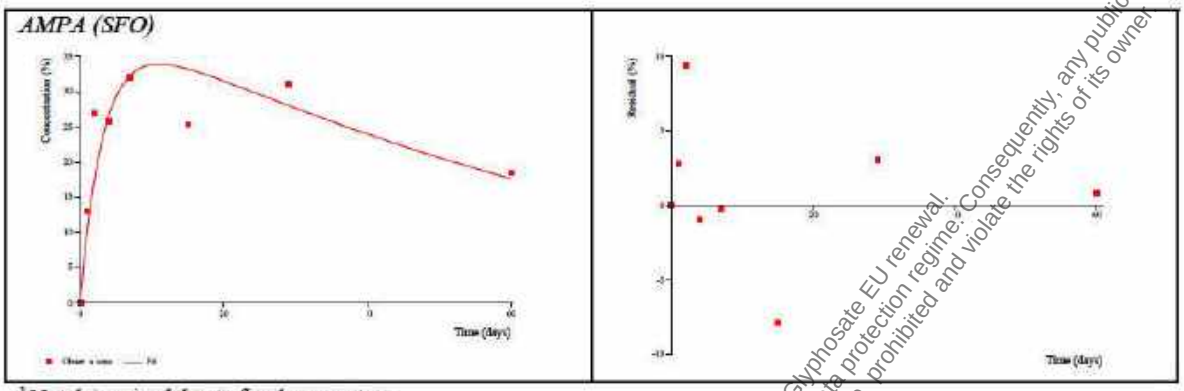


Table 7.1.2.1.1-24: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.3 (20 °C) of study [redacted] (1996, CA 7.1.1.1/004) – trigger endpoints

AMPA (SFO)



[1] Not determined due to fixed parameters

Table 7.1.2.1.1-25: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.3 (20 °C) of study [redacted] (1996, CA 7.1.1.1/004) – modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate SFO	Accep-table	99.4	k: 0.2476	8.9	k: <0.001	k: 0.1904	k: 0.305	2.8	9.3	-
AMPA: SFO	Accep-table	-	k: 0.0161	18.8	k: 0.022	k: <0.001	k: 0.032	43.1	143	0.424 (±0.065)

The degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

Conclusion: DFOP-SFO to be used for deriving modelling endpoints for glyphosate and AMPA.

Glyphosate (SFO)

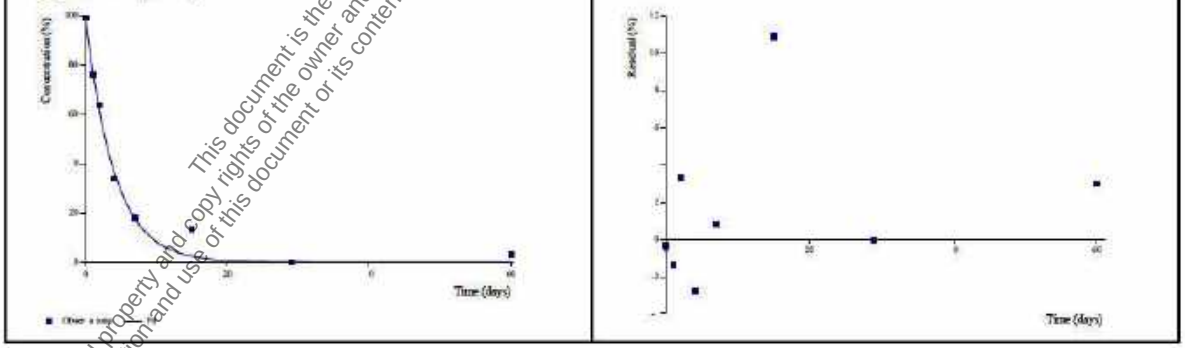


Table 7.1.2.1.1-25: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.3 (20 °C) of study [redacted] (1996, CA 7.1.1.1/004) – modelling endpoints

AMPA (SFO)

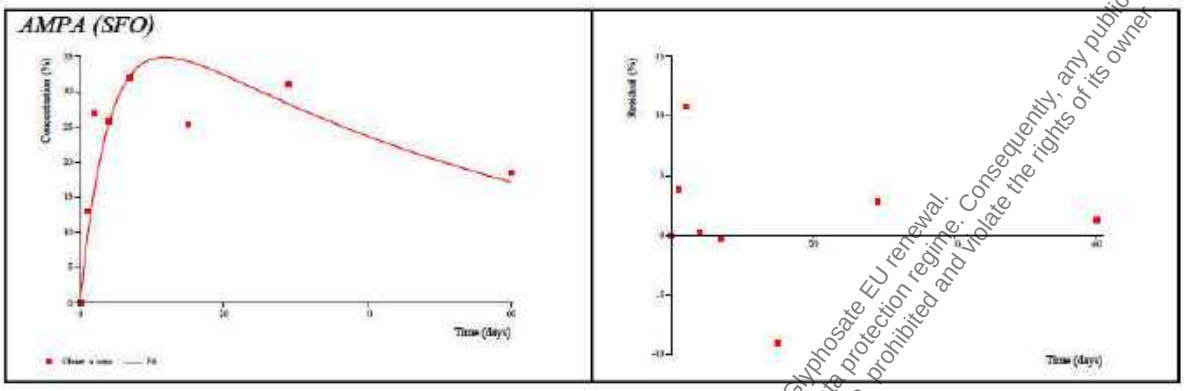


Table 7.1.2.1.1-26: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.3, 10 °C, of study [redacted] (1996, CA 7.1.1.1/004)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	91.9	k: 0.0641	7.7	k: <0.001	k: 0.0424	k: 0.086	10.8	35.9
FOMC	Good	98.3	α: 1.115 β: 9.422	3.3		β: 1.638	β: 17.21	8.1	64.9
DFOP	Good	99.5	k_1: 0.3001 k_2: 0.0361 g: 0.3756	2.3	k_1: 0.010 k_2: 0.001	k_1: 0.0807 k_2: 0.0221	k_1: 0.52 k_2: 0.05	8.1	50.8
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (the residues at the last three sampling dates) and the lowest χ^2 error.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

SFO

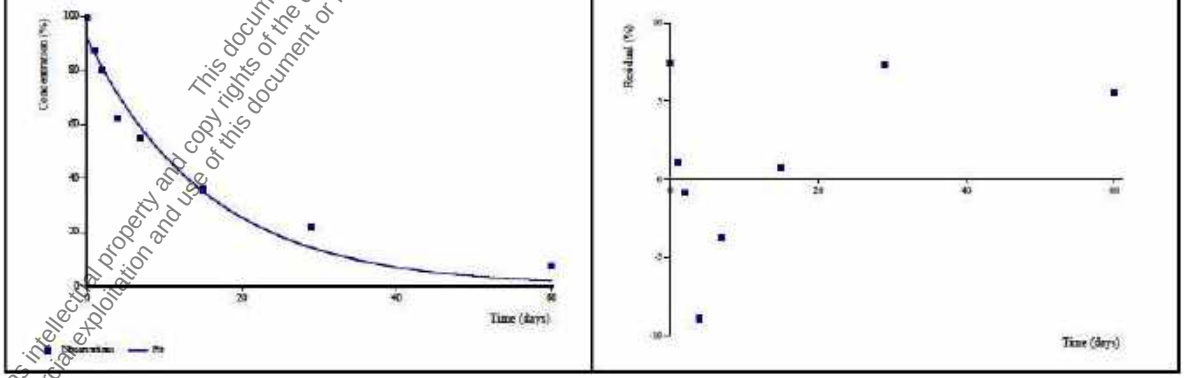
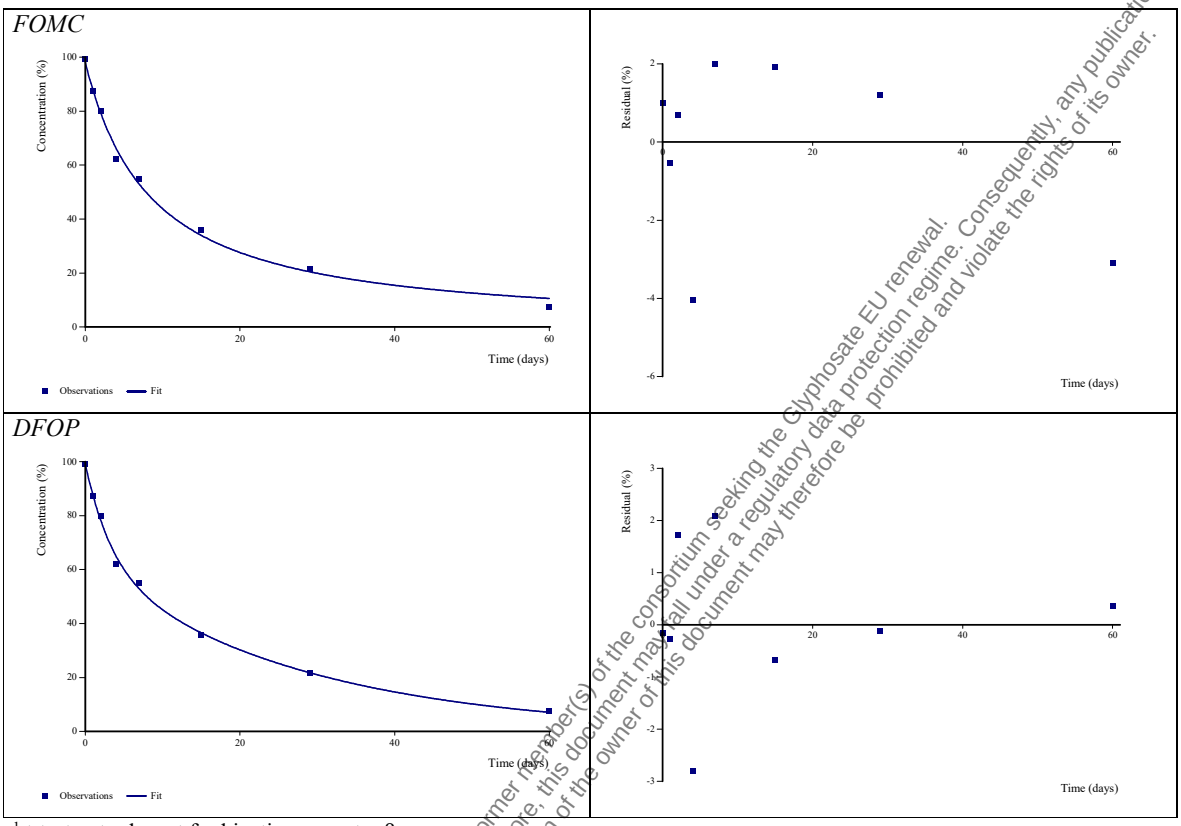


Table 7.1.2.1.1-26: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.3, 10 °C, of study [redacted] (1996, CA 7.1.1.1/004)



[1] t-test not relevant for kinetic parameter β

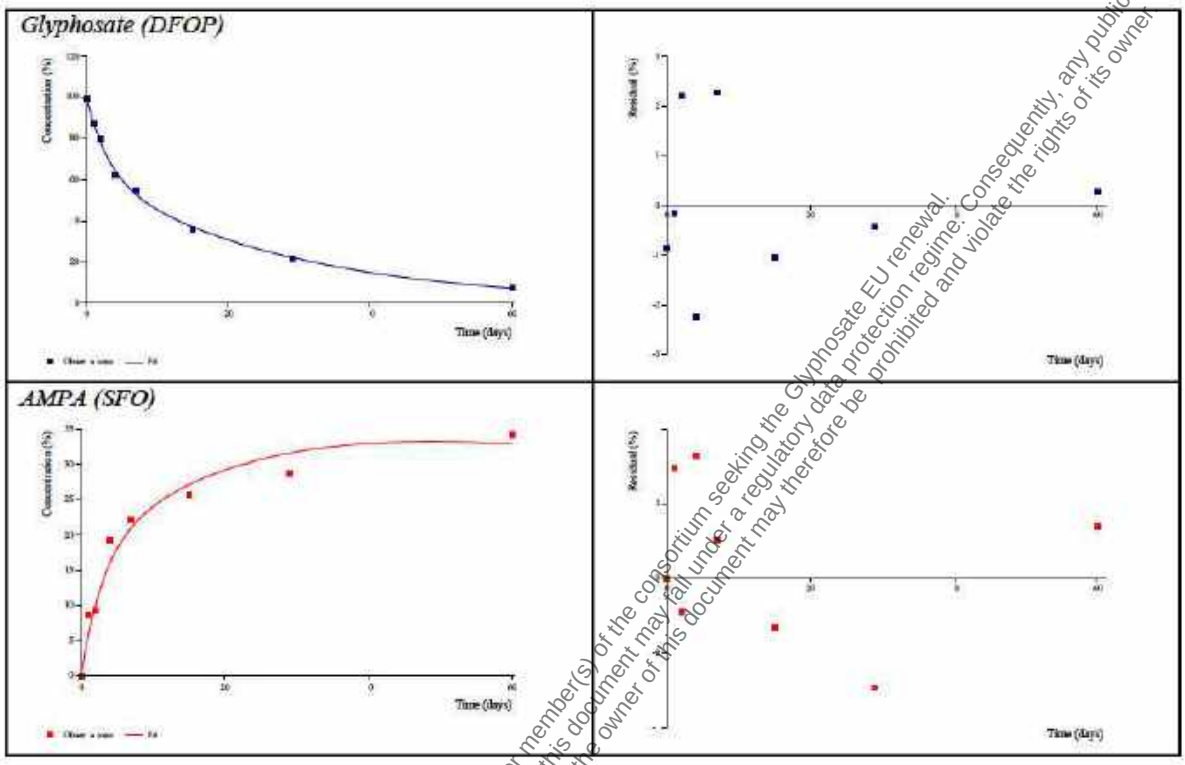
Table 7.1.2.1.1-27: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.3 (10 °C) of study [redacted] (1996, CA 7.1.1.1/004) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	100.2	k_1: 0.3317 k_2: 0.0361 g: 0.37	2.4	k_1: 0.001 k_2: <0.001	k_1: 0.157 k_2: 0.0260	k_1: 0.506 k_2: 0.046	7.9	50.9	-
AMPA: SFO	Good	-	k: 0.0054	8.2	k: 0.047	k: -0.0011	k: 0.012	129	429	0.454 (±0.040)

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

Table 7.1.2.1.1-27: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.3 (10 °C) of study [redacted] (1996, CA 7.1.1.1/004) – trigger and modelling endpoints

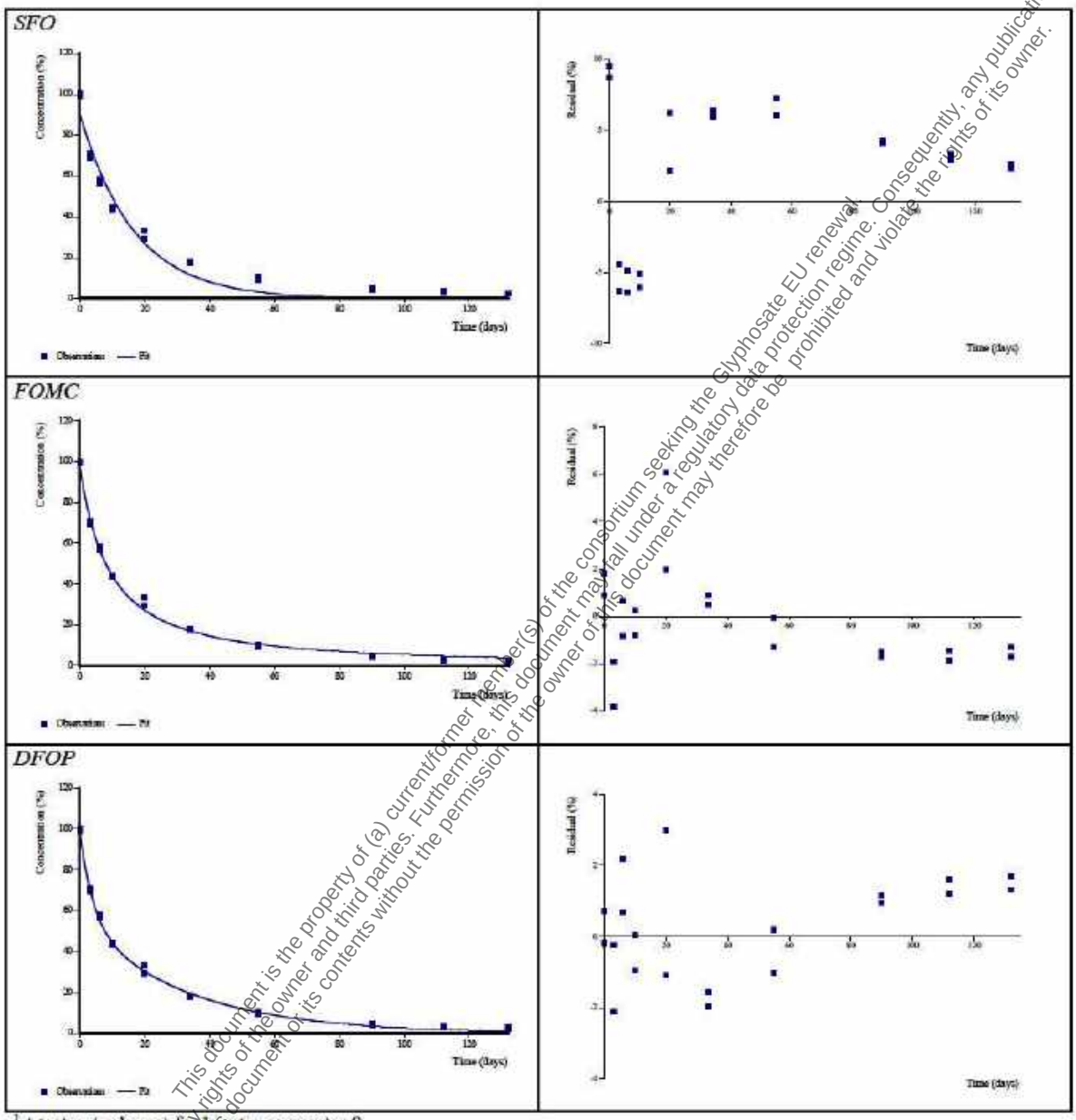


[redacted] (2010, CA 7.1.1.1/001)

Table 7.1.2.1.1-28: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Gartenacker of study [redacted] (2010, CA 7.1.1.1/001)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.5	k: 0.0604	13.1	k: <0.001	k: 0.0488	k: 0.072	11.5	38.1
FOMC	Good	98.3	α: 1.2563 β: 11.2442	4.6	-[1]	β: 7.6729	β: 14.815	8.3	59.1
DFOP	Good	99.4	k_1: 0.2486 k_2: 0.0305 g: 0.4446	3.0	k_1: <0.001 k_2: <0.001	k_1: 0.1837 k_2: 0.0259	k_1: 0.313 k_2: 0.035	7.9	56.2
HS	Not calculated								
Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide similar visual fits but the DFOP model provides the lowest χ^2 error. **Conclusion:** DFOP to be used in pathway fit for trigger endpoints DFOP to be used in pathway fit for modelling endpoints									

Table 7.1.2.1.1-28: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Gartenacker of study [redacted] (2010, CA 7.1.1.1/001)



[1] t-test not relevant for kinetic parameter β

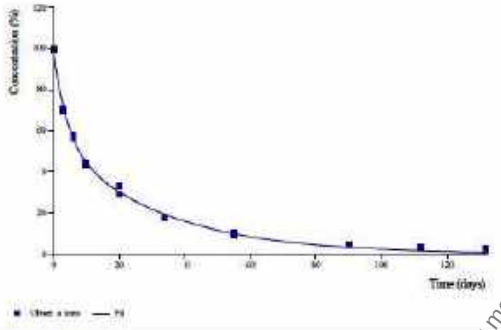
Table 7.1.2.1.1-29: Kinetic models and goodness-of-fit statistics of pathway fits for soil Gartenacker of study [redacted] (2010, CA 7.1.1.1/001) – trigger and modelling endpoints

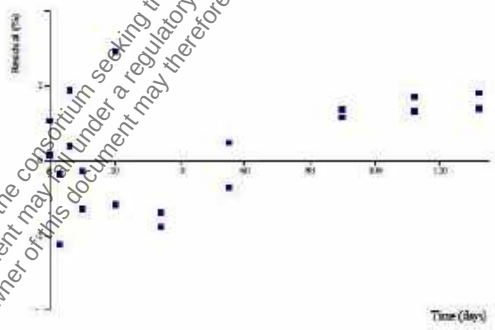
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	99.0	k_1: 0.2501 k_2: 0.0314 g: 0.4307	3.1	k_1: <0.001 k_2: <0.001	k_1: 0.185 k_2: 0.0269	k_1: 0.315 k_2: 0.036	8.1	553	-
AMPA: SFO	Accep-table	-	k: 0.0058	8.2	k: <0.001	k: 0.0042	k: 0.007	119	396	0.183 (±0.009)

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

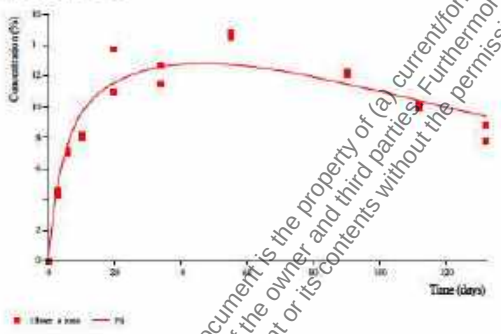
Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

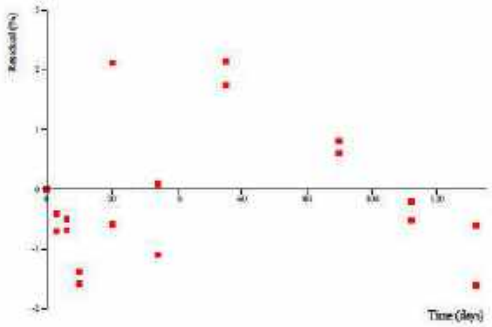
Glyphosate (DFOP)





AMPA (SFO)





[redacted] (1992, CA 7.1.2.1.1/005)

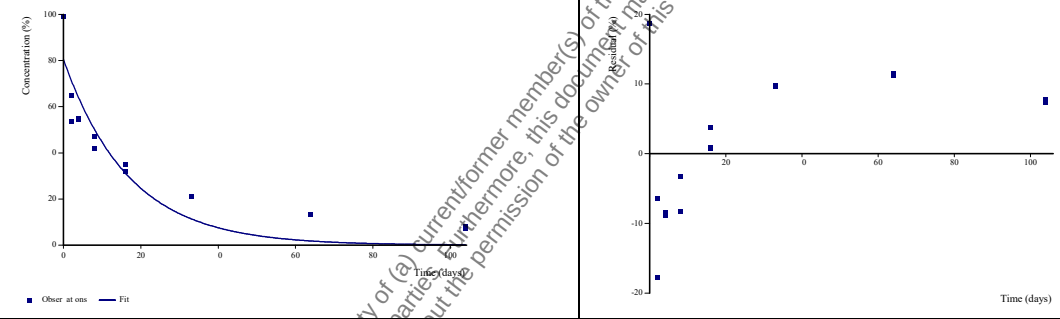
Table 7.1.2.1.1-30: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1, dose group A (20° C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	80.5	k: 0.0592	20.2	k: <0.001	k: 0.0322	k: 0.086	11.7	38.9
FOMC	Good	98.0	α: 0.4984 β: 1.6154	7.2	-[1]	β: 0.5095	β: 2.7210	4.9	162
DFOP	Good	99.0	k_1: 0.7469 k_2: 0.0245 g: 0.4592	5.6	k_1: <0.001 k_2: <0.001	k_1: 0.3338 k_2: 0.0176	k_1: 1.16 k_2: 0.031	4.5	68.9
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide similar visual fits but the DFOP model provides the lowest χ^2 error.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

SFO



FOMC

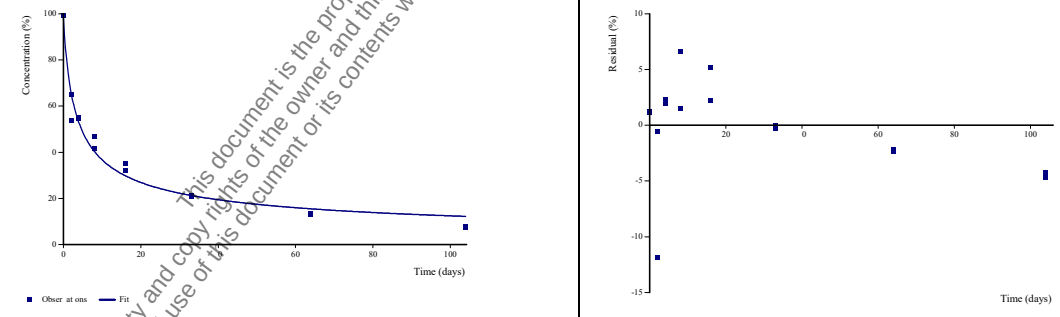
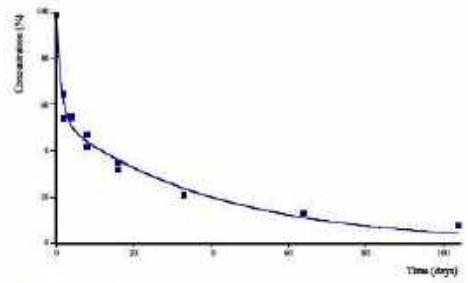
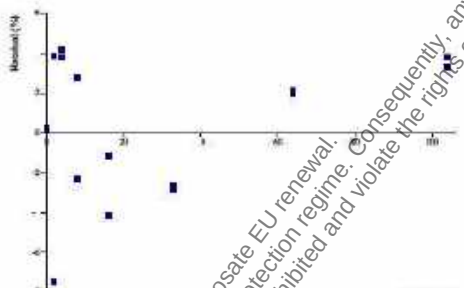


Table 7.1.2.1.1-30: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1, dose group A (20° C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005)

DFOP



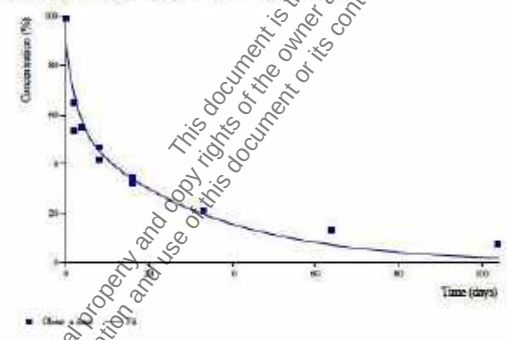


[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-31: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group A (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Initial fitting										
Glypho-sate: DFOP	Poor	90.6	k_1: 0.3946 k_2: 0.0322 g: 0.3697	12.0	k_1: <0.001 k_2: <0.001	k_1: 0.1762 k_2: 0.0202	k_1: 0.613 k_2: 0.044	8.1	57.2	-
AMPA: SFO	Good	-	k: 0.003	3.2	k: 0.002	k: 0.0011	k: 0.005	228	757	0.441 (±0.042)
For the parent, the visual fit is poor (M_0 is underestimated compared to parent-only DFOP fit). As the residue data of AMPA are well described, the fitting was repeated with initial parameters for parent (M_0, k_1, k_2 and g) fixed to results from parent-only DFOP fit.										

Initial fitting: Glyphosate (DFOP)



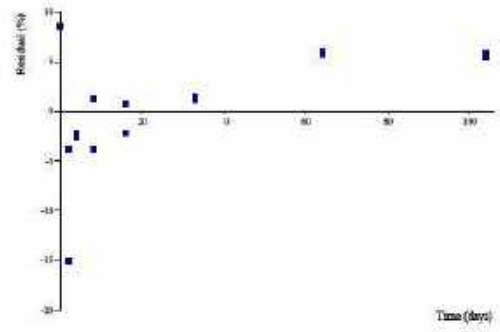
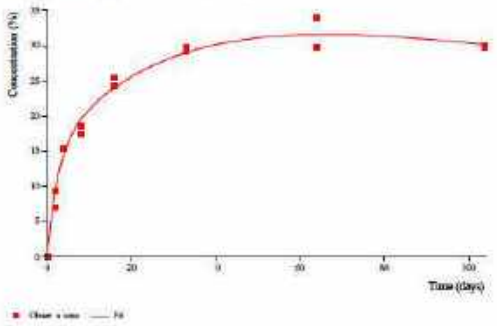
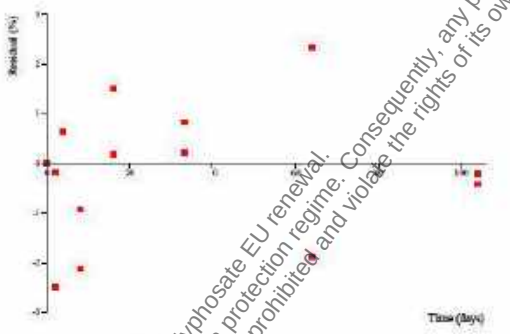


Table 7.1.2.1.1-31: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group A (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

Initial fitting: AMPA (SFO)





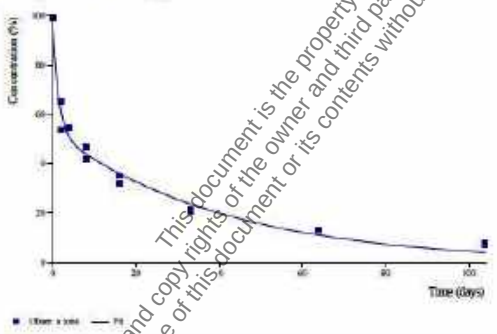
Repeated fitting: initial parameters for parent fixed to results from parent-only fit

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 99.0	fixed to k_1: 0.7469 k_2: 0.0245 g: 0.4592	5.7	- [1]		- [1]	4.5	68.9	-
AMPA: SFO	Poor	-	k: 0.0008	10.1	0.251	k: -0.0015	k: 0.003	906	>1000	0.357 (0.021)

For AMPA, the degradation rate is not significantly different from zero and the visual fit is poor (the fitted curve still increases towards the end of the study while the measured residue data starts to decrease). Thus, the pathway fit is not acceptable. A decline fit for AMPA was not performed due to the limited number of data points after the peak.

Conclusion: Parent-only DFOP fit to be used for deriving trigger and modelling endpoints for glyphosate
No trigger and modelling endpoints can be derived for AMPA

Glyphosate (DFOP) – fixed input parameters for parent from parent-only fit



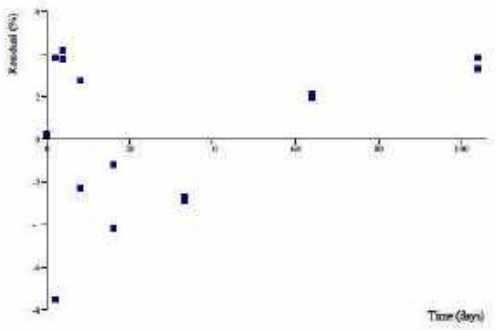
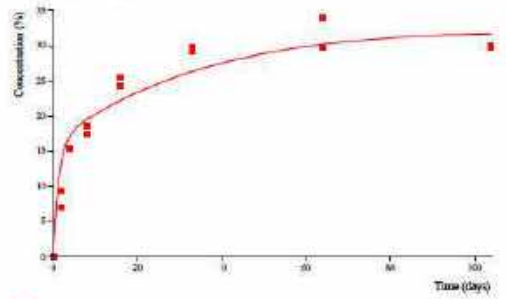
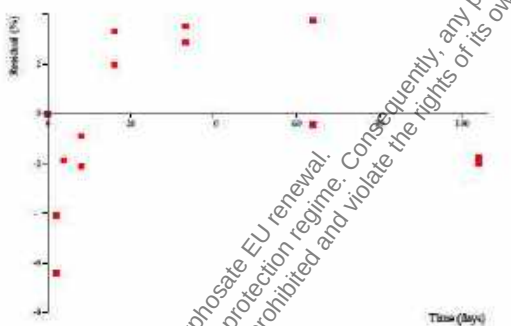


Table 7.1.2.1.1-31: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group A (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

AMPA (SFO)





[1] Not determined due to fixed parameters

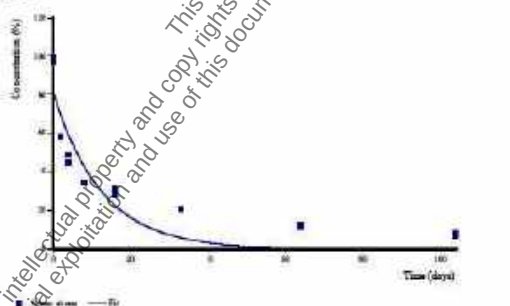
Table 7.1.2.1.1-32: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1, dose group B (20° C, 20 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (95 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	81.4	k: 0.0804	24.6	<0.001	k: 0.0362	k: 0.125	8.6	28.6
FOMC	Good	98.6	α: 0.4762 β: 1.0519	4.8	-[1]	β: 0.5975	β: 1.506	3.5	131
DFOP	Good	98.6	k_1: 0.5105 k_2: 0.0182 g: 0.5962	2.8	k_1: <0.001 k_2: <0.001	k_1: 0.4200 k_2: 0.0148	k_1: 0.601 k_2: 0.022	3.2	76.7
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (the residues at the last four sampling dates) and the lowest χ^2 error.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints period

SFO



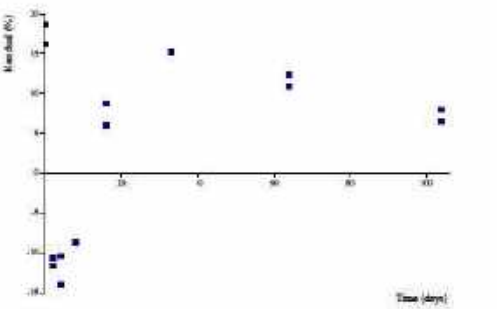
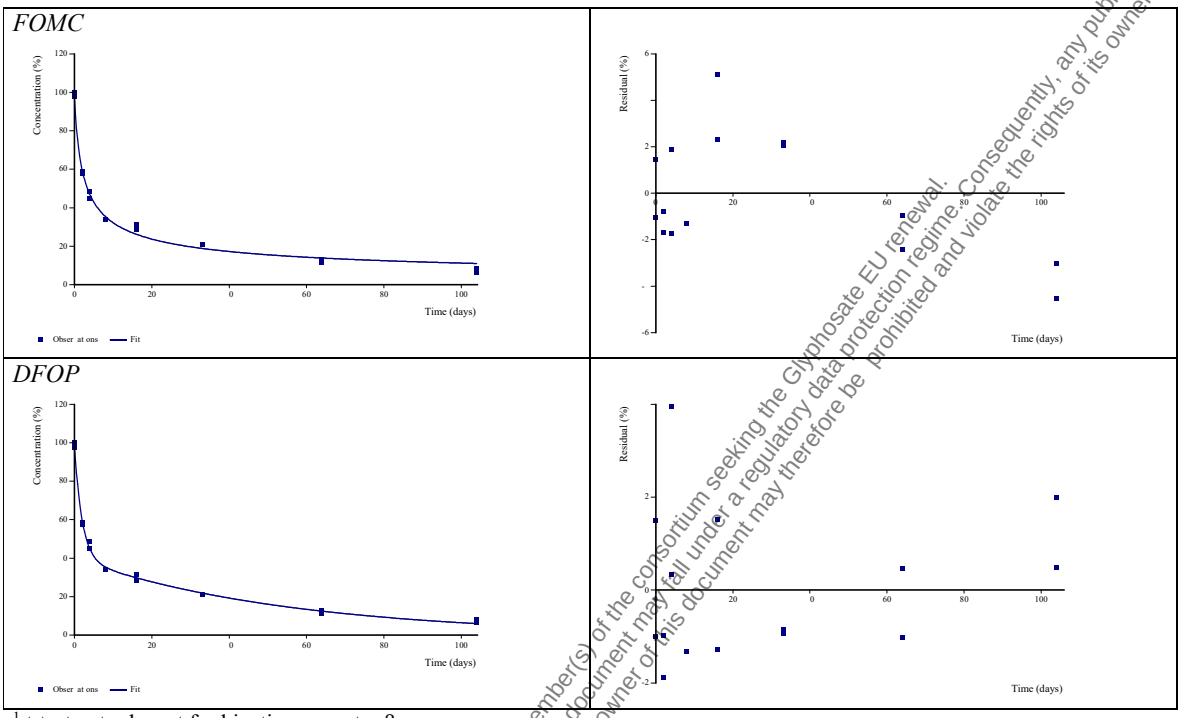


Table 7.1.2.1.1-32: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1, dose group B (20° C, 20 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005)



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-33: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group B (20 °C, 20 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	98.4	k_1: 0.5067 k_2: 0.0185 g: 0.5927	2.8	k_1: <0.001 k_2: <0.001	k_1: 0.4222 k_2: 0.0153	k_1: 0.591 k_2: 0.022	3.2	75.8	-
AMPA: SFO	Poor		k: <0.0001	9.3	k: 0.5	k: -0.0024	k: 0.002	>1000	>1000	0.306 (±0.020)

For AMPA, the degradation rate is not significantly different from zero and the visual fit is poor (the fitted curve still increases towards the end of the study while the measured residue data starts to decrease). Thus, the pathway fit is not acceptable. A decline fit for AMPA was not performed due to the limited number of data points after the peak.

Conclusion: Parent-only DFOP fit to be used for deriving trigger and modelling endpoints for glyphosate
No trigger and modelling endpoints can be derived for AMPA

Table 7.1.2.1.1-33: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group B (20 °C, 20 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

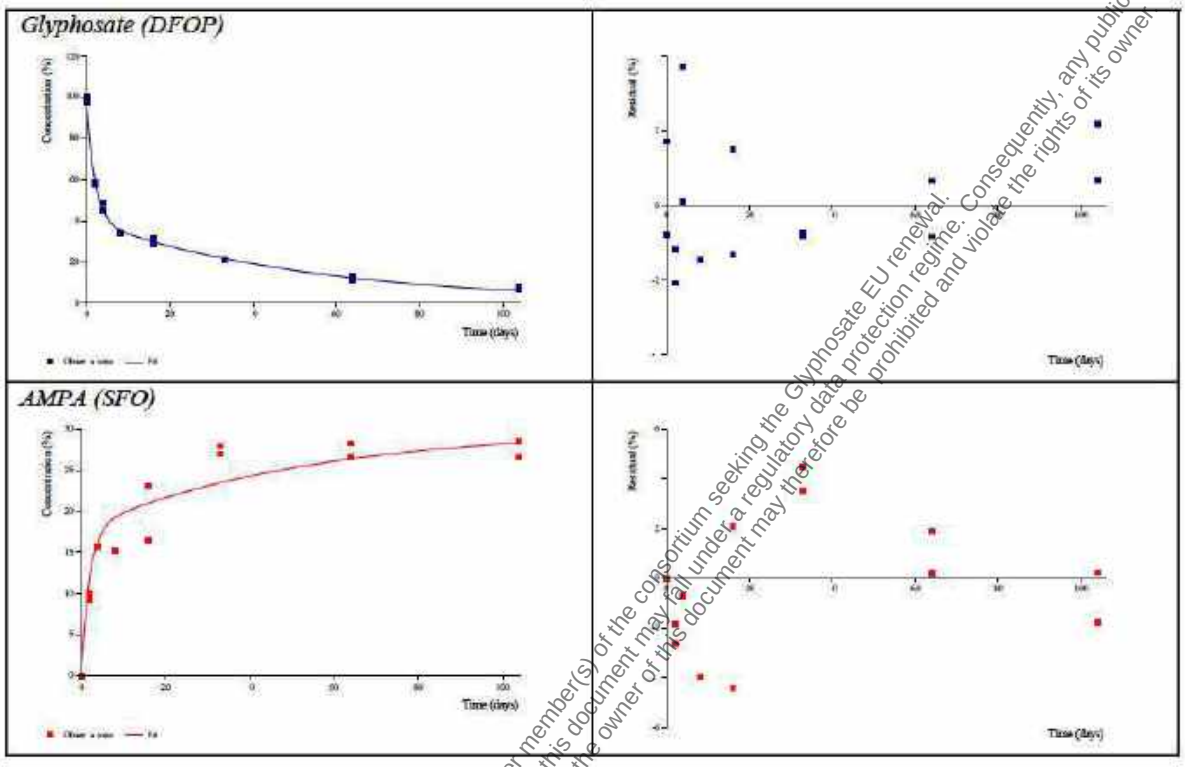


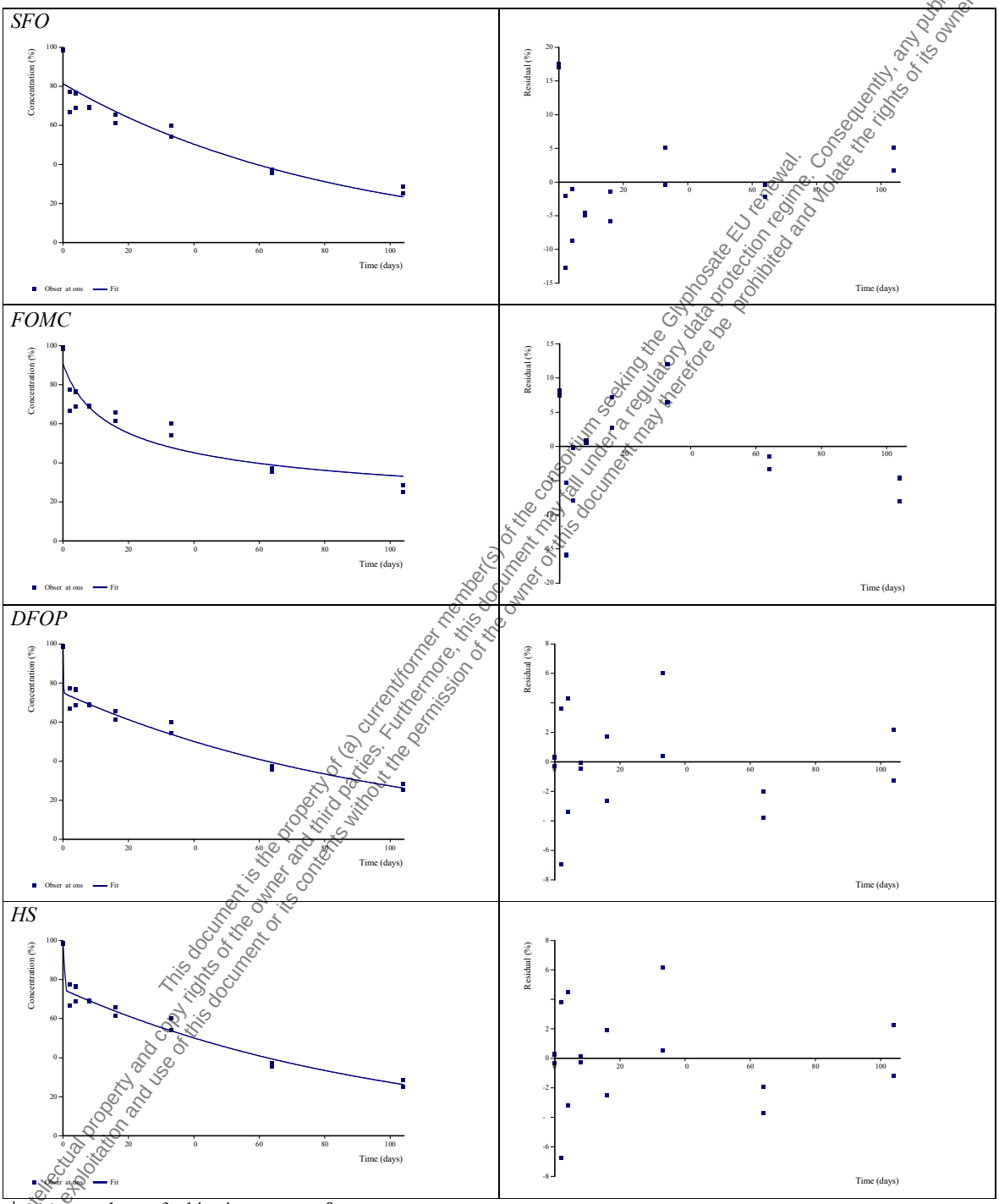
Table 7.1.2.1.1-34: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1, dose group C (8° C, 40 MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005)

Kinetic model	Visual assessment	M_0	Parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	81.3	k: 0.0120	9.4	k: <0.001	k: 0.0083	k: 0.016	57.9	192
FOMC	Acceptable	90.8	α: 0.3595 β: 6.689	9.0	-[1]	β: -5.079	β: 18.46	39.3	>1000
DFOP	Good	98.6	k_1: 8.463 k_2: 0.0101 g: 0.2397	2.5	k_1: n.c.[2] k_2: <0.001	k_1: n.c.[2] k_2: 0.0085	k_1: n.c.[2] k_2: 0.012	41.6	201
HS	Good	98.6	k_1: 0.2841 k_2: 0.0101 t_b: 1.0[3]	2.3	k_1: <0.001 k_2: <0.001	k_1: 0.2149 k_2: 0.0085	k_1: 0.353 k_2: 0.012	41.6	201

Degradation of glyphosate was best described by bi-phasic models. As the FOMC and DFOP model did not provide statistically reliable parameters, the HS model has additionally been tested and provided the best fit with statistically reliable parameters.

Conclusion: HS to be used in pathway fit for trigger endpoints
HS to be used in pathway fit for modelling endpoints

Table 7.1.2.1.1-34: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1, dose group C (8° C, 40 MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005)



[1] t-test not relevant for kinetic parameter β

[2] Errors and t-test values could not be calculated because the covariance matrix could not be created

[3] Breakpoint (t_b) was manually adjusted and fixed as CAKE did not estimate the breakpoint correctly

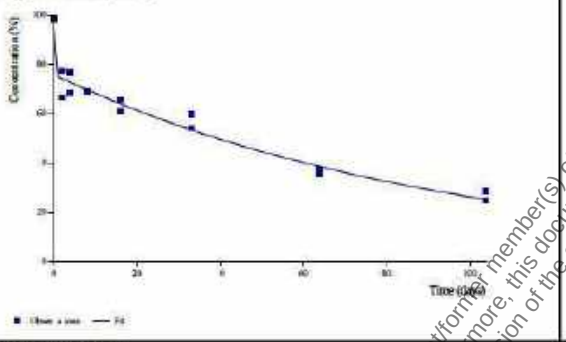
Table 7.1.2.1.1-35: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group C (8 °C, 40 % MWHC, 4 mg/kg), of study Lewis & Turnbull (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

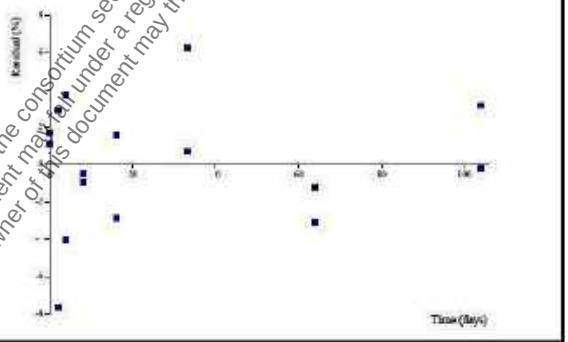
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: HS	Good	97.2	k_1: 0.2568 k_2: 0.0106 t_b: 1.0[1]	2.5	k_1: <0.001 k_2: <0.001	k_1: 0.1919 k_2: 0.0090	k_1: 0.322 k_2: 0.012	42.3	195	-
AMPA: SFO	Accep-table	-	k: 0.0013	10.4	k: 0.243	k: -0.0024	k: 0.0050	533	>1000	0.355 (±0.043)

The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable.

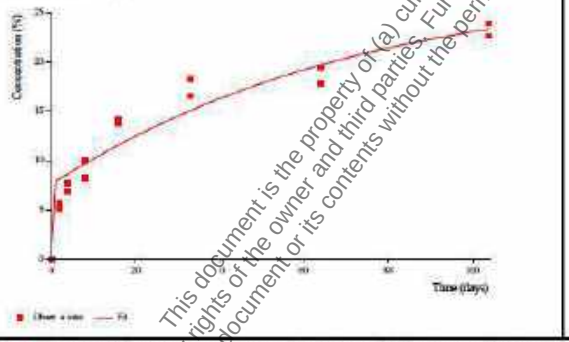
Conclusion: Parent-only HS fit to be used for deriving trigger and modelling endpoints for glyphosate
No trigger and modelling endpoints can be derived for AMPA

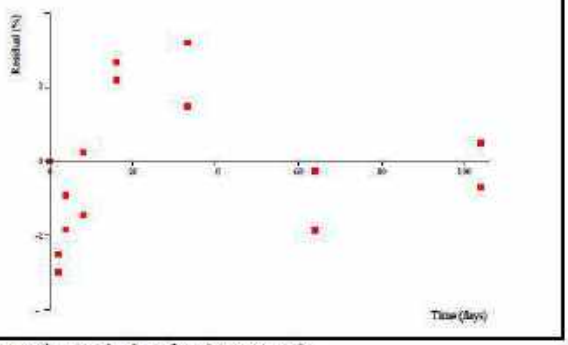
Glyphosate (HS)





AMPA (SFO)





[1] Breakpoint (t_b) was manually adjusted and fixed as CAKE did not estimate the breakpoint correctly

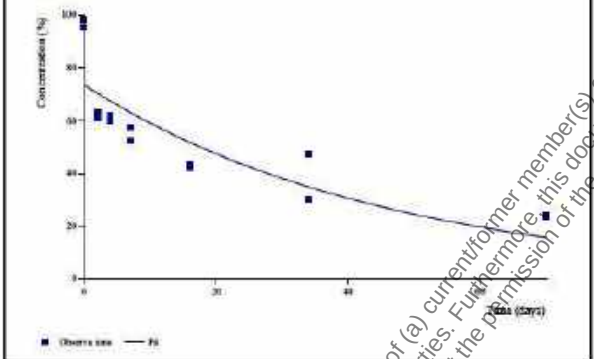
Table 7.1.2.1.1-36: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1, dose group D (20 °C, 40 % MWHC, 4 mg/kg, sterile), of study [redacted] (1992, CA 7.1.2.1.1/005)

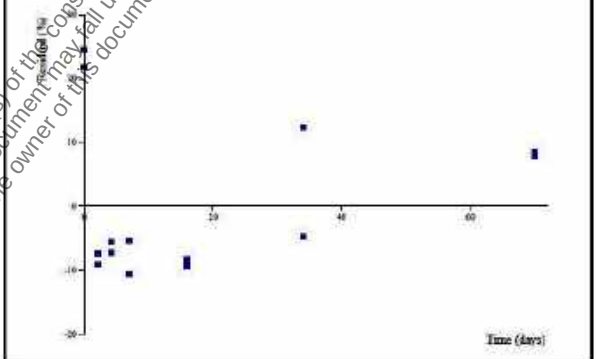
Kinetic model	Visual assessment	M_0	Parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	73.5	k: 0.0219	16.3	k: 0.001	k: 0.0097	k: 0.0340	31.7	105
FOMC	Good	96.2	α: 0.2475 β: 0.6142	5.6	-[1]	β: -0.1447	β: 1.3730	9.5	>1000
DFOP	Good	96.6	k_1: 1.029 k_2: 0.0137 g: 0.3794	4.4	k_1: 0.033 k_2: <0.001	k_1:-0.0857 k_2: 0.0086	k_1: 2.145 k_2: 0.019	15.8	134
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (the residues at the last two sampling dates) and the lowest χ^2 error.

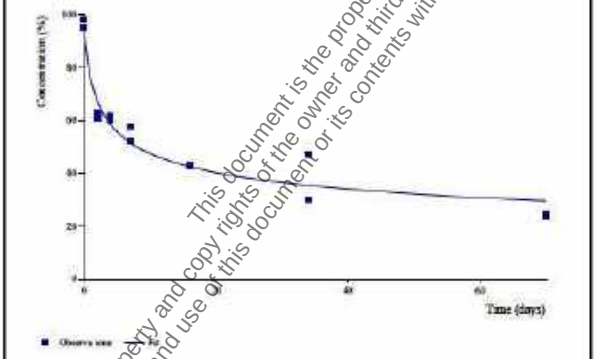
Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

SFO





FOMC



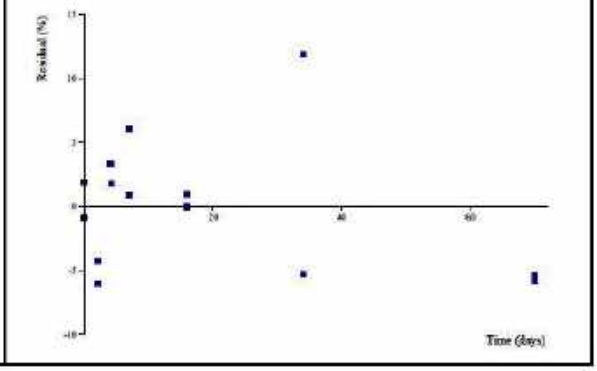
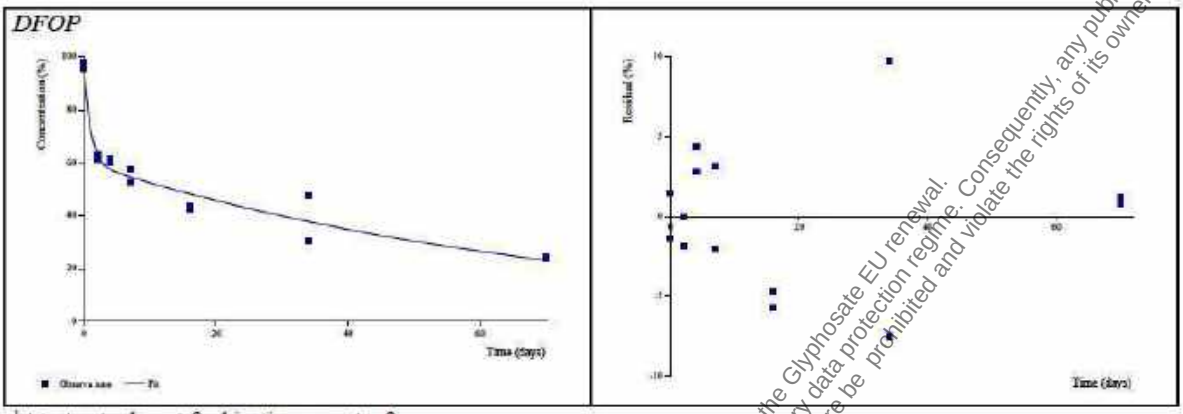


Table 7.1.2.1.1-36: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1, dose group D (20 °C, 40 % MWHC, 4 mg/kg, sterile), of study [redacted] (1992, CA 7.1.2.1.1/005)

DFOP



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-37: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group D (20 °C, 40 % MWHC, 4 mg/kg, sterile), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (95 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	93.8	k_1: 0.7952 k_2: 0.015 g: 0.3468	3.4	k_1: 0.014 k_2: <0.001	k_1: 0.0957 k_2: 0.0096	k_1: 1.495 k_2: 0.020	17.8	125	-
AMPA: SFO	Accep-table	-	k:<0.0001	9.2	k: 0.5	k: -0.0066	k: 0.0070	>1000	>1000	0.261 (±0.039)

The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable.

Conclusion: Parent-only DFOP fit to be used for deriving trigger and modelling endpoints for glyphosate
No trigger and modelling endpoints can be derived for AMPA

Glyphosate (DFOP)

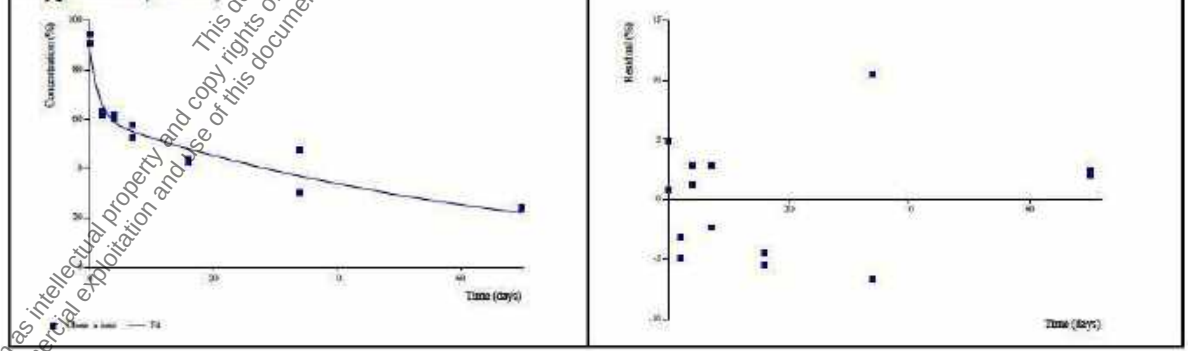
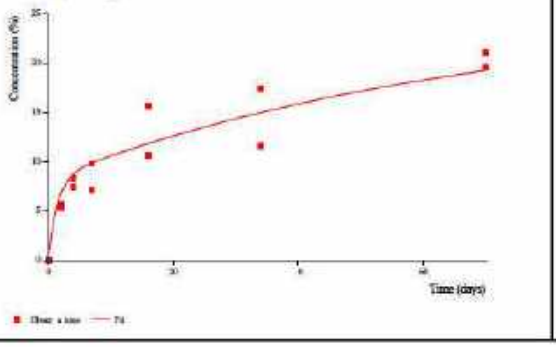


Table 7.1.2.1.1-37: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group D (20 °C, 40 % MWHC, 4 mg/kg, sterile), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

AMPA (SFO)



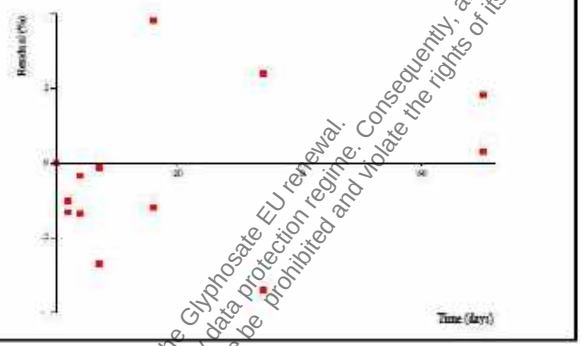


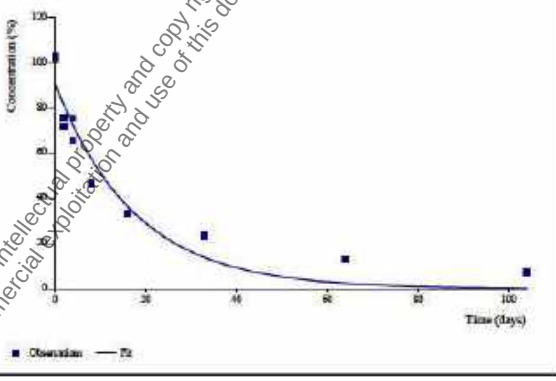
Table 7.1.2.1.1-38: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1 (20° C, 40 MWHC, 0.4 mg/kg), dose group E, [redacted] (1992, CA 7.1.2.1.1/005)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	91.0	k: 0.0573	14.5	k: <0.001	k: 0.0384	k: 0.076	12.1	40.2
FOMC	Good	101.0	α: 0.8032 β: 5.523	5.4	-[1]	β: 2.278	β: 8.767	7.6	91.6
DFOP	Good	100.5	k_1: 0.2003 k_2: 0.0177 g: 0.5861	3.7	k_1: <0.001 k_2: <0.001	k_1: 0.109 k_2: 0.0084	k_1: 0.292 k_2: 0.027	7.3	80.3
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide equally reliable and visually acceptable results. The DFOP model provides slightly lower residuals for the data points from day 8 onwards.

Conclusion: DFOP to be used in pathway fits for trigger endpoints
DFOP to be used in pathway fits for modelling endpoints

SFO



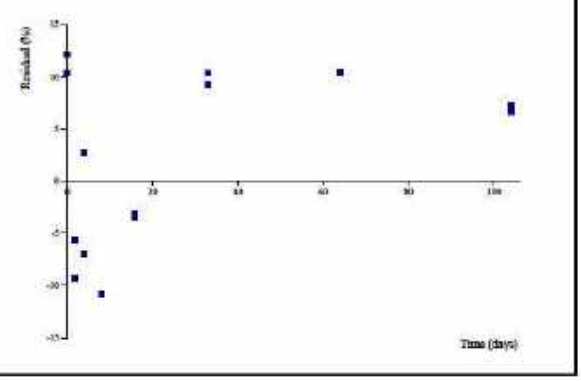
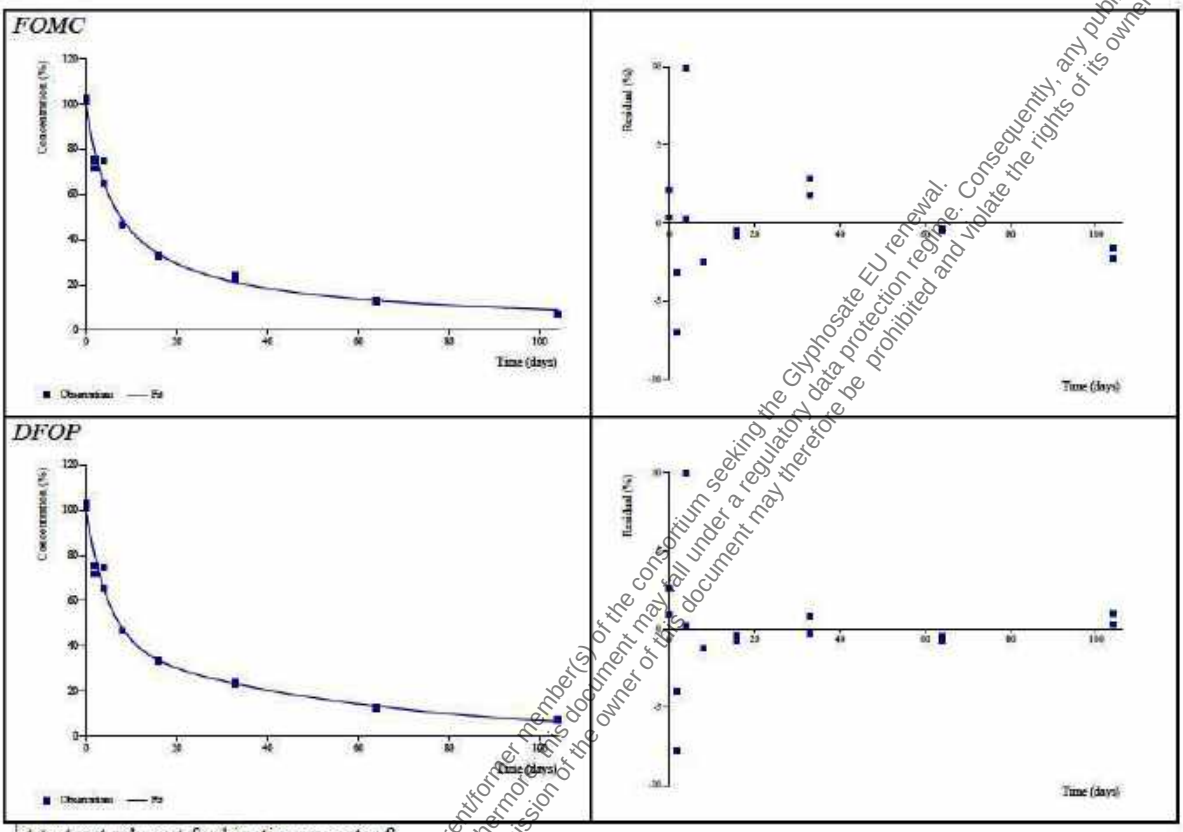


Table 7.1.2.1.1-38: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1 (20° C, 40 MWHC, 0.4 mg/kg), dose group E, [redacted] (1992, CA 7.1.2.1.1/005)



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-39: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group E (20 °C, 40 % MWHC, 0.4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	101.8	k_1: 0.2635 k_2: 0.0216 g: 0.5149	6.2	k_1: <0.001 k_2: <0.001	k_1: 0.16 k_2: 0.0131	k_1: 0.367 k_2: 0.03	7.0	73.2	-
AMPA: SFO	Good	-	k: 0.0024	6.4	k: 0.019	k: 0.0002	k: 0.005	283	940	0.393 (±0.030)

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

Table 7.1.2.1.1-39: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1, dose group E (20 °C, 40 % MWHC, 0.4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

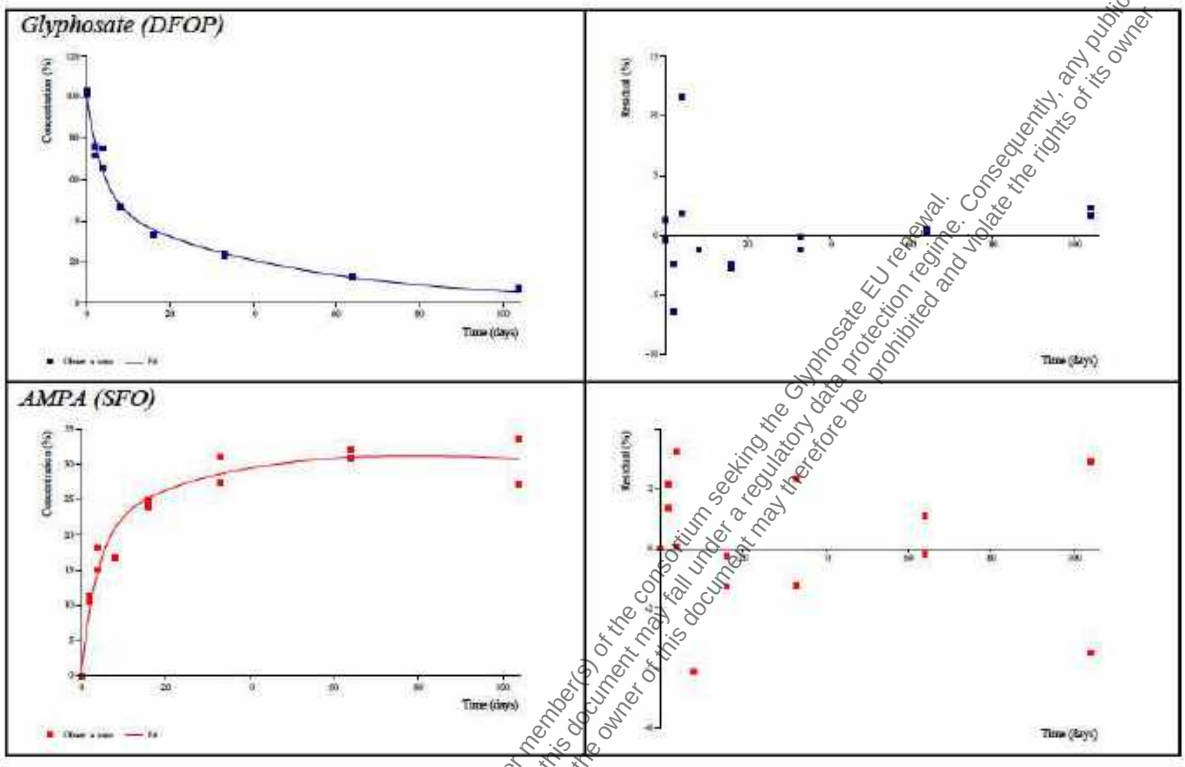


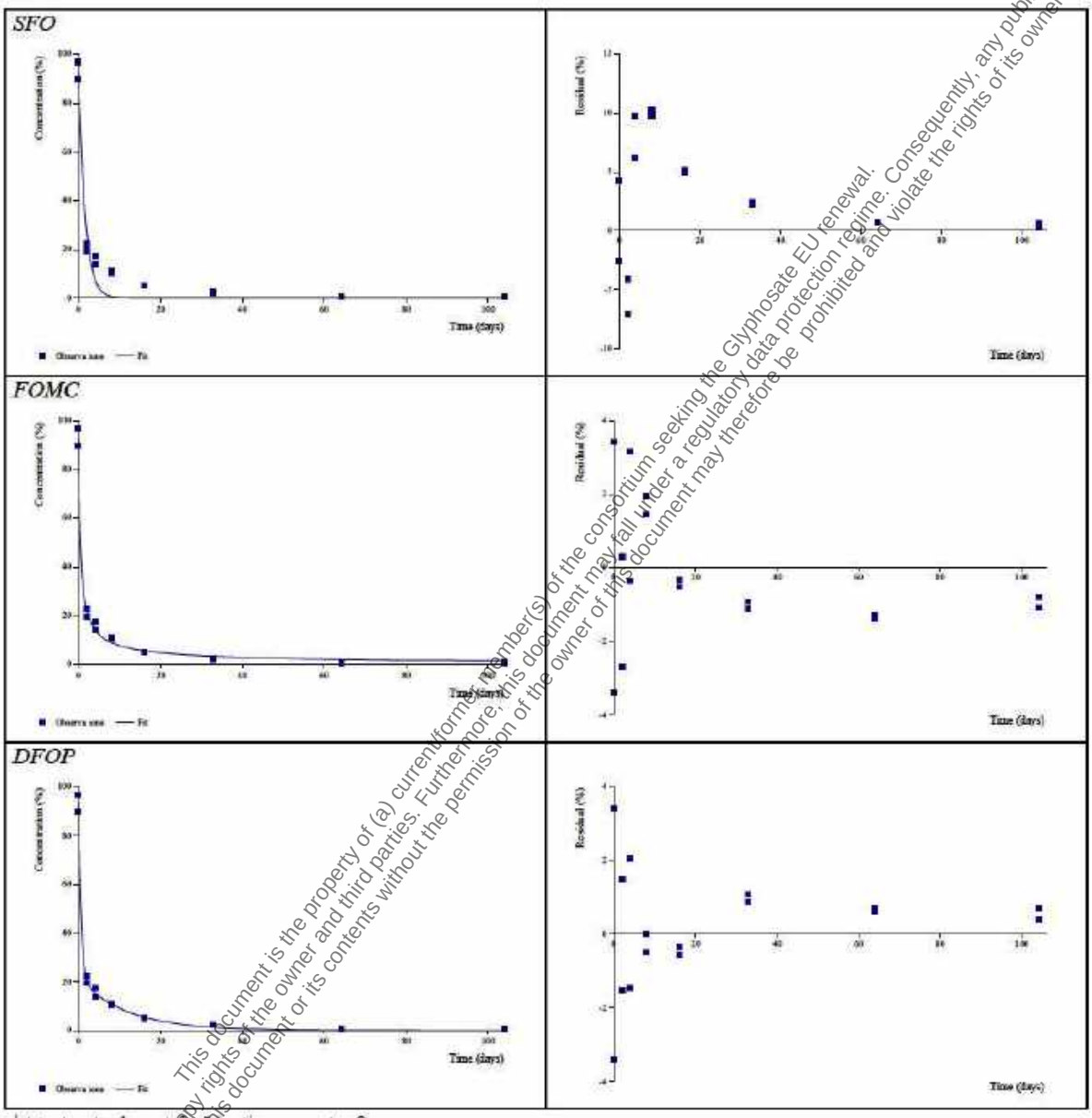
Table 7.1.2.1.1-40: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Beedon manor, dose group F (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005)

Kinetic model	Visual assessment	M_0	Parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	92.3	k: 0.6228	22.8	k: <0.001	k: 0.4709	k: 0.7750	1.1	3.7
FOMC	Good	93.2	α: 0.7097 β: 0.3056	5.2	-[1]	β: 0.0640	β: 0.5470	0.5	7.5
DFOP	Good	93.2	k_1: 1.588 k_2: 0.0839 g: 0.7714	2.5	k_1: <0.001 k_2: <0.001	k_1: 0.7635 k_2: 0.0463	k_1: 2.412 k_2: 0.121	0.6	9.9
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. Both models provide similar visual fits but the DFOP model provides the lowest χ^2 error.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

Table 7.1.2.1.1-40: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Beedon manor, dose group F (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005)



[1] t-test not relevant for kinetic parameter β

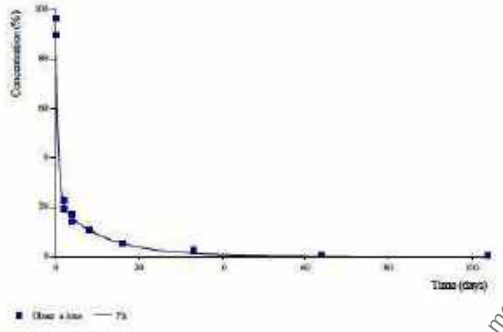
Table 7.1.2.1.1-41: Kinetic models and goodness-of-fit statistics of pathway fits for soil Beedon manor, dose group F (20 °C, 40 % MWHC, 4 mg/kg), of study [redacted] (1992, CA 7.1.2.1.1/005) – trigger and modelling endpoints

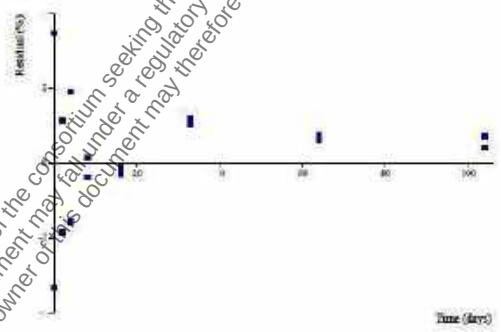
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	g_f (± std. dev.)
Glypho-sate: DFOP	Good	93.1	k_1: 1.58 k_2: 0.0885 g: 0.7649	2.6	k_1: <0.001 k_2: <0.001	k_1: 0.7877 k_2: 0.0510	k_1: 2.3720 k_2: 0.1260	0.6	2.7	-
AMPA: SFO	Accep-table	-	k: 0.0103	16.4	k: <0.001	k: 0.0050	k: 0.016	67.3	224	0.149 (±0.011)

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

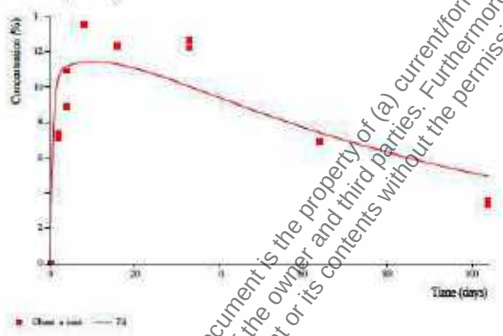
Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

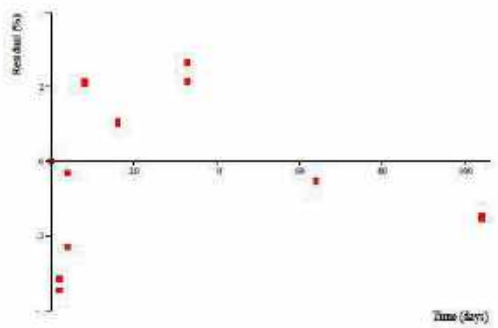
Glyphosate (DFOP)





AMPA (SFO)





[redacted] (1993, CA 7.1.2.1.1/004)

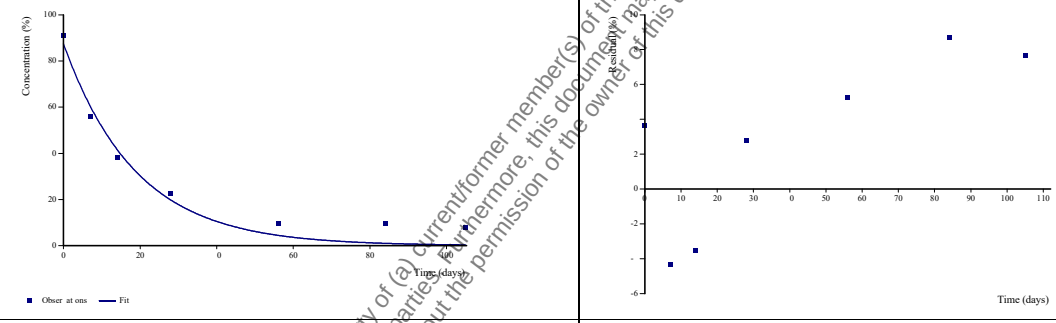
Table 7.1.2.1.1-42: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1 of study [redacted] (1993, CA 7.1.2.1.1/004)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	87.5	k: 0.0531	13.1	k: <0.001	k: 0.0316	k: 0.075	13.1	43.4
FOMC	Good	91.3	α: 1.255 β: 14.09	3.5	-[1]	β: 5.349	β: 22.83	10.4	74.2
DFOP	Good	90.7	k_1: 0.0848 k_2: 0.008 g: 0.8063	3.3	k_1: 0.002 k_2: 0.105	k_1: 0.0491 k_2:-0.0079	k_1: 0.121 k_2: 0.024	10.8	83.8
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (residues at the last sampling dates) and the lowest χ^2 error. The parameter k_2 is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k_1 as indicated by a high value for parameter g (0.8063).

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

SFO



FOMC

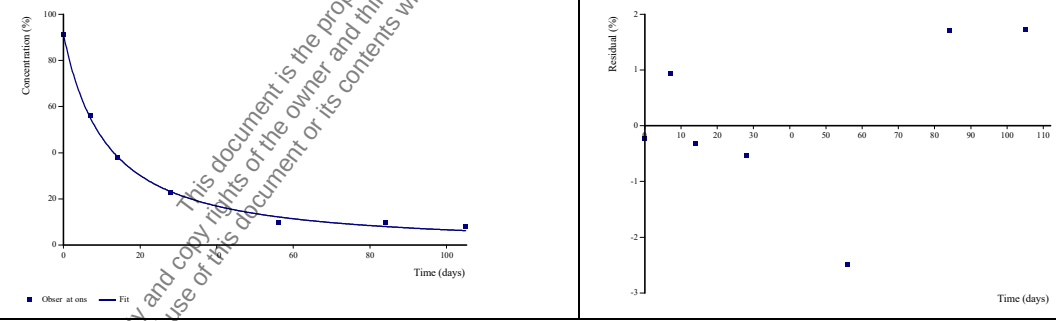
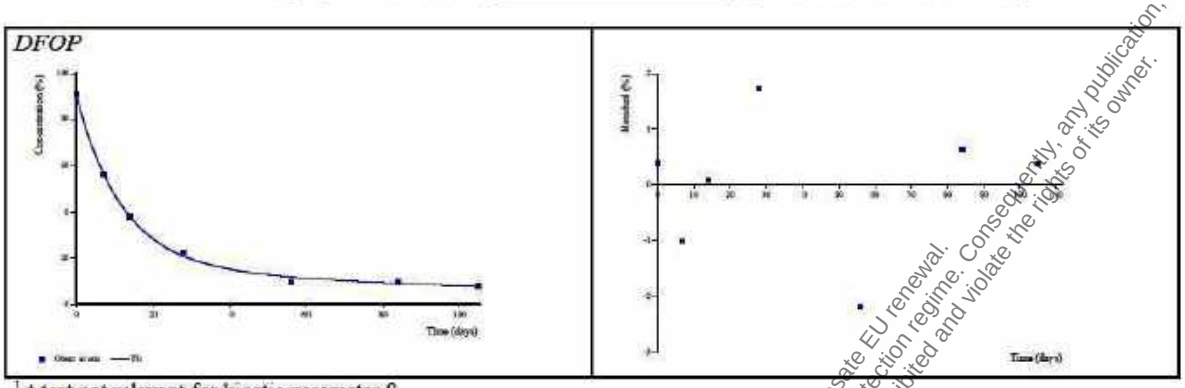


Table 7.1.2.1.1-42: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.1 of study [redacted] (1993, CA 7.1.2.1.1/004)

DFOP



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-43: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1 of study [redacted] (1993, CA 7.1.2.1.1/004) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	90.8	k_1: 0.0859 k_2: 0.0084 g: 0.7999	3.3	k_1: < 0.001 k_2: 0.054	k_1: 0.0610 k_2: -0.0024	k_1: 0.111 k_2: 0.019	10.8	84	-
AMPA: SFO	Good	-	k: 0.0080	13.7	k: < 0.05	k: 3.81×10^{-5}	k: 0.016	86.5	288	0.687 (±0.108)

The visual fits for glyphosate and AMPA are good and degradation parameters for AMPA are reliable. For glyphosate, the p-value of the t-test for parameter k_2 is still slightly above >0.05 (0.054) but this again can be accepted as the overall degradation of glyphosate is dominated by k_1 as indicated by a high value for parameter g (0.7999), and the modelling endpoint for glyphosate is derived from DT_{90}/3.32 as 10 % of the initial concentration was reached within the experimental period.

Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

Glyphosate (DFOP)

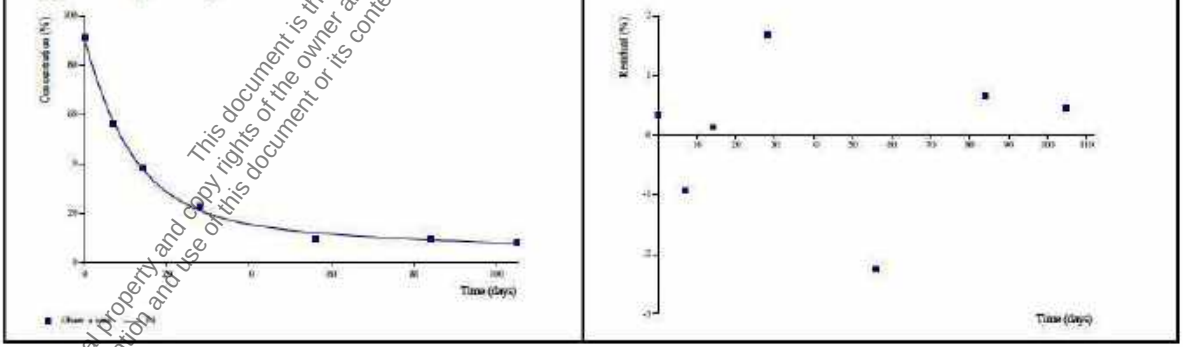


Table 7.1.2.1.1-43: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.1 of study [redacted] (1993, CA 7.1.2.1.1/004) – trigger and modelling endpoints

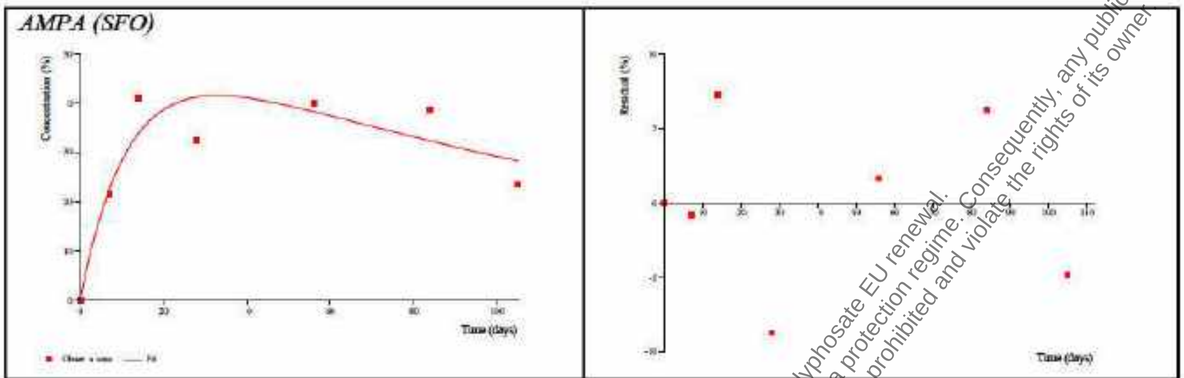


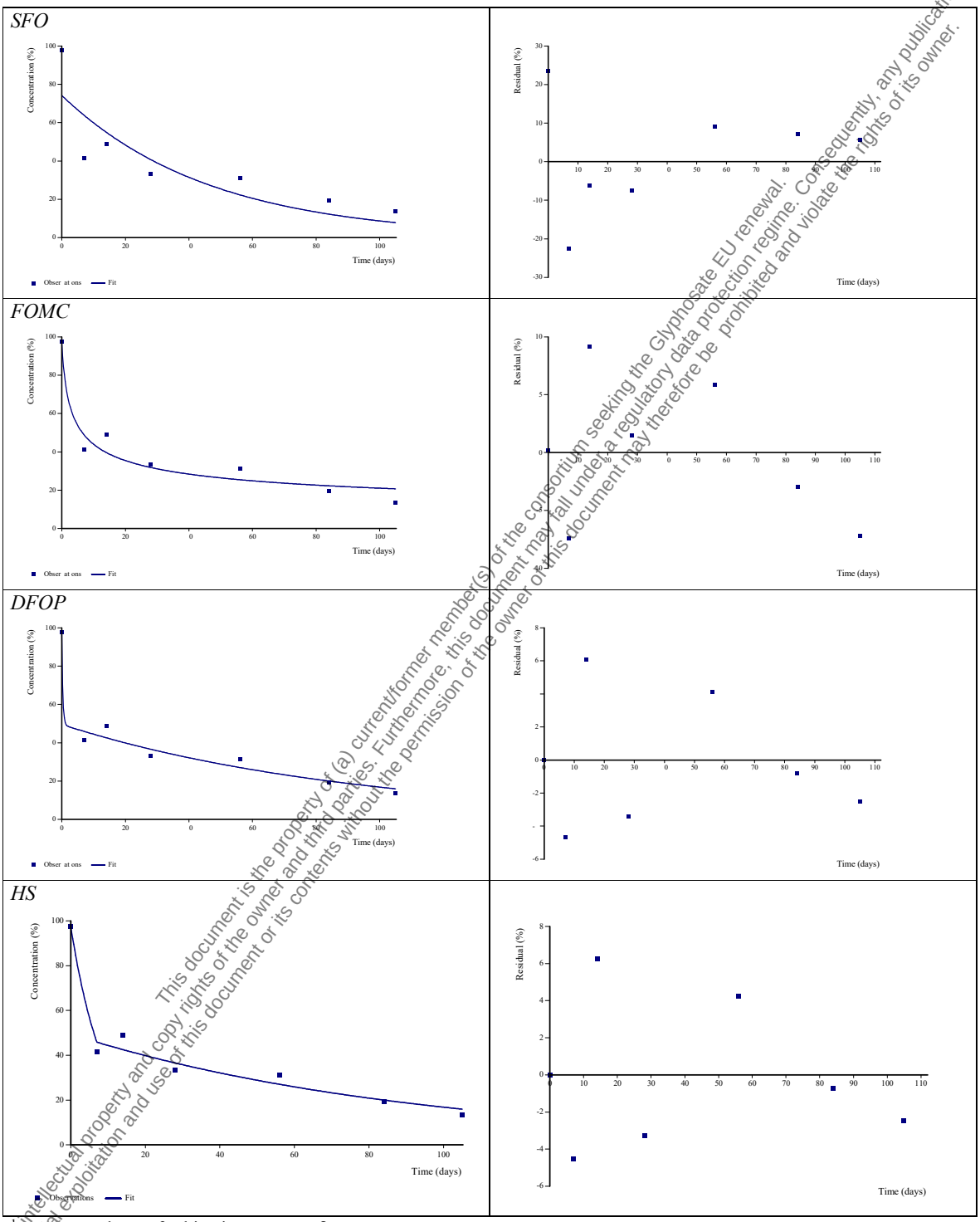
Table 7.1.2.1.1-44: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.2 of study [redacted] (1993, CA 7.1.2.1.1/004)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	74.2	k: 0.0214	26.7	k: 0.0027	k: -0.0005	k: 0.043	32.3	107
FOMC	Good	97.4	α: 0.3322 β: 1.001	12.3	-1	β: -2.613	β: 4.615	7.1	>1000
DFOP	Good	97.6	k_1: 3.186 k_2: 0.0108 g: 0.4929	8.5	k_1: 0.493 k_2: 0.012	k_1: -540.8 k_2: 0.0027	k_1: 547.2 k_2: 0.019	1.7	151
HS	Good	97.6	k_1: 0.1079 k_2: 0.0108 t_b: 7.03	7.8	k_1: <0.001 k_2: 0.005	k_1: 0.0721 k_2: 0.0045	k_1: 0.144 k_2: 0.017	6.4	151

Degradation of glyphosate was best described by bi-phasic models. As the FOMC and DFOP model did not provide statistically reliable parameters, the HS model has additionally been tested and provided the best fit with statistically reliable parameters.

Conclusion: HS to be used in pathway fit for trigger endpoints
HS to be used in pathway fit for modelling endpoints

Table 7.1.2.1.1-44: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.2 of study [redacted] (1993, CA 7.1.2.1.1/004)



1 t-test not relevant for kinetic parameter β

2 Breakpoint (t_b) was manually adjusted and fixed as CAKE did not estimate the breakpoint correctly.

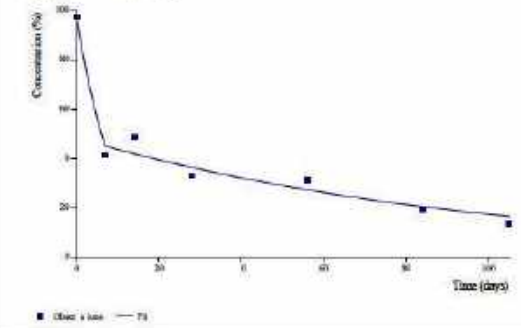
Table 7.1.2.1.1-45: Kinetic models and goodness-of-fit statistics of pathway fit for soil Speyer 2.2 of study [redacted] (1993, CA 7.1.2.1.1/004) – trigger and modelling endpoints

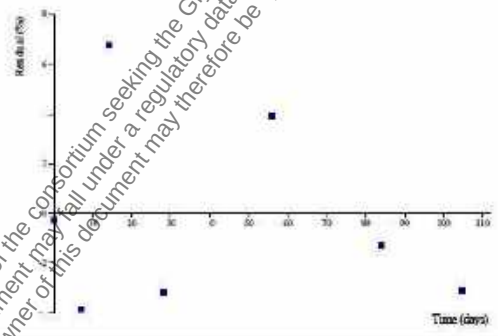
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	f (± std. dev.)
Glypho-sate: HS	Good	97.9	k_1: 0.1105 k_2: 0.0102 t_b: 7.0[1]	7.8	k_1: <0.001 k_2: <0.001	k_1: 0.0806 k_2: 0.0051	k_1: 0.14 k_2: 0.015	6.3	137	-
AMPA: SFO	Good	-	k: 0.0063	8.9	k: 0.016	k: 0.0007	k: 0.012	110	365	0.683 (±0.098)

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

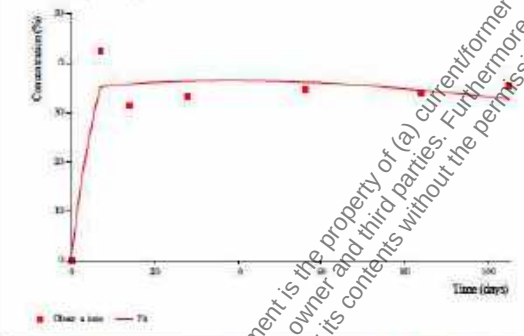
Conclusion: HS-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

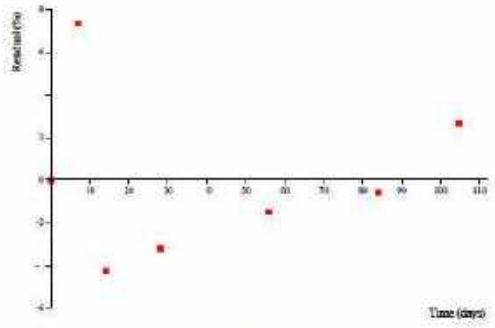
Glyphosate (HS)





AMPA (SFO)





[1] Breakpoint (t_b) was manually adjusted and fixed as CAKE did not estimate the breakpoint correctly.

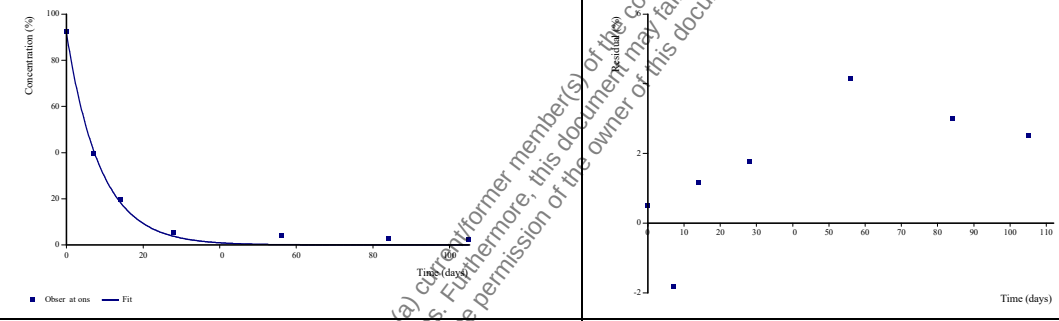
Table 7.1.2.1.1-46: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Speyer 2.3 of study [redacted] (1993, CA 7.1.2.1.1/004)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	91.8	k: 0.1143	8.0	k: <0.001	k: 0.0943	k: 0.134	6.1	20.1
FOMC	Good	92.4	α: 2.467 β: 16.42	5.8	-[1]	β: -4.695	β: 37.53	5.3	25.3
DFOP	Good	92.2	k_1: 0.1296 k_2: 0.0056 g: 0.9474	2.5	k_1: <0.001 k_2: 0.201	k_1: 0.1104 k_2:-0.0126	k_1: 0.149 k_2: 0.024	5.8	22.2
HS	Not calculated								

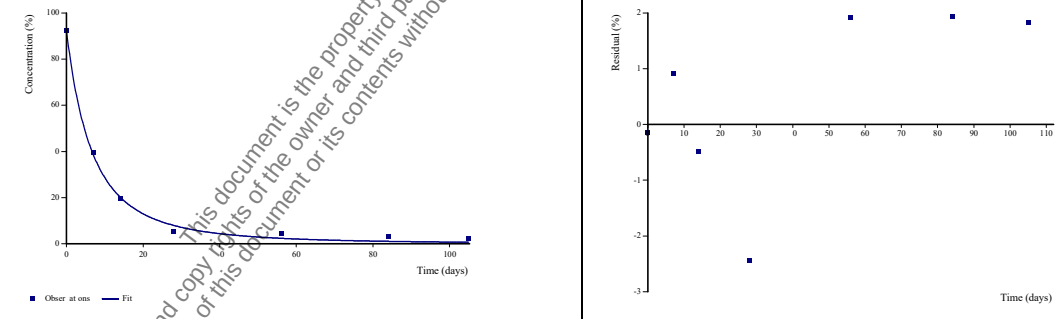
Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit (residues at the last four sampling dates) and the lowest χ^2 error. The parameter k_2 is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k_1 as indicated by a high value for parameter g (0.9474).

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

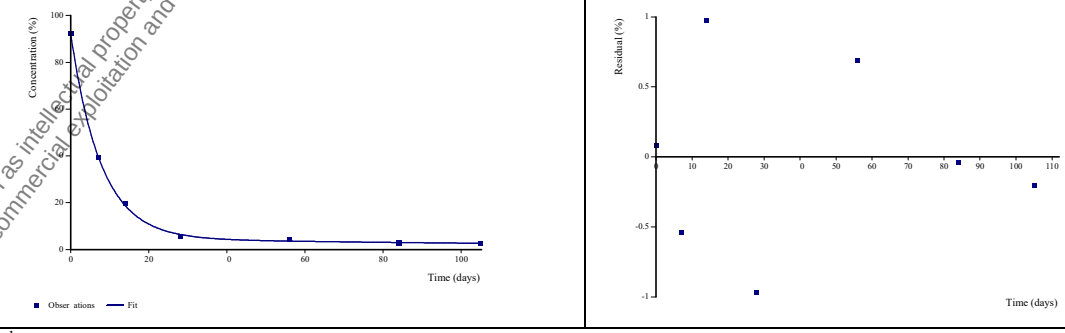
SFO



FOMC



DFOP



[1] t-test not relevant for kinetic parameter β

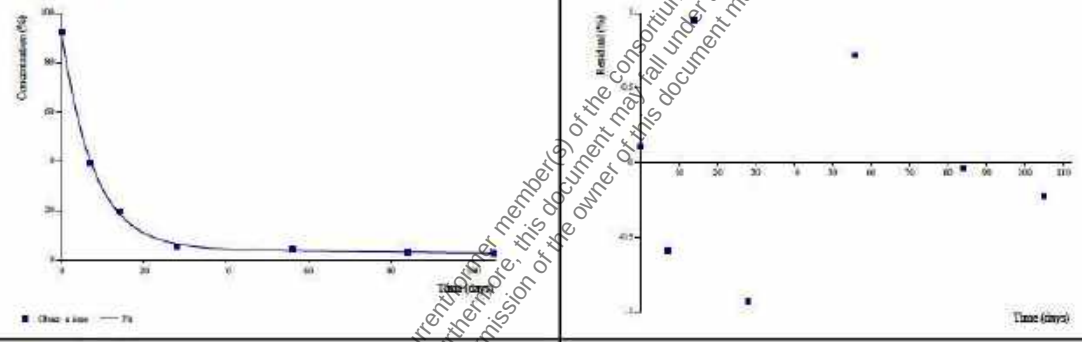
Table 7.1.2.1.1-47: Kinetic models and goodness-of-fit statistics of pathway fits for soil Speyer 2.3 of study [redacted] (1993, CA 7.1.2.1.1/004) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	92.2	k_1: 0.1291 k_2: 0.0052 g: 0.9488	2.5	k_1: <0.001 k_2: 0.179	k_1: 0.1161 k_2:-0.0073	k_1: 0.142 k_2: 0.018	5.8	223	-
AMPA: SFO	Good	-	k: 0.0082	8.8	k: 0.002	k: 0.0035	k: 0.013	85.0	282	0.336 (±0.030)

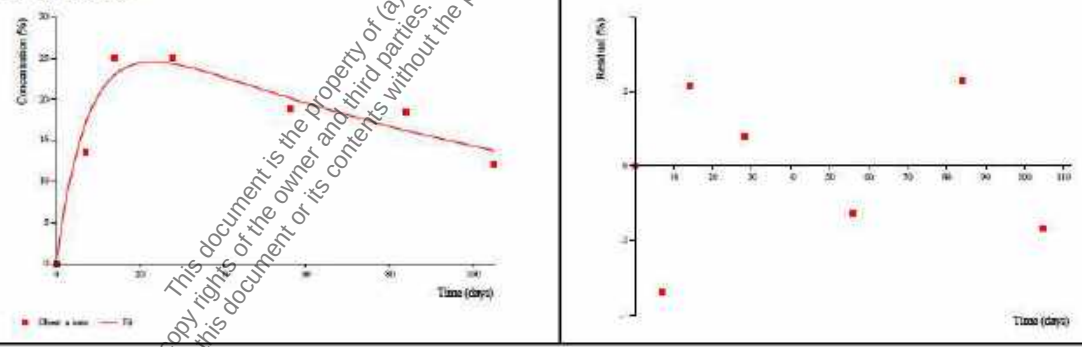
The visual fits for glyphosate and AMPA are good and degradation parameters for AMPA are reliable. For glyphosate, the parameter k_2 is not significantly different from zero which again can be accepted as the overall degradation of glyphosate is dominated by k_1 as indicated by a high value for parameter g (0.9488), and the respective modelling endpoint is derived from DT_{90}/3.32 as 10 % of the initial concentration was reached within the experimental period.

Conclusion: DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

Glyphosate (DFOP)



AMPA (SFO)



[redacted] (2010, CA 7.1.2.1.1/002)

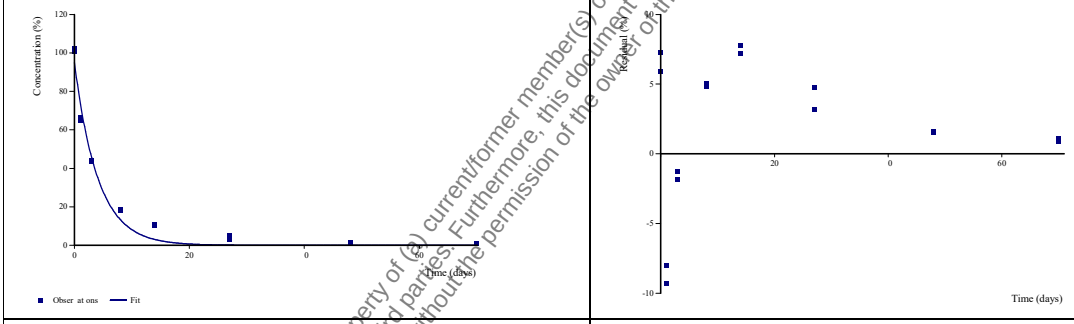
Table 7.1.2.1.1-48: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Drusenheim of study [redacted] (2010, CA 7.1.2.1.1/002)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	95.0	k: 0.2463	13.6	k: <0.001	k: 0.1939	k: 0.299	2.8	9.3
FOMC	Good	100.6	α: 1.271 β: 2.863	4.9	-[1]	β: 1.878	β: 3.849	2.1	14.7
DFOP	Good	101.5	k_1: 1.295 k_2: 0.1403 g: 0.3711	4.8	k_1: <0.001 k_2: <0.001	k_1: 0.6895 k_2: 0.1145	k_1: 1.9 k_2: 0.166	2.0	13.1
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The FOMC model provides the best visual fit (residues at the last five sampling dates) and a similar χ^2 error compared to DFOP. Thus, the FOMC model is selected as the best-fit model for parent-only fit. As 10 % of the initial concentration was reached within the experimental period, the FOMC model can also be used for derivation modelling endpoints.

Conclusion: FOMC to be used in pathway fit for trigger endpoints
FOMC to be used in pathway fit for modelling endpoints

SFO



FOMC

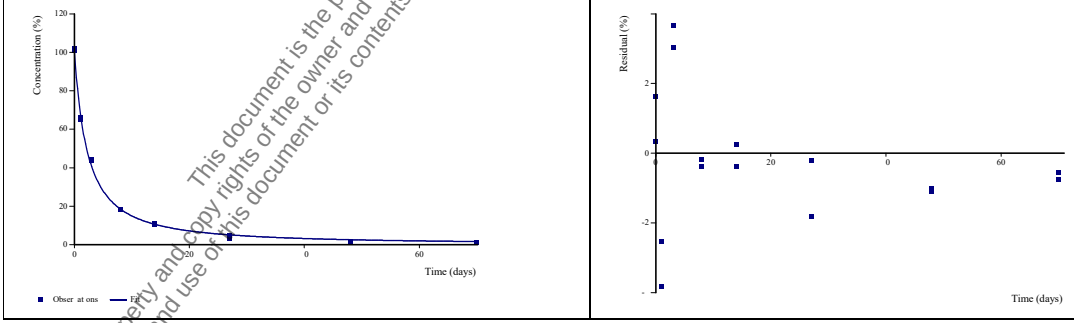
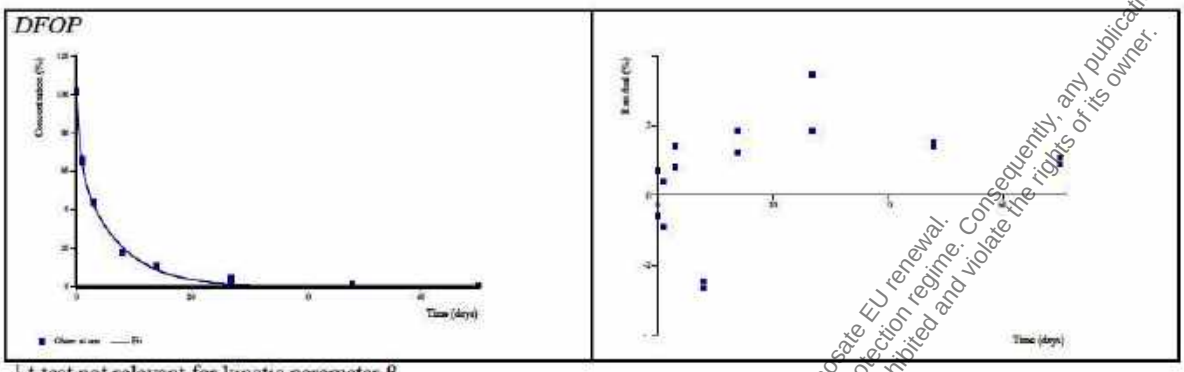


Table 7.1.2.1.1-48: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Drusenheim of study [redacted] (2010, CA 7.1.2.1.1/002)

DFOP



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-49: Kinetic models and goodness-of-fit statistics of pathway fits for soil Drusenheim of study [redacted] (2010, CA 7.1.2.1.1/002) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	99.9	α: 1.362 β: 3.26	5.0	-[1]	β: 2.279	β: 4.241	2.2	14.4	-
AMPA: SFO	Good	-	k: 0.0236	3.8	k: <0.001	k: 0.0210	k: 0.026	29.4	97.7	0.285 (±0.009)

Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable.

Conclusion: FOMC-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA

Glyphosate (FOMC)

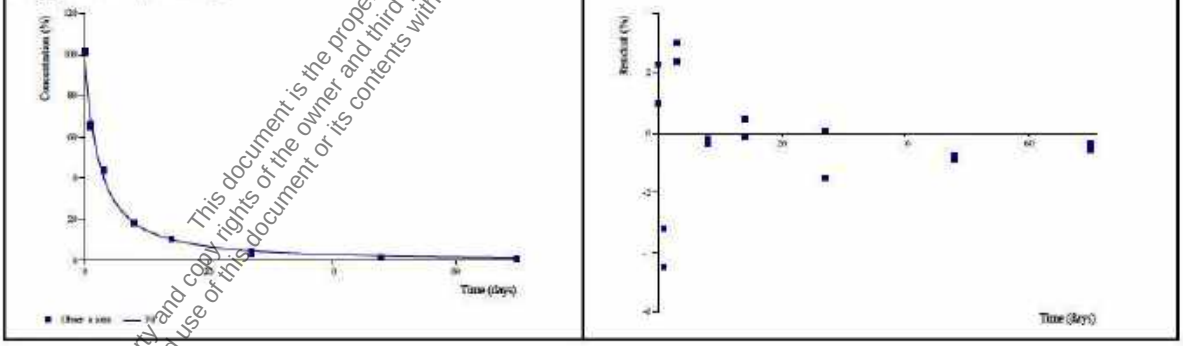
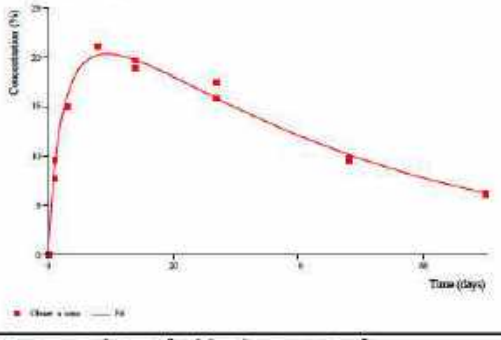
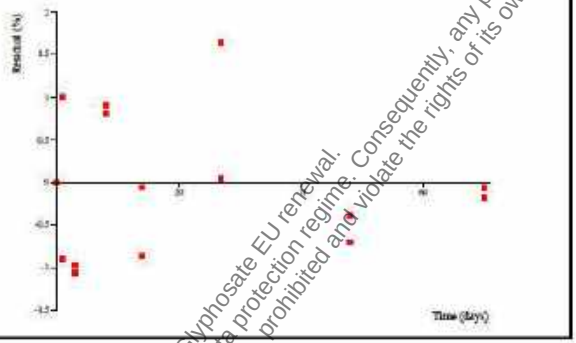


Table 7.1.2.1.1-49: Kinetic models and goodness-of-fit statistics of pathway fits for soil Drusenheim of study [redacted] (2010, CA 7.1.2.1.1/002) – trigger and modelling endpoints

AMPA (SFO) 	

[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-50: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Pappelacker, [redacted] (2010, CA 7.1.2.1.1/002)

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	93.8	k: 0.1283	16.3	k: <0.001	k: 0.0977	k: 0.159	5.4	18.0
FOMC	Good	101.1	α: 1.022 β: 3.827	6.2	[1]	β: 1.946	β: 5.708	3.7	32.6
DFOP	Good	100.8	k_1: 0.3443 k_2: 0.0338 g: 0.6505	5.5	k_1: <0.001 k_2: <0.001	k_1: 0.232 k_2: 0.0193	k_1: 0.457 k_2: 0.048	3.6	37.3
HS	Not calculated								

Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The DFOP model provides the best visual fit and the lowest χ^2 error.

Conclusion: DFOP to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

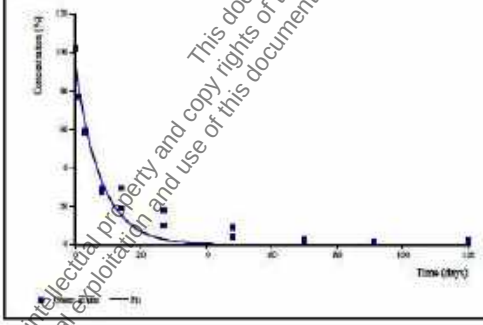
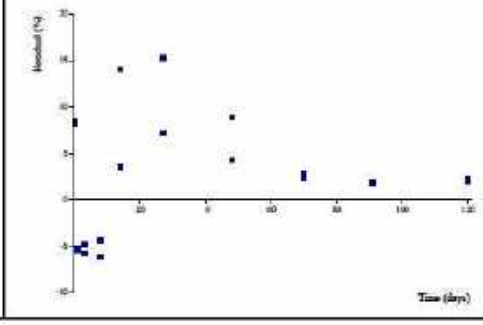
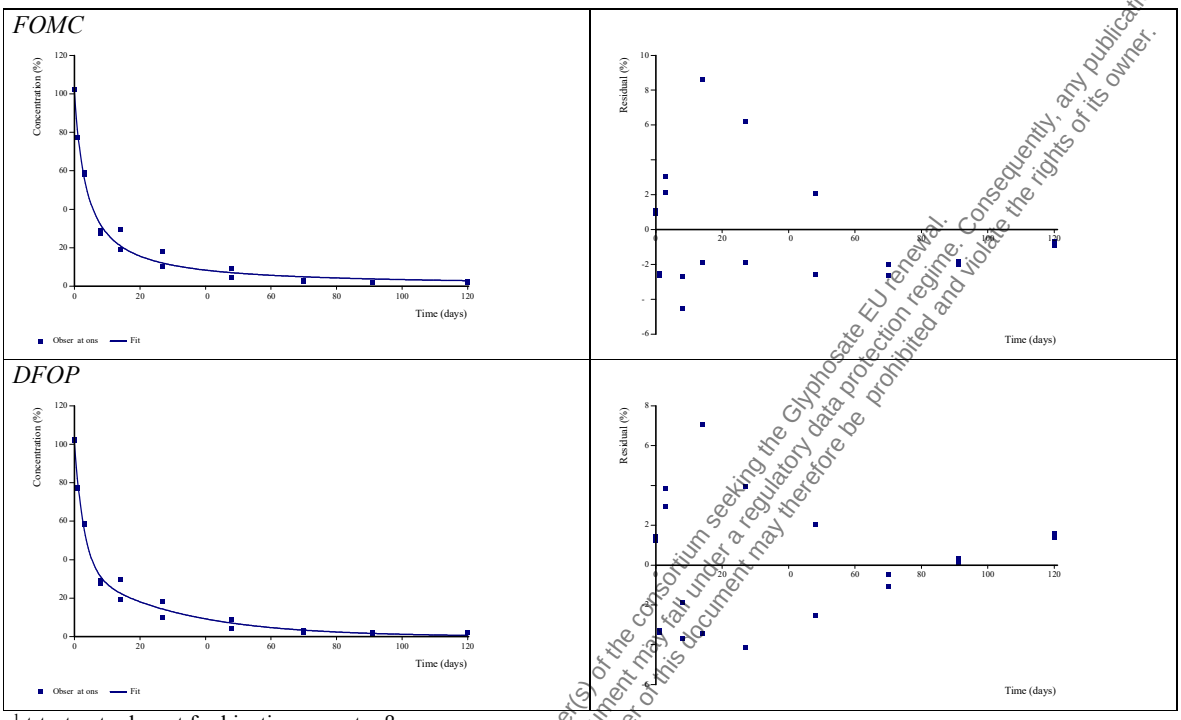
SFO 	

Table 7.1.2.1.1-50: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Pappelacker, [redacted] (2010, CA 7.1.2.1.1/002)



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.1-51: Kinetic models and goodness-of-fit statistics of pathway fits for soil Pappelacker of study [redacted] (2010, CA 7.1.2.1.1/002) – trigger and modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	100.6	k_1: 0.3322 k_2: 0.0325 g: 0.6609	5.5	k_1: <0.001 k_2: <0.001	k_1: 0.2464 k_2: 0.0199	k_1: 0.418 k_2: 0.045	3.6	37.6	-
AMPA: SFO	Good	-	k: 0.0076	6.2	k: <0.001	k: 0.006	k: 0.009	90.9	302	0.192 (±0.009)
Degradation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The visual fits and the statistical parameters are acceptable. **Conclusion:** DFOP-SFO to be used for deriving trigger and modelling endpoints for glyphosate and AMPA										

Table 7.1.2.1.1-51: Kinetic models and goodness-of-fit statistics of pathway fits for soil Pappelacker of study [redacted] (2010, CA 7.1.2.1.1/002) – trigger and modelling endpoints

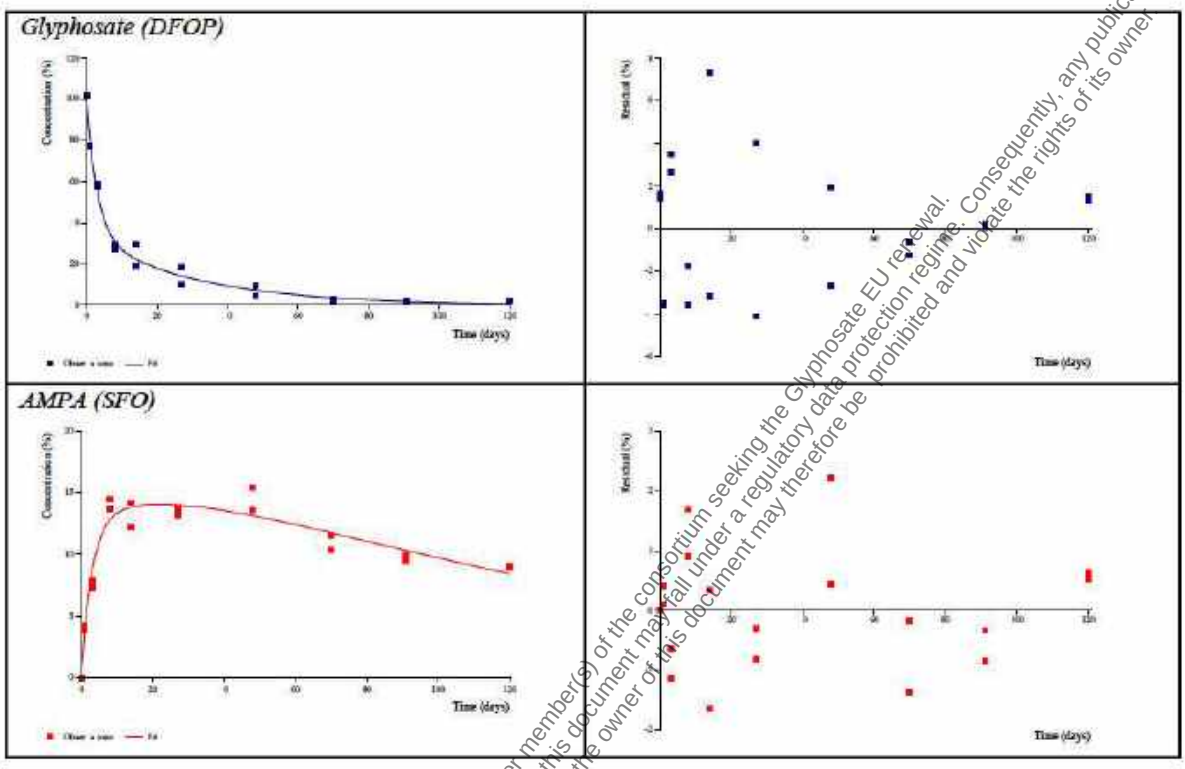


Table 7.1.2.1.1-52: Kinetic models and goodness-of-fit statistics of parent-only fits for soil 18-Acres of study [redacted] (2010, CA 7.1.2.1.1/002)

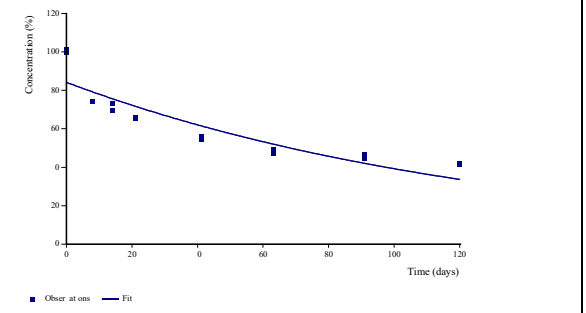
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	84.1	k: 0.0076	9.9	k: <0.001	k: 0.0053	k: 0.01	90.9	302
FOMC	Good	100.1	α: 0.2605 β: 4.522	2.0	-[1]	β: 2.626	β: 6.418	60.2	>1000
DFOP	Good	99.7	k_1: 0.1125 k_2: 0.0040 g: 0.3458	2.9	k_1: <0.001 k_2: <0.001	k_1: 0.0630 k_2: 0.0026	k_1: 0.162 k_2: 0.005	67.7	473
HS	Not calculated								

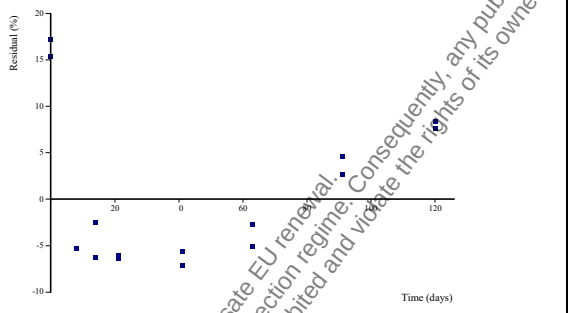
Degradation of glyphosate was best described by the FOMC and DFOP bi-phasic models. The FOMC model provides the best visual fit (residues at the last two sampling dates) and the lowest χ^2 error. Thus, the FOMC model is selected as the best-fit model for parent-only fit. As 10 % of the initial concentration was not reached within the experimental period, the DFOP model is selected for derivation of modelling endpoints.

Conclusion: FOMC to be used in pathway fit for trigger endpoints
DFOP to be used in pathway fit for modelling endpoints

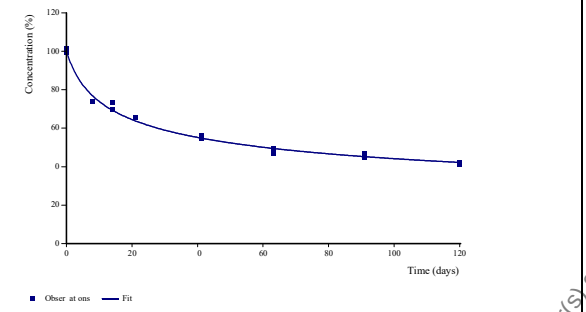
Table 7.1.2.1.1-52: Kinetic models and goodness-of-fit statistics of parent-only fits for soil 18-Acres of study [redacted] (2010, CA 7.1.2.1.1/002)

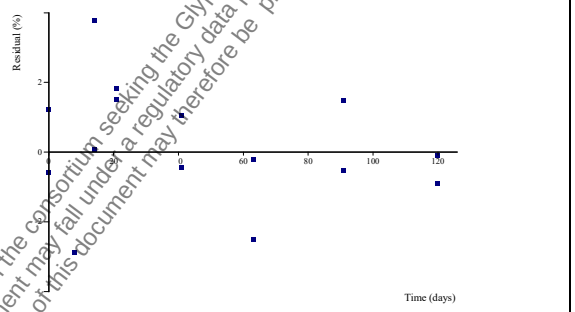
SFO



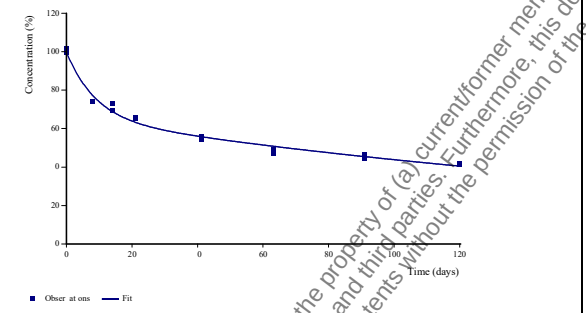


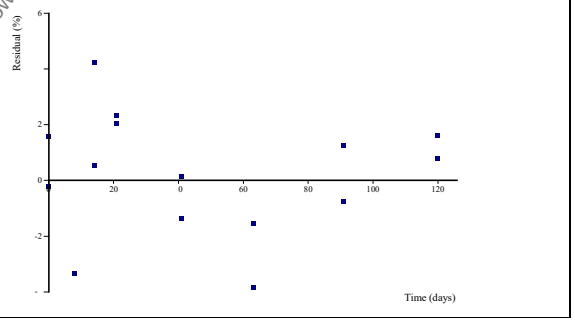
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

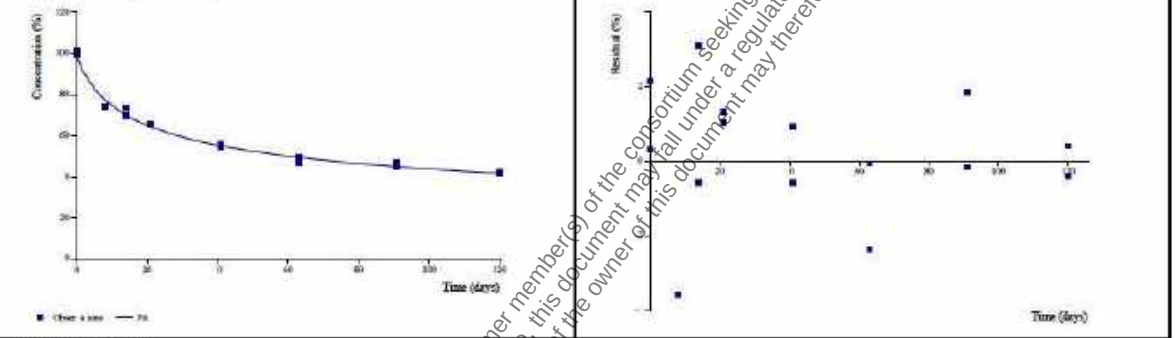
Table 7.1.2.1.1-53: Kinetic models and goodness-of-fit statistics of pathway fits for soil 18-Acres of study [redacted] (2010, CA 7.1.2.1.1/002) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	99.2	α: 0.2789 β: 5.624	2.1	-[1]	β: 3.288	β: 7.96	61.9	>1000	-
AMPA: SFO	Accep-table	-	k: <0.0001	13.6	k: 0.5	k: -0.0033	k: 0.003	>1000	>1000	0.217 (±0.024)

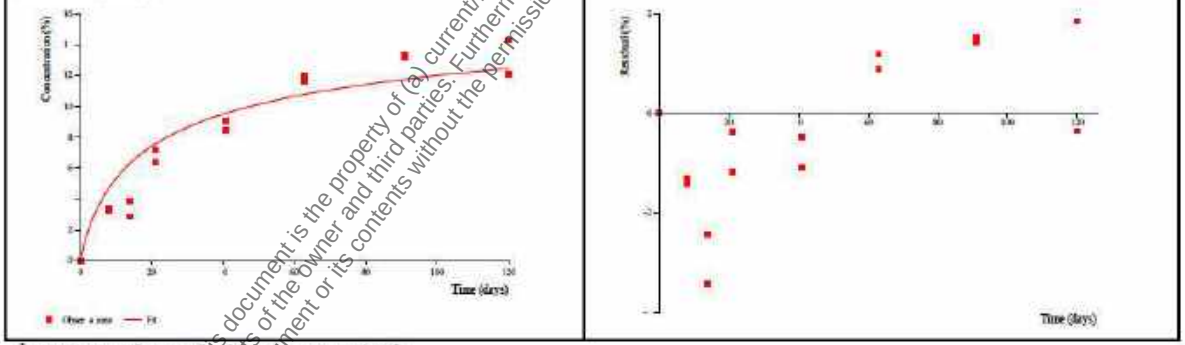
The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable.

Conclusion: Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate
No trigger endpoints can be derived for AMPA

Glyphosate (FOMC)



AMPA (SFO)



[1] t-test not relevant for kinetic parameter β

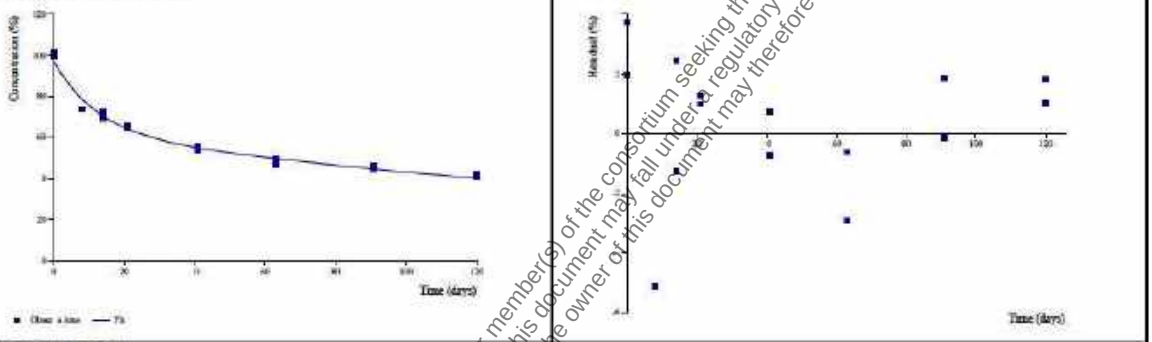
Table 7.1.2.1.1-54: Kinetic models and goodness-of-fit statistics of pathway fits for soil 18-Acres of study [redacted] (2010, CA 7.1.2.1.1/002) – modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 er-ror (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	g (± st.d. dev.)
Glypho-sate DFOP	Good	97.5	k_1: 0.0817 k_2: 0.0037 g: 0.3569	3.3	k_1: <0.001 k_2: <0.001	k_1: 0.0450 k_2: 0.0019	k_1: 0.118 k_2: 0.005	68.9	504	
AMPA: SFO	Accep-table	-	k: <0.0001	12.0	k: 0.5	k: -0.0034	k: 0.003	>1000	>1000	0.225 (±0.027)

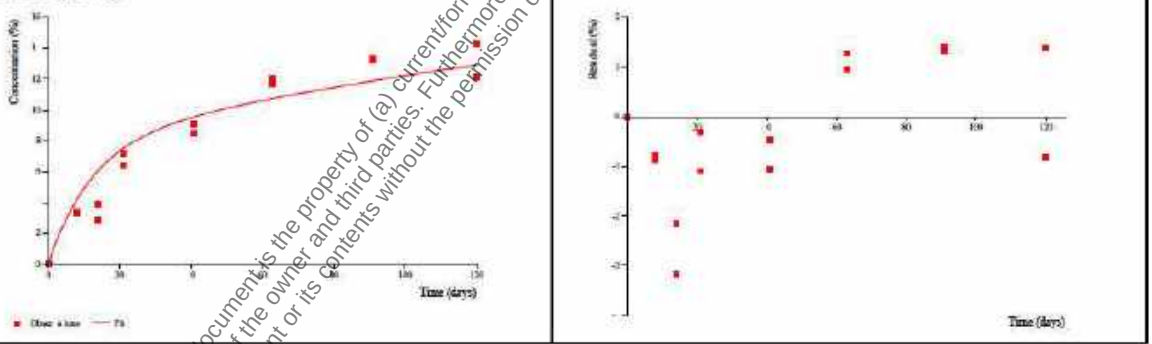
The degradation of glyphosate is well described by the pathway fit. For AMPA, the visual fit is acceptable but the parameter k is not significantly different from zero as metabolite concentration is still increasing towards the end of the study. Thus, the pathway fit is not acceptable.

Conclusion: Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate
No trigger endpoints can be derived for AMPA

Glyphosate (DFOP)



AMPA (SFO)



Overview of trigger and modelling endpoints

Table 7.1.2.1.1-55: Laboratory trigger and modelling endpoints of glyphosate

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (1993, CA 7.1.1.1/006): Les Evouettes	Silt loam	6.1[2]	20 / 40	9.7 / 184	6.5	DFOP	26.0[5]	6.5	DFOP
[redacted] (1995, CA 7.1.1.1/005): Arrow	Sandy loam	5.9[3]	20 / 40	37.8 / >1000	2.3	FOMC	126.2[6]	3.6	DFOP
[redacted] (1996, CA 7.1.1.1/003): Soil B	Sandy loam	6.7	25 / 75[4]	1.1 / 21.3	7.0	FOMC	6.9[5]	7.0	FOMC
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.1	Sand	5.9[3]	20 / 45	8.3 / 51.3	2.5	DFOP	15.5[5]	2.5	DFOP
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.2	Loamy sand	5.6[3]	20 / 45	18.1 / 162	5.9	DFOP	64.2[6]	5.9	DFOP
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	6.4[3]	20 / 45	2.7 / 13.0	7.5	DFOP	2.8	8.9	SFO
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	6.4[3]	10 / 45	7.9 / 50.9	2.4	DFOP	5.9[5]	2.4	DFOP
[redacted] (2010, CA 7.1.1.1/001): Gartenacker	Loam	7.1	20 / 50	8.1 / 55.4	3.1	DFOP	9.2[5]	3.1	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group A	Sand	6.9	20 / 40	4.5 / 68.9	5.6	DFOP	20.8[5]	5.6	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group B	Sand	6.9	20 / 20	3.2 / 76.7	2.8	DFOP	15.2[5]	2.8	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group C	Sand	6.9	8 / 40	41.6 / 201	2.3	HS	22.0[6]	2.3	HS

Table 7.1.2.1.1-55: Laboratory trigger and modelling endpoints of glyphosate

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group D (sterile)	Sand	6.3	20 / 40	15.8 / 134	4.4	DFOP	50.6[6]	4.4	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group E	Sand	6.9	20 / 40	7.0 / 73.2	6.2	DFOP	22.0[5]	6.2	DFOP
[redacted] (1992, CA 7.1.2.1.1/005): Beedon manor, dose group F	Clay loam	7.8	20 / 40	0.6 / 9.7	2.6	DFOP	2.6[5]	2.6	DFOP
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.1	Sand	6.1[2]	20 / 40	10.8 / 84.0	3.3	DFOP	13.9[5]	3.3	DFOP
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.2	Sand	6.0[2]	20 / 40	6.3 / 157	7.8	HS	47.0[6]	7.8	HS
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.3	Loamy sand	6.9[2]	20 / 40	5.8 / 22.2	2.5	DFOP	3.5[5]	2.5	DFOP
[redacted] (2010, CA 7.1.2.1.1/002): Drusenheim	Loam	7.4	20 / 50	2.2 / 14.4	5.0	FOMC	2.1[5]	5.0	FOMC
[redacted] (2010, CA 7.1.2.1.1/002): Pappelacker	Loamy sand	7.0	20 / 50	3.6 / 37.6	5.5	DFOP	6.4[5]	5.5	DFOP
[redacted] (2010, CA 7.1.2.1.1/002): 18-Acres	Sandy clay loam	5.7	20 / 50	60.2 / >1000	2.0	FOMC	98.7[6]	2.9	DFOP

[1] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
[2] Buffer solution unknown
[3] Measured in $CaCl_2$ solution
[4] % moisture at 1/3 bar
[5] Calculated as $DT_{90}/3.32$ as 10 % of initially measured concentration reached within experimental period
[6] Calculated as $\ln(2)/k_2$ as 10 % of initially measured concentration not reached within experimental period

Table 7.1.2.1.1-56: Laboratory trigger and modelling endpoints of AMPA

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	ff (from parent)	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (1993, CA 7.1.1.1/006): Les Evouettes	Silt loam	6.1[2]	20 / 40	424 / >1000	15.4	DFOP-SFO	0.346	199	15.4	DFOP-SFO
[redacted] (1995, CA 7.1.1.1/005): Arrow	Sandy loam	5.9[3]	20 / 40	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1996, CA 7.1.1.1/003): Soil B	Sandy loam	6.7	25 / 75[4]	99.4 / 330	8.9	FOMC-SFO	0.264	106	8.9	FOMC-SFO
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.1	Sand	5.9[3]	20 / 45	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.2	Loamy sand	5.6[3]	20 / 45	497[6] / >1000[6]	8.8	DFOP-SFO	0.548[6]	497[6]	8.8	DFOP-SFO
[redacted] (1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	6.4[3]	20 / 45	41.4[6] / 137[6]	15.8	DFOP-SFO	0.424	43.1	18.2	SFO-SFO
[redacted] 1996, CA 7.1.1.1/004): Speyer 2.3	Loamy sand	6.4[3]	10 / 45	129 / 429	8.2	DFOP-SFO	0.454	50.0	8.2	DFOP-SFO
[redacted] (2010, CA 7.1.1.1/001): Gartenacker	Loam	7.1	20 / 50	119 / 396	8.2	DFOP-SFO	0.183	65.7	8.2	DFOP-SFO
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group A	Sand	6.9	20 / 40	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group B	Sand	6.9	20 / 20	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group C	Sand	6.9	8 / 40	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-

Table 7.1.2.1.1-56: Laboratory trigger and modelling endpoints of AMPA

Reference	Soil type	pH (H_2O)	t. °C / % MWHC	DT_{50} / DT_{90} (d)	St. (χ^2)	Method of calc.	ff (from parent)	DT_{50} (d) 20 °C pF2/10kPa[1]	St. (χ^2)	Method of calc.
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group D (sterile)	Sand	6.3	20 / 40	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-
[redacted] (1992, CA 7.1.2.1.1/005): Speyer 2.1, dose group E	Sand	6.9	20 / 40	283 / 940	6.4	DFOP-SFO	0.393	283	6.4	DFOP-SFO
[redacted] (1992, CA 7.1.2.1.1/005): Beedon manor, dose group F	Clay loam	7.8	20 / 40	67.3 / 224	16.4	DFOP-SFO	0.149	59.0	16.4	DFOP-SFO
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.1	Sand	6.1[2]	20 / 40	86.5 / 288	13.7	DFOP-SFO	0.687	47.7	13.7	DFOP-SFO
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.2	Sand	6.0[2]	20 / 40	110 / 365	8.9	HS-SFO	0.683	76.0	8.9	HS-SFO
[redacted] (1993, CA 7.1.2.1.1/004): Speyer 2.3	Loamy sand	6.9[2]	20 / 40	85.0 / 282	8.8	DFOP-SFO	0.336	44.8	8.8	DFOP-SFO
[redacted] (2010, CA 7.1.2.1.1/002): Drusenheim	Loam	7.4	20 / 50	29.4 / 97.7	3.8	FOMC-SFO	0.285	14.2	3.8	FOMC-SFO
[redacted] (2010, CA 7.1.2.1.1/002): Pappelacker	Loamy sand	7.0	20 / 50	90.9 / 302	6.2	DFOP-SFO	0.192	51.4	6.2	DFOP-SFO
[redacted] (2010, CA 7.1.2.1.1/002): 18-Acres	Sandy clay loam	5.7	20 / 50	n.a.[5]	-	-	n.a.[5]	n.a.[5]	-	-

[1] Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
[2] Buffer solution unknown
[3] Measured in $CaCl_2$ solution
[4] % moisture at 1/3 bar
[5] No reliable endpoints were derived as no real decline phase was observed
[6] Endpoint derived from modified pathway fit with fixed parameters

3. Assessment and conclusion

Assessment and conclusion by applicant:
The kinetic evaluation was conducted according to current guidance. Therefore, the study and the endpoints derived are considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/002
Report author	[redacted]
Report year	2010
Report title	Rate of degradation of [14C]glyphosate in three soils incubated under aerobic conditions
Report No	1946W-1
Document No	
Guidelines followed in study	OECD 307 US EPA OPPTS 835.4100
Deviations from current test guideline	From OECD 307: - one soil (Drusenheim) was stored slighly longer than 3 months (sampling 15/07/2009; application 03/11/2009)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate was investigated in three soils under aerobic conditions in the dark in the laboratory at 20 ± 1 °C and 50 ± 10 % of the water holding capacity at pF 2.5 for up to 120 days.

Three soils were used for the study: the loam soil Drusenheim from France, the loamy sand soil Pappelacker from Switzerland and the sandy clay loam soil 18-Acres from the UK. The soils had an organic carbon contents ranging from 1.7 to 2.5 % and the soil pH in water ranged from 5.7 to 7.4.

The test was performed in flow-through systems, purged with moistened, CO_2-free air and connected to an ethylene glycol trap to collect volatile organic compounds and two successive 1 N NaOH traps to collect carbon dioxide.

The [^{14}C]-glyphosate dose rate was 3.80 mg/kg of soil, equivalent to a single application rate of 2.88 kg a.s./ha in the field, based on a soil density of 1.5 g/cm^3 and a penetration depth of 5 cm.

Duplicate samples were removed for work-up immediately after treatment (time 0) and, after 1, 3, 8, 14, 27, 48 and 70 days after treatment (DAT) for soil Drusenheim; 1, 3, 8, 14, 27, 48, 70, 91 and 120 DAT for soil Pappelacker; and 8, 14, 21, 41, 63, 91 and 120 DAT for soil 18-Acres. Solutions for trapping of volatile radioactivity were exchanged at each sampling point.

Material balances of radioactivity ranged from 86.3 to 101.6 % of applied radioactivity (% AR) for soil Drusenheim, from 79.7 to 102.1 % AR for soil Pappelacker and from 93.1 to 100.4 % AR for soil 18-Acres.

^{14}C-carbon dioxide was formed at 62.1 % AR in maximum at 70 DAT in soil Drusenheim, 54.4 % AR at 120 DAT in soil Pappelacker and 16.9 % AR at 120 DAT in soil 18-Acres. Formation of other organic volatile components was insignificant (<0.1 % AR).

The radioactivity extractable from soil decreased from 92.5 to 9.3 % AR from 0 DAT to the end of the incubation period at 70 DAT for soil Drusenheim, from 100.3 to 14.2 % AR from 0 DAT to 120 DAT for soil Pappelacker and from 96.6 to 58.7 % AR from 0 DAT to 120 DAT for soil 18-Acres. In turn, non-extractable radioactivity (NER) increased from 9.1 to 15.2 % AR in soil Drusenheim, from 1.9 to 20.2 % AR in soil Pappelacker and from 3.8 to 21.6 % AR in soil 18-Acres.

The portion of ^{14}C-glyphosate extractable from soil decreased from 0 DAT to 70 DAT from 91.0 to 1.0 % AR in soil Drusenheim, from 0 DAT to 120 DAT from 98.7 to 2.1 % AR in soil Pappelacker and from 0 DAT to 120 DAT from 94.4 to 41.7 % AR in soil 18-Acres.

The metabolite aminomethylphosphonic acid (AMPA) was identified in soil Drusenheim at a maximum of 21.2 % AR at 8 DAT to decrease to 6.2 % AR at 70 DAT. In soil Pappelacker, AMPA had a maximum of 14.5 % AR at 48 DAT to decrease to 9.1 % AR at 120 DAT. In soil 18-Acres AMPA had a maximum of 13.3 % AR at 91 DAT to decrease to 13.2 % AR at 120 DAT. No other radioactive components were observed above 5 % AR at any time.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C-phosphonomethyl]-glyphosate
Lot No.:	53463-3-23
Specific activity:	10.28 MBq/mg (47 mCi/mmol)
Radiochemical purity:	99.8 %

2. Soil:

The soils were collected freshly in France, Switzerland and the UK, no fertilizers or pesticides have been applied to the soils for 5 years. The soils were sieved to ≤ 2 mm. Following arrival at the testing facility, the soils were stored refrigerated in the dark in containers with free access to air for less than three months for soils Pappelacker and 18-acres, while soil Drusenheim was stored for 111 days. Characteristics of the test soils are presented in the table below.

Table 7.1.2.1.1-57: Characteristics of test soils

Parameter	Results		
Soil	Drusenheim	Pappelacker	18-Acres
Country	France	Switzerland	UK
Textural Class (USDA)	Loam	Loamy Sand	Sandy clay loam
Sand (50 µm – 2 mm) (%)	47	75	51
Silt (2 µm – 50 µm) (%)	28	20	24
Clay (< 2 µm) (%)	25	5	25
pH (water)	7.4	7.0	5.7
Organic carbon (%)	1.7	1.9	2.5
Organic matter (%)	2.9	3.2	4.4
Cation exchange capacity (meq/100 g)	23.6	11.7	18.1
Maximum Water Holding Capacity (%)	34.3	40.7	51.5
Water Holding Capacity at 0.33 bar (%)	17.6	12.4	19.7
Bulk Density (disturbed) (g/cm^3)	1.14	0.98	1.03
Microbial biomass (µg C/g)			
Experimental Start (0 DAT)	255.2	164.4	487.8
During study	-	256.3 (91 DAT)	615.7 (91 DAT)
Study end	134.8(70 DAT)	157.3 (120 DAT)	305.2 (120 DAT)

DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems, purged with moistened, CO_2-free air were used. After leaving the test vessels, the air was passed through a trap containing ethylene glycol to trap volatile organic compounds and two traps containing 1 N aqueous NaOH to collect carbon dioxide.

50 g of sieved soil (dry weight equivalents) were weighed into each test vessel and soil moisture was adjusted to 50 %±10 % of the water holding capacity at pF 2.5 and the test systems were acclimated for one week at test conditions.

The target dose for all samples was 3.8 mg/kg, corresponding to a maximum recommended field application rate of 2.88 kg glyphosate/ha, based on a soil bulk density of 1.5 g/cm^3 and a penetration depth of 5 cm. A test solution of [^{14}C]-, [^{13}C]- and [^{12}C]-glyphosate was prepared in water. 0.5 mL of this solution were applied to each test system, resulting in a final concentration of 3.8 mg/kg.

Test systems were incubated under aerobic conditions in the dark for up to 120 days at 20 °C and 50 % of the water holding capacity at pF 2.5.

2. Sampling

For all soils, duplicate samples were collected immediately after treatment (time 0) and at 7 to 9 subsequent sampling times up to 120 days after treatment (DAT, soil Drusenheim: 0, 1, 3, 8, 14, 27, 48 and 70 DAT, soil Pappelacker: 0, 1, 3, 8, 14, 27, 48, 70, 91 and 120 DAT, soil 18-Acres: 0, 8, 14, 21, 41, 63, 91 and 120 DAT). Trapping solutions were exchanged at each sampling point.

3. Analytical procedures

At each sampling interval, soil samples were extracted 3 times successively with 100 mL 0.5 M NH_4OH solution. The extracts were pooled, and radioactivity was determined by LSC.

The soil extracts were adjusted to pH of 2 to 3 by dropwise adding concentrated phosphoric acid prior to further workup. 0.01 M EDTA was added prior to concentration to breakdown any possible glyphosate-metal ions chelation. Soil extracts were concentrated under reduced pressure via roto-vac, Savant Speed-Vac or by rotary evaporation followed up by HPLC analysis. The average workup-recoveries were 98.6 ± 8.4 %, 98.2 ± 8.3 % and 95.5 ± 6.5 % for soils Drusenheim, Pappelacker and 18-Acres, respectively. The LOD for glyphosate and metabolites observed in the HPLC radio chromatograms was 3 µg/kg soil.

Identification and quantitation of radioactive glyphosate soil residues was done by cation-exchange HPLC analysis. Confirmatory HPLC analysis with anion-exchange HPLC method was carried for representative extracts. Peak assignment for glyphosate was based on co-elution with the reference standard injected with each sample. Peak assignment for AMPA was by comparison of retention time with a [^{14}C]-AMPA reference standard using the corresponding HPLC method.

The non-extractable radioactivity in post-extracted soil was determined by combustion/LSC.

For the two replicate samples from the last sampling date of all experiments, NER were fractionated into fulvic acid, humic acid and humin fractions. The previously extracted soil sample was extracted with 0.1 M aqueous NaOH. The extract was acidified with aqueous 12 N HCl. After precipitation overnight, the precipitated humic acid fraction was separated by centrifugation, and the fulvic acid fraction (supernatant) was decanted. The humic acid fraction was re-dissolved in aqueous 0.1 M NaOH. The two fractions were analysed by LSC.

Aliquots of the trapping solutions were analyzed by LSC. The identification of CO_2 in the NaOH traps was determined by the addition of $BaCl_2$ to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and metabolites in soil extracts as well as results from fractionation of NER are summarised in the tables below.

Table 7.1.2.1.1-58: Degradation of [^{14}C]glyphosate in soil Drusenheim under aerobic conditions (expressed as percent of applied radioactivity)

		DAT							
Compound	**Replicate**	**0**	**1**	**3**	**8**	**14**	**27**	**48**	**70**
Glyphosate	A	91.4	64.9	43.5	18.3	10.2	4.9	1.6	1.1
	B	90.5	66.2	44.1	18.1	10.8	3.3	1.5	0.9
	Mean	91.0	65.6	43.8	18.2	10.5	4.1	1.6	1.0
AMPA	A	0.5	9.6	15.0	21.2	19.7	17.5	9.5	6.2
	B	0.3	7.7	15.1	21.1	18.9	15.9	9.8	6.1
	Mean	0.4	8.7	15.1	21.2	19.3	16.7	9.7	6.2
Carbon Dioxide	A	NA	6.7	16.3	31.9	42.1	51.4	60.6	62.1
	B	NA	6.7	16.3	31.9	42.1	51.4	59.8	62.1
	Mean	NA	6.7	16.3	31.9	42.1	51.4	60.2	62.1
Volatile organic compounds	A	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total extractable residues	A	93.1	75.9	61.6	42.4	32.8	25.5	13.4	9.2
	B	91.8	75.6	61.2	41.8	34.4	22.1	13.8	9.4
	Mean	92.5	75.8	61.4	42.1	33.6	23.8	13.6	9.3
Non-extractable Residues	A	9.1	13.4	14.1	13.4	13.5	14.3	11.9	15.8
	B	9.1	12.5	14.2	13.5	13.8	13.2	13.1	14.6
	Mean	9.1	13.0	14.2	13.5	13.7	13.8	12.5	15.2

Table 7.1.2.1.1-58: Degradation of [^{14}C]glyphosate in soil Drusenheim under aerobic conditions (expressed as percent of applied radioactivity)

Mass balance	A	102.2	96.0	92.0	87.7	88.4	91.2	85.9	87.1
	B	100.9	94.8	91.7	87.2	90.3	86.7	86.7	86.2
	Mean	101.6	95.4	91.9	87.5	89.4	89.0	86.3	86.6

DAT: days after treatment; NA: not applicable

Table 7.1.2.1.1-59: Degradation of [^{14}C]glyphosate in soil Pappelacker under aerobic conditions (expressed as percent of applied radioactivity)

		DAT									
Compound	**Replicate**	**0**	**1**	**3**	**8**	**14**	**27**	**48**	**70**	**91**	**120**
Glyphosate	A	99.4	77.1	59.0	27.4	19.1	10.1	4.5	2.3	2.0	2.0
	B	98.0	77.2	58.1	29.2	29.6	18.2	9.1	2.9	1.8	2.2
	Mean	98.7	77.2	58.6	28.3	24.4	14.2	6.8	2.6	1.9	2.1
AMPA	A	0.4	4.2	7.4	14.5	14.2	13.7	13.6	10.4	10.0	9.1
	B	0.3	3.9	7.9	13.7	12.2	13.2	15.4	11.6	9.5	9.0
	Mean	0.4	4.1	7.7	14.1	13.2	13.5	14.5	11.0	9.8	9.1
Carbon Dioxide	A	NA	4.8	12.1	27.2	36.3	46.0	53.2	49.7	52.0	54.4
	B	NA	4.8	12.1	27.2	36.3	46.0	45.4	49.7	52.0	54.4
	Mean	NA	4.8	12.1	27.2	36.3	46.0	49.3	49.7	52.0	54.4
Volatile organic compounds	A	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total extractable residues	A	100.4	83.3	68.9	44.5	36.8	27.9	21.7	15.6	14.6	14.3
	B	100.1	82.2	68.3	45.5	46.0	36.1	28.4	17.9	13.8	14.7
	Mean	100.3	82.8	68.6	45.0	41.4	32.0	25.1	16.8	14.2	14.5
Non-extractable Residues	A	1.8	10.1	13.9	14.7	17.6	15.0	15.9	16.0	13.7	18.4
	B	1.9	9.7	14.1	13.6	18.0	16.8	17.3	18.8	13.3	21.9
	Mean	1.9	9.9	14.0	14.2	17.8	15.9	16.6	17.4	13.5	20.2
Mass balance	A	102.2	98.2	94.9	86.4	90.7	88.9	90.8	81.3	80.3	87.1
	B	102.0	96.7	94.5	86.3	100.3	98.9	91.1	86.4	79.1	91.0
	Mean	102.1	97.5	94.7	86.4	95.5	93.9	91.0	83.9	79.7	89.1

DAT: days after treatment; NA: not applicable

Table 7.1.2.1.1-60: Degradation of [^{14}C]glyphosate in soil 18-Acres under aerobic conditions (expressed as percent of applied radioactivity)

		DAT							
Compound	**Replicate**	**0**	**8**	**14**	**21**	**41**	**63**	**91**	**120**
Glyphosate	A	95.5	73.9	69.4	65.6	55.9	47.0	44.7	42.1
	B	93.3	73.9	73.1	65.3	54.4	49.3	46.7	41.3
	Mean	94.4	73.9	71.3	65.5	55.2	48.2	45.7	41.7
AMPA	A	0.6	3.3	3.9	6.4	9.1	11.7	13.3	14.3
	B	1.0	3.4	2.9	7.2	8.5	12.0	13.2	12.1
	Mean	0.8	3.4	3.4	6.8	8.8	11.9	13.3	13.2
Carbon Dioxide	A	NA	4.0	5.9	7.7	10.6	13.7	15.5	16.9
	B	NA	4.0	5.9	7.7	10.6	13.7	15.5	16.9
	Mean	NA	4.0	5.9	7.7	10.6	13.7	15.5	16.9
Volatile organic compounds	A	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total extractable residues	A	97.5	78.6	74.3	75.1	68.1	61.8	59.9	59.0
	B	95.7	78.5	76.5	75.3	67.8	64.4	61.9	58.3
	Mean	96.6	78.6	75.4	75.2	68.0	63.1	60.9	58.7

Table 7.1.2.1.1-60: Degradation of [^{14}C]glyphosate in soil 18-Acres under aerobic conditions (expressed as percent of applied radioactivity)

Non-extractable Residues	A	3.8	13.0	14.8	15.4	18.5	16.9	15.6	22.6
	B	3.8	12.8	16.0	15.2	18.2	17.0	17.7	20.6
	Mean	3.8	12.9	15.4	15.3	18.4	17.0	16.7	21.6
Mass balance	A	101.3	95.6	95.0	98.2	97.2	92.4	91.0	98.5
	B	99.5	95.3	98.4	98.2	96.6	95.1	95.1	95.8
	Mean	100.4	95.5	96.7	98.2	96.9	93.8	93.1	97.2

DAT: days after treatment; NA: not applicable

Table 7.1.2.1.1-61: Fractionation post extracted soil from last sampling dates (in percent of applied radioactivity)

Experiment	DAT	Replicate	Fulvic acid	Humic acid	Humins
Drusenheim	70	A	2.5	2.4	10.9
		B	2.1	3.1	9.4
		Mean	2.3	2.8	10.2
Pappelacker	120	A	4.2	3.2	11.0
		B	4.3	2.9	14.7
		Mean	4.3	3.1	12.9
18-Acres	120	A	2.8	10.8	9.0
		B	2.9	10.6	7.1
		Mean	2.9	10.7	8.1

DAT: days after treatment

B. MASS BALANCE

Material balances ranged from 86.3 to 101.6 % of applied radioactivity (% AR) for soil Drusenheim, from 79.7 to 102.1 % AR in soil Pappelacker and from 93.1 to 100.4 % AR in soil 18-Acres (mean of two replicates).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

In all soils, the amount of radioactivity extractable from soil decreased from 0 DAT to the end of the experiments at 70 DAT (soil Drusenheim) or 120 DAT (soils Pappelacker and 18-Acres) from 92.5 to 9.3 % AR in soil Drusenheim, from 100.3 to 14.2 % AR in soil Pappelacker and from 96.6 to 58.7 % AR in soil 18-Acres. Accordingly, the amount of non-extractable residues (NER) increased from 9.1 to 15.2 % AR in soil Drusenheim, from 1.9 to 20.2 % AR in soil Pappelacker and from 3.8 to 21.6 % AR in soil 18-Acres.

D. VOLATILE RADIOACTIVITY

The maximum amount of carbon dioxide reached at study end was 62.1 % AR at 70 DAT in soil Drusenheim, 54.4 % AR at 120 DAT in soil Pappelacker and 16.9 % AR at 120 DAT in soil 18-Acres (mean of two replicates). There were no organic volatiles determined in all soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in soil extracts decreased from 0 DAT to the end of the experiments at 70 DAT (soil Drusenheim) or 120 DAT (soils Pappelacker and 18-Acres) from 91.0 to 1.0 % AR in soil Drusenheim from 98.7 to 2.1 % AR in soil Pappelacker and from 94.4 to 41.7 % AR in soil 18-Acres. Besides carbon dioxide, major metabolite aminomethylphosphonic acid (AMPA) was detected. In soil Drusenheim, the maximum amount of 21.2 % AR was reached at 8 DAT and then decreased to 6.2 % AR at 70 DAT. In soil Pappelacker, AMPA was detected with a maximum amount of 14.5 % AR at 48 DAT and decreased to 9.1 % AR at 120 DAT. In soil 18-Acres AMPA was detected with a maximum amount of 13.3 % AR at 91 DAT and decreased to 13.2 % AR until the end of the study (120 DAT). No other metabolites were detected above 5 % AR at any time.

NER were further partitioned for the last sampling dates of the experiments. For soils Drusenheim and Pappelacker, the insoluble humin fraction was the largest component representing an average of 10.2 and 12.9 % AR while the fulvic and humic acid fractions represented below 4.3 % AR. For soil 18-Acres, the humic acid and humin fraction represented 10.7 and 8.1 % AR, respectively, while the fulvic acid fractions represented 2.9 % AR.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found under CA7.1.2.1.1/001.

III. CONCLUSIONS

The study on aerobic soil degradation rate was conducted on three soils using [^{14}C]glyphosate at a dose rate of 3.80 mg/kg at 20 °C for 70 to 120 days. Material balances, averaged 90.9 ± 5.2 % AR for soil Drusenheim, 91.4 ± 7.0 % AR for soil Pappelacker, and 96.5 ± 2.6 % AR for soil 18-Acres. The main degradate observed in the study was $^{14}CO_2$, with a maximum average of 62.1 % AR for soil Drusenheim, 54.4 % AR for soil Pappelacker and 16.9 % AR for soil 18-Acres at the end of the study. The metabolite AMPA occurred with maximum 21.2 % AR in soil Drusenheim, 14.5 % AR in soil Pappelacker and 13.3 % AR in soil 18-Acres.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was conducted according to the current guidelines, showing minor deviations. Storage of soil Drusenheim was for more than 3 months (sampling 15/07/2009; application 03/11/2009, i.e. 111 days). The material balance was below 90 % AR for some samples of soil Drusenheim (min: 86.1 %) and soil Pappelacker (min: 79.1 %). Losses can be attributed to incomplete trapping of CO_2 as the container had to be opened at each sampling point, which allowed CO_2 to escape. The deviations are considered to have no influence on the overall outcome of the study.
Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/003
Report author	[redacted]
Report year	1993
Report title	Degradation of 14C-glyphosate in three soils incubated under aerobic conditions
Report No	271618
Document No	
Guidelines followed in study	BBA Guideline Part IV, 4-1
Deviations from current test guideline	From OECD 307: - no information on soil history prior to arrival at test site
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

Data point:	CA 7.1.2.1.1/004
Report author	[redacted]
Report year	2002
Report title	First amendment (addendum) to report - Degradation of 14C-glyphosate in three soils incubated under aerobic conditions
Report No	271618
Document No	
Guidelines followed in study	BBA Guideline Part IV, 4-1
Deviations from current test guideline	From OECD 307: no information on soil history prior to arrival at test site
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C] glyphosate was investigated in three soils under aerobic conditions in the dark in the laboratory at 20 ± 2°C and 40 % of the maximum water holding capacity for 105 days.

The following three German soils were used: the sand soil Speyer 2.1, the sand soil Speyer 2.2, and the loamy sand soil Speyer 2.3. The amount of organic carbon of the soils ranged from 0.70 to 2.29 % and the pH ranged from 6.0 to 6.9.

The test was performed in flow-through systems purged with moistened air and connected to two successive 2 N aqueous NaOH traps to collect carbon dioxide and an ethylene glycol trap to collect volatile organic compounds.
The application rate was 4.8 mg/kg dry soil, corresponding to the anticipated use rate of 3.6 kg glyphosate/ha.

Duplicate samples from each system were taken at 0, 7, 14, 28, 56, 84, and 105 days after treatment (DAT) single replicates per sampling point were processed and analysed. The volatile traps were assayed at each sampling interval to determine the amount of carbon dioxide and volatile organic compounds.

Mass balances ranged from 75.6 to 99.7 % of applied radioactivity (% AR) for soil Speyer 2.1, from 88.8 to 99.7 % AR for soil Speyer 2.2, and from 84.7 to 97.7 % AR for soil Speyer 2.3. The partly low recoveries as well as the decrease over time were attributed to loss of ^{14}C-CO_2 that could have escaped from the incubation cylinders each time they had to be opened for removing the soil samples.

Maximum amounts of carbon dioxide reached at study end (105 DAT) were 26.1, 23.5, and 61.4 % AR in soils Speyer 2.1, Speyer 2.2, and Speyer 2.3, respectively. Organic volatiles determined were ≤0.1 % AR for all soils at all sampling points.

The amount of radioactivity extractable from the soil decreased from 0 DAT to 105 DAT from 90.8 to 47.9 % AR in soil Speyer 2.1, from 96.8 to 62.6 % AR in soil Speyer 2.2, and from 90.8 to 18.3 % AR in soil Speyer 2.3.

Non-extractable residues (NER) were <10 % in all soils. In soil Speyer 2.1, they increased from 0.3 % AR at 0 DAT to 2.5 % AR at 14 DAT and 56 DAT, and then slightly decreased to 1.6 % AR at 105 DAT. In soil Speyer 2.2, increased from 0.8 % AR at 0 DAT to 8.6 % AR at 105 DAT. In soil Speyer 2.3, NER increased from 1.4 % AR at 0 DAT to 8.6 % AR at 56 DAT, and then slightly decreased to 5.0 % AR at 105 DAT.

The amount of glyphosate in soil extracts decreased from 0 DAT to 105 DAT from 86.7 to 8.0 % AR in soil Speyer 2.1, from 91.3 to 13.5 % AR in soil Speyer 2.2 and from 90.9 to 2.5 % AR in soil Speyer 2.3.

The major metabolite formed, aminomethylphosphonic acid (AMPA), was detected with a maximum amount of 41.2 % AR at 14 DAT in soil Speyer 2.1 where it subsequently decreased to 23.5 % AR at 105 DAT. In soil Speyer 2.2, the maximum amount was 42.4 % AR at 7 DAT followed by a decrease to 35.4 % AR at 105 DAT. In soil Speyer 2.3, the maximum amount was 25.1 % AR at 14 DAT followed by a decrease to 12.1 % AR at 105 DAT.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C-phosphonomethyl]-glyphosate
Lot No.:	Not provided
Specific activity:	11.1 MBq/mg (298.8 µCi/mg)
Radiochemical purity:	99 %
Chemical purity:	Not provided

2. Soil:
Soils were sieved to ≤2 mm. The soils arrived at the testing facility about 4 months prior to start of the study and were stored in concrete cylinders under outdoor conditions. The soils have not been subjected to any pesticide or organic nor inorganic fertilizer treatment since their arrival. Characteristics of the test soils are presented in the table below.

Table 7.1.2.1.1-62: Characteristics of test soils

Parameter	Results		
Soil	Speyer 2.1	Speyer 2.2	Speyer 2.3
Country	Germany	Germany	Germany
Textural Class (BBA)	Sand	Sand	Loamy sand
Sand (> 20 µm) (%)	92.1	89.4	80.5
Silt (2 µm – 20 µm) (%)	4.4	5.6	11.4
Clay (< 2 µm) (%)	3.5	5.1	8.3
pH [1]	6.1	6.0	6.9
Organic carbon (%)	0.70	2.29	1.34
Cation exchange capacity (meq/100 g soil)	4.9	9.7	9.5
Maximum Water Holding Capacity (%)	31.9	44.3	34.9
Microbial biomass (mg C /100 g soil)			
Start	11.7	40.3	37.2
Completion of incubation	7.8	32.8	26.4

[1] Medium not stated

B. STUDY DESIGN

1. Experimental conditions

Flow-through test systems were used, consisting of test vessels (Petri dishes) filled with soil, which were placed in glass cylinders equipped with air inlets and outlets. Air entering the system was moistened with a water trap. After leaving the cylinders, the air was passed through two traps containing 50 mL of 2 N NaOH to collect carbon dioxide and a trap containing 50 mL of ethylene glycol to trap volatile organic compounds.

50 g of sieved soil (dry weight equivalents) were weighed into each test vessel and soil moisture was adjusted to 40 % of the maximum water holding capacity (MWHC).

The study application rate corresponded to the anticipated use rate of 3.6 kg glyphosate/ha. A test solution of [^{14}C]glyphosate with a concentration of 0.9 mg/mL was prepared in water. 0.860 mL of this solution were applied to each test vessel, resulting in a final concentration of 4.8 mg/kg. After application, the test vessels (except 0 DAT) were placed in the glass cylinders, and the cylinders were closed with trap attachments.

Test vessels were incubated under aerobic conditions in the dark for 105 days at 20 °C and 40 % MWHC.

2. Sampling

Duplicate samples from each system were taken at 0, 7, 14, 28, 56, 84, and 105 days after treatment (DAT) one replicate per sampling point was processed and analysed. The volatiles traps were assayed at each sampling interval to determine the amount of carbon dioxide and volatile organic compounds.

3. Analytical procedures

At each sampling interval, soil samples were extracted 4-5 times with 0.5 N ammonia solution, and the extract was analysed by LSC. Exhaustive extraction was confirmed by subsequent extraction tests with 0.5 N KCl or HCl solution of pH 2.0. Extraction was performed on the day of sampling, and extracts were analysed within 4 to 14 days. Extracts were stored at -20 °C.

The soil debris resulting from the extractions were combusted, and the resulting $^{14}CO_2$ was determined by LSC.

Aliquots of the combined extracts were either treated by centrifugation or by ultrafiltration (PM10 membranes, Amicon), and concentrated by evaporation at 50°C on a rotary evaporator. Thin layer chromatography (TLC) analyses were performed as well with supernatants of centrifugation as with ultrafiltrates at sampling intervals 0, 7, 14 and 28 DAT, with ultrafiltrates additionally at the intervals 56, 84 and 105 DAT. Since no differences due to the workup procedure could be detected, the best TLCs were used for the evaluation.

Glyphosate was identified by thin layer chromatography (TLC) co-chromatography with a reference item using two different sets of stationary/ mobile phase. High performance liquid chromatography (HPLC) with fluorescence spectrometry detection was used to confirm glyphosate concentrations derived from TLC analysis.

Sodium hydroxide trapping solutions were mixed with water and analysed by LSC. Ethylene glycol was radioassayed directly. The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium hydroxide to aliquots of the trap contents. The presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of glyphosate and its metabolites in soil extracts are summarised in the tables below for the respective soils.

Table 7.1.2.1.1-63: Degradation of [^{14}C]glyphosate in soil Speyer 2.1 under aerobic conditions (expressed as percent of applied radioactivity)

Fraction	DAT						
	0	7	14	28	56	84	105
Glyphosate	86.7	56.0	38.1	22.6	9.7	9.7	8.0
AMPA	1.4	21.7	41.2	32.6	40.0	38.7	23.5
Carbon Dioxide	ND	12.3	15.1	20.5	23.7	25.2	26.1
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total extractable radioactivity	90.8	77.7	81.9	58.6	55.2	53.3	47.9
Non-extractable radioactivity	0.3	2.3	2.5	2.0	2.5	1.6	1.6
Mass balance	91.1	92.3	99.6	81.1	81.3	80.1	75.6

DAT: Days after treatment
ND = Not determined

Table 7.1.2.1.1-64: Degradation of [^{14}C]glyphosate in soil Speyer 2.2 under aerobic conditions (expressed as percent of applied radioactivity)

Fraction	DAT						
	0	7	14	28	56	84	105
Glyphosate	91.3	41.4	48.8	33.3	31.3	19.3	13.5
AMPA	0.0	42.4	31.4	33.1	34.6	33.9	35.4
Carbon Dioxide	ND	5.8	9.0	13.9	18.9	20.9	23.5
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total extractable radioactivity	96.8	83.8	83.4	77.6	73.0	63.0	62.6
Non-extractable radioactivity	0.8	6.8	7.3	7.1	7.3	4.9	8.6
Mass balance	97.6	96.4	99.7	98.6	99.2	88.8	94.7

DAT: Days after treatment
ND = Not determined

Table 7.1.2.1.1-65: Degradation of [^{14}C]glyphosate in soil Speyer 2.3 under aerobic conditions (expressed as percent of applied radioactivity)

Fraction	DAT						
	0	7	14	28	56	84	105
Glyphosate	90.9	39.4	19.7	5.5	4.3	3.0	2.5
AMPA	0.0	13.6	25.1	25.1	18.9	18.5	12.1
Carbon Dioxide	ND	31.0	39.8	50.3	56.9	58.9	61.4
Volatile organic compounds	ND	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total extractable radioactivity	90.9	59.0	44.8	32.7	24.6	21.5	18.3
Non-extractable radioactivity	1.4	7.7	7.0	7.0	8.6	6.0	5.0
Mass balance	92.3	97.7	91.6	89.9	90.1	86.4	84.7

DAT: Days after treatment
ND = Not determined

B. MASS BALANCE

Mass balances ranged from 75.6 to 99.6 % of applied radioactivity (% AR) for soil Speyer 2.1, from 88.8 to 99.7 % AR for soil Speyer 2.2, and from 84.7 to 97.7 % AR for soil Speyer 2.3. The partly low mass balances as well as the decrease over time can most likely be explained by the configuration of the test system. $^{14}CO_2$ is supposed to have escaped from the cylinders each time they had to be opened and these losses probably explain the observed recoveries.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity extractable from the soil decreased from 0 DAT to 105 DAT from 90.8 to 47.9 % AR in soil Speyer 2.1, from 96.8 to 62.6 % AR in soil Speyer 2.2, and from 90.9 to 18.3 % AR in soil Speyer 2.3.

Non-extractable residues (NER) were <10 % in all soils. In soil Speyer 2.1, they increased from 0.3 % AR at 0 DAT to 2.5 % AR at 14 DAT and 56 DAT, and then slightly decreased to 1.6 % AR at 105 DAT. In soil Speyer 2.2, increased from 0.8 % AR at 0 DAT to 8.6 % AR at 105 DAT. In soil Speyer 2.3, NER increased from 1.4 % AR at 0 DAT to 8.6 % AR at 56 DAT, and then slightly decreased to 5.0 % AR at 105 DAT.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (105 DAT) were 26.1, 23.5, and 61.4 % AR in soils Speyer 2.1, Speyer 2.2, and Speyer 2.3, respectively. Organic volatiles determined were ≤0.1 % AR for all soils at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in soil extracts decreased from 0 DAT to 105 DAT from 86.7 to 8.0 % AR in soil Speyer 2.1, from 91.3 to 13.5 % AR in soil Speyer 2.2 and from 90.9 to 2.5 % AR in soil Speyer 2.3.

The major metabolite formed, aminomethylphosphonic acid (AMPA), was detected with a maximum amount of 41.2 % AR at 14 DAT in soil Speyer 2.1 where it subsequently decreased to 23.5 % AR at 105 DAT. In soil Speyer 2.2, the maximum amount was 42.4 % AR at 7 DAT followed by a decrease to 35.4 % AR at 105 DAT. In soil Speyer 2.3, the maximum amount was 25.1 % AR at 14 DAT followed by a decrease to 12.1 % AR at 105 DAT.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found under CA7.1.2.1.1/001.

III. CONCLUSIONS

The fastest degradation of [^{14}C]-glyphosate was found in soil Speyer 2.3 and the slowest degradation was found in soil Speyer 2.2. The degradation rates could not be correlated to the characteristics of the soil types used. The only metabolite formed was AMPA. The mineralization of glyphosate was rather high and can be considered one of the important pathways of disappearance of [^{14}C]-glyphosate from standard Speyer soils.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted in accordance to the current guidelines with minor deviations.
For the two soils showing highest mineralisation, mass balances were below 90 % AR for a number of samples. The losses can be assigned to a loss of $^{14}CO_2$ when the test vessels had to be opened for sampling while significant radioactivity was in the gas phase of the test vessels.
The deviations in study conduct are not regarded to influence the results and general outcome of the study.
Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/005
Report author	[redacted]
Report year	1992
Report title	Glyphosate-Trimesium: Soil dissipation study (incl. addendum to final report)
Report No	7043-38/165
Document No	
Guidelines followed in study	Not stated
Deviations from current test guideline	From OECD 307: - No history is reported for soil Beedon Manor - No analysis was conducted for volatiles - Determination of non-extractable residues was only performed for day 0, hence full material balance is only available for that sampling interval
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate-trimesium salt, radiolabelled in the phosphonomethylglycine (PMG) anion, was investigated in two soils under various aerobic laboratory conditions in the dark for 104 days in maximum. The conditions varied in temperature (20 ± 2°C or 8 ± 2°C), nominal test concentration (0.4 or 4 mg glyphosate-trimesium/kg soil dry weight) and soil moisture (40 % or 20 % of the maximum water holding capacity, MWHC). For investigation of microbiological influence on degradation, some tests were performed with sterilised Speyer 2.1 soil. The latter was sterilised by gamma irradiation prior to incubation.

In addition, experiments were performed with glyphosate-trimesium radiolabelled in the trimethylsulphonium cation (TMS). These are not subject of this summary.

The two test soils were sand soil Speyer 2.1 and clay loam Beedon Manor. The organic matter content of soils ranged from 0.8 to 3.7 % and the pH from 6.3 to 7.8, respectively.

The tests were performed in 'static' test systems consisting of flasks filled with 50 g soil and plugged with glass wool.

For non-sterile samples of Speyer 2.1 and Beedon Manor soil, the application rate was either 0.4 or 4 mg/kg soil (dry weight), corresponding to the anticipated use rate of 0.4 or 4 kg glyphosate-trimesium/ha.

Duplicates of each system were processed and analysed at 0, 2, 4, 8, 16, 33, 64, and 104 days after treatment (DAT).

For the sterile samples of Speyer 2.1 soil, duplicate samples were processed and analysed at 0, 2, 4, 7, 16, 34, and 70 DAT.

The overall material balance of radioactivity at 0 DAT ranged from 93.21 to 102.26 % for all incubation series.

At a nominal test concentration of 4 mg/kg in soil Speyer 2.1, the radioactivity extractable from soil decreased from 0 to 104 DAT from 95.31 to 44.41 % AR at 20°C and 40 % MWHC, from 95.18 to 41.68 % AR at 20°C and 20 % MWHC, and from 95.13 to 59.73 % AR at 8°C and 40 % MWHC.

Following incubation at 20 °C and 40 % MWHC, the amount of glyphosate in soil extract decreased from 0 to 104 DAT from 74.88 to 7.75 % AR, from 77.00 to 7.24 % AR at 20 °C and 20 % MWHC, and from 79.51 to 26.77 % AR at 8 °C and 40 % MWHC.

At a nominal test concentration of 4 mg/kg in soil Beedon Manor, the radioactivity extractable from soil decreased from 0 to 104 DAT from 62.32 to 9.28 % AR at 20°C and 40 % MWHC. The amount of glyphosate in soil extract decreased from 0 to 104 DAT from 33.74 to 0.57 % AR.

At a nominal test concentration of 0.4 mg/kg in soil Speyer 2.1, the radioactivity extractable from soil decreased from 0 to 104 DAT from 97.08 to 48.08 % AR at 20°C and 40 % MWHC. The amount of glyphosate in soil extract decreased from 0 to 104 DAT from 75.59 to 7.26 % AR.

At a nominal test concentration of 4 mg/kg in sterile soil Speyer 2.1, the radioactivity extractable from soil decreased from 0 to 70 DAT from 92.80 to 56.76 % AR at 20°C and 40 % MWHC.

The amount of glyphosate in soil extract decreased from 0 to 70 DAT from 72.46 to 24.11 % AR.

Following application of 4 mg/kg to soil Speyer 2.1, the amount of aminomethylphosphonic acid (AMPA) in the soil extract increased to a maximum of 31.80 % AR at 64 DAT at 20 °C and 40 % MWHC, 27.55 % AR at 104 DAT at 20 °C and 20 % MWHC, and 23.19 % AR at 104 DAT at 8 °C and 40 % MWHC.

Following application of 4 mg/kg to soil Beedon Manor, the amount of AMPA in the soil extract increased to a maximum 13.54 % AR at 8 DAT at 20 °C and 40 % MWHC. The amount of AMPA subsequently decreased to 3.46 % AR at 104 DAT.

Following application of 0.4 mg/kg to soil Speyer 2.1, the amount of AMPA in the soil extract increased to a maximum of 31.42 % AR at 64 DAT at 20 °C and 40 % MWHC.

Following application of 4 mg/kg to sterile soil Speyer 2.1, the amount of AMPA in the soil extract increased to a maximum of 20.35 % AR at the end of the experiment (70 DAT) at 20 °C and 40 % MWHC.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate-trimesium, [^{14}C]-methylene labelled in the glyphosate anion moiety
Lot No.: 88-J30 and 91-J19
Specific activity: 2.07 GBq mmol^{-1} (228 µCi/mg)
Radiochemical purity: 95.1 and 98.2 % (for two batches)
Chemical purity: Not provided

The experiments with [^{14}C]glyphosate-trimesium, radiolabelled in the trimethylsulphonium cation (TMS) are not presented in this summary.

2. Soil:

Soil Speyer 2.1 (initial study and addendum) was stored in a storage plot and shipped to the test site eight days before the start of the experiment. The soil had received no pesticide treatment prior to the study. Soil Beedon Manor was collected from Beedon Manor Farm (UK). Upon receipt, the soil was stored for about two weeks covered under outdoor conditions at the test site. Soils were sieved to ≤2 mm. Characteristics of the test soils are presented in the table below

Table 7.1.2.1.1-66: Characteristics of test soils

Parameter	Results		
Soil	Speyer 2.1		Beedon Manor
Country	Germany		UK
Textural Class (USDA)	Sand		Clay loam
Sand (50 µm – 2 mm) (%)	88 [1]	96 [2]	33
Silt (2 µm – 50 µm) (%)	6 [1]	1 [2]	33
Clay (< 2 µm) (%)	6 [1]	3 [2]	34
pH (water)	6.9 [1]	6.3 [2]	7.8
Organic matter (%)	0.9 [1]	0.8 [2]	3.7
Organic carbon [3] (%)	0.5	0.5	2.1
Cation exchange capacity (meq/100 g)	2.7 [1]	2.6 [2]	20.9
Maximum Water Holding Capacity (%)	32.95 [1]	31.31 [2]	57.94
Water Holding Capacity at 0.33 bar (%)	4.5 [1]	14.2 [2]	23.4
Water Holding Capacity at 15.0 bar (%)	1.55 [1]	6.70 [2]	11.8
Microbial biomass (mg C/kg)			
Pre-experiment	78 [1]		483
Post-experiment	66 [1]		634

USDA: United States Department for Agriculture
[1] Soil collected on 16 April 1991 and used in initial study (incubation groups A, B, C and E, see below).
[2] Soil collected on 02 March 1992 and used in addendum (incubation group D, see below).
[3] Calculated from organic matter according to OC = OM/1.724

B. STUDY DESIGN

1. Experimental conditions

'Static' test systems were used, consisting of Erlenmeyer flasks filled with soil. The tests were performed at different conditions as summarised in the table below.

Table 7.1.2.1.1-67: Incubation groups

Incubation group	Soil	Moisture content [% of MWHC]	Incubation Temperature [±2 °C]	Nominal Application rate [2] [mg/kg]
A	Speyer 2.1	40	20	4.0
B	Speyer 2.1	20	20	4.0
C	Speyer 2.1	40	8	4.0
D [1]	Speyer 2.1, sterile	40	20	4.0
E	Speyer 2.1	40	20	0.4
F [1]	Beedon Manor	40	20	4.0

[1] Experiments conducted in the addendum to the study
[2] Application rate expressed as mg glyphosate-trimesium/kg soil dry weight

The flasks containing non-sterile soil were incubated at 8 or 20°C, and deionised water was added as appropriate to maintain the moisture content.

Two flasks with soil Speyer 2.1 were gamma irradiated for sterilisation. The sterilised soil samples were incubated at 20 °C in the dark. Moistened air was passed through as appropriate to maintain the moisture content.
50 g of sieved soil (dry weight equivalents) were weighed into each test vessel. The non-sterile test systems were acclimated for approximately two months for Speyer 2.1 soil and approximately one week for Beedon Manor soil at test conditions. The sterile test systems were acclimated for approximately 5-7 days at test conditions.

The study application rate was either 0.4 or 4 mg/kg soil (dry weight), corresponding to the anticipated use rate of 0.4 or 4 kg glyphosate-trimesium/ha. A test solution of [^{14}C]glyphosate-trimesium, radiolabelled in the phosphonomethylglycine anion (PMG), with a concentration of 0.02 or 0.2 mg/mL was prepared in water. 1 mL of this solution was applied to each test system.

Test systems were incubated under aerobic conditions in the dark for 104 days for the non-sterile soils Speyer 2.1 and Beedon Manor or 70 days for the sterile soil Speyer 2.1.

2. Sampling

Duplicate samples of each system were processed and analysed at 0, 2, 4, 8, 16, 33, 64, and 104 days after treatment (DAT) for the non-sterile Speyer 2.1 and Beedon Manor soil. Duplicate samples from each system were processed and analysed at 0, 2, 4, 7, 16, 34, and 70 DAT for the sterile Speyer 2.1 soil.

3. Analytical procedures

At each sampling interval, samples of soil Speyer 2.1 were extracted three times successively with 50 mL of 0.5 M aqueous ammonia solution using a mechanical shaker for 30 minutes.

Samples of soil Beedon Manor were extracted once with 1 M aqueous ammonia solution and five times with 0.5 M aqueous ammonia solution for 15 minutes using a mechanical shaker.

Extracts and soil were separated each by centrifugation and decantation. Aliquots of the extracts were filtered, and the filtered extracts were neutralised with formic acid.

Extracts of Speyer 2.1 soil were freeze-dried, re-suspended in 1 M formic acid and basified with ammonia. The homogenised suspension was directly used for chromatography.

Extracts of Beedon Manor soil were freeze-dried, re-suspended in 1 M ammonia solution, and the suspension was used for chromatography. The original flasks were rinsed with 1 M formic acid and the rinsings were weighed and analysed for radioactivity by LSC. Procedural recoveries for the work-up steps filtration and freeze-drying are presented in the table below.

Table 7.1.2.1.1-68: Procedural recoveries for filtration and freeze drying

Incubation group	Procedural recoveries for filtration [%]	Procedural recoveries for freeze-drying [%]
A	71.29 - 100.09	99.70 - 122.01
B	87.39 - 94.78	84.44 - 111.48
C	82.03 - 95.13	100.50 - 121.21
D	89.65 - 99.42	81.87 - 103.38
E	91.39 - 97.83	88.33 - 134.28
F	85.30 - 101.46	77.96 - 94.34

For each dose group, portions of extracted soil at 0 DAT were combusted and analysed by LSC.

Residues in soil extracts were quantified by TLC on silica plates using two different solvent systems (Solvent system 1: methanol/ammonia/10 % trichloroacetic acid solution/water, 12/3/1/6; Solvent system 5: methanol/ethanol/ammonia/water, 3/3/3/3).

Glyphosate and AMPA were identified by normal phase TLC co-chromatography with reference items using the two different solvent systems described above.

II. RESULTS AND DISCUSSION

A. DATA

Recovery of radioactivity in soil extracts and combusted soil for 0 DAT is presented in Table 7.1.2.1.1-69. Extractable radioactivity and results of TLC analysis of soil extracts are summarised in Table 7.1.2.1.1-70 to Table 7.1.2.1.1-75 for the respective soils and test conditions.

Soil extracts were analysed by two TLC solvent systems but it is not reported which method was used as primary method. The results of analysis of extractable residues with the two TLC solvent systems were found to be very similar at each sampling interval. Therefore, further discussion and kinetic evaluation refer to average values of the two TLC solvent systems.

Table 7.1.2.1.1-69: Recovery of radioactivity for soils Speyer 2.1 and Beedon Manor at 0 DAT from extracts and extracted soil after combustion (% AR)

Fraction	Replicate	Incubation group					
		A, Speyer 2.1	B, Speyer 2.1	C, Speyer 2.1	D, Speyer 2.1	E, Speyer 2.1	F, Beedon Manor
Soil extract	A	95.29	96.20	95.51	94.21	96.15	65.84
	B	95.33	94.15	94.74	91.39	98.01	58.80
	Mean	95.31	95.18	95.13	92.80	97.08	62.32
Residual Combusted Soil	A	3.89	3.86	3.38	3.91	5.28	30.79
	B	3.73	3.49	3.54	3.91	5.08	30.98
	Mean	3.81	3.68	3.46	3.91	5.18	30.89
Total	A	99.18	100.06	98.89	98.12	101.43	96.63
	B	99.06	97.64	98.28	95.30	103.09	89.78
	Mean	99.12	98.86	98.59	96.71	102.26	93.21

DAT: days after treatment

Table 7.1.2.1.1-70: Soil Speyer 2.1, incubation group A: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	95.29	84.22	77.52	70.80	64.15	56.53	48.24	43.79
	B	95.33	84.74	79.31	70.51	63.63	56.50	50.74	45.02
	Mean	95.31	84.48	78.42	70.66	63.89	56.52	49.49	44.41
Total in TLC sample	A	86.02	65.15	74.29	69.38	65.00	55.17	50.05	39.67
	B	80.87	81.60	74.99	62.76	61.07	55.07	45.37	40.54
	Mean	83.45	73.38	74.64	66.07	63.04	55.12	47.71	40.11
	Results for TLC solvent system 1								
Glyphosate	A	73.08	53.19	52.25	45.19	34.75	20.62	11.52	7.98
	B	75.48	63.76	51.86	40.38	31.72	20.63	13.22	8.63
	Mean	74.28	58.48	52.06	42.79	33.23	20.62	12.37	8.30
AMPA	A	11.02	7.29	17.30	20.10	25.60	29.13	35.46	29.57
	B	4.71	9.24	17.67	18.56	24.53	30.02	29.91	29.48
	Mean	7.87	8.26	17.48	19.33	25.06	29.57	32.68	29.53
Other	A	0.00	1.66	1.09	1.47	2.63	2.69	1.57	0.77
	B	0.00	2.27	0.61	1.29	1.29	1.79	0.83	0.99
	Mean	0.00	1.96	0.85	1.38	1.96	2.24	1.20	0.88
Origin	A	1.68	2.98	3.63	2.53	1.82	2.66	1.51	1.15
	B	0.49	6.07	4.25	2.41	3.31	2.52	1.20	1.43
	Mean	1.09	4.52	3.94	2.47	2.56	2.59	1.35	1.29
Unresolved background	A	0.24	0.04	0.02	0.09	0.20	0.07	0.00	0.19
	B	0.18	0.26	0.60	0.13	0.23	0.11	0.21	0.01
	Mean	0.21	0.15	0.31	0.11	0.21	0.09	0.11	0.10
	Results for TLC solvent system 5								
Glyphosate	A	76.83	54.17	56.90	48.52	35.15	21.43	14.65	6.93
	B	74.11	66.20	58.04	43.29	32.34	21.77	13.39	7.46
	Mean	75.47	60.19	57.47	45.91	33.74	21.60	14.02	7.19
AMPA	A	4.77	6.89	13.31	17.14	25.30	29.33	32.37	30.28
	B	3.77	9.58	13.01	16.23	23.83	29.60	29.45	29.85
	Mean	4.27	8.24	13.16	16.69	24.57	29.47	30.91	30.06
Other	A	0.94	1.21	1.74	1.26	2.41	1.73	1.31	1.14
	B	0.67	0.92	0.98	0.85	1.66	1.06	1.05	1.29
	Mean	0.80	1.06	1.36	1.06	2.03	1.39	1.18	1.21
Origin	A	3.27	2.40	2.22	2.39	1.98	2.67	1.57	1.16
	B	2.16	4.63	2.66	2.09	3.15	2.38	1.33	1.84
	Mean	2.71	3.51	2.44	2.24	2.56	2.53	1.45	1.50
Unresolved background	A	0.21	0.49	0.13	0.06	0.16	0.01	0.15	0.15
	B	0.17	0.28	0.28	0.29	0.10	0.25	0.14	0.11
	Mean	0.19	0.38	0.21	0.18	0.13	0.13	0.15	0.13

Table 7.1.2.1.1-70: Soil Speyer 2.1, incubation group A: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
	Mean of solvent system 1 and 5								
Glyphosate	A	*74.96*	*53.68*	*54.58*	*46.86*	*34.95*	*21.03*	*13.09*	*7.46*
	B	*74.80*	*64.98*	*54.95*	*41.84*	*32.03*	*21.20*	*13.31*	*8.05*
	Mean	*74.88*	*59.33*	*54.76*	*44.35*	*33.49*	*21.11*	*13.20*	*7.75*
AMPA	A	*7.90*	*7.09*	*15.31*	*18.62*	*25.45*	*29.23*	*33.92*	*29.93*
	B	*4.24*	*9.41*	*15.34*	*17.40*	*24.18*	*29.81*	*29.68*	*29.67*
	Mean	*6.07*	*8.25*	*15.32*	*18.01*	*24.82*	*29.52*	*31.80*	*29.80*
Other	A	*0.47*	*1.44*	*1.42*	*1.37*	*2.52*	*2.21*	*1.44*	*0.96*
	B	*0.34*	*1.60*	*0.80*	*1.07*	*1.48*	*1.43*	*0.94*	*1.14*
	Mean	*0.40*	*1.52*	*1.11*	*1.22*	*2.00*	*1.82*	*1.19*	*1.05*
Origin	A	*2.48*	*2.69*	*2.93*	*2.46*	*1.90*	*2.67*	*1.54*	*1.16*
	B	*1.33*	*5.35*	*3.46*	*2.25*	*3.23*	*2.45*	*1.27*	*1.64*
	Mean	*1.90*	*4.02*	*3.19*	*2.36*	*2.52*	*2.56*	*1.40*	*1.40*
Unresolved background	A	*0.23*	*0.27*	*0.08*	*0.08*	*0.18*	*0.04*	*0.08*	*0.17*
	B	*0.18*	*0.27*	*0.44*	*0.21*	*0.17*	*0.18*	*0.18*	*0.06*
	Mean	*0.20*	*0.27*	*0.26*	*0.14*	*0.17*	*0.11*	*0.13*	*0.12*

DAT: days after treatment
Values calculated in the course in writing this summary are given in *italics*.

Table 7.1.2.1.1-71: Soil Speyer 2.1, incubation group B: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 20 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	96.20	82.88	76.74	69.82	62.88	55.83	48.16	41.37
	B	94.15	82.72	77.43	NS	62.91	55.48	48.71	41.98
	Mean	95.18	82.80	77.09	69.82	62.90	55.66	48.44	41.68
Total in TLC sample	A	88.70	75.96	70.26	52.87	54.56	53.00	43.15	38.66
	B	86.50	76.66	67.29	NS	55.71	53.01	42.75	38.57
	Mean	87.60	76.31	68.77	52.87	55.14	53.01	42.95	38.62
	Results for TLC solvent system 1								
Glyphosate	A	75.20	55.95	46.05	32.89	32.36	20.59	11.24	7.86
	B	75.50	55.79	40.81	NS	26.85	20.57	12.26	6.81
	Mean	75.35	55.87	43.43	32.89	29.60	20.58	11.75	7.33
AMPA	A	3.93	10.63	17.97	15.88	16.51	27.66	28.54	26.74
	B	4.33	10.41	18.20	NS	25.11	28.09	27.02	28.08
	Mean	4.13	10.52	18.08	15.88	20.81	27.88	27.78	27.41
Origin	A	6.03	7.31	5.22	2.06	2.36	2.65	1.09	1.74
	B	3.85	7.96	7.33	NS	2.67	2.10	0.96	1.71
	Mean	4.94	7.64	6.27	2.06	2.52	2.38	1.03	1.72
Other	A	2.98	1.82	0.95	1.54	3.01	1.86	2.18	2.04
	B	2.77	2.04	0.92	NS	1.01	2.21	2.25	1.75
	Mean	2.87	1.93	0.93	1.54	2.01	2.03	2.21	1.90
Unresolved background / 1	A	0.56	0.25	0.07	0.51	0.32	0.25	0.10	0.28
	B	0.06	0.45	0.04	NS	0.07	0.04	0.25	0.22
	Mean	0.31	0.35	0.06	0.51	0.19	0.14	0.18	0.25

Table 7.1.2.1.1-71: Soil Speyer 2.1, incubation group B: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 20 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
	Results for TLC solvent system 5								
Glyphosate	A	79.96	59.38	51.15	35.39	30.14	21.16	11.65	8.21
	B	77.35	61.46	49.24	NS	30.23	21.33	13.59	6.09
	Mean	78.66	60.42	50.20	35.39	30.18	21.25	12.62	7.15
AMPA	A	3.26	9.28	13.35	14.73	16.59	26.27	28.08	26.36
	B	4.06	8.04	13.10	NS	21.17	27.92	26.27	29.03
	Mean	3.66	8.66	13.22	14.73	18.88	27.10	27.17	27.70
Origin	A	3.59	4.20	3.25	1.51	3.65	2.60	1.51	1.90
	B	3.70	4.83	2.99	NS	2.21	1.92	0.87	1.26
	Mean	3.65	4.51	3.12	1.51	2.93	2.26	1.19	1.58
Other	A	1.50	1.88	1.97	1.19	2.19	2.36	1.86	1.79
	B	1.38	1.90	1.51	NS	1.96	1.59	1.97	1.58
	Mean	1.44	1.89	1.74	1.19	2.07	1.98	1.91	1.69
Unresolved background	A	0.38	1.20	0.53	0.06	2.00	0.62	0.05	0.40
	B	0.02	0.42	0.44	NS	0.14	0.25	0.06	0.61
	Mean	0.20	0.81	0.49	0.06	1.07	0.44	0.06	0.51
	Mean of solvent system 1 and 5								
Glyphosate	A	*77.58*	*57.67*	*48.60*	*34.14*	*31.25*	*20.88*	*11.45*	*8.04*
	B	*76.43*	*58.63*	*45.03*	*0.00*	*28.54*	*20.95*	*12.93*	*6.45*
	Mean	*77.00*	*58.15*	*46.81*	*22.76*	*29.90*	*20.91*	*12.19*	*7.24*
AMPA	A	*3.60*	*9.96*	*15.66*	*15.31*	*16.55*	*26.97*	*28.31*	*26.55*
	B	*4.20*	*9.23*	*15.65*	*0.00*	*23.14*	*28.01*	*26.65*	*28.56*
	Mean	*3.90*	*9.59*	*15.66*	*10.20*	*19.85*	*27.49*	*27.48*	*27.55*
Origin	A	*4.81*	*5.76*	*4.24*	*1.79*	*3.01*	*2.63*	*1.30*	*1.82*
	B	*3.78*	*6.40*	*5.16*	*0.00*	*2.44*	*2.01*	*0.92*	*1.49*
	Mean	*4.29*	*6.08*	*4.70*	*1.19*	*2.72*	*2.32*	*1.11*	*1.65*
Other	A	*2.24*	*1.85*	*1.46*	*1.37*	*2.60*	*2.11*	*2.02*	*1.92*
	B	*2.08*	*1.97*	*1.22*	*0.00*	*1.49*	*1.90*	*2.11*	*1.67*
	Mean	*2.16*	*1.91*	*1.34*	*0.91*	*2.04*	*2.01*	*2.07*	*1.79*
Unresolved background	A	*0.47*	*0.73*	*0.30*	*0.29*	*1.16*	*0.44*	*0.08*	*0.34*
	B	*0.04*	*0.44*	*0.24*	*0.00*	*0.11*	*0.15*	*0.16*	*0.42*
	Mean	*0.26*	*0.58*	*0.27*	*0.19*	*0.63*	*0.29*	*0.12*	*0.38*

DAT: days after treatment
NS: no sample taken
Values calculated in the course in writing this summary are given in *italics*.

Table 7.1.2.1.1-72: Soil Speyer 2.1, incubation group C: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 8 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	95.51	92.04	90.96	85.43	83.08	74.10	67.93	60.01
	B	94.74	91.98	91.02	86.16	82.68	73.33	68.04	59.44
	Mean	95.13	92.01	90.99	85.80	82.88	73.72	67.99	59.73
Total in TLC sample	A	91.50	78.94	89.15	84.58	85.58	75.29	57.19	53.05
	B	88.85	89.85	80.34	80.54	80.55	82.64	60.09	53.78
	Mean	90.18	84.40	84.75	82.56	83.07	78.97	58.64	53.41

Table 7.1.2.1.1-72: Soil Speyer 2.1, incubation group C: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 8 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
	Results for TLC solvent system 1								
Glyphosate	A	81.61	63.88	74.91	65.24	63.60	53.68	34.90	22.81
	B	78.78	77.01	67.37	68.48	58.80	59.96	36.62	28.50
	Mean	80.20	70.44	71.14	66.86	61.20	56.82	35.76	25.66
AMPA	A	4.52	6.53	8.50	12.00	15.10	16.85	18.86	25.98
	B	4.27	5.17	7.50	8.64	16.30	18.30	20.45	22.37
	Mean	4.40	5.85	8.00	10.32	15.70	17.57	19.66	24.17
Origin	A	5.29	5.84	4.48	5.26	4.90	2.37	1.33	2.29
	B	5.49	4.72	3.42	1.80	3.84	2.30	1.54	1.41
	Mean	5.39	5.28	3.95	3.53	4.37	2.33	1.44	1.85
Other	A	0.00	2.54	1.25	2.01	1.33	2.21	1.92	1.21
	B	0.00	2.21	1.84	1.59	1.35	1.77	1.45	1.34
	Mean	0.00	2.38	1.54	1.80	1.34	1.99	1.68	1.28
Unresolved background	A	0.08	0.15	0.02	0.06	0.65	0.18	0.17	0.75
	B	0.30	0.75	0.22	0.03	0.26	0.32	0.04	0.15
	Mean	0.19	0.45	0.12	0.05	0.45	0.25	0.10	0.45
	Results for TLC solvent system 5								
Glyphosate	A	80.01	69.55	78.04	72.49	67.88	54.89	36.23	27.35
	B	77.65	77.60	70.31	70.10	63.84	59.83	38.20	28.43
	Mean	78.83	73.57	74.17	71.30	65.86	57.36	37.22	27.89
AMPA	A	3.99	4.61	6.86	7.70	12.26	16.12	16.79	21.66
	B	5.15	5.01	6.03	7.68	12.07	18.01	18.26	22.75
	Mean	4.57	4.81	6.44	7.69	12.17	17.06	17.53	22.20
Origin	A	6.37	2.39	2.70	2.87	3.71	2.57	2.26	1.63
	B	4.79	4.60	2.94	1.27	3.17	2.59	1.48	1.81
	Mean	5.58	3.50	2.82	2.07	3.44	2.58	1.87	1.72
Other	A	1.13	1.98	1.45	1.37	1.57	1.44	1.82	2.07
	B	0.95	2.02	1.03	1.26	1.45	1.45	1.92	0.67
	Mean	1.04	2.00	1.24	1.32	1.51	1.45	1.87	1.37
Unresolved background	A	0.02	0.42	0.11	0.16	0.16	0.28	0.07	0.33
	B	0.31	0.62	0.04	0.22	0.02	0.75	0.23	0.10
	Mean	0.16	0.52	0.07	0.19	0.09	0.52	0.15	0.22
	Mean of solvent system 1 and 5								
Glyphosate	A	*80.81*	*66.72*	*76.48*	*68.87*	*65.74*	*54.29*	*35.57*	*25.08*
	B	*78.22*	*77.31*	*68.84*	*69.29*	*61.32*	*59.90*	*37.41*	*28.47*
	Mean	*79.51*	*72.01*	*72.66*	*69.08*	*63.53*	*57.09*	*36.49*	*26.77*
AMPA	A	*4.26*	*5.57*	*7.68*	*9.85*	*13.68*	*16.49*	*17.83*	*23.82*
	B	*4.71*	*5.09*	*6.77*	*8.16*	*14.19*	*18.16*	*19.36*	*22.56*
	Mean	*4.48*	*5.33*	*7.22*	*9.01*	*13.93*	*17.32*	*18.59*	*23.19*
Origin	A	*5.83*	*4.12*	*3.59*	*4.07*	*4.31*	*2.47*	*1.80*	*1.96*
	B	*5.14*	*4.66*	*3.18*	*1.54*	*3.51*	*2.45*	*1.51*	*1.61*
	Mean	*5.49*	*4.39*	*3.39*	*2.80*	*3.91*	*2.46*	*1.65*	*1.79*
Other	A	*0.57*	*2.26*	*1.35*	*1.69*	*1.45*	*1.83*	*1.87*	*1.64*
	B	*0.48*	*2.12*	*1.44*	*1.43*	*1.40*	*1.61*	*1.92*	*1.01*
	Mean	*0.52*	*2.19*	*1.39*	*1.56*	*1.43*	*1.72*	*1.89*	*1.32*
Unresolved background	A	*0.05*	*0.29*	*0.07*	*0.11*	*0.41*	*0.23*	*0.12*	*0.54*
	B	*0.31*	*0.69*	*0.13*	*0.13*	*0.14*	*0.54*	*0.14*	*0.13*
	Mean	*0.18*	*0.49*	*0.10*	*0.12*	*0.27*	*0.38*	*0.13*	*0.33*

DAT: days after treatment
Values calculated in the course in writing this summary are given in *italics*.

Table 7.1.2.1.1-73: Soil Speyer 2.1, incubation group D: Distribution of radioactivity in soil extracts following TLC analysis for sterile soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT						
		0	2	4	7	16	34	70
Soil extract	A	94.21	84.99	85.62	78.41	77.58	74.63	55.43
	B	91.39	79.47	85.42	82.28	75.34	60.35	58.09
	Mean	92.80	82.23	85.52	80.35	76.46	67.49	56.76
Total in TLC sample	A	81.38	75.71	75.33	67.51	60.38	65.06	50.42
	B	84.39	72.28	72.18	70.41	63.40	53.29	50.23
	Mean	82.88	73.99	73.76	68.96	61.89	59.17	50.33
	Results for TLC solvent system 1							
Glyphosate	A	71.52	61.81	62.02	51.41	41.94	45.62	23.23
	B	72.97	60.38	59.46	56.93	41.36	29.46	23.87
	Mean	72.25	61.10	60.74	54.17	41.65	37.54	23.55
AMPA	A	4.42	5.61	7.50	10.82	14.65	12.98	21.84
	B	4.01	6.19	9.42	7.43	16.81	17.55	19.45
	Mean	4.21	5.90	8.46	9.12	15.73	15.26	20.65
Origin	A	3.64	5.76	3.03	2.58	4.17	4.22	2.61
	B	4.36	3.19	1.83	3.15	2.09	3.48	3.17
	Mean	4.00	4.47	2.43	2.87	3.13	3.85	2.89
Other	A	0.89	1.20	1.48	1.24	1.33	1.22	1.74
	B	1.78	1.07	1.39	1.36	1.53	1.63	2.26
	Mean	1.33	1.14	1.44	1.30	1.43	1.42	2.00
Unresolved background	A	0.91	1.32	1.30	1.47	1.29	1.01	1.00
	B	1.27	1.45	0.08	1.53	1.61	1.17	1.49
	Mean	1.09	1.39	0.69	1.50	1.45	1.09	1.25
	Results for TLC solvent system 5							
Glyphosate	A	70.56	64.28	61.53	53.64	45.01	49.13	24.64
	B	74.77	61.96	61.01	58.55	43.55	30.90	24.68
	Mean	72.66	63.12	61.27	56.09	44.28	40.01	24.66
AMPA	A	4.20	5.25	7.27	8.70	9.76	10.14	20.41
	B	4.19	5.28	7.32	6.89	14.67	17.18	19.68
	Mean	4.19	5.27	7.30	7.79	12.22	13.66	20.04
Origin	A	3.99	3.84	3.53	2.44	3.05	3.76	2.36
	B	3.23	2.55	1.47	2.92	2.02	3.39	3.19
	Mean	3.61	3.19	2.50	2.68	2.53	3.57	2.78
Other	A	1.52	1.50	1.73	1.59	1.64	1.52	2.17
	B	1.35	1.46	1.54	1.32	2.31	1.35	1.90
	Mean	1.44	1.48	1.64	1.45	1.98	1.44	2.04
Unresolved background	A	1.11	0.85	1.29	1.15	0.91	0.51	0.84
	B	0.85	1.02	0.84	0.74	0.85	0.46	0.78
	Mean	0.98	0.93	1.06	0.95	0.88	0.49	0.81

Table 7.1.2.1.1-73: Soil Speyer 2.1, incubation group D: Distribution of radioactivity in soil extracts following TLC analysis for sterile soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT						
		0	2	4	7	16	34	70
	Mean of solvent system 1 and 5							
Glyphosate	A	*71.04*	*63.05*	*61.78*	*52.53*	*43.48*	*47.38*	*23.94*
	B	*73.87*	*61.17*	*60.24*	*57.74*	*42.46*	*30.18*	*24.28*
	Mean	*72.46*	*62.11*	*61.01*	*55.13*	*42.97*	*38.78*	*24.11*
AMPA	A	*4.31*	*5.43*	*7.39*	*9.76*	*10.71*	*11.56*	*21.13*
	B	*4.10*	*5.74*	*8.37*	*7.16*	*15.74*	*17.37*	*19.57*
	Mean	*4.21*	*5.58*	*7.88*	*8.46*	*13.22*	*14.46*	*20.35*
Origin	A	*3.82*	*4.80*	*3.28*	*2.51*	*3.61*	*3.99*	*2.49*
	B	*3.80*	*2.87*	*1.65*	*3.04*	*2.06*	*3.44*	*3.18*
	Mean	*3.81*	*3.84*	*2.47*	*2.77*	*2.83*	*3.71*	*2.83*
Other	A	*1.21*	*1.35*	*1.61*	*1.42*	*1.49*	*1.37*	*1.96*
	B	*1.57*	*1.27*	*1.47*	*1.34*	*1.92*	*1.49*	*2.08*
	Mean	*1.39*	*1.31*	*1.54*	*1.38*	*1.70*	*1.43*	*2.02*
Unresolved background	A	*1.01*	*1.09*	*1.30*	*1.31*	*1.10*	*0.76*	*0.92*
	B	*1.06*	*1.24*	*0.46*	*1.14*	*1.23*	*0.82*	*1.14*
	Mean	*1.04*	*1.16*	*0.88*	*1.22*	*1.17*	*0.79*	*1.03*

DAT: days after treatment
Values calculated in the course in writing this summary are given in *italics*.

Table 7.1.2.1.1-74: Soil Speyer 2.1, incubation group E: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 0.4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	96.15	86.79	83.24	73.48	67.66	60.91	55.78	47.79
	B	98.01	86.70	81.46	NS	67.43	60.42	54.80	48.36
	Mean	97.08	86.75	82.35	73.48	67.55	60.67	55.29	48.08
Total in TLC sample	A	101.29	90.92	101.61	72.32	63.58	59.19	52.20	48.72
	B	79.67	95.27	88.49	NS	66.80	63.55	55.24	42.30
	Mean	90.48	93.09	95.05	72.32	65.19	61.37	53.72	45.51
	Results for TLC solvent system 1								
Glyphosate	A	84.18	71.36	74.71	44.62	33.06	22.47	12.68	7.99
	B	65.74	76.06	64.88	NS	31.51	23.13	12.61	6.90
	Mean	74.96	73.71	69.80	44.62	32.28	22.80	12.65	7.45
AMPA	A	6.13	10.37	17.32	17.43	23.78	27.22	30.58	32.79
	B	4.37	11.42	14.16	NS	25.13	29.79	31.77	26.64
	Mean	5.25	10.90	15.74	17.43	24.46	28.50	31.17	29.71
Origin	A	10.55	6.36	6.92	7.71	5.67	6.06	4.97	4.04
	B	9.00	6.07	6.42	NS	7.55	6.53	6.91	6.30
	Mean	9.78	6.22	6.67	7.71	6.61	6.30	5.94	5.17
Other	A	0.00	2.39	2.45	2.10	0.93	3.41	3.55	3.62
	B	0.00	1.54	2.64	NS	2.43	3.64	3.66	2.17
	Mean	0.00	1.97	2.54	2.10	1.68	3.52	3.61	2.90
Unresolved background	A	0.43	0.43	0.21	0.46	0.15	0.02	0.41	0.27
	B	0.56	0.18	0.39	NS	0.18	0.47	0.30	0.29
	Mean	0.49	0.30	0.30	0.46	0.16	0.25	0.35	0.28

Table 7.1.2.1.1-74: Soil Speyer 2.1, incubation group E: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 0.4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
	Results for TLC solvent system 5								
Glyphosate	A	86.68	72.26	75.43	48.82	32.96	23.52	12.71	7.25
	B	65.76	75.55	65.88	NS	35.27	25.03	13.12	6.88
	Mean	76.22	73.91	70.66	48.82	34.11	24.28	12.91	7.07
AMPA	A	5.26	10.81	19.02	16.12	24.31	27.62	30.97	34.58
	B	4.02	11.37	15.75	NS	24.83	32.21	32.34	27.94
	Mean	4.64	11.09	17.39	16.12	24.57	29.92	31.66	31.26
Origin	A	8.00	6.17	5.54	5.29	3.41	6.00	6.51	5.71
	B	8.27	6.33	4.62	NS	4.42	4.04	8.03	6.12
	Mean	8.14	6.25	5.08	5.29	3.92	5.02	7.27	5.92
Other	A	1.34	1.40	1.47	2.00	2.79	1.97	1.26	0.98
	B	1.59	1.91	1.28	NS	1.67	1.83	1.54	1.02
	Mean	1.47	1.65	1.38	2.00	2.23	1.90	1.40	1.00
Unresolved background	A	0.01	0.27	0.14	0.11	0.10	0.09	0.76	0.20
	B	0.03	0.10	0.94	NS	0.61	0.44	0.21	0.34
	Mean	0.02	0.18	0.54	0.11	0.35	0.26	0.48	0.27
	Mean of solvent system 1 and 5								
Glyphosate	A	*85.43*	*71.81*	*75.07*	*46.72*	*33.01*	*23.00*	*12.70*	*7.62*
	B	*65.75*	*75.81*	*65.38*	*0.00*	*33.39*	*24.08*	*12.87*	*6.89*
	Mean	*75.59*	*73.81*	*70.23*	*31.15*	*33.20*	*23.54*	*12.78*	*7.26*
AMPA	A	*5.70*	*10.59*	*18.17*	*16.78*	*24.05*	*27.42*	*30.78*	*33.69*
	B	*4.20*	*11.40*	*14.96*	*0.00*	*24.98*	*31.00*	*32.06*	*27.29*
	Mean	*4.95*	*10.99*	*16.56*	*11.18*	*24.51*	*29.21*	*31.42*	*30.49*
Origin	A	*9.28*	*6.27*	*6.23*	*6.50*	*4.54*	*6.03*	*5.74*	*4.88*
	B	*8.64*	*6.20*	*5.52*	*0.00*	*5.99*	*5.29*	*7.47*	*6.21*
	Mean	*8.96*	*6.23*	*5.88*	*4.33*	*5.26*	*5.66*	*6.61*	*5.54*
Other	A	*0.67*	*1.90*	*1.96*	*2.05*	*1.86*	*2.69*	*2.41*	*2.30*
	B	*0.80*	*1.73*	*1.96*	*0.00*	*2.05*	*2.74*	*2.60*	*1.60*
	Mean	*0.73*	*1.81*	*1.96*	*1.37*	*1.96*	*2.71*	*2.50*	*1.95*
Unresolved background	A	*0.22*	*0.35*	*0.18*	*0.29*	*0.13*	*0.06*	*0.59*	*0.24*
	B	*0.30*	*0.14*	*0.67*	*0.00*	*0.40*	*0.46*	*0.26*	*0.32*
	Mean	*0.26*	*0.25*	*0.42*	*0.19*	*0.26*	*0.26*	*0.42*	*0.28*

DAT: days after treatment
NS: no sample taken
Values calculated in the course in writing this summary are given in *italics*.

Table 7.1.2.1.1-75: Soil Beedon Manor, incubation group F: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
Soil extract	A	65.84	48.39	47.88	39.53	30.75	27.45	16.73	9.12
	B	58.80	47.96	48.14	39.60	32.03	22.37	16.49	9.43
	Mean	62.32	48.18	48.01	39.57	31.39	24.91	16.61	9.28
Total in TLC sample	A	47.59	37.16	37.13	28.51	23.47	20.02	11.22	6.03
	B	48.47	35.18	31.07	29.44	23.80	18.17	10.94	6.66
	Mean	48.03	36.17	34.10	28.98	23.63	19.10	11.08	6.34

Table 7.1.2.1.1-75: Soil Beedon Manor, incubation group F: Distribution of radioactivity in soil extracts following TLC analysis for soil incubated at 4 mg/kg, 20 °C and 40 % MWHC (% AR)

Radioactive fraction	Replicate	DAT							
		0	2	4	8	16	33	64	104
	Results for TLC solvent system 1								
Glyphosate	A	30.21	22.10	16.95	10.72	5.17	2.26	0.79	0.23
	B	35.53	19.67	13.49	10.47	5.45	2.10	0.56	0.45
	Mean	32.87	20.88	15.22	10.60	5.31	2.18	0.67	0.34
AMPA	A	3.92	7.34	10.79	13.76	12.23	12.54	6.67	3.55
	B	3.51	6.70	9.07	13.46	12.10	11.95	6.80	3.70
	Mean	3.72	7.02	9.93	13.61	12.17	12.25	6.73	3.62
Origin	A	11.56	6.15	8.22	2.91	4.51	3.17	2.46	1.37
	B	8.26	7.50	7.54	4.21	5.08	2.34	2.11	1.78
	Mean	9.91	6.82	7.88	3.56	4.80	2.76	2.29	1.58
Other	A	0.88	1.24	0.93	1.08	1.55	2.00	1.28	0.88
	B	0.95	1.01	0.92	1.28	1.12	1.75	1.45	0.69
	Mean	0.92	1.12	0.93	1.18	1.34	1.87	1.37	0.79
Unresolved background	A	1.01	0.35	0.25	0.04	0.00	0.05	0.02	0.00
	B	0.20	0.31	0.05	0.02	0.04	0.02	0.03	0.04
	Mean	0.61	0.33	0.15	0.03	0.02	0.03	0.02	0.02
	Results for TLC solvent system 5								
Glyphosate	A	33.63	22.81	17.94	11.05	4.76	2.60	0.83	0.59
	B	35.60	19.23	14.28	10.39	4.94	2.37	0.76	0.99
	Mean	34.62	21.02	16.11	10.72	4.85	2.49	0.79	0.79
AMPA	A	3.84	7.42	11.02	13.39	12.52	12.80	7.08	3.32
	B	3.75	7.43	8.77	13.56	12.43	12.35	6.97	3.26
	Mean	3.79	7.43	9.90	13.48	12.47	12.58	7.03	3.29
Origin	A	8.46	5.53	6.89	2.49	4.84	3.28	2.20	1.21
	B	8.01	7.58	7.08	4.10	4.91	2.15	2.04	1.55
	Mean	8.23	6.55	6.98	3.29	4.87	2.71	2.12	1.38
Other	A	1.49	0.99	1.17	1.57	1.34	1.33	1.05	0.89
	B	0.99	0.76	0.92	1.36	1.52	1.30	1.15	0.85
	Mean	1.24	0.88	1.04	1.47	1.43	1.31	1.10	0.87
Unresolved background	A	0.17	0.42	0.10	0.01	0.01	0.00	0.06	0.02
	B	0.11	0.17	0.02	0.03	0.00	0.00	0.02	0.01
	Mean	0.14	0.29	0.06	0.02	0.00	0.00	0.04	0.01
	Mean of solvent system 1 and 5								
Glyphosate	A	*31.92*	*22.46*	*17.45*	*10.89*	*4.97*	*2.43*	*0.81*	*0.41*
	B	*35.57*	*19.45*	*13.89*	*10.43*	*5.20*	*2.24*	*0.66*	*0.72*
	Mean	*33.74*	*20.95*	*15.67*	*10.66*	*5.08*	*2.33*	*0.74*	*0.57*
AMPA	A	*3.88*	*7.38*	*10.91*	*13.58*	*12.38*	*12.67*	*6.88*	*3.44*
	B	*3.63*	*7.07*	*8.92*	*13.51*	*12.27*	*12.15*	*6.89*	*3.48*
	Mean	*3.76*	*7.22*	*9.91*	*13.54*	*12.32*	*12.41*	*6.88*	*3.46*
Origin	A	*10.01*	*5.84*	*7.56*	*2.70*	*4.68*	*3.23*	*2.33*	*1.29*
	B	*8.14*	*7.54*	*7.31*	*4.16*	*5.00*	*2.25*	*2.08*	*1.67*
	Mean	*9.07*	*6.69*	*7.43*	*3.43*	*4.84*	*2.74*	*2.20*	*1.48*
Other	A	*1.19*	*1.12*	*1.05*	*1.33*	*1.45*	*1.67*	*1.17*	*0.89*
	B	*0.97*	*0.89*	*0.92*	*1.32*	*1.32*	*1.53*	*1.30*	*0.77*
	Mean	*1.08*	*1.00*	*0.99*	*1.32*	*1.38*	*1.60*	*1.23*	*0.83*
Unresolved background	A	*0.59*	*0.39*	*0.18*	*0.03*	*0.01*	*0.03*	*0.04*	*0.01*
	B	*0.16*	*0.24*	*0.04*	*0.03*	*0.02*	*0.01*	*0.03*	*0.03*
	Mean	*0.37*	*0.31*	*0.11*	*0.03*	*0.01*	*0.02*	*0.03*	*0.02*

DAT: days after treatment
Values calculated in the course in writing this summary are given in *italics*.

B. MATERIAL BALANCE

The material balance of radioactivity for all incubation groups at 0 DAT ranged from 93.21 to 102.26 % AR. No full material balance was determined for soil samples beyond DAT 0 of all incubation series, i.e. nor non-extractable radioactivity (NER) neither volatile radioactivity was determined.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

Following application of 4 mg/kg to soil Speyer 2.1, the amount of radioactivity in the soil extract decreased from 0 to 104 DAT from 95.31 to 44.41 % AR at 20°C and 40 % MWHC, from 95.18 to 41.68 % AR at 20 °C and 20 % MWHC, and from 95.13 to 59.73 % AR at 8°C and 40 % MWHC.

Following application of 4 mg/kg to soil in Beedon Manor, the amount of radioactivity in the soil extract decreased from 0 to 104 DAT from 62.32 to 9.28 % AR at 20°C and 40 % MWHC.

Following application of 0.4 mg/kg to soil Speyer 2.1, the amount of radioactivity in the soil extract decreased from 0 to 104 DAT from 97.08 to 48.08 % AR at 20°C and 40 % MWHC.

Following application of 4 mg/kg to sterile soil Speyer 2.1, the amount of radioactivity in the soil extract decreased from 0 to 70 DAT from 92.80 to 56.76 % AR at 20°C and 40 % MWHC.

Non-extractable radioactivity (NER) in soils was not determined.

D. TRANSFORMATION OF THE TEST ITEM

All values provided are the mean values of the results of analysis by TLC with two different solvent systems.

Following application of 4 mg/kg to soil Speyer 2.1, the amount of glyphosate in the soil extract decreased from 0 to 104 DAT from 74.88 to 7.75 % AR at 20 °C and 40 % MWHC, from 77.00 to 7.24 % AR at 20 °C and 20 % MWHC, and from 79.51 to 26.77 % AR at 8 °C and 40 % MWHC.

Following application of 4 mg/kg to soil Beedon Manor, the amount of glyphosate in the soil extract decreased from 0 to 104 DAT from 33.74 to 0.57 % AR in soil at 20 °C and 40 % MWHC.

Following application of 0.4 mg/kg to soil Speyer 2.1, the amount of glyphosate in the soil extract decreased from 0 to 104 DAT from 75.59 to 7.26 % AR at 20 °C and 40 % MWHC.

Following application of 4 mg/kg to sterile soil Speyer 2.1, the amount of glyphosate in the soil extract decreased from 0 to 70 DAT from 72.46 to 24.11 % AR at 20 °C and 40 % MWHC.

Following application of 4 mg/kg to soil Speyer 2.1, the amount of aminomethylphosphonic acid (AMPA) in the soil extract increased to a maximum of 31.80 % AR at 64 DAT at 20 °C and 40 % MWHC, 27.55 % AR at 104 DAT at 20 °C and 20 % MWHC, and 23.19 % AR at 104 DAT at 8 °C and 40 % MWHC.

Following application of 4 mg/kg to soil Beedon Manor, the amount of AMPA in the soil extract increased to a maximum 13.54 % AR at 8 DAT at 20 °C and 40 % MWHC. The amount of AMPA subsequently decreased to 3.46 % AR at 104 DAT.

Following application of 0.4 mg/kg to soil Speyer 2.1, the amount of AMPA in the soil extract increased to a maximum of 31.42 % AR at 64 DAT at 20 °C and 40 % MWHC.

Following application of 4 mg/kg to sterile soil Speyer 2.1, the amount of AMPA in the soil extract increased to a maximum of 20.35 % AR at the end of the experiment (70 DAT) at 20 °C and 40 % MWHC.

E. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found under CA7.1.2.1.1/001.

III. CONCLUSIONS

Glyphosate was rapidly degraded in viable soil at an incubation temperature of 20 °C. AMPA was observed as the only significant metabolite of glyphosate to account for a 31.80 % AR in maximum (mean of two replicates).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted overall consistent with current guidelines, showing minor deviations. Determination of non-extractable radioactivity was performed for day 0, hence, a full material balance is available just for day 0. There was no determination of volatile radioactivity for the other sampling intervals. Furthermore, pesticide soil history was not reported for soil Beedon Manor. However, when putting the results of this study into context with overall information available on aerobic degradation in soil, the deviations are regarded of minor influence on the general outcome.
Therefore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/006
Report author	[redacted]
Report year	1991
Report title	Glyphosate-Trimesium: Laboratory degradation in four soils
Report No	RJ1064B
Document No	
Guidelines followed in study	BBA guidelines Part IV, 4-1
Deviations from current test guideline	From OECD 307: - no information on test item reported ("technical material") - residues were corrected for procedural recoveries (55 – 82 %) to depend on soil type - only average recoveries of fortified controls reported, i.e. 82 % for soil Speyer 2.1, 80 % for soil Speyer 2.2, 60 % for soil Jubilee and 55 % for soil 18-Acres, being in particular low for soils Jubilee and 18-Acres - storage times and conditions of soils after sampling prior to use is not reported - no information on storage of samples
Previous evaluation	Yes, accepted in RAR (2015)
GLP	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary
The degradation of glyphosate-trimesium was investigated in four soils under aerobic conditions in the dark in the laboratory at 20 °C and 40 % of the soil moisture holding capacity for 108 days. Only the methods

and results for glyphosate (PMG) are presented in this summary and reported information for the trimesium cation (TMS+) are not considered here.

The soils used were two loamy sands (Speyer 2.1 and Speyer 2.2) from Germany and a sandy loam (East Jubilee) and a sandy clay loam (18 Acres) from England. The amount of organic carbon in the soils was between 0.5 % and 5.2 % and the pH in water was between 5.7 and 6.6.

The test was performed in static systems sealed with a plastic foam plug.

The application rate of glyphosate-trimesium was 5.0 mg/kg soil (dry weight), corresponding to an application rate of 5 kg glyphosate-trimesium /ha.

Duplicate test systems were processed and analysed 0, 2, 4, 8, 16 or 18, 32 or 46, 64 or 72 and 108 days after treatment (DAT).

The amount of glyphosate decreased from 3.3 mg/kg at 0 DAT to 0.17 mg/kg at 108 DAT in soil Speyer 2.1, from 3.0 mg/kg at 0 DAT to 0.54 mg/kg at 108 DAT in soil Speyer 2.2, from 2.3 mg/kg at 0 DAT to 0.62 mg/kg at 108 DAT in soil East Jubilee and from 2.3 mg/kg at 0 DAT to 0.64 mg/kg at 108 DAT in soil 18 Acres (mean of duplicates).

The DT_{50} value for the degradation of glyphosate was calculated to be 24 days in Speyer 2.1 soil, 46 days in Speyer 2.2 soil, 58 days in East Jubilee soil and 62 days in 18 Acres soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: glyphosate-trimesium

No details on purity or lot no. of the test item are reported. It is stated that a solution of technical glyphosate-trimesium was used. Methods and results for glyphosate (PMG) are presented in this summary and reported information for the trimesium cation (TMS+) are not considered here.

2. Soil:
Soils were obtained from stocks. No herbicide treatment was applied to the soils for 5 years. Soils were air-dried and sieved to ≤ 2 mm. The soils were each freshly sampled to a depth of 15 cm (after removal of the turf). Characteristics of the test soils are presented in the table below.

Table 7.1.2.1.1-76: Characteristics of test soils

Parameter	Results			
Soil	Speyer 2.1	Speyer 2.2	East Jubilee	18 Acres
Country	Germany	Germany	England	England
Textural Class (USDA)	Loamy sand	Loamy sand	Sandy loam	Sandy clay loam
Sand (%)	87	85	66	52
Silt (%)	7	8	17	22
Clay (%)	6	7	17	26
pH (water)	6.6	6.0	5.7	6.2
Organic carbon (%) [1]	0.5	2.4	3.0	5.2
Organic matter (%)	0.8	4.1	5.1	9.0
Moisture at 0.33 bar	4.91	11.85	12.76	23.68
Moisture at 15 bar	2.55	5.72	8.67	16.52

Table 7.1.2.1.1-76: Characteristics of test soils

Cation exchange capacity (meq/100 g)	2.9	7.8	9.6	17.6
Microbial biomass (mg C/100 g soil)				
Begin of study	13.9	39.6	59.0	>112.5
Study end	7.9	24.8	25.0	69.1

USDA: United States Department for Agriculture
[1] Calculated from organic carbon according to OC = OM x 0.58

B. STUDY DESIGN

1. Experimental conditions

Static test systems were used, consisting of Erlenmeyer flasks filled with soil and sealed with a plastic foam plug. As a non-radiolabelled test item was used, volatiles were not collected.

50 g of sieved soil (dry weight equivalents) were weighed into each test vessel and the moisture was adjusted to 40 % of the determined moisture holding capacity. The soils were pre-incubated at 20 °C for 14 days before application.

The study application rate was 5.0 mg/kg, corresponding to an application rate of 5 kg glyphosate-trimesium/ha. The test item glyphosate-trimesium was applied to each test vessel in 0.25 mL of an aqueous test solution.

Test systems were incubated under aerobic conditions in the dark for 108 days at 20 °C. The moisture was maintained at 40 % moisture holding capacity during the study by addition of de-ionised water.

2. Sampling

Duplicate test systems were processed and analysed 0, 2, 4, 8, 16 or 18, 32 or 46, 64 or 72 and 108 days after treatment (DAT).

3. Analytical procedures

At each sampling interval, soil samples were extracted with 0.5 M NH_4OH solution. After centrifugation an aliquot of the extract was evaporated to dryness and re-suspended in acidic solution. The pH was adjusted to 9-10 by addition of 2 M NaOH and the samples were derivatised with 9-fluorenylmethylchloroformate.

Glyphosate in the derivatised samples was quantified by HPLC using anion exchange chromatography and fluorescence detection. Residue concentrations were quantified by external standardisation and corrected for recoveries of fortified control samples (for values <100 %). The mean recoveries were 82 % for soil Speyer 2.1, 80 % for soil Speyer 2.2, 60 % for soil Jubilee and 55 % for soil 18-Acres. The limit of determination (LOD) of the method was 0.05 mg/kg.

Control samples were incubated alongside treated samples and analysed at the 0, 64/72 and 108 day intervals. No residues of glyphosate above the limit of determination (LOD) were determined in any of the control samples for any of the four soils.

II. RESULTS AND DISCUSSION

A. DATA

Degradation of glyphosate in the tested soils is summarised in the tables below. Values were corrected for recoveries of fortified control samples.

Table 7.1.2.1.1-77: Residues of glyphosate in Speyer 2.1 soil under aerobic conditions (values expressed as mg/kg)

		DAT							
Compound	**Replicate**	**0**	**2**	**4**	**8**	**16**	**32**	**64**	**108**
Glyphosate	A	3.1	3.3	2.4	1.9	1.7	1.6	0.29	0.17
	B	3.5	3.6	2.2	1.9	1.8	1.5	0.29	0.16
	Mean	3.3	3.4	2.3	1.9	1.8	1.6	0.29	0.17

DAT: days after treatment

Table 7.1.2.1.1-78: Residues of glyphosate in Speyer 2.2 soil under aerobic conditions (values expressed as mg/kg)

		DAT							
Compound	**Replicate**	**0**	**2**	**4**	**8**	**16**	**32**	**64**	**108**
Glyphosate	A	3.0	2.5	2.5	2.1	1.9	1.8	0.97	0.64
	B	3.0	2.7	2.5	2.3	1.9	1.9	0.90	0.44
	Mean	3.0	2.6	2.5	2.2	1.9	1.9	0.94	0.54

DAT: days after treatment

Table 7.1.2.1.1-79: Residues of glyphosate in East Jubilee soil under aerobic conditions (values expressed as mg/kg)

		DAT							
Compound	**Replicate**	**0**	**2**	**4**	**8**	**18**	**46**	**72**	**108**
Glyphosate	A	2.1	2.1	2.2	2.0	1.7	1.6	0.81	0.63
	B	2.6	2.2	1.8	1.8	1.6	1.3	0.76	0.60
	Mean	2.3	2.1	2.0	1.9	1.7	1.5	0.79	0.62

DAT: days after treatment

Table 7.1.2.1.1-80: Residues of glyphosate in 18 Acres soil under aerobic conditions (values expressed as mg/kg)

		DAT							
Compound	**Replicate**	**0**	**2**	**4**	**8**	**18**	**46**	**72**	**108**
Glyphosate	A	2.2	2.1	1.8	2.1	1.9	1.6	0.99	0.67
	B	2.4	2.1	2.4	1.9	1.6	1.6	0.95	0.61
	Mean	2.3	2.1	2.1	2.0	1.7	1.6	0.97	0.64

DAT: days after treatment

B. MASS BALANCE

No material balances were established.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of extractable and non-extractable residues was not determined.

D. VOLATILE RADIOACTIVITY

The amount of volatiles was not determined.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate decreased from 3.3 mg/kg at 0 DAT to 0.17 mg/kg at 108 DAT in soil Speyer 2.1, from 3.0 mg/kg at 0 DAT to 0.54 mg/kg at 108 DAT in soil Speyer 2.2, from 2.3 mg/kg at 0 DAT to 0.62 mg/kg at 108 DAT in soil East Jubilee and from 2.3 mg/kg at 0 DAT to 0.64 mg/kg at 108 DAT in soil 18 Acres (mean of duplicates).

F. KINETICS

The DT_{50} value for the degradation of glyphosate was calculated by a second order model to be 24 days in Speyer 2.1 soil, 46 days in Speyer 2.2 soil, 58 days in East Jubilee soil and 62 days in 18 Acres soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study shows major deficiencies. A major deficiency is that residues of glyphosate were quantified by external standardisation and corrected for external recoveries of fortified control samples in case the measured recovery of glyphosate was below 100 % - While residues were not corrected for recoveries higher than 100 %. The report just gives tables with values corrected, no tables are included with initial, uncorrected values. For the external recoveries mean values are reported only. Consequently, the original uncorrected amount of glyphosate in the sample cannot be assessed. The mean external recoveries were below 70 % for two soils.

Therefore, the study is considered invalid and therefore not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/007
Report author	[redacted]
Report year	1991
Report title	Behaviour of Glyphosate in water and soil, Part 5 Degradation in soil
Report No	PR90/002
Document No	
Guidelines followed in study	BBA-guideline for testing of pesticides, Part IV 4-1
GLP	Yes
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: aerobic rate of degradation Test item: glyphosate, non-labelled (99 % purity) Test soil (origin/soil pH/organic carbon content): LUFA F1 (Speyer, Germany/ 5.7/ 0.70), LUFA F2 (Speyer, Germany/ 6.4/ 1.34), LUFA 2.2 (Speyer, Germany/ 5.6/ 2.29), Eigenboden (Goch, Germany/ 6.2/ not reported) Test concentration: 1 mg/kg soil Test design: static system with flasks loosely closed with cotton wool Volatile trapping: None Incubation: 22-26 °C (ambient temperature) in the dark, soil moisture adjusted to 40 % of maximum water holding capacity

	Sampling: 0, 2, 7, 15, 30, 60 and 100 days after treatment (DAT), duplicate samples Workup: soil extracted with water/phosphoric acid at ambient temperature, derivatization to trifluoroacetyl ester, clean-up by HPLC, quantification by GC-ECD; recoveries of analytical method for glyphosate were from 89 to 142 % (mean values). Limit of detection: 20 µg a.s./kg soil Identification of glyphosate and AMPA residues: calibration of GC-system with reference substances
Short description of results:	No full mass balances and information about non-extractable residues owing to the non-labelled character of the test substance. Derivatisation of test item (GC-ECD analysis of TFA derivate, values for AMPA estimated from figures): Soil LUFA F1: Glyphosate: 0.84 mg/kg at 0 DAT, 0.02 mg/kg at 100 DAT AMPA: 0.09 mg/kg at 0 DAT, 0.25 mg/kg at 30 DAT, 0.2 mg/kg at 100 DAT Soil LUFA F2: Glyphosate: 0.36 mg/kg at 0 DAT, 0.001 mg/kg at 100 DAT AMPA: not detected at 0 DAT, 0.15 mg/kg at 7 DAT, 0.12 mg/kg at 100 DAT Soil LUFA 2.2: Glyphosate: 0.63 mg/kg at 0 DAT, 0.05 mg/kg at 100 DAT AMPA: not detected at 0 DAT, 0.15 mg/kg at 15 DAT, 0.15 mg/kg at 100 DAT Soil Eigenboden: Glyphosate: 0.56 mg/kg at 0 DAT, 0.02 mg/kg at 100 DAT AMPA: not detected at 0 DAT, 0.23 mg/kg at 30 DAT and 100 DAT The half-life for glyphosate (square root first order) was estimated to 3.78 days for LUFA F1, 1.57 days for LUFA F2, 8.04 days for LUFA 2.2 and 10.36 days for soil Eigenboden.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The data were not considered for environmental risk assessment for the following reasons: - Incubation temperature was rather variable (22-26°C) - Information on study conduct and results given in the report is very limited (e.g. no information on application solution, application technique) - No numeric results reported for metabolite AMPA (only graphs) - Tabulated results for glyphosate only shown in DT_{50} evaluation tables, only mean values reported (no individual replicates, no standard deviation) - Recovery at day 0 rather variable from 36 – 84 % - Recoveries of fortified samples outside the range of 70-110 % (89-142 % for glyphosate and 117-181 % for AMPA) - No soil history data provided - No pre-equilibration of soil prior to application - No information on soil storage condition and length prior to use - No information on storage of soil extracts prior to and after analysis
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.2.1.1/008
Report author	[redacted]
Report year	1980
Report title	Soil dissipation of Glyphosate following multiple applications under laboratory conditions
Report No	MSL-1173
Document No	
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: aerobic soil metabolism Test item: glyphosate, non-labelled Test soil: Two, i.e. Drummer and Spinks Soil type: silty clay loam (Drummer), sandy loam (Spinks) pH: 6.2, 4.7 (method not reported) Organic matter: 5.6 %, 2.3 % (combustion) and 3.4 %, 1.8 % (Walkeley-Black) Application rate: 12.5 mg/kg, reflecting three seasonal applications Test design: pans in a plant growth chamber Volatiles trapping: no trapping of volatiles Incubation: light/dark cycles each of 12 hours including temperature change (30 °C during day, 25 °C at night), soils kept moist with no control of moisture Sampling: 0, 1.5, 3, 6, 12 and 24 weeks after treatment, single samples Workup/Analysis: three successive extraction steps each with 0.5 N aqueous NH_4OH at ambient temperature, purification of soil extracts by ion exchange chromatography, i.e. elution from anion exchange resin followed by cation exchange resin, conversion to N-trifluoroacetyl methyl ester derivative and its quantification by GC-FPD; recovery of analytical method 43.8-98.0 % for glyphosate, 49.7-98.5 % for AMPA, overall average 63.8 to 78.7 %
Short description of results:	Results based on GC-FPD analysis for Drummer soil: Glyphosate: 11.3 mg/kg at 0 DAT, 0.4 mg/kg at 24 weeks AMPA: 0.4 mg/kg at 0 DAT, 2.4 mg/kg at 6 weeks, 1.0 mg/kg at 24 weeks Spinks soil: Glyphosate: 14.1 mg/kg at 0 DAT, 0.2 mg/kg at 24 weeks AMPA: 1.0 mg/kg at 0 DAT, 4.3 mg/kg at 6 weeks, 2.3 mg/kg at 24 weeks No analysis for other metabolites. The half-life for glyphosate was reported as 2.2 weeks (*ca.* 15 days) for Drummer soil and 1.6 weeks (*ca.* 11 days) for Spinks soil.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not considered for environmental risk assessment due to the following reasons: - The incubation included phases of light – being no more standard design in soil degradation testing - Temperature varied significantly during incubation. Again, this is no more standard in soil degradation testing - Recoveries of the analytical procedures below actual standards - Soil origin, collection, pesticide history, handling, storage till incubation were not reported - Soil moisture not reported and not controlled during incubation
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.2.1.1/009
Report author	[redacted]
Report year	1972
Report title	The rate of dissipation of MON-0573 in soil
Report No	271
Document No	
Guidelines followed in study	U.S. Department of Agriculture (ARS, Pesticides Regulation Division): Pesticide Registration (PR) Notice 70-15 "Guidelines For Studies to Determine the Impact of Pesticides on the Environment." June 23, 1970
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: aerobic soil metabolism Test item: [^{14}C]-glyphosate, phosphonomethyl-label (97 % radiochemical purity) and non-labelled glyphosate Test soils (soil type): Ray (silt loam), Drummer (silty clay loam), Norfolk (sandy loam) pH: 6.5, 7.0, 5.7 (medium not stated) Organic matter: 1.0 %, 6 %, 1 % Test containers were planted with corn seeds immediately after application. Plants were also analysed. Large scale-experiments for characterization of metabolites conducted with each soil type. Application rate: single application of 4 mg/kg or 8 mg/kg, corresponding to 4.48 kg/ha and 8.96 kg/ha; 202.32 mg/kg for large scale experiment Test design: blackened pyrex planters, planted with four corn seeds per test vessel, soil moisture set to 11 % water content prior to application, addition of Hoagland solution 10, 11 and 12 weeks after application Volatiles trapping: no trapping of volatiles Incubation: 32 °C, greenhouse Sampling: 0, 7, 14, 28, 41/42, 55/56, 83/84, 111/112 days after treatment (DAT), single samples per application rate Workup: threefold extraction with 0.5 N aqueous NH_4OH solution at ambient temperature (five extractions for soil Drummer); extraction efficiency tested 76.3 to 100.1 %; clean-up by

	diethylaminoethylcellulose (DEAE) and ion exchange chromatography Determination of radioactivity: Extracts: LSC NER: combustion/LSC Volatiles: not collected Identification of radioactive residues: TLC co-chromatography with reference items; additional characterization by NMR
Short description of results:	Recovery of radioactivity: not applicable due to open test systems Mineralization: not applicable due to open test systems Extractable radioactivity: For the 4 mg/kg application rate: 67.8 % AR at 0 DAT to 10.9 % AR at 112 DAT for soil Ray, 88.8 % AR at 0 DAT to 74.2 % AR at 112 DAT for soil Norfolk, 60.3 % AR at 0 DAT to 13.5 % AR at 112 DAT for soil Drummer. For the 8 mg/kg application rate: 78.5 % AR at 0 DAT to 9.6 % AR at 111 DAT for soil Ray, 89.8 % AR at 0 DAT to 77.6 % AR at 111 DAT for soil Norfolk, 60.3 % AR at 0 DAT to 12.8 % AR at 111 DAT for soil Drummer. Non-extractable radioactivity: For the 4 mg/kg application rate: 29.9 % AR at 0 DAT to 12.5 % AR at 112 DAT for soil Ray, 8.8 % AR at 0 DAT to 18.6 % AR at 112 DAT for soil Norfolk, 33.8 % AR at 0 DAT to 22.3 % AR at 112 DAT for soil Drummer. For the 8 mg/kg application rate: 20.6 % AR at 0 DAT to 9.6 % AR at 111 DAT for soil Ray, 8.6 % AR at 0 DAT to 12.4 % AR at 111 DAT for soil Norfolk, 35.4 % AR at 0 DAT to 20.0 % AR at 111 DAT for soil Drummer. Transformation of test item (TLC analysis): 4 mg/kg application rate Glyphosate: 62.5 % AR at 0 DAT, 3.1 % AR at 112 DAT for soil Ray; 86.0 % AR at 0 DAT, 57.5 % AR at 112 DAT for soil Norfolk, 56.6 % AR at 0 DAT, 1.1 % AR at 112 DAT for soil Drummer. AMPA: 5.1 % AR at 0 DAT, 25.6 % AR at 14 DAT, 7.8 % AR at 112 DAT for soil Ray; 2.8 % AR at 0 DAT, 16.7 % AR at 112 DAT for soil Norfolk, 3.7 % AR at 0 DAT, 23.1 % AR at 84 DAT, 12.4 % AR at 112 DAT for soil Drummer 8 mg/kg application rate Glyphosate: 76.1 % AR at 0 DAT, 1.8 % AR at 55 DAT, 2.9 % AR at 111 DAT for soil Ray; 87.4 % AR at 0 DAT, 67.0 % AR at 111 DAT for soil Norfolk, 58.4 % AR at 0 DAT, not detected at 111 DAT for soil Drummer. AMPA: 2.4 % AR at 0 DAT, 26.7 % AR at 14 DAT, 6.7 % AR at 111 DAT for soil Ray; 2.4 % AR at 0 DAT, 10.5 % AR at 111 DAT for soil Norfolk, 1.9 % AR at 0 DAT, 17.5 % AR at 83 DAT, 12.7 % AR at 111 DAT for soil Drummer No unknown metabolites observed at >5 % AR.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	- 'mixed design' as soil degradation and plant test - planting pots as test vessels - incubated in greenhouse under unspecified conditions of light and moisture - incubation temperature (32 °C) out of standard range of testing (20 to 25°C) - influence of corn plants on degradation in soil - addition of media like Hoagland solution during the study with unknown effects on outcome of test - no full material balance established due to open test systems - microbial activity of soils not reported
Category study in AIR 5 dossier (L docs)	Category 3b

Relevant published articles from Literature Search Report

1. Information on the study

Data point:	CA 7.1.2.1.1/010
Report author	[redacted]
Report year	2017
Report title	Effect of Biochar Amendment and Ageing on Adsorption and Degradation of Two Herbicides
Document No	DOI 10.1007/s11270-017-3392-7 ISSN 0049-6979
Guidelines followed in study	Degradation experiment: none Adsorption experiment: OECD 106 (2000)
Deviations from current test guideline	Not applicable: insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Biochar amendment can alter soil properties, for instance, the ability to adsorb and degrade different chemicals. However, ageing of the biochar, due to processes occurring in the soil over time, can influence such biochar-mediated effects. This study examined how biochar affected adsorption and degradation of two herbicides, glyphosate (N-(phosphonomethyl)-glycine) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) in soil and how these effects were modulated by ageing of the biochar. One sandy and one clayey soil that had been freshly amended with a wood-based biochar (0, 1, 10, 20 and 30 % w/w) were studied. An ageing experiment, in which the soil-biochar mixtures were aged for 3.5 months in the laboratory, was also performed. Adsorption and degradation were studied in these soil and soil-biochar mixtures, and compared to results from a soil historically enriched with charcoal. Biochar amendment increased the pH in both soils and increased the water-holding capacity of the sandy soil. Adsorption of diuron was enhanced by biochar amendment in both soils, while glyphosate adsorption was decreased in the sandy soil. Ageing of soil-biochar mixtures decreased adsorption of both herbicides in comparison with freshly biochar-amended soil. Herbicide degradation rates were not consistently affected by biochar amendment or ageing in any of the soils. However, glyphosate half-lives correlated with the Freundlich Kf values in the clayey soil, indicating that degradation was limited by availability there.

Materials & Methods

Soil Sampling and Processing

The soil samples were collected in September 2015 from arable fields at two locations: Länna (L) (59° 52′ N, 17° 58′ E) and Ulleråker (U) (59° 49′ N, 17° 39′ E). Soil sampling at L was performed in two parts of the arable field: an untreated part (L) and a historically charcoal-enriched part (LB). Because of the long-term charcoal amendment, the latter soil was characterised by lower bulk density and higher loss on ignition and water-holding capacity (WHC) than the unamended soil from the same field, which leads to higher yields in dry years. In each soil, about 10 samples were taken from the upper layer (5–15 cm below surface) and pooled. After sieving, the Ø < 2 mm fraction was homogenised and stored at −20 °C in plastic bags until the start of the experiment. Moisture content and WHC were measured for all soil samples. Moisture content was determined by drying at 110 °C for 10 h, while WHC was defined as the moisture content after saturation of 30 g soil with distilled water for 10 h followed by 4 h of free drainage. Chemical and physical properties of the three soils studied (L, LB, U) were determined by a commercial laboratory and are presented in Table 7.1.2.1.1-81 and Table 7.1.2.1.1-82.

Preparation and Ageing of Soil-Biochar Mixtures

The biochar used was the commercial product *Skogens kol*, which is produced from a mixture of about 80 % hardwood, mainly birchwood (*Betula* sp.) and 20 % wood from Norway spruce (*Picea abies*), by slow pyrolysis with a maximum process temperature of 380– 430 °C (Cederlund *et al.* 2016). Soil-biochar mixtures were prepared by mixing soil (L and U) with sieved biochar (Ø < 2 mm) at a rate of 1, 10, 20 and 30 % biochar per unit soil dry weight (designated L1, L10, L20 and L30 and U1, U10, U20 and U30). WHC was determined as described above and pH for all mixtures was measured in a 1:2 slurry of soil and distilled water (*w*/*v*) after shaking and stabilisation for 10 h. Biochar ageing was performed with soil-biochar mixtures made from U soil. These mixtures were incubated in darkness at 20 °C for 3.5 months. The moisture content was adjusted to 55 % of WHC and monitored and adjusted weekly by addition of deionised water.

Chemicals Used in Herbicide Adsorption and Degradation Experiments

Glyphosate (N-(phosphonomethyl)-glycine, CAS [1071-83-6], 98 %) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), CAS [330-54-1], 99.0 %) were provided by Dr. Ehrenstorfer GmbH, Augsburg, Germany. ^{14}C-labelled diuron ([ring-U-^{14}C], 96.4 %, 5.71 MBq/mg) and glyphosate ([P- methylene-^{14}C], 4.87 MBq/mg) were provided by the Institute of Isotopes Co. Ltd., Budapest, Hungary.

Measurement of Herbicide Adsorption in Soils and Soil-Biochar Mixtures

Adsorption was determined in a batch equilibrium system according to OECD guideline 106 (OECD 2000). A pre-study was performed to estimate the time when the equilibrium between adsorbed herbicide and herbicide in solution was reached (8, 24 and 32 h). In all cases, equilibrium was reached within 24 h. For high-percentage soil-biochar mixtures with U soil, an additional pre-study was performed to estimate an appropriate soil to solution ratio as defined in the OECD guideline. Soil and soil-biochar mixtures, corresponding to 1 g of soil or mixture dry weight, were weighed into tubes (15-mL glass tubes for diuron and 50-mL polypropylene tubes for glyphosate) and adjusted with 0.01 M $CaCl_2$ to reach the appropriate soil-solution ratio. This was 1:40 for all samples with glyphosate and for U20, U30, U20a and U30a with diuron and 1:4 for all other samples with diuron. The samples were shaken for 24 h (20 °C, 200 revolutions/min). After that, herbicides were added to reach concentrations of 1, 5, 10, 50 and 100 µg/g dry weight (dw) soil for glyphosate and 0.1, 0.5, 1, 5 and 10 µg/g dw soil for diuron, due to its lower water solubility. In addition, a fixed amount (10 µL for glyphosate and 20 µL for diuron) of ^{14}C- labelled herbicide was added to each tube to reach an activity of 2000 DPM (3.333×10^{-5} MBq) per sample. There were two replicate tubes of each concentration. After 24 h, the tubes were centrifuged (3000 revolutions/min for 30 min), samples of supernatant were transferred to scintillation vials (4 mL for diuron and 10 mL for glyphosate samples) and Quicksafe A (Scintvaruhuset, LAB-service, Uppsala, Sweden) was added directly before measurement of scintillation. ^{14}C activity was measured on a Beckman LS 6000TA liquid scintillation counter (Beckman Counter Inc., Fullerton, CA). Controls without herbicides were measured for all samples to exclude the level of background radioactivity. The data obtained were fitted using the linear form of the Freundlich isotherm.

Herbicide Degradation Experiment

The herbicides were dissolved in water (glyphosate) or methanol (diuron) and added dropwise to a fraction (10 %) of the soils and soil-biochar mixtures. Water and methanol were allowed to evaporate from the samples for 10 h. The herbicide-treated part was then mixed with the rest of each sample to give an initial

nominal concentration of 10 mg/kg soil dry weight. Portions of soil corresponding to 5 g of dry weight were weighed into 50-mL plastic tubes and the water content was adjusted to 60 % of WHC and kept at this level for the duration of the experiment. The tubes were sealed with caps and were incubated at 20 °C in the dark. After 1, 2, 5, 8, 16, 23 (only for U samples) and 31 days of incubation, two replicate tubes from each treatment were placed in the freezer (−20 °C) for future extraction and analysis.

Table 7.1.2.1.1-81: Chemical properties of the soils studied

Soil	Code	HCl extracted K (mg 100 g^{-1})	HCl extracted P (mg 100 g^{-1})	Al-K[a] (mg 100 g^{-1})	Al-P[a] (mg 100 g^{-1})	Total C (%)	Total N (%)	pH
Charcoal-amended soil from Länna	LB	68.35	85.02	3.82	16.48	[illegible]	0.37	5.57
Untreated soil from Länna	L	229.36	78.77	37.28	16.67	[illegible]	0.34	5.27
Soil from Ulleråker	U	287.59	68.16	34.95	4.87	1.36	0.1	6.41

[a] Al–K/Al-P = ammonium lactate-extractable K and P—Swedish standard method for estimation of plant available K and P fractions ([redacted] 2009)

Table 7.1.2.1.1-82: Physical properties of the soils studied

Soil code	Clay Ø < 0.002 mm	Fine silt 0.002–0.006 mm	Medium silt 0.006–0.02 mm	Coarse silt 0.02–0.06 mm	Fine sand 0.06–0.2 mm	Medium sand 0.2–0.6 mm	Coarse sand 0.6–2 mm	Loss on ignition %
LB	n.d.	n.d.	n.d.	n.d.	5.9	3.3	4.1	39.4
L	66.5	14.8	9.1	6.5	2.1	0.7	0.3	13.7
U	7.5	3.2	2.4	3.2	12.1	63.8	7.8	3.3

n.d. not determined

Data from the degradation experiment after recovery correction were used to estimate herbicide half-life. Recovery was calculated as:

$$\text{Recovery} = \left(\frac{C_0}{C_{\text{nominal}}}\right) \times 100$$

where *C0* is the herbicide concentration determined at day 0. Natural logarithms of remaining concentrations for days 0–31 were plotted against time, giving the first- order rate constant *k* as the slope of the linear regression line. Half-life (*T*½) was calculated as:

$$T^{1/2} = \frac{\ln 2}{k}$$

Analysis of Diuron

For diuron extraction from soil and soil-biochar mixtures, the following protocol was used: 10 mL methanol were added using a Vogel pipette to the tubes with sample. The tubes were shaken at 200 revolutions/min for 60 min, centrifuged at 4000 revolutions/min for 10 min and the supernatant was filtered (OOH Whatman; 11 cm). Portions (1 mL) of filtrate were transferred to sample vials and HPLC analysis was performed according to the protocol in [redacted] 2007). Standard solutions with concentration range 0.05–50 µg/mL were analysed with extracts from samples. The HPLC was equipped with a G1314A UV detector, a G1311A pump, a G1329A auto injector (Agilent Technologies AB; 1100 Series; Sweden) and a Zorbax SB-C18 column (12.5 × 4.6 mm, 5 mm; ChromTech AB, Sundbyberg, Sweden).

Analysis of Glyphosate

Extraction of glyphosate, derivatisation and measurement on GC-MS were performed using the same reagents for analytical standards, glyphosate extraction and internal standards as previously described (Bergström, Börjesson, and Stenström, 2011).

Results & Discussion

Effect of Biochar on Soil Water-Holding Capacity and pH

The studied soils had different physical texture: the dominant particle fractions in the L soil were clay and fine silt, while the U soil was dominated by medium and fine sand. The texture of the LB soil could not be fully determined due to its high organic matter content, as traces of organic C remained in the sample after digestion (oxidation by H_2O_2). Coming from the same field as L, it is likely that the LB soil was also dominated by clay. However, the proportion of sand was higher (Table 7.1.2.1.1-82). This agrees with Kihlberg *et al.* (unpublished), who also reported a coarser particle size distribution in LB compared with L soil, but also did not subdivide particles with Ø < 0.06 mm. The WHC of the clayey L soil (53 %) was higher than in the sandy U soil, where it was only 27 %, and was not affected by biochar addition. However, the LB soil, which was historically amended by charcoal, had a higher WHC (57 %) than the L soil with or without fresh biochar amendment. In the sandy soil, the WHC increased from 27 to 42 % with biochar addition and was correlated positively (r = 0.98) with the biochar percentage (Figure 7.1.2.1.1-1). Biochar addition increased the pH from 5.27 to 6.07 in the L soil and from 6.41 to 7.69 in the U soil (Figure 7.1.2.1.1-2). Ageing of the biochar led to a further pH increase in most of the soil-biochar mixtures (U10a- U30a). In the LB soil, the pH was higher (5.77) than in the L soil. The pH of soil-biochar mixtures was correlated with the percentage of biochar added in all cases (r = 0.99 for L soil-biochar mixtures; r = 0.99 for fresh U soil-biochar mixtures; r = 0.98 for aged U soil- biochar mixtures).

Figure 7.1.2.1.1-1: Water-holding capacity (WHC) of the soil samples ± standard deviations plotted against biochar percentage added

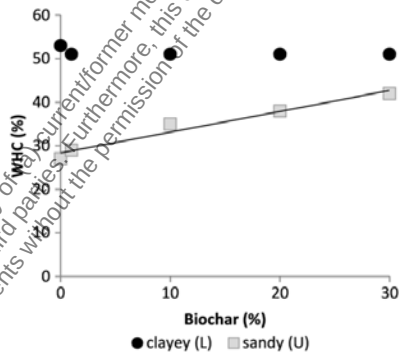
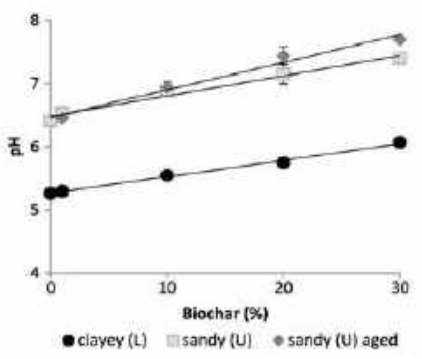


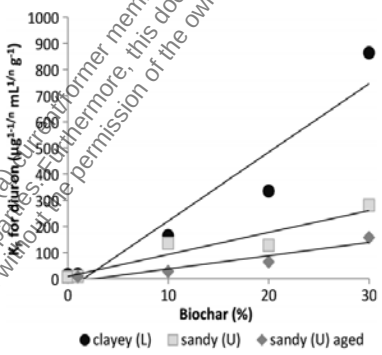
Figure 7.1.2.1.1-2: pH of the soil samples (N = 2) ± standard deviations plotted against biochar percentage added



Adsorption of Diuron

Biochar amendment increased diuron adsorption in both the L and U soils (Fig. 3). In the LB soil, K_F was 364 $\mu g^{1-1/n}(mL)^{1/n} g^{-1}$, which is quite close to the K_F value of the L20 soil-biochar mixture. K_F values in the aged soil-biochar mixtures were lower than in mixtures with fresh biochar addition. There were positive correlations between the diuron K_F values and biochar percentage for L, U and aged U soils ($r = 0.96$, $r = 0.95$, and $r = 0.95$, respectively).

Figure 7.1.2.1.1-3: Freundlich K_F values for diuron plotted against biochar percentage added in samples from Länna (L) and Ulleråker (U)



Adsorption of Glyphosate

Glyphosate was more strongly adsorbed in the L soil (K_F = 1218 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}) than in the U soil (K_F = 146 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}). No consistent effect of biochar amendment on glyphosate adsorption in L soil was observed (Figure 7.1.2.1.1-4). A very high K_F value was observed for the sample with 1 % biochar addition (K_F = 1892 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}), while the K_F values for the unamended L soil and the other soil-biochar mixtures varied between 1099 and 1294 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}. The LB soil had a much lower *KF* value (539 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}) than the L soil and soil-biochar mixtures. However, in the U soil, glyphosate adsorption was correlated negatively ($r = -0.99$) with the biochar percent- age (Figure 7.1.2.1.1-4). Ageing of the biochar decreased adsorption further.

Figure 7.1.2.1.1-4: Freundlich K_F values for glyphosate plotted against biochar percentage added in samples from Länna (L) and Ulleråker (U)

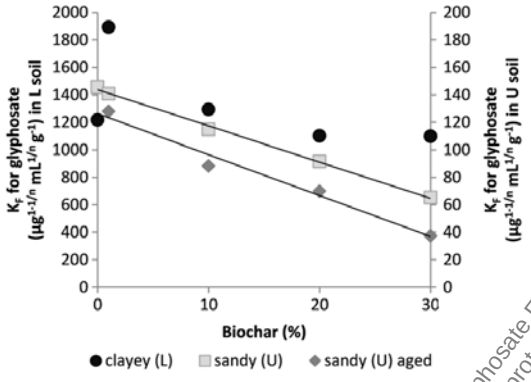


Table 7.1.2.1.1-83: Freundlich parameters (K_F, 1/n and R^2 value) for adsorption and half-life of diuron and glyphosate

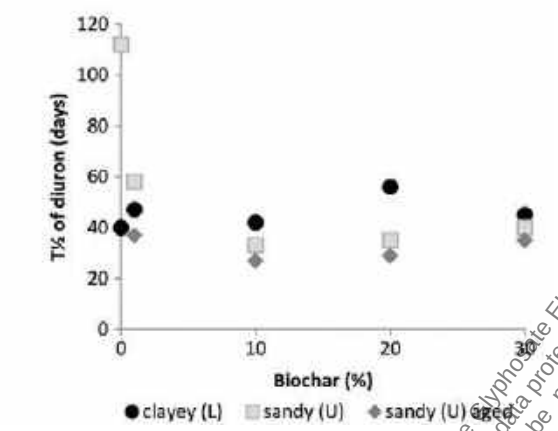
Sampling site		Adsorption						Degradation			
		Diuron			Glyphosate			Diuron		Glyphosate	
		K_F[a]	1/n	R^2	K_F[a]	1/n	R^2	$T½$[b]	R^2	$T½$[b]	R^2
Länna	LB	364	0.859	0.99	539	0.890	0.99	36	0.965	17	0.97
	L	15.21	0.863	0.99	1218	0.842	0.99	40	0.963	87	0.606
	L1	17.10	0.807	0.99	1892	0.872	0.99	47	0.708	187	0.333
	L10	164	0.859	0.99	1294	0.806	0.99	42	0.853	151	0.385
	L20	335	0.822	0.98	1102	0.796	0.99	56	0.918	131	0.402
	L30	863	0.978	0.96	1099	0.780	0.98	45	0.86	51	0.945
Ulleråker	U	5.73	0.798	0.99	145.5	0.783	0.99	112	0.663	182	0.482
	U1	8.60	0.586	0.95	140.9	0.765	0.99	58	0.718	83	0.767
	U10	135	0.889	0.99	114.8	0.754	0.99	33	0.866	66	0.674
	U20	127	0.721	0.85	91.6	0.780	0.99	35	0.868	78	0.621
	U30	281	0.953	0.97	65.2	0.750	0.99	40	0.888	53	0.861
	U1a	6.44	0.760	0.99	127.9	0.761	0.99	37	0.71	51	0.716
	U10a	27.9	0.824	0.99	88.3	0.776	0.99	27	0.785	81	0.683
	U20a	9.4	0.547	0.94	70.0	0.788	0.99	29	0.849	49	0.917
	U30a	155	0.686	0.97	37.4	0.751	0.99	35	0.871	68	0.885

[a] The unit of K_F is $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}
[b] The unit of $T½$ is days

Degradation of Diuron

In the L and U soils and soil-biochar mixtures, from 20 to 50 % of the added diuron was degraded during the experimental period. Diuron half-life varied between 40 to 56 days in the L soil, was 36 days in the LB soil and varied between 26 to 112 days in the U soil (Figure 7.1.2.1.1-5). No correlation was seen between the biochar percentage and diuron half-life in any of the soils. However, in the U soil, the half-life was shorter in all samples with biochar addition compared with the unamended soil. Here, it should be noted that the half-life of 112 days found for the U soil without biochar may be a less accurate estimation, since the dynamics of diuron degradation did not fit well with a first-order kinetic model in this sample. The degradation kinetics of all other samples followed first-order kinetics reasonably well, with R^2 values of 0.7–0.96. Ageing of the biochar consistently decreased diuron half-life in the U soil.

Figure 7.1.2.1.1-5: Diuron half-life in the Länna (L) and Ullerâker (U) soils and soil-biochar mixtures



Degradation of Glyphosate

In the L and U soils and soil-biochar mixtures, 10–70 % of the added glyphosate was degraded during the experimental period. Glyphosate half-life in the L soil varied between 51 and 187 days. However, in the L, L1, L10 and L20 samples, the data fitted poorly to the first-order kinetic model (R^2 = 0.33–0.61), mostly due to great variation in glyphosate concentrations during the first week of degradation. This fact can explain the some-what inconsistent pattern of half-life variation for the soil-biochar mixes. However, degradation in the LB and L30 samples followed first-order kinetics well (R^2 = 0.97 and 0.94). The shortest glyphosate half-life (19 days) was observed in the LB soil. Degradation of glyphosate was relatively slow in the unamended U soil, but was faster in all samples with biochar amendment. In the unamended U soil, the half- life of glyphosate was 182 days, while in the U soil- biochar mixtures, it varied between 49 and 83 days. However, as in the case of diuron, data from the un-amended U soil were a poor fit to the first-order model (R^2 = 0.48) and the degradation rate in the biochar-amended samples did not appear to be related to the biochar percentage added. The fastest degradation was observed in the U1a and U20a soil-biochar mixtures, but ageing of the biochar did not consistently affect degradation rates (Figure 7.1.2.1.1-6). No correlations between glyphosate half-life and amount of added biochar were found for any of the L and U soils (Fig. 6). However, the half-life was correlated with the K_F value for glyphosate (r = 0.88) in samples of the L soil when the LB sample was included (Fig. 7). In the U soil and soil-biochar mixtures, the adsorption coefficient of glyphosate was generally lower and its half-life was not correlated with the K_F value (Fig. 7).

Figure 7.1.2.1.1-6: Glyphosate half-life in the Länna (L) and Ullerâker (U) soils and soil-biochar mixtures

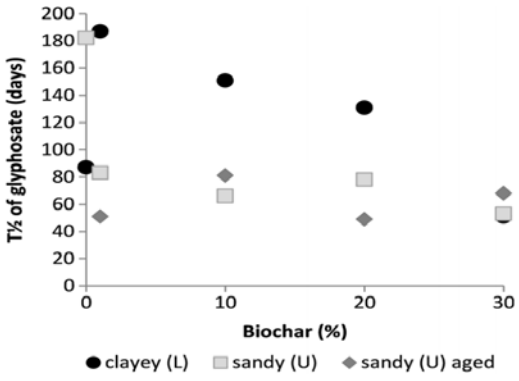
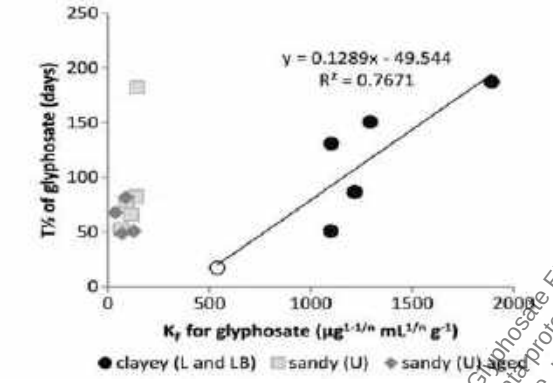


Figure 7.1.2.1.1-7: Correlation between glyphosate half-life and adsorption coefficient (KF). The open circle is for the LB soil



Effects of Biochar on Herbicide Adsorption

Diuron adsorption increased after biochar amendment in both the L and U soils. This effect of biochar addition has been observed in previous studies for silty loam and sandy soil. Biochar contains many adsorption sites that can bind non-polar herbicides, so diuron adsorption increased with amount of biochar added, and the risk of it leaching is lower. The increased pH obtained with biochar addition is not likely to have contributed to the increased sorption since diuron is uncharged at relevant soil pH-levels. In a previous study, we also found that pH has no effect on diuron adsorption when studying this particular biochar without soil ([redacted] 2016).

Biochar addition decreased glyphosate adsorption in the sandy U soil, but not in the clayey L soil. The difference in effects of biochar on glyphosate adsorption between the L and U soils may be explained by the different soil texture and physical properties of these soils. The decreased glyphosate adsorption in the U soil is likely to be related to the induced pH changes. According to several studies, soil pH is negatively correlated with glyphosate adsorption ([redacted] 2004b; [redacted] 2005; [redacted] 2005). Increased soil pH can increase the negative charge of both soil surfaces and glyphosate itself, which leads to enhanced repulsion. Glyphosate has a pH-dependent OH− group with a pKa value of 5.7, so its charge is likely to have been affected in the pH range studied here. The same relationship with pH has been observed for glyphosate adsorption on pure biochar: [redacted] (2016) studied the effect of pH on adsorption of glyphosate on a rice husk biochar and found that the adsorption percentage varied from 75 to 85 % at pH 3–5, decreased to 75–65 % at pH 6–8 and then significantly dropped to 55 % at pH 9. However, in a previous study, we showed that glyphosate adsorption by the studied biochar was low at both low and high pH ([redacted] 2016). In the L soil, there was no linear relationship between glyphosate adsorption coefficient and biochar amendment. The overall strong adsorption in this soil possibly contributed to masking the relatively minor effects of the biochar. It is known that inorganic components of soil, such as Al- and Fe-oxides, adsorb glyphosate effectively ([redacted] 2004a) and that this herbicide is less available in soils with a high clay content. The induced pH changes in this soil also occurred over a different pH interval, which may have contributed to the less clear outcome.

Effects of Biochar Ageing on Adsorption

Short-term ageing of the biochar mixtures in the laboratory decreased adsorption of both herbicides. This suggests that processes that have the potential to reduce sorption, such as organo-mineral interactions with the biochar surface ([redacted] 2006; [redacted] 2009; [redacted] 2012), were the dominant forces affecting the biochar during our ageing experiment. For diuron, our results are consistent with findings in a field study on biochar amendment of Australian ferrosols, in which diuron and atrazine adsorption to soils amended by poultry litter and paper mill biochar was significantly reduced after 32 months of ageing. For glyphosate, it is possible that the further increase in pH during the 3 months of ageing contributed to the additional decrease observed in sorption. Although we cannot know the original properties of the charcoal applied to the historically charcoal-enriched LB soil, it may be informative to

compare the adsorption results from this soil. In LB, the K_F value for diuron was comparable to that determined in the 20 % soil-biochar mixture (L20) and, considering that the total carbon content of the LB soil is about 18 % (Table 7.1.2.1.1-81), this suggests limited effects of ageing. However, for glyphosate, the K_F value of the LB soil was only 539 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}, which is only about half the K_F value found for any of the fresh biochar mixtures or the unamended L soil (Table 7.1.2.1.1-83). Since the adsorption of glyphosate on the biochar itself is very weak, this low adsorption is difficult to explain in terms of reduced adsorptive affinity of the charcoal. It is more likely to reflect a reduced affinity for glyphosate of the soil itself. [redacted] (unpublished) suggest that the heat from the charcoal kilns in LB may have contributed to sintering the clay particles in the soil, causing a shift towards a coarser particle size distribution. Heating clay soils to 500 °C has been shown to change soil physical texture and increase the amount of silt and sand particles. Such a reduction in the proportion of clay would consequently reduce the amount of surfaces available for glyphosate adsorption. Heating may also cause other mineralogical changes in soil that affect adsorption, for instance de [redacted] (2006) reported reduced interaction between glyphosate and Al_2O_3 and Fe_2O_3 in soil after burning. Our results for glyphosate differ somewhat from those of [redacted] (2016), who found that glyphosate sorption was increased in a silty loam soil amended with the same wood-based biochar that we used (*Skogens kol*) after 7–10 months of ageing under field conditions. The application rates used in their study varied from 10 to 100 Mg biochar/ha added to the topsoil layer (0–10 cm), which corresponds to about 0.8–8 % of biochar per gramme dry weight assuming a bulk density of the soil of 1.3 g/cm³. Increases in glyphosate sorption occurred in plots amended with 10, 20 and 40 Mg/ha of biochar (i.e. corresponding to 0.8, 1.6 and 3.2 % *w*/*w*), while the plot amended with 100 Mg/ha, where the glyphosate adsorption was the same as in the unamended plots, was considered to be an outlier [redacted] 2016). In the present study, the clayey L soil with the lowest application rate was the outlier: the adsorption coefficient in the L1 soil-biochar mixture was much higher than in L soil without amendment, while the adsorption coefficient in the L10, L20 and L30 soil-biochar mixtures was the same or lower than in the unamended clayey L soil. However, we cannot offer an explanation for this pattern. In the sandy U soil, the adsorption of glyphosate was reduced after the ageing process, which can be explained by a further pH increase and low affinity to sorb glyphosate in both sandy soil and biochar itself.

Herbicide Degradation before and after Biochar Amendment

Microbial degradation of chemicals in soil has often been reported to be limited by strong sorption ([redacted] 2011; [redacted] 2004a; [redacted]. 2011). Moreover, pesticide degradation is often inhibited after fresh biochar addition ([redacted] 2010), which can be explained by a decrease in their bioavailability. In the present case, it seems that despite the fact that adsorption of diuron increased in both soils and that adsorption of glyphosate decreased in the sandy soil, biochar amendment had no clear effect on either diuron or glyphosate degradation. However, even though neither the K_F value nor the half-life of glyphosate was clearly correlated with the added biochar percentage in the clayey L soil, the half-life was correlated with the K_F value (Figure 7.1.2.1.1-7). This indicates that in the case of glyphosate in the clayey L soil, which had K_F values >1000 $\mu g^{1-1/n}$ $mL^{1/n}$ g^{-1}, availability of glyphosate may have been a rate-limiting factor for its degradation, while in the other cases adsorption was too weak to have an effect.

Conclusion

As hypothesised, fresh biochar addition increased diuron adsorption in both clayey (L) and sandy (U) soils. However, glyphosate adsorption decreased only in the sandy U soil. These effects are most likely due to adsorption of diuron on the biochar itself, while in the case of glyphosate the decreased sorption may be explained by an increase in soil pH after biochar addition. No consistent effect of biochar amendment on herbicide degradation was observed in the studied soils, which contradicts our initial hypothesis. However, there was a positive relationship between adsorption and glyphosate half-life in the clayey soil-biochar mixtures, indicating that availability may be the rate-limiting step, but only where adsorption is strong. The consequences of biochar ageing under laboratory conditions were further increases in soil pH and a reduction in adsorption of both herbicides. Changes in biochar adsorptive properties during ageing in soil should be taken into consideration when planning its use in agriculture and for soil remediation purposes.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the adsorption and degradation behavior of two agrochemicals in two agricultural soils from Northern Europe following amendment of biochar.
The tests resulted in data on adsorption and degradation of glyphosate in the presence and absence of biochar amended to soil samples.
The tests designs are described and the adsorption parameters are sufficiently reported. For adsorption experiments, conduct according to OECD guideline 106 is claimed for. However, validity criteria in terms of OECD Guideline 106 and the EU Evaluators Checklist could not checked due to a lack of such detail in reporting.
For the evaluation of the degradation tests, no information was reported in the publication whether a specific guideline was followed including details in design, conduct and analysis. The results were kinetically evaluated against Single First Order kinetics only to partly result in poor fits. No detailed information on findings at the different time points is reported thus preventing kinetic re-evaluation based on the presented data.
The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/011
Report author	Cassigneul, A. *et al.*
Report year	2016
Report title	Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study
Document No	DOI 10.1016/j.scitotenv.2015.12.052 E-ISSN: 1879-1026
Guidelines followed in study	None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The increasing use of cover crops (CC) may lead to an increase in glyphosate application for their destruction. Sorption and degradation of ^{14}C–glyphosate on and within 4 decaying CC–amended soils were compared to its fate in a bare soil. ^{14}C–Glyphosate and its metabolites distribution between mineralized, water–soluble, NH_4OH–soluble and non–extractable fractions was determined at 5 dates during a 20 °C/84-d period. The presence of CC extends ^{14}C–glyphosate degradation half–life from 7 to 28 days depending on the CC. ^{14}C–Glyphosate dissipation occurred mainly through mineralization in soils and through mineralization and bound residue formation in decaying CC. Differences in sorption and degradation levels were attributed to differences in composition and availability to microorganisms. CC– and soil–specific dissipation patterns were established with the help of explicit relationships between extractability and microbial activity.

Materials and Methods

Soil and mulch sampling

Common vetch (*Vicia sativa*), white mustard (*Sinapis alba*), hybrid ryegrass (*Lolium hybridum*) and a mixture of common vetch + oat (*Avena sativa*) were grown as cover crops (CC) on the Lamothe INP–EI Purpan experimental station (near Toulouse, SW France) on a clay loam soil from June to September 2012. Prior to this cover crop, the whole field had grown a durum wheat–sunflower rotation without glyphosate application for more than 10 years. Aerial parts of the 4 cover crops were collected, dried at 40 °C and cut into 1 cm square pieces. The underlying 0–5 cm topsoil was collected, sieved (5 mm) and stored at 4 °C. CC–associated soils were sampled on each CC plot to record any possible plant–specific soil–borne microbial populations.

Herbicides

Both experiments were conducted with a mixture of technical–grade and [phosphonomethyl–^{14}C] glyphosate (Sigma–Aldrich), prepared in 0.01 M $CaCl_2$. Specific radioactivity and radiochemical purities of GLY were 81.4 MBq/mmol and 98.8 %, respectively.

Experiment 1: glyphosate adsorption on decaying cover crop residues
Incubations and CC characterization/description

CC– were subjected to accelerated decomposition in the dark for 6, 28 or 56 days at 28 °C and in non–limiting moisture conditions. Each CC–was moistened and placed on top of its associated soil in a plastic tray (24 ∗ 37 ∗ 7 cm). Soils had been previously brought to field capacity (pF 2.5). At days 0, 6, 28 and 56 of the incubation, CC were dried, ground and analyzed (i) in duplicate for their carbon and nitrogen content and (ii) on a single aliquot for their biochemical composition as assessed by Van Soest fractionation ([redacted], 1979). CC and soils characteristics are described in Table 7.1.2.1.1-84.

Table 7.1.2.1.1-84: Cover crops (a) and associated soils (b) characteristics at different incubation times. OM: organic matter, SOL: water–soluble, NDF: neutral detergent fiber soluble, HEM: hemicellulose–like, CEL: cellulose–like, LIC: lignin–like, C: carbon, N: nitrogen

(a)

CC	Incubation Time (days)	OM	SOL	NDF	HEM	CEL	LIC	C (mg·g^{-1})	N (mg·g^{-1})	C/N
Vetch + oat	0	89.1	31.3	7.1	21.1	33.7	6.8	427.6	34.7	12.3
	6	88.0	29.4	12.0	12.7	38.7	7.1	427.5	34.8	12.3
	28	73.8	27.8	18.7	16.2	23.7	13.6	370.6	37.7	9.8
	56	75.6	18.8	24.3	18.2	23.5	15.2	374.0	36.5	10.3
Vetch	0	88.6	27.4	18.5	13.9	31.1	9.1	432.0	44.3	9.8
	6	86.8	29.6	13.0	11.6	33.9	11.9	352.7	27.9	12.6
	28	62.8	19.0	20.1	14.7	23.8	22.3	321.2	33.5	9.6
	56	54.2	7.4	30.3	11.5	27.1	23.6	290.3	29.9	9.7
White mustard	0	64.0	14.4	23.0	24.3	31.4	6.9	391.9	40.4	9.7
	6	62.8	35.9	24.7	7.8	22.1	9.6	323.4	29.5	11.0
	28	63.9	16.1	19.8	14.8	26.8	22.5	317.6	32.8	9.7
	56	69.9	23.1	20.8	15.2	25.5	15.4	336.1	30.5	11.0
Ryegrass	0	87.8	37.7	3.1	28.8	26.8	3.6	423.9	34.9	12.1
	6	72.1	24.4	26.1	15.1	18.2	16.1	377.6	44.6	8.5
	28	74.9	39.5	9.6	14.2	28.1	8.6	382.3	33.9	11.3
	56	72.6	23.5	27.3	15.7	19.2	14.3	380.9	43.2	8.8

(b)

CC	pH	OM (%)	C/N	N (%)	Organic C (%)	CEC (méq/100 g)	$CaCO_3$ (%)	CaO exangeable (mg·kg^{-1})	K_2O exangeable (mg·kg^{-1})	MgO exangeable (mg·kg^{-1})	P_2O_5 Olsen (mg·kg^{-1})
Vetch + oat	7.4	2.1	8.2	0.148	1.21	19.5	<0.1	4671	158	734	29.4
Vetch	7.5	1.8	7.4	0.138	1.02	16.2	<0.1	4255	153	582	20.3
White mustard	7.4	1.8	7.5	0.138	1.04	17.5	<0.1	4721	147	631	33.3
Ryegrass	7.4	2.2	9.2	0.140	1.28	17.0	<0.1	4387	184	645	24.0

Sorption characterization

Sorption of glyphosate onto CC residues and soil was determined using a batch equilibration technique, as detailed in [redacted]. (2015). The sorbent:glyphosate solution ratio was 1:9 (g/mL) for soil and 1:5.8 for CC residues. Amounts of sorbed glyphosate were described using the partition coefficient K_d (L/kg) and the normalised organic carbon content K_d i.e. K_{oc} (L/kg OC)

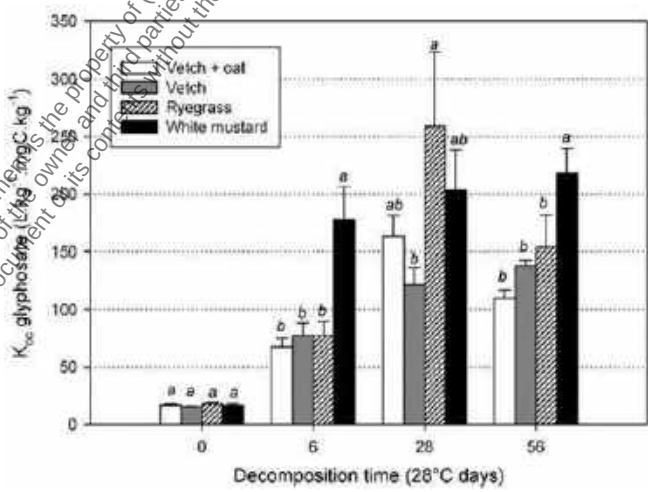
Experiment 2: glyphosate degradation in microcosms of soil and cover crop residues
Microcosm setup/construction/description – Microcosms, i.e. cylinders containing soil (118 g dw) covered by CC mulch (2.5 g dw), were set up as detailed in [redacted] (2014). The amount of mulch corresponds to 8 t/ha of biomass in the field, soil and mulch densities being 1.2 g/cm^3 and 0.05 g/cm^3 respectively. This amount of biomass was chosen to ensure a sufficient soil coverage given our objectives. After determination of their retention curve using pressure plates, water content of both soil and mulch was brought to field capacity (pF 2.5) in order to ensure water availability to microorganisms. Microcosms were placed in a 2 L hermetically sealed jar and incubated in the dark (20 ± 1°C). To maintain constant soil moisture, a 10 mL vial filled with deionized water was placed in each jar and water content was adjusted weekly by weighing and adding water as necessary. Two 84–days incubations were performed, both with treatments including a bare soil (control) and 4 studied CC amended soils, but with and without ^{14}C–glyphosate application. The aim was to characterize separately (i) glyphosate fate in 'soil + mulch' and (ii) carbon mineralization from mulch. Each treatment was repeated thrice.

Organic C mineralization – CO_2–C produced by soil respiration and mulch decomposition was trapped in a vial containing 20 mL of 0.1 M NaOH, which was replaced weekly throughout the incubation. From a 1 mL aliquot, CO_2–C was analyzed by colorimetry on a continuous flow–analyzer. Net mineralization of CC carbon was calculated by subtracting the mineralization measured in the control soil treatment from that of the CC–amended treatment, and expressing the difference as a percentage of the initially–introduced organic carbon content.

Degradation study: pesticide monitoring in soil and mulch samples – At day 0, the recommended rate of glyphosate (2 L/ha) was applied at the microcosm surface (soil or mulch) in 2 mL of aqueous solution with a micropipette. The water volume thus added had been subtracted from the total amount of water that had to be added to reach the targeted water content.

Mineralized fraction – $^{14}CO_2$–C originating from glyphosate mineralization in the mulch and/or underlying soil was trapped by the same procedure as total CO_2–C. The vials containing 20 mL of 0.1 M NaOH were replaced weekly throughout the incubation.

Figure 7.1.2.1.1-8: Sorption of glyphosate on cover crop residues. Letters correspond to LSD grouping within a single incubation time



Extractable fractions – At 0, 7, 22, 49 and 84 DAT (days after treatment), microcosms were destructively sampled. Soils (top 1 cm) and mulches were separately submitted to 4 sequential extractions. Substrates were placed in polypropylene tubes containing solvent and shaken in a rotary shaker for 24 h in the dark at room temperature. The substrate:solvent ratio was 1:20 (g/g) and 1:3 (g/g) for mulches and soils respectively. Extractions were performed first with $CaCl_2$ (0.01 M) and then 3 times with NH_4OH (0.1 M), providing access to the weakly–sorbed and to the strongly–sorbed ^{14}C–glyphosate. Between each

extraction, tubes were centrifuged for 10 min at 10,000 g and 6000 g for the mulch and the soil respectively. Supernatants were sampled for radioactivity counting, and the remaining volumes were stored at 4 °C until HPLC analysis.

Non–extractable fraction – CC or soil material pellets remaining after the last extraction were oven–dried for 72 h (40 °C) and ground for 10 min (Retsch GmbH, Germany). Duplicate aliquots of 500 mg were burnt in a Sample Oxidizer 307 where evolved $^{14}CO_2$ was trapped in a scintillation vial containing Oxysolve T. The vial was immediately subjected to scintillation counting.

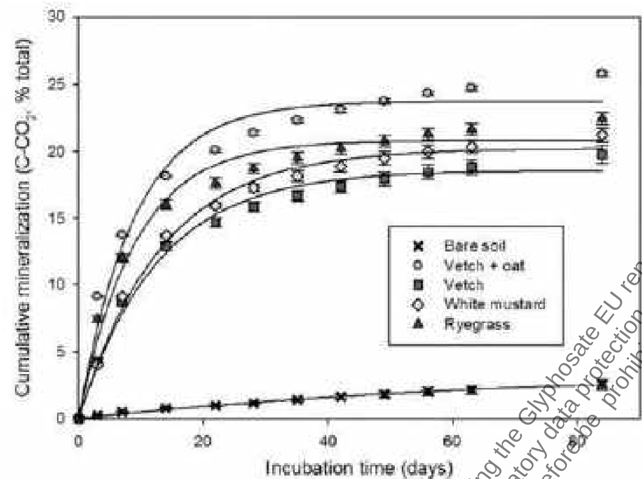
Analytical determinations – Radioactivity content in the liquid samples was measured by scintillation counting from a 1 mL aliquot mixed with 10 mL of scintillation liquid (Ultima Gold™ XR, Perkin Elmer, USA), using a Packard Tri–Card counter (GMI, Inc., USA). To prevent a chemiluminescence reaction, NaOH and NH_4OH scintillation vials were submitted to a 24 h period in the dark prior to counting. A blank sample containing solvent or NaOH solution was inserted in each counting series. To determine the amount of glyphosate and metabolites, soil and mulch extracts containing sufficient radioactivity (83.3 Bq/mL) were previously filtered, concentrated by evaporation under vacuum at 50°C (Rotavapor ®, Büchi, Switzerland), and centrifuged to ensure maximum particle removal. Samples of NH_4OH extracts included the extracts of the 3 successive extractions. HPLC analysis was performed coupled with a Flexar (PerkinElmer, USA) coupled with a radioactive flow detector (Radiomatic Flow Scintillation Analyzer 150TR, PerkinElmer, USA). Samples (200–500 µL) were injected into an Allsep ™ A–2 anion exchanger column (100 mm × 4.6 mm, 7 µm, Grace Davison Discovery Science, USA) preceded by a GA–1 Anion guard column (7.5 × 4.6 mm, Grace Davison Discovery Science, USA) to ensure an efficient separation, eluted with a KH_2PO_4 solution (0.34 g/L) adjusted to pH 2 with a 85 % H_3PO_4 solution. The mobile phase flow was 10^{-3} L/min. Under these conditions, the retention time was 3–5 min for GLY and 1–3 min for its main metabolite.

Glyphosate in the extracts was identified by comparison with the standard solution on the basis of retention time. Other detected peaks were considered as "main metabolite" (MM) or "unidentified" (UI) peaks. The main metabolite was suspected to be AMPA from previous experience with the same analytical method but, in the absence of a radiolabeled standard, this could not be verified in this particular experiment. The area of each peak was integrated (Chromera ® chromatography Data System, PerkinElmer) and expressed as a percentage of initial radioactivity applied in the microcosm.

Degradation half–life modeling.
The percentage of glyphosate in the extractable fraction in the microcosm (corresponding to the extractable fraction in the mulch + the extractable fraction in the underlying soil) was fitted to a single first–order kinetic model with untransformed data, $C(t) = C_0 e–kt$ where C(t) is the measured concentration in glyphosate at time t, C_0 is the initial concentration measured immediately after application, and k is the first–order rate constant (day^{-1}). Following this model, the degradation half–life (DT_{50}), time (days) for 50 % disappearance of the initial amount of glyphosate, was calculated for $C = C_0/2$ and corresponds to the ln 2/k ratio.

Data analysis
Data handling and modeling – The radioactivity measured in the different glyphosate fractions was expressed as a percentage of the initially applied radioactivity. Cumulative net CO_2–C and $^{14}CO_2$–C mineralization were fitted to an exponential model that describes mineralization at incubation time t as $a_{MIN} * (1–exp.(–k_{MIN} * t))$. The parameters α_{MIN} and k_{MIN} describe the maximum % C mineralized, and the rate at which it is reached, respectively. The kinetics data for extractable and non–extractable fractions proportions were fitted to an exponential model with 2 ($y = a_{EXT} * exp.(–k_{EXT} * t)$) and 3 ($y = y_0 + a_{NER} * (1–exp.(–k_{NER} * t))$) parameters respectively. In the former case, α_{EXT} is the initial extractable proportion and k_{EXT} the rate of decrease, and in the latter case y_0 is the initial NER proportion, a_{NER} the direction of variation, increase or decrease in NER and k_{NER} the rate of NER variation.

Figure 7.1.2.1.1-9: Organic carbon mineralization. Error bars represent the standard error of the mean of 3 replicates



Statistical analysis
Analyses of variance were performed to ascertain whether each glyphosate fraction proportion was influenced by the incubation time, the treatment or the compartment (soil or mulch) at/on which it was measured. Then, for each fraction, a Fisher's LSD test was used to rank the treatments or compartments. Additionally, an analysis of the correlations between the different glyphosate fractions was carried out at the column and compartment level, with treatments considered together and alone. Parameters of the different kinetic models were also subjected to analysis of variance and post–hoc LSD Fisher test to rank the different treatments, with a level of significance set at 0.05.

Results
Adsorption
Sorption was significantly higher on soil than on cover crop residues (Figure 7.1.2.1.1-8). K_d was 53 and 8 times higher on soil than on fresh and decomposed (56–d) CC residues, respectively. Furthermore, the statistical analysis performed within the CC revealed a significant effect of both decomposition degree and CC type. Sorption increased with the decomposition degree of cover crops ($p < 0.0001$), K_d and K_{oc} for decomposed CC (56 d) being on average 8 or 9 times higher than those measured on fresh CC (0 d). K_d was significantly higher on white mustard than on other CC for 6– and 56–d old CC, being 57 L/kg and 75 L/kg while other CC averaged 28 and 50 L/kg, respectively. For 28–d old CC, K_d was significantly higher on ryegrass (99 L/kg) than on vetch (39 L/kg), other CC being intermediate (66 L/kg). In CC, the analysis of correlations between sorption coefficients and organic matter descriptors did not show any significant relations for K_d. K_{oc} was inversely correlated with the hemicellulose–like fraction ($r = -0.55$, $p < 0.05$).

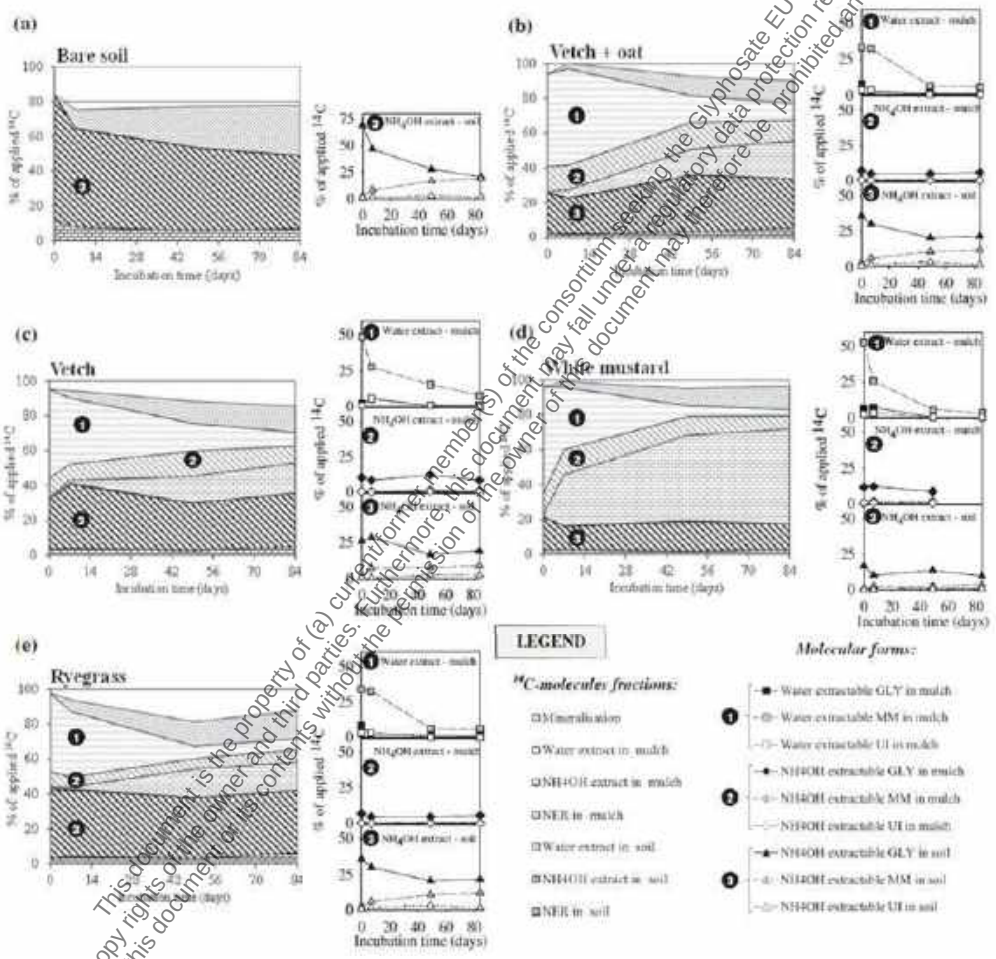
Degradation study
Mulch characteristics during incubation – During the whole incubation period, 2.6 % of the microcosms' carbon content was mineralized from the bare soil. In CC–amended soils, carbon mineralization ranged from 19 % (vetch) to 25 % (vetch + oat) (Figure 7.1.2.1.1-9), corresponding to a weight loss of approximately 35 %. Water content remained constant, being 17 % (w:w) in the soil and 60–72 % according to the mulch (data not shown).

Variability across intercepting material, plant type and time – Glyphosate recovery in the microcosms averaged 90 % of the initially applied dose (Figure 7.1.2.1.1-10). ^{14}C glyphosate fractions were significantly influenced by time, intercepting material (i.e. decaying residue or soil) and plant type (i.e. cover crop species).

Mineralized fraction – Glyphosate mineralization started immediately after application, without any lag phase. It fitted the chosen exponential model well ($R^2 > 0.95$), with parameter k_{MIN}, the speed at which the

maximum is reached, and a, the maximum value reached. Treatments differed significantly from each other for the parameter a_{MIN} (Table 7.1.2.1.1-85), with a higher cumulative glyphosate mineralization in the bare soil microcosms, compared to the mineralisation in the CC–amended microcosms, with values ranging from 13.0 to 15.8 % (Table 7.1.2.1.1-85). Analysis of the plant type effect showed that the maximum mineralized glyphosate was reached significantly faster in ryegrass (Table 7.1.2.1.1-85).

Figure 7.1.2.1.1-10: Fate of glyphosate in the microcosms. Letters indicate the treatment (a: bare soil, b: vetch + oat, c: vetch, d: white mustard, e: ryegrass) and numbers 1, 2 or 3 indicate the fraction within which molecular forms were analyzed (water- or NH_4OH–extractable in mulch and/or soil). Results are expressed as % of applied ^{14}C



Extractable fraction – Total extractable fraction (corresponding to the water and NH_4OH extracts) was well fitted by the chosen exponential model, with R^2 >0.8 and R^2 = 0.65 for CC–amended and bare soil treatments respectively. The extractable fraction decreased over time for all treatments (Figure 3). Differences were observed between the treatments, extractability falling faster in white mustard than in other treatments (param b_{EXT}) (Table 7.1.2.1.1-85). At the end of the experiment, significantly less glyphosate was extractable in the white mustard treatment. The extractable fraction is separated into a water–extractable and an ammonia–extractable fraction, for which more details of molecular forms are given below. The water–extractable fraction decreased rapidly from 52.7 ± 3.4 to 7.0 ± 1.3 % of the applied ^{14}C between 0 DAT and 84 DAT in the mulch compartment (Figure 3, fraction ❶) while it remained low in the soil compartment (<1 % of applied ^{14}C). A larger proportion of ^{14}C was extracted with water in the vetch + oat microcosms, until 49 days of incubation (Figure 3b). In mulches, more metabolites than

glyphosate (GLY) were found in the water extracts. Both GLY and its main metabolite decreased during incubation, averaging 7.9 ± 2.7 % to 0.9 ± 0.3 % and 45.0 ± 4.7 % to 6.7 ± 1.2 % of the applied ^{14}C between 0 DAT and 84 DAT, respectively. The ammonia–extractable fraction remained stable with time in the mulch compartment and varied with no clear trend in the soil compartment while it decreased during incubation in the bare soil treatment (Figure 7.1.2.1.1-10, fractions ❷ and ❸). In the bare soil treatment, GLY proportions decreased from 70 to 20 % of the applied dose between 0 DAT and 84 DAT, whereas MM proportions increased from 2 to 19 % in the same period (Figure 7.1.2.1.1-10a). In all CC–amended treatments, the GLY proportion decreased from an average of 26.5 ± 4.7 % to 14.4 ± 2.1 % and from 11.9 ± 0.1 % to 8.8 ± 1.3 % in soil and in mulch compartments between 0 DAT and 84 DAT, respectively. Meanwhile, MM proportion (i) increased from 1.5 ± 0.3 % to 8.5 ± 1.9 % in soils and (ii) averaged 1.01 ± 0.15 % in mulches.

Table 7.1.2.1.1-85: Fraction–dynamics model parameters. Letters correspond to LSD groups

Fraction	Treatment	y_0			$a_{(i = MIN, EXT, NER)}$			$k_{(i = MIN, EXT, NER)}$		
		Value	Effect of mulch vs. bare soil	Effect of plant type	Value	Effect of mulch vs. bare soil	Effect of plant type	Value	Effect of mulch vs. bare soil	Effect of plant type
Mineralized fraction $y = a \cdot (1\text{-}\exp(-kt))$	Bare soil	–	–	–	27.1 ± 0.9	a	–	0.06 ± 0.01	ab	–
	Vetch + oat	–	–	–	15.0 ± 2.5	b	ns	0.04 ± 0.01	b	b
	Vetch	–	–	–	15.8 ± 1.4	b	ns	0.04 ± 0.01	b	b
	White mustard	–	–	–	13.0 ± 0.4	b	ns	0.04 ± 0.01	b	b
	Ryegrass	–	–	–	15.1 ± 0.2	b	ns	0.08 ± 0.01	a	a
Extractable fraction $y = a \cdot \exp(-kt)$	Bare soil	–	–	–	66.5 ± 0.6	b	–	0.01 ± 0.00	b	–
	Vetch + oat	–	–	–	92.2 ± 0.5	a	ns	0.00 ± 0.00	b	b
	Vetch	–	–	–	89.6 ± [illegible]	[illegible]	ns	0.01 ± 0.00	b	b
	White mustard	–	–	–	84.9 ± 7.8	ab	ns	0.02 ± 0.00	a	a
	Ryegrass	–	–	–	89.0 ± [illegible]	[illegible]	ns	0.01 ± 0.00	b	b
Non-extractable fraction $y = y_0 + a \cdot (1\text{-}\exp(-kt))$	Bare soil	8.9 ± 1.0	a	–	−[illegible] ± [illegible]	[illegible]	–	0.12 ± 0.04	a	–
	Vetch + oat	4.1 ± 1.0	b	ns	[illegible] ± 2.5	a	ns	0.01 ± 0.01	b	b
	Vetch	3.1 ± 0.7	b	ns	[illegible]	b	ns	0.02 ± 0.01	b	b
	White mustard	3.4 ± 1.0	b	ns	[illegible] ± 2.3	ab	ns	0.05 ± 0.01	b	a
	Ryegrass	3.6 ± 0.9	b	ns	[illegible] ± 5.9	ab	ns	0.02 ± 0.01	b	b

Non–extractable fraction – The NER fraction increased with time for CC–amended treatments, especially in the mulch compartment. On the contrary, NER decreased in the bare soil from 11 to 7 % of the applied dose between 0 and 84 DAT (*a* <0, Table 7.1.2.1.1-85). By comparison, in the soil compartment below the mulch NER increased from 3 to 5 % or remained constant (white mustard) (Figure 3, bricks symbols). At the end of the experiment, three statistical groups differing in their NER proportions were distinguished: (i) white mustard with 59.7 ± 2.8 %, (ii) the 3 other mulches with 27.2 ± 0.8 % and (iii) bare soil with 9.0 ± 1.1 % of the initially applied ^{14}C. The modeling of NER formation showed that NER formation rate was significantly greater in white mustard (k_{NER} parameter) than in other mulches (Table 7.1.2.1.1-85).

Glyphosate degradation half–life – In presence of a cover crop mulch, glyphosate degradation half–life was longer than in bare soil, being respectively 28–47 days and 20 days (Table 7.1.2.1.1-86). DT_{50} values showed that glyphosate persistence was increased in the presence of a mulch layer at the soil surface, whatever the type of mulch.

Table 7.1.2.1.1-86: Glyphosate half–life (DT_{50}) calculated from fitting of experimental data to $C = C_0.e^{-kt}$ model. Data are mean ± standard–error

Treatment	DT_{50} (days)	LSD group	R^2
Bare soil	21 ± 1	*b*	0.91
Vetch + oat	28 ± 10	*ab*	0.80
Vetch	47 ± 4	*a*	0.74
White mustard	43	*ab*	0.77
Ryegrass	38 ± 5	*ab*	0.73

Correlation between processes – Considering all treatments, glyphosate mineralization was (i) positively correlated with carbon mineralization (r = 0.80 for CC– amended and r = 0.99 for bare soil) and non–

extractable fraction (r = 0.54 for CC–amended and r = 0.99 for bare soil, p <0.05); (ii) negatively correlated with water–extractable fraction (r = −0.69 for CC–amended and r = −0.97 for bare soil, p <0.01). Furthermore, NER fraction was (i) positively correlated with mineralized glyphosate fraction (r >0.96, p <0.01) and carbon mineralization (r = 0.55 for CC–amended and r = 0.99 for bare soil, p <0.05) and (ii) negatively correlated with water–extractable fraction (r <−0.96, p <0.05) in bare soil and all CC–treatments except vetch. Vetch specific correlations were found between the ammonia–extractable fraction and carbon (r = −0.98) and glyphosate (r = −0.99) mineralization. At the compartment level, the analysis revealed a correlation of NER formation either with water–extractable fraction in mulch (r = −0.98) or with ammonia extractable fraction in soil (r = −0.94).

Discussion

Glyphosate fate depends on the intercepting material

After application, glyphosate fate presented specificities according to the intercepting material, i.e. soil or CC mulch. It was much strongly retained by soil than by mulch, being mainly extractable with ammonia and with water, respectively. These results are in agreement with the sorption measurements (Figure 7.1.2.1.1-8) and are mainly explained by the sorption affinity of glyphosate to soil mineral constituents (clays, oxides). Furthermore, despite a high microbial activity in the mulches, reflected by the carbon mineralization (Figure 7.1.2.1.1-9), glyphosate mineralization in the presence of mulch was lowered as compared to the bare soil treatment. These observations partly suggest a difference in glyphosate accessibility to microorganisms in the two compartments. In soil, although glyphosate is strongly retained, as stated above, the herbicide remains accessible for a complete biological degradation. These results are in agreement with those of Schnurer *et al.* (2006) who observed biodegradation of soil–sorbed glyphosate. In mulches, the absence of change in the molecular forms with time in the ammonia extracts (Figure 7.1.2.1.1-10, fraction ❷) suggests that mulch–sorbed molecules were not available for microorganisms. In contrast, soluble glyphosate and its degradates are available in the mulch–water extracts, as shown by the decrease in their respective proportion (Figure 7.1.2.1.1-10, fraction ❶). However, microbial populations that colonize the decaying mulch are not as efficient as soil microbial population in mineralizing glyphosate. NER formation is generally considered to be the result of either microbial incorporation of pesticide, physical entrapment in the nanoporosity, chemical stabilization by bounding, or diffusion to less accessible sites during a long period of contact. In this study, NER formation was positively correlated to glyphosate mineralization by microorganisms in both soil and mulch compartments. As the mulch compartment is prone to a higher microbial activity (Figure 7.1.2.1.1-9), NER formation is clearly one of the main dissipation pathways of glyphosate in mulches, while it is a minor pathway for soils. In our study, NER proportion was negatively correlated either to sorbed (ammonia extracted) glyphosate fraction in soil or to soluble (water extracted) glyphosate fraction in mulches. In CC mulches, the decrease with time in soluble glyphosate is combined with a weak mineralization and its nearly constant sorbed proportion (recovery of glyphosate in the ammonia fraction). This supports the hypothesis of a direct transfer from the 'soluble' to the 'NER' fraction.

Glyphosate fate as influenced by the nature of the intercepting plant material

Glyphosate fate in the mulch compartment is similar whatever the mulch, i.e. the time evolutions of different fractions are generally similar. However, two of the four cover crop species stand out from the others. Glyphosate was less mineralized in ryegrass than in other cover crops, which we cannot explain, and NER formation is maximal in white mustard. This latter result was not expected but can be explained in view of the results of the sorption study where white mustard was the mulch which maximized sorption at day 6 and 56.

Glyphosate fate in cover crop residues and environmental risk assessment

In this study, glyphosate fate was studied at a fine scale by considering several fractions. The results can be interpreted at a broader scale by considering only two fractions: (i) the dissipated glyphosate i.e. the glyphosate mineralized as CO_2 and immobilized as NER; and (ii) the available glyphosate and metabolites i.e. the molecules which remain available and could be leached in field conditions. At this scale, except for the white mustard treatment, both dissipated and available glyphosate were statistically the same in all treatments. This does not lead to the conclusion that glyphosate fate is not influenced by the presence of a cover crop since (i) dissipation pathways are treatment–specific, i.e. mineralization and metabolites (AMPA) formation are greater in bare soil and more non–extractable residues are formed in CC–amended

treatments; and (ii) the NER formation pathway in mulch is time–dependent, leading to a potential decrease in availability of glyphosate in CC–amended treatment. According to the mechanisms potentially involved in NER formation routes we have proposed for mulch, such release cannot be excluded. The extent to which these results can be extrapolated to field conditions will be determined by (i) weather conditions, especially during the time between application and the first rain and the temperature; (ii) agricultural practices, especially cover crop incorporation and fertilization; and (iii) mulch biomass, coverage, and contact with soil as well as soil type.

Conclusions
This study aimed at evaluating the effects of a mulch of cover crop residues located at the soil surface on the environmental behavior of glyphosate. In the presence of a cover crop mulch, glyphosate and its metabolite remained mainly water–soluble, but with time, a higher proportion of the herbicide became non–extractable. Unlike in soil conditions, bound residue formation was the main process involved in glyphosate dissipation in cover crop mulches. Variations in the intensity of each process were observed among the four cover crop residues studied, but remained unexplained by the biochemical composition of the residues. Finally, degradation half–life of glyphosate was increased with all type of mulches.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes investigations on the degradation in and of adsorption of Glyphosate to soil under the potential influence by cover crops. The article is well described and provides potential endpoints for degradation and sorption. However, the available information does not allow to check the validity against current guidelines, and not enough parameters are provided to evaluate the kinetic behavior.
The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/012
Report author	Norgaard, T. *et al.*
Report year	2015
Report title	Can Simple Soil Parameters Explain Field-Scale Variations in Glyphosate-, Bromoxyniloctanoate-, Diflufenican-, and Bentazone Mineralization?
Document No	DOI 10.1007/s11270-015-2518-z ISSN 0049-6979
Guidelines followed in study	None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary
The large spatial heterogeneity in soil physico-chemical and microbial parameters challenges our ability to predict and model pesticide leaching from agricultural land. Microbial mineralization of pesticides is an

important process with respect to pesticide leaching since mineralization is the major process for the complete degradation of pesticides without generation of metabolites. The aim of our study was to determine field-scale variation in the potential for mineralization of the herbicides glyphosate, bromoxyniloctanoate, diflufenican, and bentazone and to investigate whether this variation can be predicted by variations in basic soil parameters. Sixty-five soil samples were sampled from an agricultural, loamy field in Silstrup, Denmark, from a 60×165 m rectangular grid. The mineralization potential of the four pesticides was determined using a 96-well microplate ^{14}C-radiorespirometric method. Initial mineralization rates were determined using first-order kinetics for glyphosate and bromoxyniloctanoate and zero-order kinetics for diflufenican and bentazone. The mineralization rates of the four pesticides varied between the different pesticides and the different soil samples, but we could not establish correlations between the pesticide mineralization rates and the measured soil parameters. Only the glyphosate mineralization rates showed slightly increasing mineralization potentials towards the northern area of the field, with increasing clay and decreasing OC contents. The mineralization potentials for glyphosate and bentazone were compared with 9-years leaching data from two horizontal wells 3.5 m below the field. The field-scale leaching patterns, however, could not be explained by the pesticide mineralization data. Instead, field-scale pesticide leaching may have been governed by soil structure and preferential flow events.

Materials and Methods

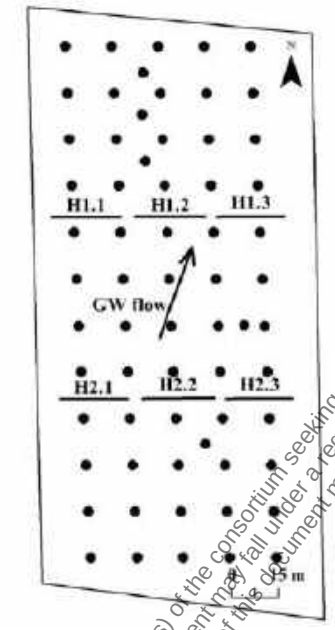
Field Site

The agricultural test field (Silstrup, northwestern Jutland, Denmark) was a conventionally cultivated, loamy field with a cultivated area of 1.69 ha. The climate is coastal, cold temperate. The field has been cultivated as part of a routine agricultural practice with management and pesticide records dating back to 1983. Glyphosate was sprayed on the field five times since 1983, bentazone was sprayed four times, bromoxyniloctanoate only once, and diflufenican not at all. Application dates for the pesticides and their commercial formulations are shown in Table 7.1.2.1.1-87. Two horizontal wells, H1 and H2 (Figure 7.1.2.1.1-11), are located 3.5 m below the surface, and each consists of three screen sections of 18 m. Water samples from the middle screen section of each well (H1.2 and H2.2) have been analyzed for pesticides every month, and the samples from the outer screen sections (H1.1, H1.3, H2.1, and H2.3) have been analyzed twice a year (Rosenbom *et al.* 2010). During 9 years screening (2000–2009), pesticides were detected in 44 % of the water samples from the middle section of the northern horizontal well (H1) whereas only 5 % of the water samples from the middle screen section of the southern horizontal well (H2) contained detectable pesticide concentrations. In the outer screen sections of the northern well (H1.1 and H1.3), pesticides were detected in 30 and 27 % of the water samples whereas there were no pesticide detections in the outer screen sections of H2 (Norgaard *et al.* 2012). Consequently, pesticides seem to leach only from the northern part of the field.

Table 7.1.2.1.1-87: Pesticide application history. There is no record of which Roundup formulation was applied in 1988 and 1999

	Application date	Formulation
Glyphosate	25 October 1988	Roundup (unknown)
	10 October 1994	Touchdown
	5 August 1999	Roundup (unknown)
	25 October 2001	Roundup Bio
	15 September 2003	Roundup Bio
Bromoxyniloctanoate	20 April 1999	Oxitril
Bentazone	24 May 1994	Basagran 480
	17 June 1994	Basagran 480
	17 May 2003	Basagran 480
	19 May 2009	Fighter 480

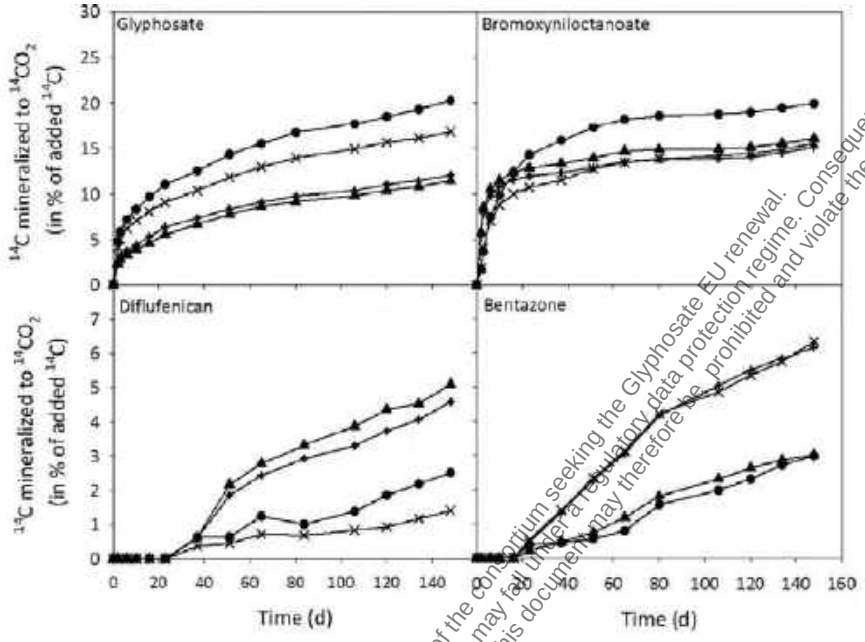
Figure 7.1.2.1.1-11: Schematic presentation of the Silstrup field. Sample positions are indicated by the black dots. The horizontal wells (H1 and H2) and the screen sections in each well are indicated by the lines. The arrow indicates the groundwater flow direction



Soil Sampling

Sixty-five samples were sampled from a 60 × 165 m rectangular field with a distance of 15 m between sampling points (Figure 7.1.2.1.1-111) on 6 December 2011. Soil was sampled from the plough layer at a depth of approximately 8–16 cm. First, the upper 8-cm top soil was removed and then a sample was taken by pounding a sterile 50-ml centrifuge tube (upside down) into the ground until the tube was almost full and then the tube was sealed. In the lab, each sample was homogenized by sieving twice through a sterile 4-mm mesh. The soil was further mixed thoroughly and stored at 2 °C for 1 week.

Figure 7.1.2.1.1-12: Mineralization curves depicting the variation in mineralization within the field. The curves represent for each herbicide the two soil samples with the lowest initial mineralization rate and the two with the highest



Physical and Chemical Soil Analyses

Soil texture was determined according to Gee and Or (2002) using a combined sieve/hydrometer method. Organic carbon was determined on a LECO analyzer coupled with an infrared CO_2 detector. Bulk density was determined from weights of 20×20 cm intact soil columns after drying at 105 °C for 2 weeks. The soil pH was measured in a soil/water suspension of 1:4 (*v/v*), and the soil electrical conductivity (EC) was measured in a soil/water suspension of 1:9 (*w/v*). Oxalate-extractable iron, aluminum, and phosphorus were determined at AGROLAB GmbH, Germany, using the procedure described by Schoumans (2000). The Dexter index (Dexter *n*) for each soil sample was calculated as the ratio (*w/w*) between clay and organic carbon.

Table 7.1.2.1.1-88: Basic soil parameters. Minimum, maximum, mean, and coefficient of variation (CV) of soil texture, organic carbon (OC), Dexter n, bulk density, oxalate-extractable aluminum (Al), oxalate-extractable iron (Fe), and oxalate-extractable phosphorus (P), pH, and electrical conductivity (EC)

	Clay (<2 µm)	Silt (2–50 µm)	Sand (0.05–2 mm)	OC	Dexter *n*	Bulk density	Al	Fe	P	pH	EC
	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}	–	g cm^{-3}	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	–	mS cm^{-1}
Min. value	0.14	0.23	0.45	0.017	6.75	1.39	24	33	7.60	6.39	0.40
Max. value	0.19	0.33	0.59	0.022	10.43	1.60	36	53	15	7.45	0.71
Mean	0.16	0.30	0.51	0.02	8.11	1.50	28.3	43.3	10.6	6.75	0.47
CV	8.4	5.5	4.2	6.9	11.7	3.4	7.7	10.4	14.2	2.2	11.0

Mineralization Potentials

The mineralization potential of the four pesticides was tested using a modified version of a radiorespirometric microplate method. [P-methylene-^{14}C]glyphosate (>99 % radiochemical purity) was purchased from IZOTOP, Institute of Isotopes (Budapest, Hungary). Radioactive pesticide solutions (10 mg/mL, approximately 870 Bq/mL) were prepared by dissolving appropriate amounts of radioactive

pesticide and the corresponding non-labeled pesticide in sterile water. For each of the 65 homogenized soil samples, subsamples of 0.5 g were transferred to microplate wells, one microplate for each of the four pesticides and one subsample per pesticide. The microplates were 96-well polypropylene microplates (Nunc 278752) with a well volume of 2.0 mL to minimize oxygen depletion. Fifty microliters of ^{14}C-labeled pesticide solution was added to all wells, corresponding to an initial pesticide concentration of 1 mg/kg soil. The microplates were sealed with PCR sealing tapes on which 96 $^{14}CO_2$ traps (Ca(OH)-amended filter paper discs) were placed in a pattern corresponding to the microplate wells. Polyurethane foam sheets (the size of a microplate lid) were placed on top of the sealing tapes, microplate lids were added, and the plates and lids were held tightly together with strong rubber bands. The sealing tapes were changed after approximately 2, 3, 6, 10 16, 23, 37, 51, 65, 80, 106, 120, 134, and 148 days of incubation at 10 °C. The trapped $^{14}CO_2$ from each well, captured on the $Ca(OH)_2$-impregnated filters, was quantified from a standard series of $NaH^{14}CO_3$ using digital autoradiography and subsequent digital image analysis as described by Hybholt *et al.* (2011).

Mineralization Kinetics

A two-parameter exponential model (first-order kinetics, Eq. 1) was used to fit the mineralization curves for glyphosate and bromoxyniloctanoate.

$$y = a \cdot (1 - e^{-bt}) \qquad (1)$$

where y is the accumulated $^{14}CO_2$ (% of added ^{14}C) released at time t (day), a is the maximum ^{14}C mineralized (% of added ^{14}C), and b is the mineralization rate constant (day^{-1}).

Since we were interested in estimating the in-situ mineralization potentials, we fitted only the first 23 days of mineralization, where the mineralization followed first-order kinetics. The initial rate at time zero was then calculated from the first derivative function (Eq. 2).

$$dy/dt_0 = ba \qquad (2)$$

A linear regression model (Eq. 3) was used to fit the mineralization curves for diflufenican and bentazone.

$$y = a + bt \qquad (3)$$

For diflufenican and bentazone, the models are based on the mineralization data from days 23–84 and 16–65, respectively. This was done in order to capture the initial, linear part of the mineralization curves from the first detection of mineralization in each of the two cases. The slope of the linear models was used as an estimate of the initial mineralization rate.

Two-Dimensional Interpolation and Statistical Analysis

The spatial, field-scale variation in soil texture, organic carbon content and the mineralization rates were mapped using minimum curvature interpolation with regularized spline interpolation in ArcMap 10.1. The number of points used in the calculation of each interpolated cell was set to 12 and the weight parameter to 0.1. The mineralization rates were correlated to soil physical and chemical parameters using the linear correlation coefficient (R^2), as it shows the fraction of the variation in the mineralization potentials that can be explained by the variation in the physical or chemical soil parameters. Coefficients of variation (CVs) for the pesticide mineralization rates and the soil parameters were calculated as the standard deviation divided by the mean and are given as percentage.

Most Probable Number of Pesticide Degraders

The most probable numbers (MPNs) of cultivable glyphosate-, bromoxyniloctanoate-, diflufenican-, and bentazone degraders were estimated by a modification of the above microplate radiotracer method. To represent the gradients in clay and organic carbon across the field, selected samples were pooled into groups with high clay and low organic carbon content, low clay and high organic carbon content, and intermediate clay and organic carbon content (five to seven subsamples for each group: group A with 17.6–18.9 % clay and 1.8–1.9 % organic carbon, group B with 14.2–14.3 % clay and 2.0–2.1 % organic carbon, and group C with 16.1– 6.2 % clay and 1.9–2.0 % organic carbon).

A well was considered mineralization-positive if the accumulated amount of $^{14}CO_2$ at the end of the experiment (148 days at 10 °C) exceeded 5 % of the initially added ^{14}C-labeled pesticide. The MPNs were calculated according to Hurley and Roscoe (1983) from the distributions of positive and negative microplate wells. The lower detection limit was calculated by assuming only one mineralization-positive well at the lowest dilution (10 - fold), and the upper limit was calculated from only one mineralization-negative well at the highest dilution (21,870-fold).

Results

Pesticide Mineralization

The MPNs of the microbial degrader populations were for glyphosate and bromoxyniloctanoate above the detection limit of 6.6×10^4 cells/g soil, which indicates a large potential for microbial degradation of these pesticides. This was reflected in the rapid mineralization without any-lag phase of both glyphosate and bromoxyniloctanoate (Figure 7.1.2.1.1-12). After a fast, immediate phase, the mineralization of glyphosate leveled off at 10–20 % and bromoxyniloctanoate at 13–26 %. Diflufenican and bentazone both showed slow linear mineralization with a lag-phase, and both pesticides reached very low mineralization levels (diflufenican 1–5 %, bentazone 3–7 %) within the 148 days of the experiment (Figure 7.1.2.1.1-12). The first mineralization was detected on day 23 for bentazone and on day 37 for diflufenican. We did not detect any microorganisms that could utilize diflufenican or bentazone as a source of carbon and energy (MPN <4 cells/g soil), which probably explains the long lag-phases in the mineralization of these two herbicides. The mineralization of bromoxyniloctanoate showed the best model fits (R^2 = 0.980–0.996, average 0.992, Fig. 3), whereas the glyphosate mineralization was slightly underestimated within the first 3 days and slightly overestimated the following 13 days (R^2 = 0.933–0.987, average 0.968). The model fits for diflufenican mineralization (R^2 = 0.734–0.995, average 0.963) and bentazone mineralization (R^2 = 0.850–1.00, average 0.992) were more variable.

Field-Scale Variation in Pesticide Mineralization Rates

The spatial variability of the initial mineralization rates, derived either from the initial rate at time zero for glyphosate and bromoxyniloctanoate or as the slope of the linear regression models for diflufenican and bentazone, is depicted in Figure 7.1.2.1.1-14. Throughout the field, the initial glyphosate mineralization rates varied from 12.1 to 26.0 µg/(kg day) (average 17.1 µg/(kg day), CV=16.7 %), with a slight indication of lower mineralization in the southern part of the field. Bromoxyniloctanoate had the largest initial mineralization rates varying from 14.9 to 42.0 µg/(kg day) (average 29.6 µg/(kg day), CV=16.5 %). Diflufenican and bentazone showed very limited mineralization of only 0.11– 0.58 µg/(kg day) (average 0.32 µg/(kg day), CV= 24.7 %) and 0.13–0.64 µg/(kg day) (average 0.47 µg/(kg day), CV= 22.4 %).

Figure 7.1.2.1.1-13: Examples of herbicide mineralizations and the corresponding model fits. The data represent for each herbicide the two soil samples with the lowest initial mineralization rate and the two with the highest initial mineralization rate within the fitted time period

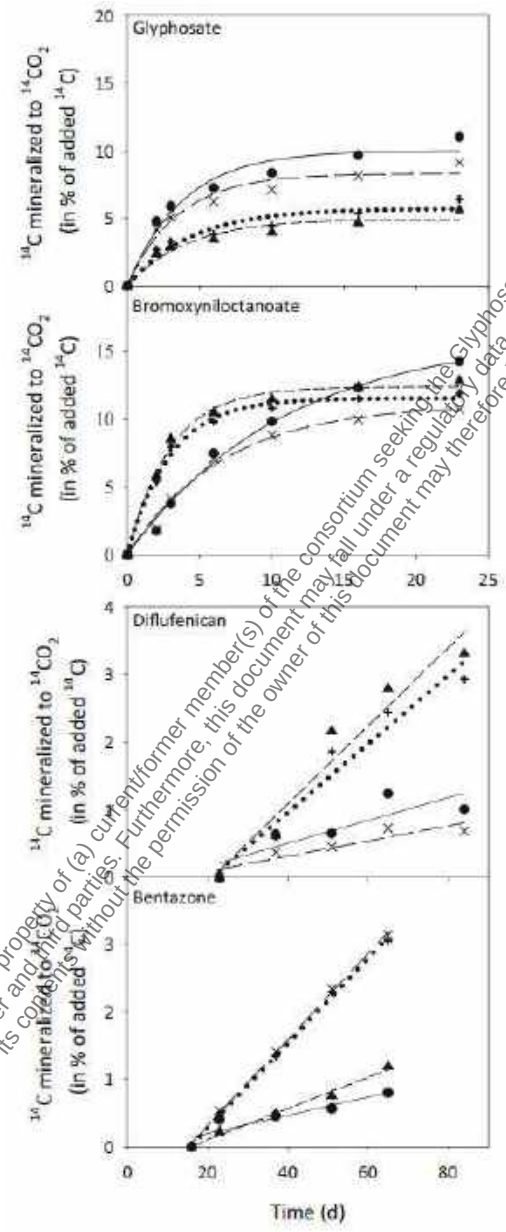


Figure 7.1.2.1.1-14: Spatial distributions of herbicide mineralization rate, clay-, silt-, sand-, and organic carbon (OC) content, and Dexter n (clay/OC ratio). The dots denote the sampling points (n=65)

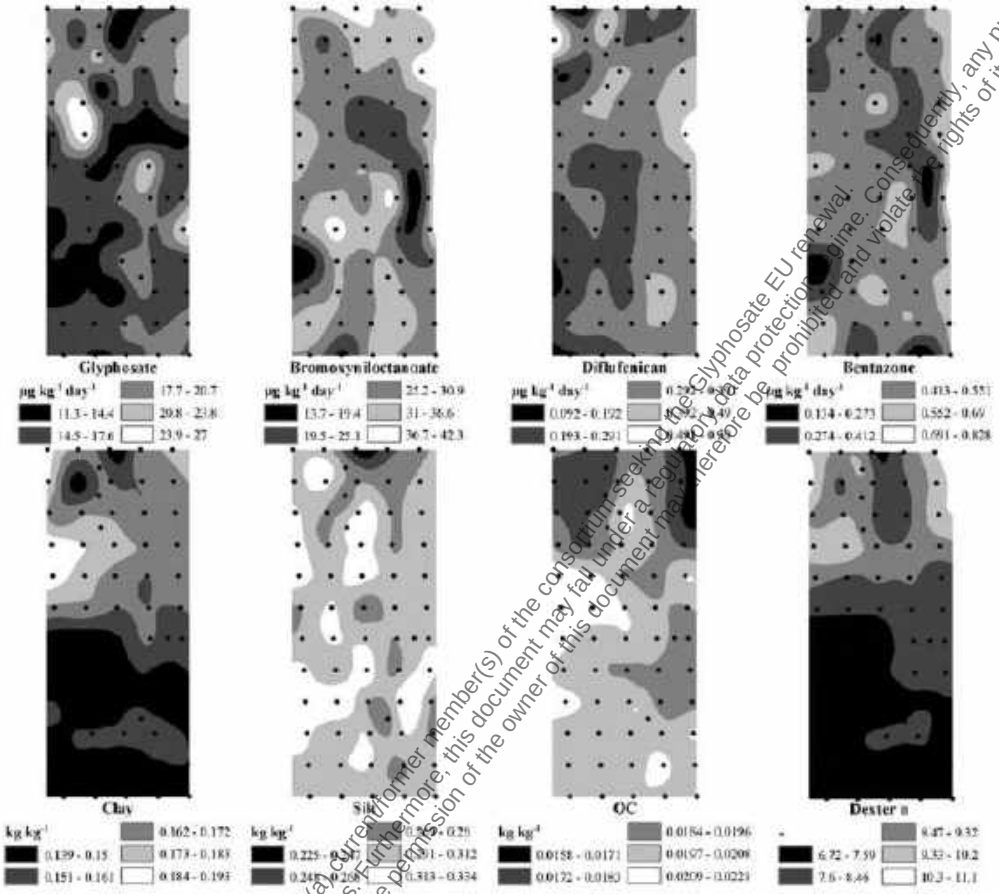


Table 7.1.2.1.1-89: The linear correlation coefficients (R^2) between the pesticide mineralization rates and the basic soil parameters

	Clay (0–2 µm)	Silt (2–50 µm)	Sand (0.05–2 mm)	OC	Dexter *n*	Bulk density	Al	Fe	P	pH	EC
Glyphosate	0.00	0.01	0.00	0.11	0.17	0.06	0.06	0.03	0.10	0.00	0.00
Bromoxynil octanoate	0.01	0.10	0.04	0.04	0.04	0.01	0.08	0.04	0.05	0.05	0.01
Diflufenican	0.06	0.02	0.00	0.06	0.10	0.03	0.01	0.01	0.07	0.00	0.00
Bentazone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01

Mineralization Rates and Soil Characteristics

The range, mean, and CV for the measured soil parameters are reported in Table 7.1.2.1.1-88. Gradients in clay and organic carbon content run in opposite directions within the field. Thus, highest clay contents and lowest organic carbon contents were found in the northern part of the field and lowest clay and highest organic carbon contents were found in the southern part of the field (Figure 7.1.2.1.1-14). The ratio between clay and organic carbon, Dexter *n*, therefore increased from south to north (Figure 7.1.2.1.1-14). The mineralization rates for each of the four pesticides generally showed no correlation or very little correlation to the soil parameters (Table 7.1.2.1.1-89). The highest correlation was between the glyphosate mineralization and the Dexter *n*, but this correlation was also weak (R^2=0.17). Linear correlations between

the mineralization rates of the four pesticides are reported in Table 4. As in Table 7.1.2.1.1-89, the correlation coefficients are weak and the strongest correlation was between the mineralization rates of bromoxyniloctanoate and bentazone (R^2=0.16).

Table 7.1.2.1.1-90: The linear correlation coefficients (R^2) between the pesticide mineralization rates

	Glyphosate	Bromoxyniloctanoate	Diflufenican	Bentazone
Glyphosate	1.00	0.07	0.08	0.01
Bromoxyniloctanoate		1.00	0.02	0.16
Diflufenican			1.00	0.03
Bentazone				1.00

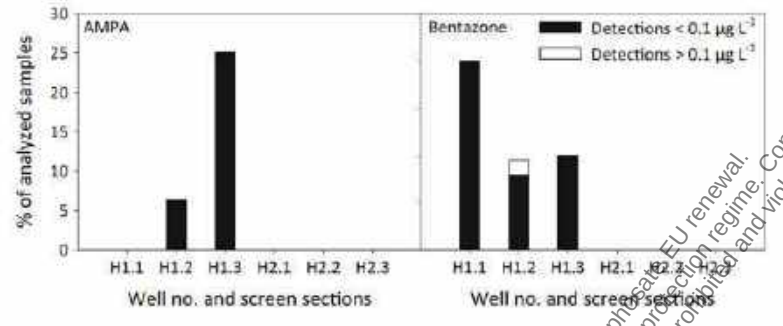
Table 7.1.2.1.1-91: Number of groundwater samples from H1 and H2 analyzed for glyphosate, aminomethylphosphonic acid (AMPA), bentazone, and 2-amino-N-isopropylbenzamide

	Number of samples, *n*					
	H1.1	H1.2	H1.3	H2.1	H2.2	H2.3
Glyphosate	8	48	8	10	46	8
AMPA	8	48	8	10	46	8
Bentazone	10	63	10	7	46	6
2-Amino-*N*-isopropylbenzamide	4	29	4	4	29	4

Field-Scale Leaching

Water from the two horizontal wells (H1 and H2, Figure 7.1.2.1.1-11) was analyzed for glyphosate and bentazone and their main metabolites aminomethylphosphonic acid (AMPA) and 2-amino-N-isopropylbenzamide (Table 7.1.2.1.1-91). Glyphosate was applied five times on the field during the period from 1988 to soil sampling in 2011 with two applications within the monitoring period (2001 and 2003, Table 7.1.2.1.1-87). The glyphosate and AMPA contents in water from different subsections of H1 and H2 were analyzed from 2001 to 2005. Glyphosate was not detected in any of the samples. The glyphosate degradation product AMPA, however, was detected in 6.3 % of the analyzed samples from H1.2 and 25 % of the analyzed samples from H1.3 (Figure 7.1.2.1.1-15). None of the AMPA concentrations exceeded the drinking water quality criterion of 0.1 µg/L. Bentazone was applied four times on the field from 1994 to 2011, and two of these applications were within the monitoring program (2003 and 2009, Table 7.1.2.1.1-87). Bentazone was analyzed for in the periods from 2003 to 2006 and from 2009 to 2011. The bentazone metabolite was analyzed for only in the period from 2003 to 2006. In total, bentazone was detected in 20 % of the samples from H1, 19.5 % of the H1.2 samples, and 10 % of the H1.3 samples (Figure 7.1.2.1.1-15). One of the detections in H1.2 was above the criterion of 0.1 µg/L. The bentazone degradation product, 2-amino-N-isopropylbenzamide, was not detected in any of the analyzed samples. The metabolite, however, was analyzed for only in the period from 2003 to 2006, whereas bentazone was analyzed for in the periods from 2003 to 2006 and from 2009 to 2011. The horizontal monitoring well, H2, was suspended from 2009.

Figure 7.1.2.1.1-15: Percentage of samples AMPA Bentazone from the two horizontal wells, H1 and H2 (Figure 7.1.2.1.1-11), containing detectable levels of the glyphosate degradation product AMPA or bentazone. Water was collected monthly (H1.2 and H2.2, n=29–63) or half yearly (remaining filter sections, n=4–10)



Discussion

In this study, we have investigated the potential mineralization of four herbicides commonly used in agriculture (Miljøstyrelsen 2014). These herbicides represent different physico-chemical properties with very different literature reports on hydrophobicity and sorption. Glyphosate was an easily mineralized, hydrophilic compound with strong sorption to clay loam. Bromoxyniloctanoate was also easily mineralized and strongly sorbing, but hydrophobic. Diflufenican was difficult to mineralize, hydrophobic, and strongly sorbing, and bentazone was also difficult to mineralize, in spite of being hydrophilic with low sorption.

It is clear from the above that bioavailability, expressed as the soil/water distribution coefficient K_d, did not determine the different mineralization patterns between the four herbicides. One reason could be that we added the Tween-80 detergent to the solutions of bromoxyniloctanoate and diflufenican to be able to handle these compounds in aqueous solution. Also, bentazone was not mineralized to any great extent in spite of high bioavailability, which suggest a microbiological limitation rather than a physico-chemical limitation. Glyphosate, in contrast, was easily mineralized in spite of a high distribution coefficient and thus low bioavailability, indicating that sorption may be less important when degraders are very numerous in the soil.

We used first-order kinetics to quantify the mineralization of glyphosate and bromoxyniloctanoate for the first 23 days. Linear regression was used to quantify the mineralization of diflufenican and bentazone covering the time periods 23–84 and 16–65 days, respectively. The linear mineralization patterns indicate that these pesticides were probably mineralized by slow co-metabolic metabolism without growth of the degrader organisms, which is consistent with the absence of bacteria that could utilize them for growth. The 2–3-week delay in mineralization may imply that the degrader organisms were fungi.

The mineralization potentials of bromoxyniloctanoate, diflufenican, and bentazone did not correlate with the gradients in clay and organic carbon across the field or any other of the measured soil parameters. Only the glyphosate mineralization rates tended to increase towards the northern part of the field, correlating slightly with increasing clay and decreasing organic carbon contents (Table 7.1.2.1.1-89). The highest correlation was, however, between the glyphosate mineralization and Dexter *n*, so that it was the ratio between clay and organic carbon more than the total contents that influenced the glyphosate mineralization.

Our results indicate that the development of generally valid models for predicting pesticide mineralization across field sites, based on simple soil characteristics and in-vitro mineralization rates, may be unrealistic. Furthermore, if the mineralization of two or more of the herbicides were determined by the same soil parameters, we would have seen correlations between these herbicides, which were not the case (Table 7.1.2.1.1-90). It seems difficult to connect pesticide mineralization (or degradation) and specific topsoil parameters, but what about pesticide mineralization and leaching? Though included in the analyses, we did not detect glyphosate in the samples from the horizontal monitoring wells, but the glyphosate

degradation product, AMPA, was detected. In contrast, only bentazone was detected, and not the degradation product, 2-amino-Nisopropylbenzamide. All detections of AMPA and bentazone were from the H1 well that collected water from the northern part of the field. Neither AMPA nor bentazone was detected in the samples from H2 which collected water from the southern part of the field. This pattern does not correspond well with the rather random distribution of mineralization potentials of the two herbicides (Figure 7.1.2.1.1-14).

Conclusion
Glyphosate was an easily mineralized, hydrophilic compound with strong sorption to clay loam. Bromoxyniloctanoate was also easily mineralized and strongly sorbing, but hydrophobic. Diflufenican was difficult to mineralize, hydrophobic, and strongly sorbing, and bentazone was also difficult to mineralize, in spite of being hydrophilic with low sorption. It is clear from the above that bioavailability, expressed as the soil/water distribution coefficient K_d, did not determine the different mineralization patterns between the four herbicides. The linear mineralization patterns indicate that these pesticides were probably mineralized by slow co-metabolic metabolism without growth of the degrader organisms, which is consistent with the absence of bacteria that could utilize them for growth.

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3. Assessment and conclusion

Assessment and conclusion by applicant:
The article deals with investigations on mineralization in aerobic soil under laboratory and field conditions. Amongst other active substances, laboratory tests were performed with glyphosate.
In parallel the leaching behavior was investigated under field conditions for the soils used in laboratory tests on mineralization.
The study did not follow guidelines in design and conduct. Moreover, the level of detail of provided data does not allow for a check of validity of the study against current guidelines.
Furthermore, nor data on glyphosate content per sampling point, neither half-lives were provided.
The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/013
Report author	Kanissery, R. G. *et al.*
Report year	2015
Report title	Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate
Document No	DOI 10.2134/jeq2014.08.0331 E-ISSN 1537-2537
Guidelines followed in study	Adsorption experiment: USEPA guidelines for adsorption studies (USEPA, 2008) Degradation experiment: None
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The adsorption, desorption, degradation, and mineralization of ^{14}C-glyphosate [*N*-(phosphonomethyl)glycine] were examined in Catlin (a fine-silty, mixed, superactive, mesic Oxyaquic Argiudoll), Flanagan (a fine, smectitic, mesic Aquic Argiudoll), and Drummer (a fine-silty, mixed, superactive, mesic Typic Endoaquoll) soils under oxic and anoxic soil conditions. With the exception of the Drummer soil, soil aeration did not significantly alter the adsorption pattern of ^{14}C-glyphosate to soils. Herbicide desorption was generally enhanced with anaerobiosis in all the soil types. Anoxic soils demonstrated slower microbial degradation and mineralization kinetics of ^{14}C-glyphosate than oxic soils in all the soil types studied. Phosphate additions significantly reduced the adsorption of ^{14}C-glyphosate to soils irrespective of soil aeration and confirmed the well-established competitive adsorption theory. The addition of soil phosphate stimulated degradation only in anoxic soils. The results from this research highlight the importance of soil redox conditions as an important factor affecting the bioavailability and mobility of glyphosate in soils.

Materials and Methods

Chemicals

Carbon-14-glyphosate (phosphonomethyl-^{14}C) (specifc activity: 1.85 × 109 Bq/mmol) was obtained from American Radiolabeled Chemicals. Unlabeled glyphosate (chemical purity: 99 %) was procured from Sigma Chemical Company. Organic solvents and water were of Optima grade from Fisher Scientifc and used without further purification.

Soils

The soils used were a Drummer silty clay loam, a Flanagan silt loam, and a Catlin silt loam. The moderately well drained Catlin, the somewhat poorly drained Flanagan, and the poorly drained Drummer soils occur in proximity in landscapes and form a soil catena; they thus have similar parent materials but vary in organic matter content, landscape position, and soil drainage class. The soils were collected (to a depth of 15 cm) from a field with no previous glyphosate application history at the University of Illinois Crop Sciences Research and Education Center in Urbana. All soil samples were sieved through a 2-mm screen and stored at 4 °C for 4 weeks. Relevant physical and chemical properties of the three soil types used in this study were analyzed at the A&L Great Lake Laboratories and are listed in Table 7.1.2.1.1-92. Each soil type had three replicates for each treatment in the subsequent experiments.

Table 7.1.2.1.1-92: Selected properties of the soils used in the experiment (analysis by A&L Great Lakes Laboratories, Inc.)

Soil	pH	Texture†			WHC‡	CEC§	Organic matter¶	P
		Sand	Silt	Clay				
		%				$cmol_c\ kg^{-1}$	%	$mg\ kg^{-1}$
Catlin	7.6	10	58	32	31.4	13.9	3.3	69
Flanagan	6.5	12	56	32	32.0	17.5	3.7	154
Drummer	7.1	14	46	40	33.0	23.8	4.9	81

† Determined by hydrometer method.
‡ Water holding capacity at 33 1/3 kPa (determined by porous plate/pressure apparatus).
§ Cation exchange capacity.
¶ Determined by loss-on-ignition.

Adsorption-Desorption Study
Adsorption isotherms of ^{14}C-glyphosate were determined using the batch equilibrium method for the three soil types. The initial concentrations of ^{14}C-glyphosate (0.1, 1, 5, and 10 mg/L) were prepared in 0.1 mol/L KCl solution and the adsorption experiment followed USEPA guidelines for adsorption studies. Two grams of air-dried soil was equilibrated with 10 mL of ^{14}C-glyphosate in 20-mL Teflon centrifuge tubes in a horizontal shaker (150 rpm) for 24 h (sufficient for apparent equilibrium in a preliminary study) at room temperature (25 ± 1°C). For the anaerobic treatments, 2-g portions of the soil samples contained in the Teflon centrifuge tubes were flooded with sterile, deoxygenated water. Preliminary studies revealed that <5 % of added glyphosate was degraded during 24 h of contact with any of the soils used (data not shown). The tubes were flushed with N_2 gas, sealed, and incubated in an anaerobic chamber from Coy Laboratory Products containing a primary headspace of $N_{2(g)}$ with 5 % CO $_{2(g)}$ and <2 % $H_{2(g)}$ at room temperature (25 °C) for 2 wk to allow reduction. To study anaerobic adsorption, ^{14}C-glyphosate was added, using a concentrated O_2-free stock solution, to the reduced (anoxic) soil to attain final concentrations of 0.1, 1, 5, and 10 mg/L. Sealed tubes were equilibrated on a shaker inside the anaerobic chamber for 24 h, where the O_2 was maintained at zero concentration. The effect of phosphate addition on glyphosate adsorption in oxic and anoxic soils was examined by incorporating $CaHPO_4$ into the Catlin, Flanagan, and Drummer soils at an overwhelming concentration (500 mg/kg soil), followed by thorough mixing of the soils before the addition of the herbicide.

At the end of the equilibration period, the soil suspension was centrifuged (15 min, 12,000 × g) and aliquots removed from each tube for a radioactivity assay using a Packard Tri-Carb (1900TR) scintillation counter. Controls (treatment without herbicide) were included for calibration and background correction purposes. The amount of ^{14}C-glyphosate adsorbed to the soil was calculated based on the difference between the initial and final concentrations of herbicide in the solution.

Following equilibration and removal of 5 mL of the initial 10 mL of supernatant, herbicide desorption from the soil was estimated by adding equal amounts of fresh 0.1 mol/L KCl solution to the centrifuge tubes, dispersing the soil aggregates by vibration, and shaking for 24 h. Sampling from the anaerobic soil treatments was handled inside the anaerobic chamber. Soil samples were centrifuged (15 min, 12,000 × g), and an aliquot of the supernatant was removed and analyzed utilizing the radioactivity assay. The desorption process was repeated four times. Desorption was estimated by determining the amount of herbicide (described below) in the soil solution following equilibration and calculated by subtracting the amount of herbicide remaining on the soil surface.

Degradation Study
Microcosm Preparation
Soil incubations were performed for 56 d under reduced (anoxic) or oxidized (oxic) conditions using serum bottle microcosms to determine the degradation kinetics of ^{14}C-glyphosate. No degradation was detected in aqueous or organic stock solutions of glyphosate during the experiment.

Anaerobic incubations: Microcosms consisting of serum bottles (60 mL) were amended with soil (10 g) and were spiked with phosphonomethyl-C-labeled ^{14}C-glyphosate (specifc activity of 3.33 x 103 Bq/mmol,

diluted with unlabeled glyphosate) in 50 mL of methanol to produce a final concentration of 2 mg/kg of soil that corresponded to the recommended agricultural application rate. The glyphosate-spiked soils were agitated on a reciprocating shaker for 24 h at room temperature to ensure thorough mixing and to evaporate the solvent. To determine the effect of soil phosphate on glyphosate degradation, $CaHPO_4$ was uniformly mixed into the soils at a concentration of 500 mg/kg soil before the addition of the herbicide. The soil was then flooded with 20 mL of sterile (autoclaved), deoxygenated water to mimic soil saturation by rainfall. The microcosm headspace was flushed with N_2 gas and immediately crimp sealed with a butyl stopper fitted with a vial containing 1 mL of 0.5 mol/L NaOH to trap the mineralized $^{14}CO_2$. These microcosms were incubated in a dark, temperature-controlled chamber at 25°C. Sterilized soil microcosms were included as controls for each soil type. Sterilization was achieved by autoclaving the soils twice at 121°C for 1 h on successive days.

Aerobic incubations: Soil microcosms were built from serum bottles as described above. Sterile, distilled water was added to the glyphosate-spiked soils to adjust the moisture content to about 60 % of the field water-holding capacity. The serum bottles were lightly capped (no crimp seal) with a butyl stopper fitted with a NaOH trap and stored in the dark at 25°C. At 1-wk intervals, the microcosms were aerated by equilibrating the headspace with the atmosphere, and the soil moisture content was adjusted by returning each vessel to its initial weight with sterile, distilled water.

Sample Extraction and Analysis

Anaerobic and aerobic microcosms were destructively sampled at consecutive intervals (0.5, 3, 7, 14, 28, 42, and 56 d) by removing the NaOH trap, followed by agitating the microcosm for 1 min and transferring the contents to a 50-mL Teflon centrifuge tube. Quantification of $^{14}CO_2$ in the NaOH traps was accomplished by direct liquid scintillation spectrometry (LSS) using a Packard Tri-Carb (1900TR) scintillation counter. The solid and liquid phases of the soil slurry were then separated by centrifugation (15 min, 12,000 × g). Aqueous samples were removed and filtered (0.2 µm), and the total aqueous radioactivity was estimated using LSS. The soil was extracted with 20 mL of NaOH (0.1 mol/L) in a Teflon centrifuge tube with horizontal shaking following the method described by Druart *et al.* (2011). Extracts were centrifuged at 12,000 rpm for 15 min, an aliquot was removed for LSS (to quantify extractable radioactivity), and the supernatant was retained for analysis of the herbicide. The recovery values of glyphosate from oxic soils were 73 to 78 % and 74 to 76 % from anoxic soils. The recovery efficiencies obtained were taken into consideration in the calculations of the results. Soil extract samples containing ^{14}C-glyphosate were analyzed using high-performance liquid chromatography with a Packard Radiomatic Flo-one Beta scintillation detector. Separation was achieved with an isocratic elution of the mobile phase composed of acetonitrile/water (10:90 v/v) through a 4.6 × 150 mm, 5-µm particle size, C_{18} column from Prontosil. Glyphosate had a reproducible retention time of 4.1 min at a flow rate of 1 mL/min.

Data Analysis

The adsorption and desorption parameters of glyphosate under oxic and anoxic conditions for each soil type were calculated using the transformed Freundlich equation; equation: $\log C_s = \log K + 1/n \log C_e$, where C_s is the amount of glyphosate adsorbed to the soil (mg/kg), C_e is the equilibrium concentration in the soil solution (mg/L), and K and $1/n$ are empirical constants that reflect the affinity of the soil for the herbicide and the degree of linearity between the amount adsorbed and the solution concentration, respectively. Regression analysis was performed on adsorption and desorption isotherms to calculate K (intercept) and $1/n$ (slope) values of glyphosate in oxic and anoxic soils. Hereafter, K_{ads} and $1/n_{ads}$ will indicate Freundlich parameters for adsorption, and K_{des} and $1/n_{des}$ will refer to desorption parameters. The data on the degradation of glyphosate in soils were fitted into the first-order kinetics model $C_t = C_0 \exp(-kt)$, where C_0 is the initial concentration (mg/kg soil) of the herbicide in the soil, C_t is the herbicide concentration (mg/kg soil) detected in the soil at time t, and k is the first-order rate constant. Degradation rate constants were calculated by linear regression of the natural logarithm of the percentage of herbicide remaining against the time. The aerobic and anaerobic degradation half-lives ($T_{1/2}$) for each soil type were calculated using the equation $T_{1/2} = \ln 2/k$. The statistical program SAS Version 9.3 from SAS Institute was used to calculate the treatment means and standard errors ($n = 3$). The experiments were set up as a completely randomized design, and the differences between treatments were evaluated using one-way analysis of variance followed by a least significant difference test at $p < 0.05$.

Results

Adsorption-Desorption

Adsorption data from the experiment were very well fitted by the Freundlich equation ($R^2 = 1$) for the range of herbicide concentrations (0.1-10 mg/L) and soils tested regardless of the soil redox conditions (Table 7.1.2.1.1-93). Among the different soils and treatments, the slope ($1/n_{ads}$) values ranged from 0.76 to 0.93 and the Freundlich adsorption coefficient (K_{ads}) from 62.21 to 103.46. Soil redox conditions did not alter glyphosate adsorption to the Catlin and Flanagan soils, as evident from their nearly equal K_{ads} values. However, the herbicide exhibited a noticeably lower K_{ads} value in the anaerobically treated Drummer soil vs. the aerobic Drummer soil incubations. Further, K_{ads} was observed to be lowest for Catlin and highest for Drummer regardless of the soil redox conditions. A higher K_{ads} indicates a higher adsorption affinity of the herbicide to the soils. Desorption isotherms for glyphosate in all the soils fit well into the Freundlich model (R^2 >0.92). The calculated desorption parameters of glyphosate in the oxic and anoxic soils are presented in Table 7.1.2.1.1-94. Freundlich desorption coefficient (K_{des}) values of glyphosate were considerably lower in the anoxic soils than the oxic soils. Among the three soils tested, the highest K_{des} was observed in the Catlin soil irrespective of the soil redox conditions. A higher K_{des} indicates a greater retention of glyphosate on the soil surface.

Table 7.1.2.1.1-93: Adsorption (Freundlich model) of ^{14}C-glyphosate in different soil types under oxic and anoxic environmental conditions

Soil	K_{ads}†		$1/n_{ads}$‡		R^2§	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
Catlin	62.21 (±1.71)¶	72.38 (±5.80)	0.92 (±0.02)	0.76 (±0.05)	0.999	0.999
Flanagan	78.14 (±2.05)	69.64 (±4.02)	0.90 (±0.02)	0.78 (±0.04)	0.999	1.000
Drummer	103.46 (±5.11)	84.82 (±4.36)	0.93 (±0.03)	0.88 (±0.03)	0.998	0.998

† Freundlich adsorption coefficient.
‡ Adsorption isotherm slope.
§ Goodness of fit for Freundlich model.
¶ 95% confidence intervals in parentheses.

Table 7.1.2.1.1-94: Desorption (Freundlich model) of ^{14}C-glyphosate in different soil types under oxic and anoxic environmental conditions

Soil	K_{des}†		$1/n_{des}$‡		R^2§	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
Catlin	46.52 (±0.03)¶	42.85 (±0.20)	0.02 (±0.002)	0.28 (±0.003)	0.94	0.94
Flanagan	17.08 (±0.03)	5.81 (±0.02)	0.09 (±0.002)	0.25 (±0.005)	0.92	0.92
Drummer	17.95 (±0.03)	7.75 (±0.05)	0.02 (±0.003)	0.25 (±0.005)	0.92	0.92

† Freundlich desorption coefficient.
‡ Desorption isotherm slope.
§ Goodness of fit for Freundlich model.
¶ 95% confidence intervals in parentheses.

Degradation and Mineralization

Figure 7.1.2.1.1-16 a to c depict the degradation pattern of ^{14}C-glyphosate in the Catlin, Flanagan, and Drummer soils incubated under oxic and anoxic conditions. The first-order parameters including the rate constant (k) and degradation half-life ($T_{1/2}$) of the ^{14}C-glyphosate in the different soil types and redox conditions are presented in Table 4. The ^{14}C-glyphosate degradation followed first-order kinetics in all the nonsterile oxic and anoxic soils, as obvious from their R^2 values (0.83 -1.00). The loss of herbicide from the sterile soil control microcosms was not substantial in either aerobic or anaerobic incubations (Figure 7.1.2.1.1-16 a to c). In all three soil types studied, the aerobic $T_{1/2}$ values (15 – 18 d) calculated for glyphosate were significantly lower than the corresponding anaerobic values (42 -51 d). The $T_{1/2}$ of the herbicide in the Catlin, Flanagan, and Drummer soils were comparable in the aerobic incubations. On the other hand, compared with the other soils, glyphosate degradation was relatively slow in the Flanagan soil

in the anaerobic incubations. Figure 7.1.2.1.1-16 d to f illustrate the comparative microbial mineralization trends of glyphosate amendments observed as the amount of $^{14}CO_2$ measured from the alkali trap from aerobic and anaerobic soil microcosms. More than half (53 – 63 %) of the radioactivity in the applied ^{14}C-glyphosate was mineralized as $^{14}CO_2$ from the oxic soils, and only 38 to 41 % of the applied ^{14}C-glyphosate was mineralized in the anaerobic microcosms by the end of incubation. Conversely, aerobically or anaerobically incubated sterilized microcosms had little or no mineralization of the herbicide in all the soil types considered. Another interesting observation from the study is the absence of a lag phase before the evolution of $^{14}CO_2$ from the soils. The evolution of $^{14}CO_2$ from soils was evident immediately after Day Zero of the incubation in both oxic and anoxic soils. Glyphosate mineralization in oxic soils was initially rapid, followed by a gradually decreasing rate. However, in anoxic soils, mineralization of the glyphosate started out slowly and steadily increased toward the end of incubation.

Figure 7.1.2.1.1-16: (a,b,c) Degradation kinetics and (d,e,f) mineralization patterns of ^{14}C-glyphosate under oxic (Ox) and anoxic (An) soil conditions in Catlin, Flanagan, and Drummer soils. Data from oxic (Ox Ster) and anoxic (An Ster) sterilized control soils are also shown

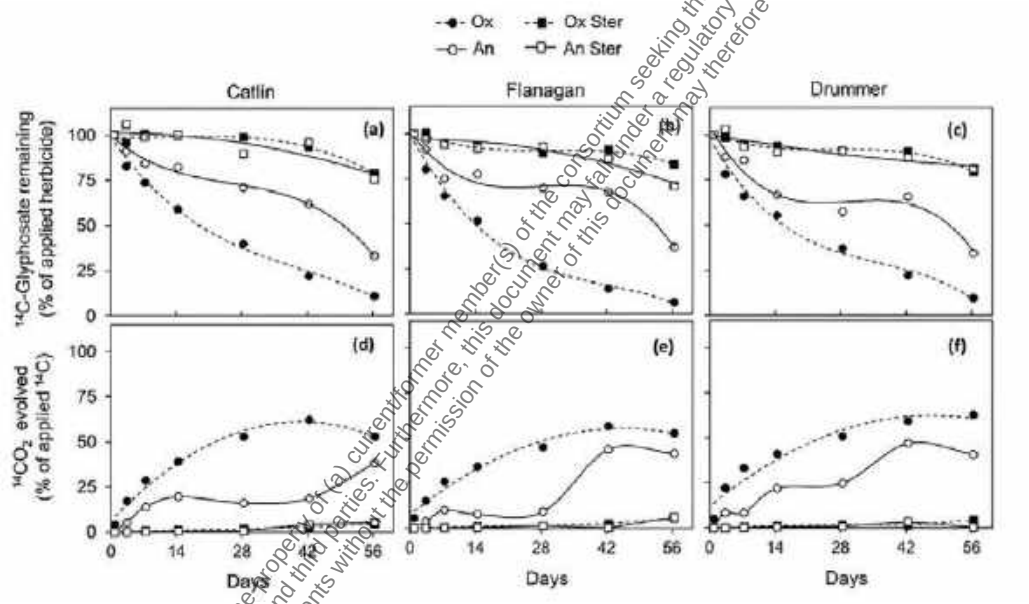


Table 7.1.2.1.1-95: Degradation (first-order kinetics) parameters of ^{14}C-glyphosate in different soil types under oxic and anoxic environmental conditions

Soil	k†		$T_{1/2}$‡		R^2§	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
	d^{-1}		d			
Catlin	0.038 (0.003)¶	0.016 (0.005)	18 c# (209)	42 b (154)	0.99 (0.73)	0.88 (0.68)
Flanagan	0.048 (0.003)	0.014 (0.005)	15 c (228)	51 a (140)	1.00 (0.84)	0.81 (0.85)
Drummer	0.038 (0.003)	0.015 (0.004)	18 c (210)	45 b (200)	1.00 (0.85)	0.83 (0.86)

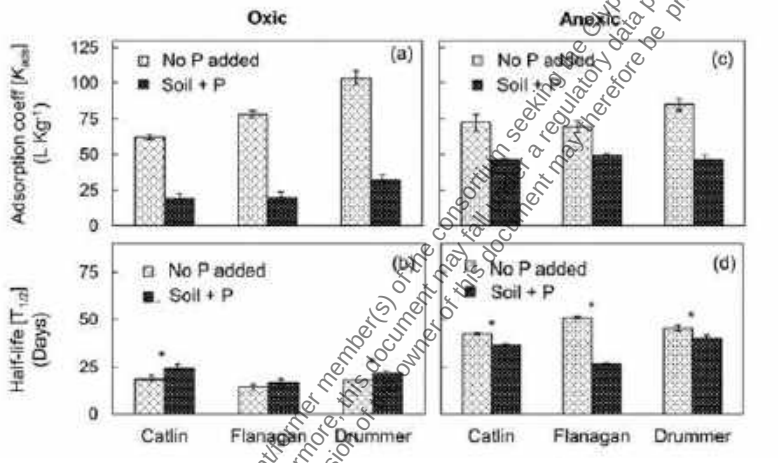
† Rate constant.
‡ Degradation half-life.
§ Goodness of fit for first-order degradation model.
¶ Corresponding values for the sterilized soil control in parentheses.
# Means followed by the same letter are not significantly different ($p < 0.05$).

Effect of Phosphate Addition on Adsorption and Degradation

The addition of phosphate to the Catlin, Drummer, and Flanagan soils significantly reduced the ^{14}C-glyphosate adsorption to oxic and anoxic soils (Figure 7.1.2.1.1-17 a - c). Moreover, the extent of the reduction in herbicide adsorption was more pronounced in the oxic soils. Phosphate additions did not improve or had no effect on the degradation of ^{14}C-glyphosate in the oxic soils, as observed from the degradation half-life values ($T_{1/2}$) of the herbicide in the respective soils (Figure 7.1.2.1.1-17 b). Conversely, the presence of soil phosphate significantly enhanced the anaerobic degradation of ^{14}C-glyphosate in all three soil types studied (Figure 7.1.2.1.1-17 d).

Figure 7.1.2.1.1-17: Effect of phosphate addition (500 mg/kg soil) on the adsorption and degradation of ^{14}C-glyphosate in oxic and anoxic soils: comparison of (a,c) the adsorption coefficient and (b,d) the degradation half-life of glyphosate in oxic and anoxic soils without (No P added) and with (Soil + P) phosphate amendment

***Signifcantly different at p <0.05. Error bars represent standard errors (n = 3)**



Discussion

Adsorption-Desorption

High K_{ads} values of ^{14}C-glyphosate from the present study (62.21-103.46) clearly indicate a strong adsorption affinity of the herbicide to the soils (Table 7.1.2.1.1-93). The results obtained from this study were comparable to reported K_{ads} values (33-152.9) for glyphosate. The greatest extent of ^{14}C-glyphosate desorption was observed in the soils having the least adsorption (Table 7.1.2.1.1-94). Relatively lower K_{des} values in the anaerobic treatments than the corresponding aerobic treatments in all the tested soils indicate that desorption of the herbicide was enhanced in the anoxic, reduced soils. Increased desorption of the herbicide under anoxic conditions may result in an enhanced bioavailability of glyphosate, increasing the risk of movement or crop damage and possibly enhancing degradation of the herbicide under anoxic soil conditions.

Degradation and Mineralization

Degradation of ^{14}C-glyphosate occurred more rapidly in the aerobically incubated Catlin, Flanagan, and Drummer soils than in the corresponding anaerobic incubations, as evident from the significantly lower aerobic $T_{1/2}$ values (Table 7.1.2.1.1-95). This concurs with previous studies. Glyphosate degradation could be inferred to be a purely microbially mediated process because practically no degradation or mineralization occurred in the sterile control soils in any soil type or redox condition. The slow start in the anaerobic mineralization may be ascribed to the acclimation of specialized herbicide degrading microbial populations in the anoxic soil.

Impact of Soil Phosphate

Suppression of glyphosate adsorption in both oxic and anoxic soils with phosphate addition explicitly demonstrated the competition for adsorption sites between glyphosate and phosphate despite differences in

redox conditions (Figure 7.1.2.1.1-17). Several studies have confirmed similar competitive adsorption of glyphosate and phosphate on Al^{3+} and Fe^{3+} surface sites in soil. The effect of phosphate addition on the enhanced microbial bioavailability of glyphosate was found only in the anoxic soils, where the $T_{1/2}$ of glyphosate was noticeably reduced in all the soil types treated anaerobically with phosphate (Figure 7.1.2.1.1-17). Phosphate addition did not stimulate glyphosate degradation in oxic soils.

Implications
This study examined the significance of oxic and anoxic soil conditions on the microbial bioavailability of glyphosate in soils. Although ^{14}C-glyphosate was highly adsorbed to the soils regardless of the soil type and redox conditions, desorption or release of the adsorbed herbicide was enhanced in anoxic soils. The degradation and mineralization of ^{14}C-glyphosate exhibited slower kinetics in anoxic soils than oxic soils in all the soil types investigated. The addition of phosphate to the soil suppressed the adsorption of glyphosate in both oxic and anoxic soils and improved the degradation rate in anoxic soils. The effects of anaerobiosis on the observed K_{ads} and K_{des} suggest greater glyphosate bioavailability in saturated soils. Significant decreases in degradation kinetics observed under anaerobiosis across soils could confer a greater potential for transport in water and subsequent environmental impacts. These findings are based on soils in corn (*Zea mays* L.) - soybean [*Glycine max* (L.) Merr.] rotations from the Upper Midwest and may not reflect outcomes in soils in warmer climates or situations involving frequent flooding cycles, such as in wetland rice (*Oryza sativa* L.) production or crop areas in river floodplains. The conflicting observations between oxic and anoxic soil conditions on the environmental fate of glyphosate in the presence of soil phosphate requires additional research attention.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the sorption and degradation behavior of glyphosate in three different US soils under consideration of aerobic and anaerobic conditions and the addition of phosphates. The sorption experiment is well described stating that USEPA guidelines was followed. However, design, conduct and results are missing details in reporting (ads/des results at each concentration not available numerically) to allow for a check of validity.
A degradation test was conducted – being non-standard compared to Guideline OECD 307 - in a microcosm while again lacking of details in description of results to allow for the calculation of degradation or dissipation rates according to current EU guidance.
The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/014
Report author	Rampoldi, E. *et al.*
Report year	2014
Report title	Carbon-14-Glyphosate Behavior in Relationship to Pedoclimatic Conditions and Crop Sequence
Document No	DOI 10.3844/ajessp.2014.94.101 E-ISSN 1558-3910
Guidelines followed in study	None
Deviations from current test guideline	Not applicable

GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The recognition of glyphosate [(*N*-phosphonomethyl) glycine] behavioral patterns can be readily examined using a pedoclimatic gradient. In the present study, glyphosate adsorption–desorption and degradation were examined under different scenarios in relationship to soil properties and soil use applications. Three sites with varied pedoclimatic conditions and two crop sequences were selected. Adsorption–desorption and glyphosate distribution in mineralized, extractable, and non-extractable fractions were assessed under laboratory conditions. Glyphosate sorption was characterized by isotherms and glyphosate degradation using the distribution of ^{14}C-glyphosate radioactivity among mineralized fractions, two extractable fractions (in water, ER1; in NH_4OH, ER2), and non-extractable fractions. Results showed sorption indices (distribution coefficient K_d and Freundlich sorption coefficient K_f: 13.4 ± 0.3–64.1 ± 0.9 L/kg and 16.2–60.6, respectively), and hysteresis increased among soil sites associated with decreasing soil particle size <2 µm, soil organic matter, and other soil properties associated with soil granulometry. A multiple stepwise regression analysis was applied to estimate the relationship between K_d values and soil properties. Cation exchange capacity, water field capacity, and Bray-1 P were the soil properties retained in the equation. Soils under continuous soybean [*Glycine max* (L.) Merr.] (monoculture) treatment exhibited reduced glyphosate adsorption and decreased hysteresis desorption relative to soils under rotation. To our knowledge, these results are the first to demonstrate that soils with identical properties exhibited different glyphosate retention capacities based on crop sequence. We propose possible explanations for this observation. Our results suggested that characterization of the variability in soil property gradients can serve to determine glyphosate behavioral patterns, which can establish a criterion for use in reducing potential environmental risks.

Materials and Methods

Soils

The province of Cordoba, Argentina, is characterized by broken relief to the west and plains in the central and eastern parts. The dominant parent materials are sediments transported by wind, called *loess*, from the mountain range of Los Andes. Three sites were selected: Pampa de Pocho (PP), Manfredi (M), and Marcos Juarez (MJ). At each site, two crop sequences were investigated, a monoculture of soybean with four glyphosate applications of 6 L/ha (2880 g a.i./ha) during the year and a soybean–maize rotation with only one glyphosate application of 2 L/ha (960 g a.i./ha). The soil was sampled at 0-5 cm. All samples were characterized by particle size determined by sedimentation, water-holding capacity (WHC) by membrane pressure plate, and the permanent wilting point (PWP) by ceramic pressure plate. Soil pH in water (soil/water, 1:1), and total organic C content (TOC) by wet combustion, extractable P by Bray 1, cation-exchange capacity (CEC) by NH_4OAc saturation, exchangeable Ca^{2+} and Mg^{2+} by complexometric titration with ethylenediaminetetraacetic acid, and exchangeable Na^+ and K^+ by flame photometer.

Table 7.1.2.1.1-96: Main characteristics of the three soils under two cropping sequences, a soybean monoculture and a soybean-maize rotation

Property	Marcos Juárez		Manfredi		Pampa de Pocho	
Altitude, m asl	110		292		1026	
Annual avg. temperature, °C	17.9		16.8		16.6	
Mean annual precipitation, mm	931		787		523	
Soil type	Typic Argiudoll		Typic Haplustoll		Entic Haplustoll	
Main textural class†	clay loam		loam		sandy loam	
	Monoculture	Rotation	Monoculture	Rotation	Monoculture	Rotation
pH	5.2 ± 0.1	5.5 ± 0.2	6.1 ± 0.1	6.3 ± 0.1	6.7 ± 0.1	6.2 ± 0.1
Water holding capacity, g kg^{-1}	300	280	220	250	80	150
Permanent wilting point, g kg^{-1}	130	130	100	100	30	70
Clay, g kg^{-1}	278 ± 2	242 ± 18	200 ± 4	216 ± 18	145 ± 15	170 ± 8
Silt, g kg^{-1}	580 ± 9	602 ± 15	558 ± 20	520 ± 28	88 ± 11	268 ± 21
Sand, g kg^{-1}	142 ± 7	156 ± 3	242 ± 5	264 ± 8	766 ± 22	562 ± 15
Total organic C, g kg^{-1}	17.2 ± 0.9	17.0 ± 0.1	14.3 ± 0.27	15.8 ± 0.5	7.3 ± 0.9	9.9 ± 0.3
Bray-1 P, mg kg^{-1}	58.0 ± 1.0	57.0 ± 1.0	53.0 ± 1.0	53.0 ± 1.0	12.0 ± 0.3	45.0 ± 1.0
Cation exchange capacity, cmol kg^{-1}	19.6 ± 0.1	18.8 ± 0.2	18.9 ± 0.1	19.0 ± 0.1	15.0 ± 0.1	9.0 ± 0.1
Ca, cmol kg^{-1}	11.8 ± 0.1	10.9 ± 0.1	12.1 ± 0.1	12.4 ± 0.1	9.4 ± 0.1	6.3 ± 0.1
Mg, cmol kg^{-1}	3.2 ± 0.1	3.4 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.0 ± 0.1	1.5 ± 0.1
K, cmol kg^{-1}	2.3 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.2 ± 0.1	0.7 ± 0.1
Na, cmol kg^{-1}	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

Soil size fractionation was done by dispersion of soils in water. The fractions 2000 to 200, 200 to 50, and <50 µm were recovered from the dispersed suspension by sieving and dried at 50 °C. The soil weight and organic C concentration in each fraction were quantified.

Carbon-14-Glyphosate Retention

A solution of [methyl-^{14}C]glyphosate was purchased from Sigma Chemical Co. (81 MBq/mmol, 99.2 % radiopurity) and was prepared in Milli-Q water by isotopic dilution with unlabeled glyphosate (>99 % purity) at six different concentrations (0.2, 0.5, 1, 2, 5, and 10 mg/L). Each solution contained 0.166 MBq/L. Two-gram subsamples of air-dried soil were placed in 25-mL Corex glass centrifuge tubes and 10 mL of ^{14}C-glyphosate solution at one of the six different concentrations was added. Blanks without soil were included and each soil and glyphosate concentration combination was prepared in triplicate. The tubes were shaken by rotation for 24 h at 20 ± 2°C in the darkness. After shaking, the tubes were centrifuged for 15 min at 1800 x g and supernatant removed. The ^{14}C-glyphosate concentrations in the supernatant solution were calculated with a Packard Tri-Carb 2100 TR liquid scintillation counter (Packard Instruments) from the supernatant radioactivity measurements. The amount of sorbed glyphosate per mass of soil was calculated from the difference in herbicide concentration before and after sorption. Desorption of ^{14}C-glyphosate was studied in all samples initially treated with 10 mg glyphosate/L during the adsorption study. After sorption equilibration, most of the supernatant was removed and replaced by an equivalent volume of Milli-Q water. The tubes were vortexed to disperse the soil pellets, and the suspensions were mechanically shaken for 24 h at 20 ± 2°C.The suspensions were then centrifuged for 15 min at 1899 x g, and the supernatant was again replaced with Milli-Q water. Five successive desorption treatments were done for each sample. The supernatant radioactivity was determined after each desorption to quantify the amount of desorbed herbicide.

Table 7.1.2.1.1-97: Freundlich sorption-desorption isotherm parameters (adsorption $K_{f,ads}$ and $n_{ads'}$, desorption $K_{f,des}$ and $n_{des'}$ and hysteretic index H) and distribution coefficients (K_d) of glyphosate in three soils under two cropping sequences

Location	Cropping sequence	Sorption					Desorption			
		$K_{f,ads}$	n_{ads}	R^2	K_d	R^2	$K_{f,des}$	n_{des}	R^2	H
		L kg^{-1}								
Marcos Juárez	monoculture	48.8 ± 0.3	0.91 ± 0.01	0.99	50.1 ± 0.5	0.98	44.9 ± 0.6	0.17 ± 0.01	0.98	0.19 ± 0.02
	rotation	60.6 ± 0.8	0.90 ± 0.02	0.99	64.1 ± 0.9	0.95	45.2 ± 0.5	0.11 ± 0.01	0.93	0.12 ± 0.03
Manfredi	monoculture	29.3 ± 0.1	0.84 ± 0.01	0.99	28.4 ± 0.5	0.97	36.3 ± 0.3	0.23 ± 0.01	0.85	0.27 ± 0.02
	rotation	42.6 ± 0.4	0.86 ± 0.02	0.99	43.7 ± 0.7	0.88	43.1 ± 0.5	0.16 ± 0.01	0.85	0.19 ± 0.03
Pampa de Pocho	monoculture	16.2 ± 0.2	0.77 ± 0.01	0.99	13.4 ± 0.3	0.96	24.7 ± 0.3	0.39 ± 0.01	0.90	0.39 ± 0.03
	rotation	20.3 ± 0.1	0.85 ± 0.01	0.99	18.7 ± 0.3	0.98	26.5 ± 0.3	0.16 ± 0.01	0.87	0.19 ± 0.02

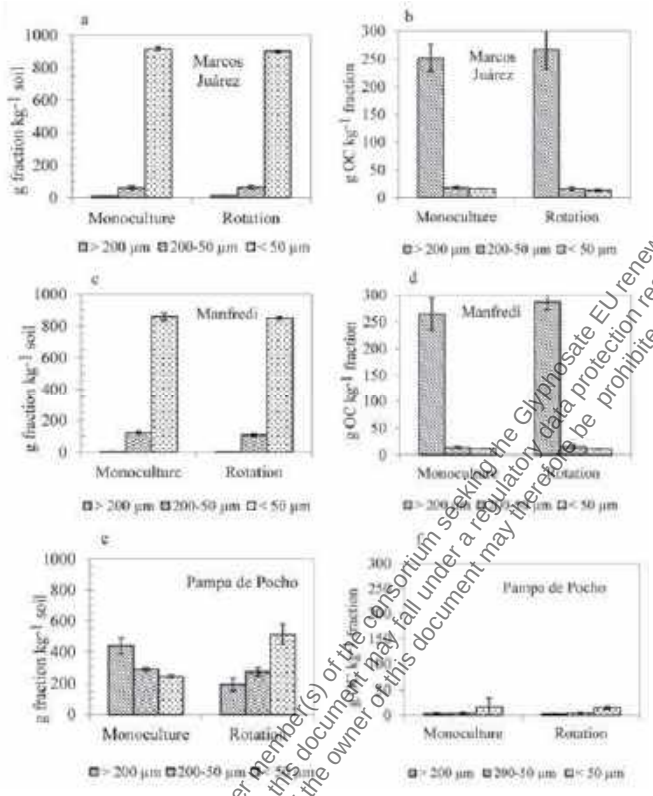
Carbon-14-Glyphosate Behavior

The mineralization of ^{14}C-glyphosate was followed during laboratory incubations (in triplicate) of 49 d at 28 ± 1°C in the dark. One milliliter of the ^{14}C-glyphosate solution was added to 10 g of each soil. The soil water content was adjusted to 85 % of WHC of each soil with Milli-Q water, taking into account the glyphosate solution. The ^{14}C-CO_2 evolved during the incubation was trapped in NaOH. The vials containing NaOH were sampled and replaced after 3, 7, 14, 21, 28, 35, 42, and 49 d. The total radioactivity content was measured by liquid scintillation counting using a Tri-Carb 2100 TR counter and with external standardization and Ultima Gold XR as a scintillation cocktail.

Extractable and Non-extractable Residues

At the 49th d of the incubation period, four sequential extractions were done for the corresponding soil samples. The extractable fraction of ^{14}C-glyphosate was obtained in two steps. The first extraction was done using 50 mL of Milli-Q water during 24 h, the supernatant was recovered, and the radioactivity was measured by scintillation counting (ER1). After that, three successive extractions were performed, each 24, 24, and 4 h, respectively, with 50 mL of 0.5 mol/L NH_4 OH in glass centrifuge tubes. The three successive extracts were pooled for each soil sample and the radioactivity was measured by scintillation counting (ER2). Radioactivity in the solid soil samples containing non-extractable ^{14}C-glyphosate residues (NER) were recovered and dried at 40°C. The radioactivity was measured on three subsamples (100–200 mg) by scintillation counting after combustion at 800°C under O_2 flow in a sampler oxidizer (Packard) followed by ^{14}C-CO_2 trapping in 8 mL of Carbosorb E (Packard) mixed with 12 mL of Permafluor E+ (Packard).

Figure 7.1.2.1.1-18: Distribution of soil mass and organic C (OC) content in three soil size fractions (2000-200, 200-50, and <50 µm) in three soils and two cropping sequences



Mathematical Adjustment and Statistical Analysis

Sorption Isotherms

The amounts of ^{14}C-glyphosate adsorbed on the soil (x/m, mg glyphosate/kg solid) were calculated as the difference between the initial ^{14}C-glyphosate concentration and the supernatant concentration (C, mg glyphosate/L supernatant solution). Glyphosate sorption isotherms were described by the Freundlich model and the linear model.

Kinetics of Degradation

Cumulative ^{14}C-CO_2 glyphosate and C-CO_2 evolved were adjusted to a first-order model:

Statistical Analysis

An ANOVA procedure was performed using the soil type (location) as the main factor, with six replicates per soil. Fisher's test of comparison of means was used. Multiple regression analysis was also performed between glyphosate K_d values and soil properties: sand, clay, silt, pH, TOC, CEC, Ca^{2+}, Mg^{2+}, Na^+, K^+, WHC, PWP, Bray-1 P, and three organic C fractions (>200, 50–200, and <50 µm). The criteria for the selection of the variables were $p < 0.05$. For each crop sequence (monoculture or rotation), a simple ANOVA by each soil with three replicates was used. The statistics software used was Infostat (Di Rienzo [redacted] 2009).

Table 7.1.2.1.1-98: Stepwise linear regression of glyphosate sorption index $K_{d'}$

Variable	Coefficient	SE	P	R^2	Adjusted R^2
	−48.04	13.22	0.0027		
Water field capacity	130.68	29.6	0.0006		
Bray-1 P	−0.54	0.14	0.0018		
Cation exchange capacity	3.53	1.4	0.0243		
				0.97	0.96

Results and Discussion

Soil Characterization

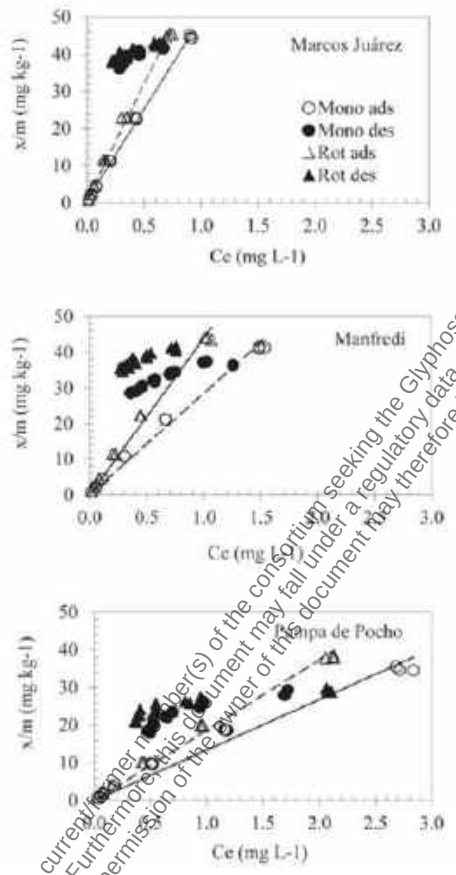
Edaphoclimatic characteristics from each of the three scenarios studied are shown in Table 7.1.2.1.1-96. The PP soils positioned at the northwestern sampling site exhibited a sandy texture, the highest pH and the lowest TOC, CEC, PWP, and WHC. At the extreme southeastern sampling site, the MJ soils showed the lowest pH and the highest TOC, CEC, PWP, WHC, and clay and silt contents. The M soils located in the geographically intermediate sampling site also exhibited intermediate edaphic properties in relationship to the other two sampling sites.

The soil size distribution among fractions ranged from 2000 to 200, 200 to 50, and <50 µm (Figure 7.1.2.1.1-18 a, c, e) and showed granulometric differences among the three sample sites. The M and MJ sites primarily differed in the fraction proportion in micrometers (i.e., 200-50), corresponding to the categories of fine sand and very fine sand. The PP soils differed from the other two sampling sites in the proportion and distribution of the three soil size categories evaluated. Rotation and monoculture treatments revealed identical soil size distributions for M and MJ. However, the two cropping sequence treatments from the PP site were not congruent with M and MJ, and significant differences in soil particle size distribution were observed ($P < 0.05$). Results showed that the coarsest soil size fraction (2000 – 200 µm) containing fresh soil organic matter (SOM) represented the largest organic C concentration in MJ and M soils and both treatments (monoculture and rotation) Figure 7.1.2.1.1-18 b and d). Nevertheless, the highest TOC proportion corresponded to humified organic matter associated with a soil size fraction <50 µm, i.e., the highest proportion of this fraction was present in these soils (between 75 and 85 %). Carbon enrichment in some of the three soil size fractions, which was associated with soil texture and granulometry, was not found in the PP soils (Figure 7.1.2.1.1-18 f).

Carbon-14-Glyphosate Sorption-Desorption

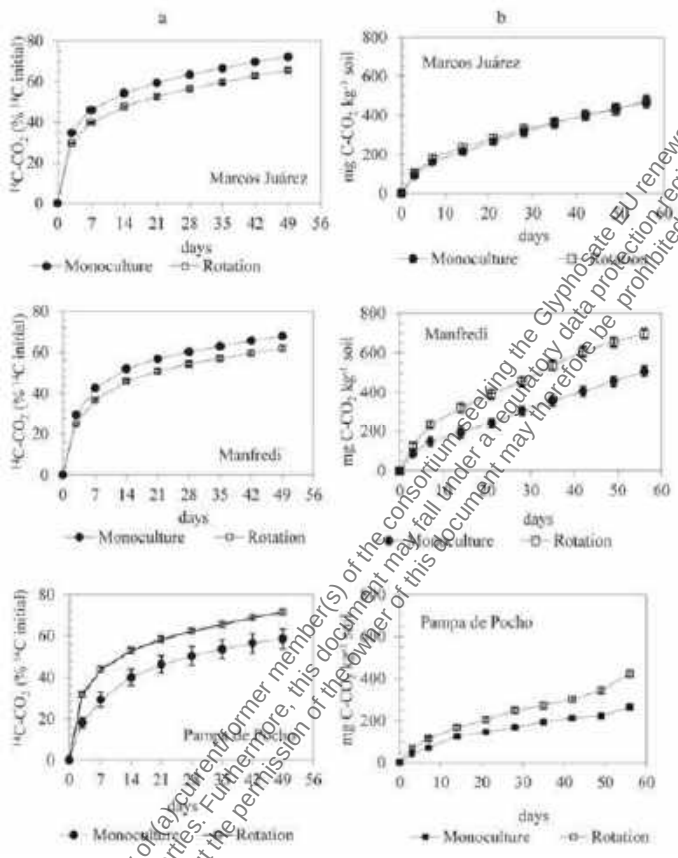
Carbon-14-glyphosate sorption-desorption isotherms obtained from the three scenarios evaluated are shown in Figure 7.1.2.1.1-19. The experimental data were fitted with two mathematical models: the Freundlich adsorption isotherm and the linear model. Empirical evidence indicated that as the value of n_{ads} decreased, the linear approximation became less satisfactory, especially at high and low concentrations, and discrepancies between K_f and K_d values occurred. Our results showed that the equilibrium concentration range was 0.1 to 1.0 mg/L (MJ soils), 0.1 to 1.5 mg/L (M soils), and 0.1 to 3 mg/L (PP soils), and $K_{f,ads}/K_d$ ranged between 0.9 and 1.1.

Figure 7.1.2.1.1-19: Glyphosate adsorption (open symbols) and desorption (filled symbols) isotherms in three soils and two cropping sequences: monoculture (circles) and rotation (triangels)



The three scenarios examined clearly differed in glyphosate adsorption. The $K_{f,ads}$ and K_d indices showed a threefold increase from PP soils (northwest position) to MJ soils (southeast position). Our results from the three sampling sites showed a geographic gradation in soil characteristics that were associated with glyphosate adsorption, i.e., we detected a relation among geographic position, edaphic characteristics, and glyphosate adsorption capacity. That behavioral pattern was confirmed by adsorption studies. The PP site collective soil characteristics were associated with low glyphosate adsorption capacity, i.e., high pH, low clay content and SOM, while the MJ site exhibited inverse soil attributes and an overall high adsorption capacity. A multiple linear regression analysis was performed to estimate the relationship between glyphosate K_d values and soil properties (Table 7.1.2.1.1-98). The PWP, CEC, and Bray-1 P were the regressed variables retained in the analysis, which explained 93 % (R^2 = 0.97) of the total variation. Desorption isotherms were fitted for the Freundlich model. An irreversible desorption process was shown by the lack of overlap in the sorption - desorption isotherms (Figure 7.1.2.1.1-19). Glyphosate desorption indices were higher than corresponding adsorption indices. Our study revealed that glyphosate desorption hysteresis increased from the northwest toward the southeast sampling sites. The *H* indices, which ranged from 0.12 to 0.39, were used to compare the hysteresis degree among soils. Between 15 and 57 % of the glyphosate initially applied was desorbed. The PP soils exhibited the highest ^{14}C-glyphosate desorption, with >50 % recovered in the first desorption step. On the contrary, for the M and MJ soils in the first desorption step, only about 30 % of the total ^{14}C-glyphosate was desorbed. Crop sequence effects were evaluated only for the M and MJ soils. These two sample sites had similar soil characteristics, while the PP site differed and was therefore excluded. The extent of glyphosate adsorption and desorption hysteresis was higher in rotation than monoculture soils (Figure 7.1.2.1.1-20; Table 7.1.2.1.1-97). To our knowledge, our results are the first to document that soils with identical properties exhibited different glyphosate retention capacities due to cropping sequence.

Figure 7.1.2.1.1-20: Kinetics of (a) ^{14}C-glyphosate mineralisation and (b) total organic carbon mineralisation interpreted as indicator of total microbial activity during laboratory incubations of three soils and the two cropping sequences monoculture and rotation; standard deviations (error bars) are shown when larger than the symbol size



Carbon-14-Glyphosate Mineralization

Carbon-14-glyphosate mineralization kinetics together with C-CO_2 evolution are shown in Fig. 3. In addition, cumulative glyphosate mineralization and oxidizable C after 49 d of incubation are shown in Table 4. At the end of the incubation period, the ^{14}C-CO_2 released ranged from 61.7 to 72.8 % of the ^{14}C applied, and the time needed to reduce the ^{14}C initially applied to 50 % was 5.0 ± 0.7 d (calculated from ln2/*k*).

Decreased ^{14}C-glyphosate mineralization detected in the MJ and M sites relative to the PP site might be associated with increased glyphosate adsorption in the MJ and M soils. We found that the TMA (total microbial activity) was significantly different ($P < 0.01$) among sampling site soils: M > PP > MJ. The M soils, with the highest TMA, did not have the highest ^{14}C-glyphosate mineralization. Glyphosate mineralization was affected by cropping sequence. At the end of incubation, the ^{14}C-CO_2 evolved was monoculture MJ = 69 % vs. rotation MJ = 63.6 % and monoculture Mm = 68 % vs. rotation M = 61.7 % ($P \leq 0.05$). These results provide additional support to our interpretations regarding glyphosate mineralization differences detected among study sites, given that monoculture soils showed reduced glyphosate adsorption and a history of glyphosate use.

Table 7.1.2.1.1-99: Carbon-^{14}C-glyphosate mineralised, C-CO_2 expressed as a percentage of the total organic C, and setting parameters for three soils and two cropping sequences using the equation $C_t=C_0[1-\exp(-kt)]$, where C_t is the percentage of ^{14}C-CO_2 or C-CO_2 mineralized at time t, C_0 is the percentage of C potentially mineralizable, k is the daily mineralization rate, and t is time in days

Soils	Cropping sequence	Mineralization of glyphosate			Mineralization of soil organic C		
		^{14}C-CO_2	C_0	k	C-CO_2	C_0	k
		%		d^{-1}	%		d^{-1}
Marcos Juarez	monoculture	69.1 ± 0.3	62.8 ± 1.3	0.178 ± 0.018	2.8 ± 0.03	3.0 ± 0.15	0.044 ± 0.003
	rotation	63.8 ± 0.2	57.7 ± 1.2	0.170 ± 0.017	2.4 ± 0.02	2.5 ± 0.09	0.053 ± 0.005
Manfredi	monoculture	68.3 ± 0.3	63.1 ± 1.0	0.163 ± 0.012	3.3 ± 0.03	3.1 ± 0.12	0.031 ± 0.004
	rotation	61.6 ± 0.1	57.4 ± 1.0	0.141 ± 0.011	4.3 ± 0.02	4.3 ± 0.21	0.044 ± 0.004
Pampa de Pocho	monoculture	65.3 ± 0.5	60.9 ± 1.2	0.117 ± 0.009	3.2 ± 0.03	3.0 ± 0.11	0.063 ± 0.006
	rotation	74.3 ± 2.8	66.9 ± 1.5	0.159 ± 0.017	3.2 ± 0.03	2.3 ± 0.11	0.054 ± 0.005

Carbon-14-Glyphosate Distribution among Mineralized, Extractable, and Nonextractable Residues

The three study sites differed in the distribution of initial radioactivity applied and in the proportion remaining in ER and NER forms. The lower proportion of ER2 and NER in the PP soils corresponded with soil properties involving low sorbent surfaces. Sequential extraction of ER and NER was conducted following 49 d of incubation; consequently equilibrium between soluble and sorbed forms of glyphosate should have occurred. The PP soil contained 19 % ER (ER1 + ER2), which clearly contrasted with 30 % ER obtained from the M and MJ soils. The ER1 fraction, extracted with water, represented the weakly adsorbed herbicide and on average was <5 % of the total ER for the three sample sites. The ER1 from the PP soils was slightly higher than that from the other two sample sites (MJ and M), indicating weak glyphosate adsorption properties and high sorption reversibility. Nonextractable residues constituted a small fraction (4-6 %) of the ^{14}C-glyphosate initially applied. Small differences among soils were observed, such as decreasing order of NER proportions: M > MJ > PP ($P < 0.05$). The M soils showed the highest TMA and NER proportion.

Conclusions

The study of glyphosate retention and degradation processes through a pedoclimatic gradient turned out to be a useful tool to recognize and establish some behavioral patterns. Identification of soil indicators that allow inference of glyphosate behavior is one of the goals in studies of sustainable soil use. We found that along a distance of approximately 280 km, gradual changes in glyphosate behavior were associated with pedoclimatic characteristics. Soil properties associated with soil surface reactivity, such as CEC, WHC, and PWP, increased in from northwest to southeast together with the increase in glyphosate adsorption and the increase in hysteresis of desorption. Changes in the glyphosate distribution between adsorbed and soluble forms establish, in part, a behavior pattern of extractable (ER) and mineralized forms. The extent of glyphosate adsorption and also the hysteresis of desorption were higher in rotation soils than monoculture soils; that is, soils with identical properties exhibited a different glyphosate retention capacity due to the cropping sequence. The results of this study contribute to our understanding of glyphosate behavioral patterns in relation to different edaphoclimatic scenarios and establish criteria for use in reducing potential environmental risks.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article investigates ^{14}C-glyphosate adsorption–desorption and degradation under different scenarios in relationship to soil properties and soil use applications. Three Argentinian sites/soils with varied pedoclimatic conditions and two crop sequences were selected. Sorption parameters and degradation in terms of mineralization are reported. Essential details to assess the quality of data, for example, in terms of the EU Evaluators Checklist, are not available, described, and there are some deviations from current guidelines. In addition, the pedo-climatic conditions do not correspond to EU conditions.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/015
Report author	Al-Rajab, A. *et al.*
Report year	2014
Report title	Behavior of the non-selective herbicide glyphosate in agricultural soil
Document No	DOI 10.3844/ajessp.2014.94.101 E-ISSN 1558-3910
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Glyphosate [*N*-phosphonomethyl]glycine is a systematic, non-selective, organophosphorus herbicide used worldwide in agriculture and industrial zones. In this study, the authors followed the degradation, stabilisation, remobilisation and leaching of ^{14}C-glyphosate in three agricultural soils in laboratory incubations and in lysimeters under field conditions. Glyphosate degradation was relatively rapid with a half-life of 14.5 days in the silt clay loam soil incubated at 20°C. Glyphosate's degradation product, aminomethylphosphonic acid (AMPA), represented more than 85 % of residues after 80 days of laboratory incubation. Leaching of glyphosate in lysimeters of three different investigated soils under outdoor conditions was very slow, less than 1 % of the initial applied amount has been detected in the leachates after 100 days of experimentation. Glyphosate rapidly formed non-extractable residues after treatment. In summary, glyphosate was removed from soil very rapidly and its leaching seems to be very slow regardless the type of treated soil. On the other hand, the contamination risk of groundwater with its metabolite AMPA at long term is probably due to the release of the non-extractable residues.

Materials & Methods

Chemicals

[Phosphonomethyl-^{14}C]-glyphosate diluted, purity 99 % was purchased from ARC-ISOBIO (Belgium). Glyphosate [*N*-phosphonomethyl]glycine, purity 99 % was purchased from Cluzeau (CIL, Paris). Aminomethylphosphonic Acid (AMPA), 10 ng µ/L in water, was purchased from Dr. Ehrenstorfer GmbH (Germany). Sarcosine [*N*-methylglycine], purity 99 % was purchased from Fluka (Germany). H_2PO_4, FMOC-chloride, Potassium hydroxide and Sodium tetraborate decahydrate were purchased from Fluka (Germany). Methanol and acetonitrile (HPLC grade) were purchased from SDS (France).

Sampling

Soils used in this study were obtained from three different agricultural lands in Lorraine region (France). Therefore, based on information provided by the landowners, these soils were never exposed to direct agricultural application of glyphosate and their properties were as following: Sandy loam soil (Sand:Silt:Clay (59:30:11), pH 5.1; % organic matter 0.82); silt clay loam soil (Sand:Silt:Clay (16:53:31), pH 6.3; % organic matter 1.45); and clay loam soil (Sand:Silt:Clay (35:30:35), pH 7.9; % organic matter 1.91).

In the laboratory studies, soils were air dried then sieved at 2 mm and stored in fridge at 4 °C until treatment. Otherwise, in the outdoor leaching study, lysimeters were prepared in site using an undisturbed soil for each type of soil separately, a total of 7 columns of each soil were used in this study. Laboratory lysimeters were polyvinyl chloride pipes of 10 cm wide and 35 cm long. Therefore, the 21 lysimeters of the three selected soils were placed in the experimental field of ENSAIA (54500 Vandoeuvre-lès-Nancy, France) for 100 days.

Extraction of Glyphosate

The efficacy of different solvents for extraction of glyphosate from soil was evaluated as follows. A 5 g portion of each soil (in triplicate) was treated with a 0.5 mL solution of H_2O (concentration of 19.4 Bq/g) of [Phosphonomethyl-^{14}C]-glyphosate and 0.1 µg/g of unlabelled glyphosate. Treated soil was placed into a 250 mL PPCO (Nalgene®, VWR, USA) centrifuge bottle and 25 mL of selected solvent were added. Five different solvents were tested separately for the glyphosate extraction efficacy: Ammonium oxalate monohydrate 0.1 M; potassium dihydrogen phosphate (KH_2PO_4) 0.1 M; a mixture of (NH_4OH 0.5 M + KH_2PO_4 0.1 M + H_3PO_4 0.5 %); $CaCl_2$ 0.1 M and distilled water. Bottles were rotary shaken for 2 h, then centrifuged at 5000 g for 20 min, the supernatant of each sample was recovered. Extraction of each sample has been repeated twice, the supernatants of the same sample were combined and a portion of 1 mL counted by Liquid Scintillation Counter (LSC). Thereafter, extraction of glyphosate from soil samples was effectuated with (KH_2PO_4) 0.1 M.

Laboratory Degradation Study

About 25-g soil samples were placed in glass jars (60 mm diameter, 40 mm high). Samples of silt clay loam soil were prepared in triplicates for each sampling time. Each sample was amended by 0.51 mg of glyphosate and 45.1 kBq in water. Final soil moisture was 80 % of the soil retention capacity. After treatment, each sample was added to a Mason jars (1.5 L). At the same time, a plastic vial of 10 mL H_2O was added to each jar in order to maintain the humidity of soil (Al-Rajab *et al.*, 2009). Another plastic scintillation vial with 10 mL of 0.5 N NaOH was placed into each jar for trapping $^{14}CO_2$. Jars were incubated at 20°C in the dark for 80 days. The radioactivity trapped in NaOH was counted at each sampling time using a Liquid Scintillation Counter LSC Packard Tri-Carb 1900 CA (Packard, USA). 1 mL of NaOH was added to 10 mL of scintillation cocktail in a plastic scintillation vial to measure the radioactivity in the LSC during 10 min. At each sampling date, the 25-g soil samples were extracted separately using KH_2PO_4 as described previously. Then, after the 3rd and last extraction, soil samples were air-dried at the lab ambient temperature for 3 days. The remaining ^{14}C-radioactivity in the samples after extraction was referred as (non-extractable residues) which was determined by combustion at 900°C using a 307 Packard Oxidiser (Packard, USA).

Leaching Study

Laboratory lysimeters were prepared and placed in the experimental field of Lorraine University (France) 3 months before the treatment. During the experimentation of 100 days, the average temperature was 10 °C; total precipitation was 235 mm; in total 8 leachates samples were collected. Leached radioactivity from each lysimeter was determined directly after collection. Therefore, water samples were stored at -18 °C until analysis.

Analytical Methods

^{14}C-Radioactivity has been determined using a Liquid Scintillation Counter LSC. Glyphosate residues were determined using a Varian HPLC (USA) equipped with two detectors: A fluorescence detector and a β-radioactivity detector. A Lichrosorb (NH_2) column (4×250 mm, 5 µm) purchased from (CIL-Cluzeau, France) was used and thermostated at 30°C. Fluorescence detector was set at (λ 260 and 310 nm), while the flow rate of 1.2 mL/min was adopted in the β-radioactivity detector with a counting cell of 500 µL. The mobile phase was a mixture of (KH_2PO_4 0.05 mol^{-1}, pH 5.7)/acetonitrile (70/30: V/V) at flow rate of 0.8 mL/min. The injected volume was 50 µL. Within these conditions, the retention times were 4.2, 6.6 and 13.3 for sarcosine, AMPA and glyphosate respectively. Determination of the non-extractable residues in soil has been effectuated by combustion of 0.5 g portions at 900°C using an oxidizer (Packard, USA). Statistical analyses were conducted using Stat Box (Version 6.4, Grimmer Software, France).

Results

Extraction of Glyphosate

Extraction recovery of glyphosate varied from 4 to 74 % of the initial applied amount (Table 7.1.2.1.1-100). $CaCl_2$ (0.1 M) and water were the less effective solvents in glyphosate extraction in the three investigated soils. However, ammonium oxalate (0.1 M) was the most efficient solvent with a recovery rate ranged from 60 to 74 %. The only issue with the extraction with ammonium oxalate was that the extracts were very dark and need an intensive clean up. On the other hand, potassium dihydrogen phosphate (KH_2PO_4 0.1 M) was adopted as a suitable solvent since the extracts were clear and it showed an acceptable recovery rate varied from 45 to 49 % in investigated soils (Table 7.1.2.1.1-100). Recovery rate with citric acid (20 %) was not high enough (less than 37 %) for the three investigated soils.

Dissipation of Glyphosate

Results showed an immediate and high degradation rate of glyphosate after its application on the soil (Figure 7.1.2.1.1-21). Mineralization of glyphosate after 17 days of incubation reached 39.7 % of the initial amount applied. Thereafter, the mineralization of glyphosate declined gradually. The half-life of glyphosate derived from the mineralization rates was 31 days for silt clay loam soil. However, the extraction curves are opposite to those of the mineralization (Figure 7.1.2.1.1-22). The percentage of extracted residues from the silt clay loam soil at T0 was only 56.9±0.7 %. This availability to extraction decreased overtime, it reached 6.9 % of the initial amount for silt clay loam soil. HPLC analysis showed the appearance of two degradation products of glyphosate AMPA and sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co-eluted and unlabeled organic compounds. The half-life of glyphosate extractable was 14.5.

Leaching of Glyphosate

Our study showed that the residues of glyphosate were detected in the first leachates samples of three soils, the cumulated precipitation was 85 mm. In the case of silt clay loam soil, the maximum residues concentration of 9.5±7 µg L-1 has been reached after 2 months of application. Concentration of leached residues decreased dramatically after 2 months until the end of experiment (Figure 7.1.2.1.1-23).

Table 7.1.2.1.1-100: Extraction efficiency of glyphosate from the selected soils using different solvents

Extraction efficiency (%: Mean±standard deviations; n = 3)			
Solvent	Sandy loam	Silt clay loam	Clay loam
KH_2PO_4 (0.1M)	48.9 (±0.3)	48.8 (±0.7)	48 (±0.5)
Ammonium oxalate (0.1 M)	59.9 (±0.7)	73.5 (±0.2)	61.1 (±0.1)
Citric acid (20%)	34.2 (±0.1)	36.4 (±0.2)	28.9 (±0.2)
$CaCl_2$ (0.1 M)	5.7 (±0.5)	3.6 (±0.9)	10.3 (±0.6)
H_2O	14.3 (±0.2)	23.5 (±0.1)	31.7 (±0.1)

Figure 7.1.2.1.1-21: Residues evolution of glyphosate and AMPA in the extractable residues in silt clay loam soil during incubation at 20 °C

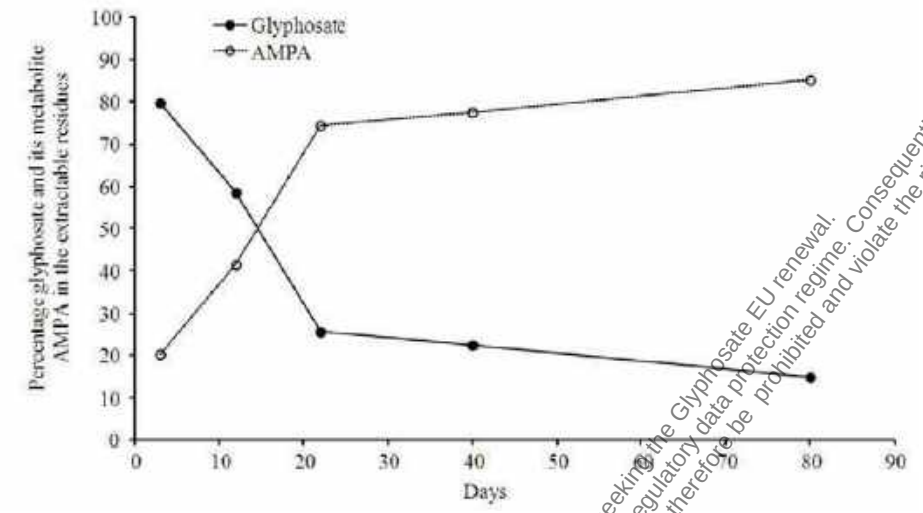


Figure 7.1.2.1.1-22: Evolution of different portions of ^{14}C-glyphosate residues (extractable, mineralization as $^{14}CO_2$ and Non-extractable) in silt clay loam soil during incubation at 20 °C

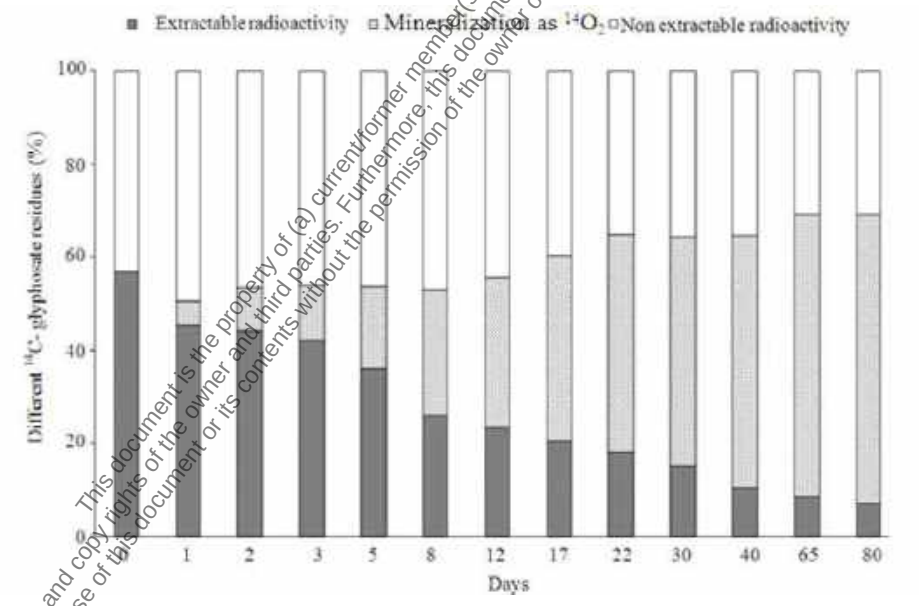
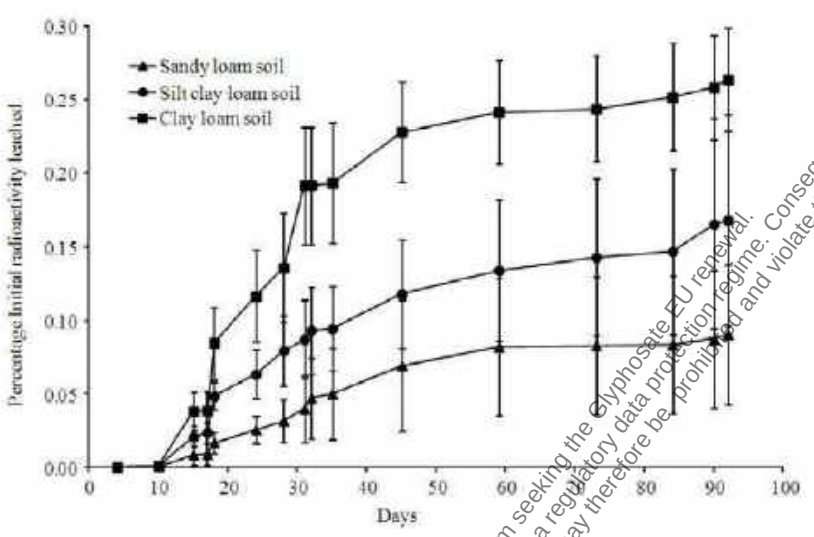


Figure 7.1.2.1.1-23: Radioactivity leached from lysimeters of the investigated soils treated with ^{14}C-glyphosate under outdoor conditions



Discussion

Extraction of Glyphosate

Extraction and determination of glyphosate in agricultural soil is problematic due to its high solubility and its physic-chemical properties (Botero-Coy *et al.*, 2013). In the present study, extraction recovery of glyphosate varied from 4 to 74 % of the initial applied amount (Table 7.1.2.1.1-100). $CaCl_2$ (0.1 M) and water were the less effective solvents in glyphosate extraction in the three investigated soils. However, ammonium oxalate (0.1 M) was the most efficient solvent with a recovery rate ranged from 60 to 74 %. The only issue with the extraction with ammonium oxalate was that the extracts were very dark and need an intensive clean up.

On the other hand, potassium dihydrogen phosphate (KH_2PO_4 0.1 M) was adopted as a suitable solvent since the extracts were clear and it showed an acceptable recovery rate varied from 45 to 49 % in investigated soils (Table 7.1.2.1.1-100), this rate was similar to that one reported by other studies (Cheah and Lum, 1998; Landry *et al.*, 2005). Recovery rate with citric acid (20 %) was not high enough (less than 37 %) for the three investigated soils. Non-extractable residues of glyphosate in soil increase with the time; consequently, glyphosate will be less available for extraction or degradation.

Dissipation of Glyphosate

Monitoring of mineralization of glyphosate labelled on the phosphonomethyl group allows assessing both the loss of glyphosate and AMPA. We observed an immediate and high degradation rate of glyphosate after its application on the soil (Figure 7.1.2.1.1-21). The absence of lag phase indicates that the microflora of soil already had an enzymatic system capable of degrading glyphosate and as such did not need an adaptation period. Mineralization of glyphosate after 17 days of incubation reached 39.7 % of the initial amount applied. Thereafter, the mineralization of glyphosate declined gradually. The fast mineralization of glyphosate in the soil appears due to its bioavailability. The half-life of glyphosate derived from the mineralization rates was 31 days for silt clay loam soil. On the other hand, the effect of organic matter content in the soil on mineralization of glyphosate was not clear under the conditions of this study. The extraction rate of glyphosate is an indication of the accessibility of the residues for microbial degradation and/or their transfer to groundwater under natural conditions. The extraction curves are opposite to those of the mineralization (Figure 7.1.2.1.1-22). The percentage of extracted residues from the silt clay loam soil at T0 was only 56.9±0.7 %. We can assume that the treatment in a dry soil may cause an entry of glyphosate into the microporisity of aggregates during the capillary invasion by the aqueous solution of treatment (Guimont *et al.*, 2005; Al-Rajab *et al.*, 2010b). The size of this compartment would be defined at the time of treatment and may depend on the physicochemical and physical properties and the moisture rate of soil

at the application moment. This availability to extraction decreased overtime, it reached 6.9 % of the initial amount for silt clay loam soil. The evolution of extraction rate with KH_2PO_4 over time in the soil is related to the mineralization of residues and the availability of non-extractable residues for mineralization or extraction. A similar behaviour of extractable residues of glyphosate over time was reported by (Getenga, 2004; Miles, 1998). HPLC analysis showed the appearance of two degradation products of glyphosate AMPA and sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co-eluted and unlabelled organic compounds.

The appearance of AMPA during the first days of incubation is due the fast mineralization of glyphosate in soil, reaching about 85.1 % of residues after 80 days of treatment (Figure 7.1.2.1.1-21). The half-life of glyphosate extractable was 14.5 days. The fraction of non-extractable residues represent the residues which cannot be extracted from the soil by the series of KH_2PO_4 extractions (exhaustive extraction) (Figure 7.1.2.1.1-22). The formation of the non-extractable residues NER in the silt clay loam soil reached 43 % of the initial applied amount at T0 and 49.4 % at T1. The rate stayed stable until T2 after which it decreased to 30.9 % by the end of experiment. The rate of non-extractable residues decreased over time unlike other pesticides such as atrazine where the rate of non-extractable residues increases gradually over dozens of days (Winkelmann, 1991). The rate of non-extractable residues is probably dependent on the properties and physical aspects of the soils including the size of the microporal compartment. This rapid formation of non-extractable residues immediately after treatment is very specific for glyphosate. The treatment of herbicide on a dry soil promotes the capillary invasion and the rapid transport of the solution of treatment in the microporisity intra aggregate, subsequently making the herbicide inaccessible for extraction (Guimont *et al.*, 2005). We also reported that the initiation of the degradation of glyphosate did not affect the evolution of extractable residues rate. The very slow decrease of non-extractable residues showed that these residues can return by diffusion and under the effect of a concentration gradient, to areas accessible to microorganisms to subsequently undergo mineralization.

Leaching of Glyphosate

This study showed that water circulation in the soil might has an important role in contamination of groundwater with glyphosate. The diminution of soil macroporosity on the surface layer (where most residues usually present) with the time slows the water infiltration and might encourage the desorption of glyphosate residues. The circulation of glyphosate residues in soil could be due to a preferential water flow regarding the presence of its residues in the 1st collected leachates (Figure 7.1.2.1.1-23). In disaccording with results reported by (Dousset *et al.*, 2004), our study showed that the residues of glyphosate were detected in the first leachates samples of three soils, the cumulated precipitation was 85 mm. Detection of glyphosate residues in the 1st leachates was due to the preferential flow (Laitinen *et al.*, 2006). In the case of silt clay loam soil, the maximum residues concentration of 9.5±7 µg/L has been reached after 2 months of application. However, (De Jonge and Jacobsen, 2000) have reported residues concentration of glyphosate much higher than what was obtained from the current study. Concentration of leached residues decreased dramatically after 2 months until the end of experiment (Figure 7.1.2.1.1-23). Our findings were in accord with results reported by (De Jonge and Jacobsen, 2000; Landry *et al.*, 2005) who detected the glyphosate residues in the soil leachates after 3 months of application. Overall, the total residues (extractable and non-extractable) of glyphosate in the soil should be considered to evaluate its persistence in the soil, not only the extractable residues.

Conclusion

The present study monitored the residue dynamics of glyphosate in agricultural soil in controlled and outdoor conditions. Results obtained for the fate study suggest that the water pollution with this herbicide is closely related to the adsorption and the formation of non-extractable residues, which are themselves dependent on soil texture and its moisture condition at the time of treatment. In case of rain following treatment, the risk of groundwater pollution by glyphosate will be low but may continue to be present for long time since the mineralization is slow. The silt clay loam soil could be less favourable for water pollution since it showed a formation of large amount of non-extractable residues. In the semi-field lysimeters study, leaching of ^{14}C-glyphosate was limited, but its metabolite AMPA seems to be the main potential pollutant of the groundwater. The water circulation mode in the soil was preferential flow which facilitate a fast leaching of residues to reach the groundwater.

In summary, these results suggest that the organophosphorus herbicide glyphosate is rapidly degradable in the agricultural soil. Leaching of glyphosate seems to be very slow regardless the type of the soil. Release of the non-extractable residues of glyphosate probably increases the risk of groundwater pollution with its metabolite AMPA at long term. More investigations are requested for a better understanding of the effect of soil content of organic carbon and soil microflora on environmental behavior of glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article investigates the degradation/dissipation and the potential for mobility of glyphosate and its metabolite AMPA in three French soils. The soil degradation tests perfomed with ^{14}C-labelled glyphosate cannot be assesses fully for their quality and deviations from current guideline due to a lack of detail in reporting. This includes, for example, that no detailed values per sampling interval are reported for all soils.
The semi-field leaching experiments were small-scale soil columns consisting of 35-cm with undisturbed soil with low diameter. It is a common observation that this design can cause preferential flow as some artifact thus having potential to result in false-positive findings in percolates of such type of 'lysimeter'. Being indicative in the best case, the results cannot be compared to those of 'full lysimeter studies' that are typically run for more than a year under outdoor conditons.
The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/016
Report author	Nghia, N.K. *et al.*
Report year	2013
Report title	Soil properties governing biodegradation of the herbicide glyphosate in agricultural soils
Document No	ISBN 978-602-96519-2-8
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary
The relationships between soil properties and glyphosate biodegradation in different agricultural soils was investigated in this study. Soils differ hugely in soil texture, soil organic matter content. pH, oxalate extractable Al^{3+} and Fe^{3+}. The biodegradation experiments were conducted under test conditions: water tension of -15 kPa as soil moisture, a soil density of 1.3 g/cm^3 and at 20 °C in the dark. The biodegradation experiments showed that the mineralization of glyphosate in 21 agricultural soils greatly varied. Between 7.6 to 68.7 % of the applied ^{14}C-glyphosate was mineralized to $^{14}CO_2$ in the 21 different soils within 32 days of incubation. The highest and lowest mineralized glyphosates were observed in Feldkirchen (68.7 %) and Brejze soil sample (7.6 %), respectively. Glyphosate was mineralized rapidly by the microorganisms in the soil solution and the highest mineralization rate was reached shortly after application. The mineralization of glyphosate in soils was individually regulated by exchangeable H^+, soil pH-$CaCl_2$, oxalate extractable

AI $^{3+}$ and bacterial cell numbers at the end of the experiments, but it was collectively controlled by exchangeable H^+, Ca^{2+} ions and plant available K. Moreover, soil textures, soil organic content, P_2O_5, Cu^{2+}, oxalate extractable Fe^{3+} and CEC were found not to have any correlation with mineralization of glyphosate. The NaOH extractable residues were bioavailable for degradation whereas the bound residues of glyphosate in soils were mostly formed by microbial activity.

Materials and Methods

Soil

The experiment was conducted using 21 agricultural soils typical of Germany and Slovenia. There was a big variation in the different soil characteristics (soil textures, organic matter content, total N, C/N, plant available P, oxalate extractable Al^{3+}, Fe^{3+}, Cu^{2+}, CEC, pH-$CaCl_2$, water content at water potential of -15 kPa and heterotrophic bacteria). All soils were taken from the upper Ap layer of arable fields (0-30 cm), sieved (2 mm) after sampling, homogenized and stored at 4°C in the dark before use. At the beginning of the experiments all soils were conditioned and moistened to a water potential close to -15 kPa at room temperature (20 ± 2 °C) for 2 weeks and compacted to the soil density of 1.4 g/cm³ to equilibrate the microbial processes and to make sure that all soils have the comparable conditions at the start of the experiments.

Table 7.1.2.1.1-101: Some characteristics of soil samples

Name of soil (site of origin)	Sand [%]	Silt [%]	Clay [%]	Water content -15 kPa (%)	pH ($CaCl_2$)	Organic matter [%]	C [%]	N [%]	P_2O_5 (mg 100g^{-1})	K_2O (mg 100g^{-1})
1 (Ada-A02)	62.5	27.4	10.1	21.9	[illegible]	2.9	1.7	0.2	17	4.1
2 (Apace-njiva)	66.4	31.2	2.4	20.7	[illegible]	2.6	1.5	0.2	4	22.6
3 (Berta-A02)	46.4	39.4	14.2	28.1	5.7	2.5	1.5	0.2	8	12.5
4 (Brezje)	8.3	73.2	18.5	32.2	5.2	2.8	1.6	0.2	5	21.1
5 (Dunja - A06)	62.4	25.9	11.7	[illegible]	5.4	2.2	1.3	0.2	11	13.2
6 (Feldkirchen)	34.8	47.0	[illegible]	20.2	7.0	3.4	2.0	0.3	39	9.4
7 (Grace - A13)	50.3	41.3	8.4	21.0	5.4	2.6	1.5	0.2	12	9.6
8 (Hanna - A15)	62.3	24.2	[illegible]	18.4	5.2	1.7	1.0	0.1	7	8.2
9 (Hohenwart)	67.2	20.5	12.3	22.4	6.2	1.7	1.0	0.1	21	21.1
10 (Joy -A19)	[illegible]	[illegible]	22.8	31.9	5.9	2.7	1.6	0.2	34	43.2
11 (Kelheim)	76.2	15.5	8.3	12.5	6.5	1.2	0.7	0.1	23	17.0
12 (Konjise)	33.8	60.2	6.0	34.6	6.9	4.5	2.6	0.2	4	7.9
13 (Lamanose)	10.3	69.6	20.1	35.8	5.8	4.3	2.5	0.3	5	18.7
14 (Lea-A18)	18.9	66.8	14.3	28.9	5.2	1.9	1.1	0.2	6	23.8
15 (Lomanosec)	21.9	60.2	17.9	25.8	5.8	1.7	1.0	0.2	11	16.8
16 (Neumark)	85.5	8.8	5.7	12.6	5.2	1.6	0.9	0.1	11	12.2
17 (Pearl - A20)	29.3	51.8	18.9	28.3	5.0	2.3	1.3	0.2	12	31.7
18 (Scheyern Lysi)	17.2	62.6	20.2	30.1	5.5	2.7	1.6	0.2	20	5.3
19 (Skrinjar)	67.5	27.0	5.5	19.2	7.1	1.6	0.9	0.1	21	24.7
20 (Zepovci)	41.3	43.1	15.6	24.0	5.7	2.9	1.7	0.2	11	24.7
21 (Zepovci (Plitv.))	11.8	72.2	16.0	27.4	5.2	1.9	1.1	0.2	8	20.2

Table 7.1.2.1.1-101: Some characteristics of soil samples (continued)

Name of soil (site of origin)	Al_{ox} (mg $100g^{-1}$)	Fe_{ox} (mg $100g^{-1}$)	Cu^{2+} (mg kg^{-1})	Ca	Mg	K	Na	H	CEC	Heterotrophic bacteria (x10^7 CFU g^{-1})*
				[$mmol_c$ $100g^{-1}$]						
1	63	198	4	8.5	0.8	1.0	0.04	5.7	16.0	0.3
2	62	248	4	11.1	2.3	0.1	0.04	1.5	15.0	0.5
3	76	265	3	9.0	1.0	0.6	0.04	5.3	15.9	0.4
4	187	518	2	7.2	0.9	0.6	0.07	11.1	19.8	0.1
5	80	211	62	7.0	0.6	0.6	0.04	5.3	13.6	0.3
6	139	310	12	26.4	2.5	0.5	0.05	3.5	32.9	1.1
7	106	259	3	8.7	0.7	0.6	0.04	7.4	17.5	0.6
8	83	215	2	7.2	0.5	0.2	0.04	5.7	13.6	0.1
9	75	206	4	5.5	1.2	0.4	0.05	3.9	11.1	0.3
10	101	320	39	13.1	1.8	0.7	0.06	6.7	22.4	0.9
11	44	132	8	5.5	1.2	0.3	0.05	2.0	9.1	0.8
12	88	381	7	10.8	4.6	0.1	0.06	3.2	18.8	0.1
13	134	456	4	16.4	3.6	0.3	0.06	9.2	29.6	0.4
14	107	345	3	6.4	0.8	0.4	0.07	6.9	14.6	0.3
15	72	252	3	9.5	1.8	0.3	0.09	5.4	17.0	0.7
16	88	110	1	2.6	0.4	0.2	0.05	4.3	7.5	0.7
17	125	319	4	6.9	0.8	0.5	0.05	8.8	17.0	0.8
18	102	349	10	9.1	1.6	0.6	0.06	7.1	18.4	0.9
19	57	257	4	10.8	0.5	0.1	0.06	1.5	13.0	0.5
20	165	476	4	7.8	0.7	0.9	0.05	10.6	20.0	0.3
21	147	430	2	4.4	0.5	0.7	0.10	9.4	15.1	0.1

* CFU = colony-forming unit at the start of degradation experiments

Chemicals

^{14}C-labelled glyphosate [N-(phosphonomethyl)glycine purity >97.0 %.] was labelled on the phosphonomethyl group. ^{14}C-glyphosate was mixed with non-labelled glyphosate (purity 98 %) resulting in a final specific radioactivity of 1.6 Bq/mg (for degradation experiments). Aminomethylphosphonic acid (AMPA) had the purity of 98 %. Sodium hydroxide (NaOH), monopotassium phosphate (KH_2PO_4), sodium chloride (NaCl), calcium chloride dihydrate ($CaCl_2x2H_20$), NH_4Cl, NH_4NO_3, methanol (CH_40), diatomaceous earth, water for chromatography were purchased commercially.

Glyphosate mineralization experiments

Application - All biodegradation experiments were performed in 4 replicates with 50 g soil (dry mass) for each replicate. ^{14}C-glyphosate was dissolved in autoclaved and distilled water and mixed with non-labeled glyphosate which was also dissolved in sterilized distilled water. This was the application standard solution with a concentration of a.i. of 5.42 µg/L and a specific radioactivity of 166.70 Bq/µg. The application standard (0 .089 mL) was applied to an oven dried, pulverized soil and carefully stirred for 1 minute with a spatula. The spiked aliquot was transferred to another glass beaker containing the rest of equilibrated soils and mixed for another 2 min. The total concentration of glyphosate was 10 µg/g in each set corresponding to a total radioactivity of 83,000 Bq.

Test system, experimental conditions and samplings - The spiked soils were compacted to a soil density of 1.3 g/cm^3 and soil water was adjusted to a water potential of -15 kPa. The flasks were covered with special rubber caps, and incubated at 20 ± 1°C in the dark for a maximum period of 32 days. The soil humidity was controlled weekly. The rubber caps were equipped with an air inlet and outlet system as well as a facility to trap the evolved CO_2. The air exchange system should prevent unaerobiosis in the incubation flasks and consisted of a canal which was made of a stainless needle with a diameter of 1 mm. To eliminate CO_2 from the ambient air entering the flasks, a 12 mL plastic syringe filled with granular CO_2 absorber (soda lime) was connected to the canal at the top of the cap. Below the cap a small plastic beaker was placed containing 0.1 M NaOH solution to capture $^{14}CO_2$ released from glyphosate mineralization from the soil samples. The NaOH solution was exchanged three times per week and from the collected solution an aliquot of 2 mL was mixed with 3 mL of scintillation cocktail Ultim a Flo AF to determine $^{14}CO_2$ in a liquid scintillation counter. At the end of the experiment, 30 g of each soil sample (dry mass) were used for pore water extraction, 7 g of each soil sample were extracted with NaOH to determine the quantity and quality of the

extractable residues as well as to quantify the non-extractable residues, while 1 g of each soil sample was used for cell counts.

NaOH extraction, clean up and HPLC analysis- For NaOH extraction, the method used by Gimsing *et al.* (2004a) was applied. At the end of the experiment, soil was extracted with 0.1 M NaOH by shaking on overhead shaker for 17 hours. The supernatant was collected after centrifuging for 10 min at 3020 rcf. Radioactivity of the filtered supernatant was measured by scintillation counting using 100 µl of supernatant aliquot and 5mL of scintillation cocktail Ultima Gold XR to quantify the NaOH extractable pesticide residues. Subsequently, extracts were concentrated and cleaned up before injecting to HPLC. Twenty µl of each sample (NaOH extract) were injected via an Auto Sampler AS50 to a HPLC system that was connected with a Radioflow detector LB 509. ^{14}C-glyphosate and its metabolites (AMPA, sarcorsine, glycine, methylamine) were identified by comparison of their retention times with standard substances. After each analysis the column was regenerated with Regenerant-RG019 at a flow velocity (isocratic) of 0.5 mL/min for 30 min.

Quantification of non-extractable ^{14}C-labelled residues - After extraction with 0.1 M NaOH, the rest of radioactivity remaining in the soil was considered as non-extractable residues. Soil material was intensively mixed and homogenized with diatomaceous earth for 2 min in a mortar. Four aliquots of each soil sample were weighed in combustion cups and mixed with 8 drops of saturated aqueous sugar solution to accelerate and ensure a complete oxidation of the ^{14}C. The oxidation step was done with an automatic sample-oxidizer 306. $^{14}CO_2$ from the combustion was trapped in Carbo-Sorb E and mixed with Permaflour E before scintillation counting. The extractable and non-extractable glyphosate residues were calculated after the combustion.

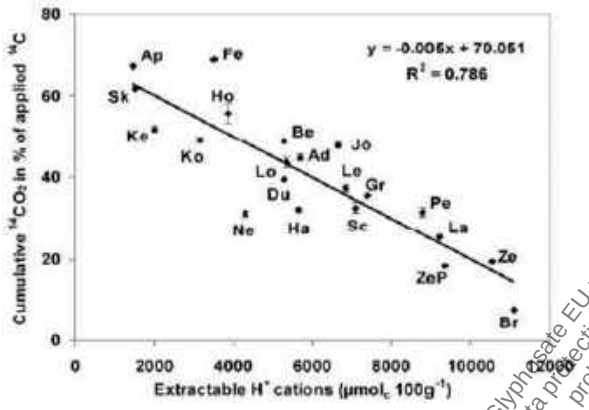
Bacterial cell counts- Bacterial cell counts were performed to count the cultivable and heterotrophic bacteria in the different soils. The method for bacterial cell counts was adapted from Ngigi *et al.* (2011). Soil bacteria were extracted from the soil by mixing soil with a buffer solution. Before use the buffer solution was autoclaved and shaken vigorously for 1 hour on a shaker at 150 rpm. The soil particles were allowed to sediment for 10 min. Then 0.1 mL of the supernatant was transferred to sterilized buffer solution for further dilution steps. A total of 4 dilutions (10^{-1} to 10^{-4}) were established. Finally, 0.1 mL of each dilution was spread in triplicates on Lysogeny broth (LB) agar media. This medium was also autoclaved before use. The number of CFU was determined after three days of incubation at 25 °C by counting.

Table 7.1.2.1.1-102: Behavior of ^{14}C-glyphosate in different soils

Soil	Cum. Min (%)[a] (1)	NaOH extract. residues (%)[a]* (2)	Non-extract. residues (%)[a] (3)	Total recovery** (%)[a] (4)	Quality of NaOH extract. residues: Glyphosate (%) (5)	AMPA[b] (%) (6)	Unknown (%) (7)
1	44.7	48.3	4.8	97.8	37.7	2.3	8.3
2	67.3	24.5	9.6	101.4	18.9	2.2	3.4
3	48.9	42.7	6.3	97.9	34.4	0.0	8.3
4	7.6	91.0	2.5	101.1	88.0	0.0	3.0
5	39.3	53.8	3.9	97.3	43.2	0.0	8.7
6	68.7	23.3	9.0	101.0	12.2	7.6	3.6
7	35.5	57.7	3.7	96.9	44.1	2.8	10.8
8	32.2	59.8	4.1	96.1	54.0	0.0	5.8
9	55.8	37.7	6.3	99.8	24.8	5.7	7.3
10	47.9	46.9	8.2	103.0	31.3	7.1	8.5
11	51.8	37.3	6.2	95.3	25.1	4.1	8.1
12	49.1	35.7	9.5	94.3	23.3	4.5	7.9
13	25.5	64.4	6.7	96.6	45.0	8.3	11.1
14	37.3	55.7	5.4	98.4	30.0	0.0	25.7
15	43.7	46.8	6.4	96.9	30.4	8.0	8.4
16	31.2	63.0	3.1	97.3	48.8	2.5	11.7
17	31.5	63.6	3.7	98.8	29.5	0.0	34.1
18	32.5	59.8	5.0	97.3	40.9	5.5	13.4
19	61.6	28.8	11.4	101.8	16.9	4.8	7.1
20	19.5	73.3	4.1	96.9	65.8	0.0	7.5
21	18.4	78.5	2.7	99.6	55.9	11.3	11.3

a) % of applied ^{14}C-glyphosate after 32 days; the mean value is presented
b) Aminomethylphosphonic acid
* Total NaOH extractable residues (2) = (5) + (6) + (7)
** Total recovery (4) = (1) + (2) + (3)

Figure 7.1.2.1.1-24: Correlation between cumulative mineralization of glyphosate and extractable H^+ cations in soils (bars indicate standard deviation of 4 samples)

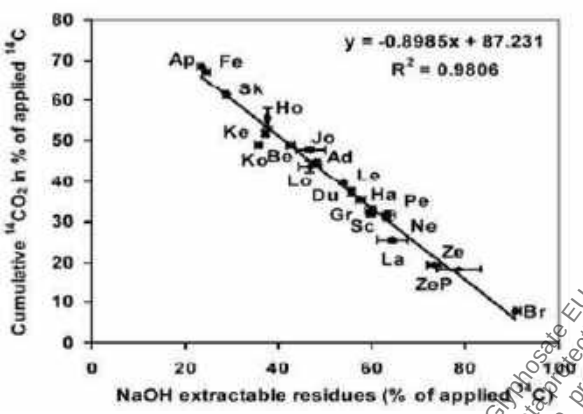


Statistical analysis
The data were statistically analysed using analysis of variance (ANOVA) and multiple regression analysis.

Results and Discussion
The main aim in this part of the study is to check the correlation between soil parameters and glyphosate mineralization. The selected soil parameters for correlation were exchangeable [H^+], silt, clay, soil organic matter, C, N, C/N, P_2O_5, Cu^{2+}, oxalate extractable Al^{3+}, oxalate extractable Fe^{3+}, K_2O, $CFU_{beginning}$ and CFU_{end}, Ca^{2+}, Mg^{2+}, K^+, Na^+, CEC, and Ph.

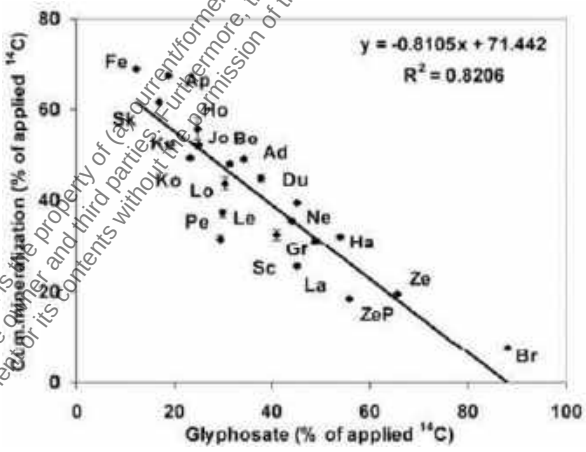
Mineralization of glyphosate
After 33 days of incubation a big variance of cumulative mineralization can be observed. Between 7.6 to 68.7 % of the applied ^{14}C -glyphosate was mineralized to $^{14}CO_2$ in the 21 different soil types (Table 7.1.2.1.1-102). Shortly after application, a high amount of glyphosate was mineralized. The lowest mineralization of ^{14}C-glyphosate was identified in Brezje soil while the highest mineralization of ^{14}C-glyphosate was obtained in Feldkirchen and Apace-njiva soils. Low mineralization of glyphosate was also observed in Zepovci, Zepovci(Pliv.) and Lamanose soils. In these 3 soils less than 30 % of the initial glyphosate was mineralized after 32 days. In contrast, other soils had a higher mineralization activity and $^{14}CO_2$ production after 32 days reached 31.2-68.7 % of the initial glyphosate. A big difference in biomineralization of glyphosate among 21 soils indicates that agricultural soils have difference in ability to degrade glyphosate. The firstly rapid mineralization of glyphosate was observed for most soils during the first 4 days without a lag phase, but mineralization rates subsequently decreased over time, as found in other earlier studies (von Wiren-Lehr *et al.*, 1997; Gimsing *et al.*, 2004a). At the end of the biodegradation experiments, mass balances were established. Mass balances of ^{14}C-glyphosate are presented in Table 7.1.2.1.1-102. In all soils, the ^{14}C mass balances were quite good: over 94 % of the totally applied ^{14}C-glyphosate was recovered at the end of the biodegradation experiments.

Figure 7.1.2.1.1-25: Correlation between cumulative mineralization of glyphosate and NaOH extractable residues (bars indicate standard deviation of 4 samples)



Identification of the parameters governing mineralization of glyphosate
In order to identify the factors which govern glyphosate mineralization in the 21 soils, soil parameters, NaOH extractable residues, ^{14}C-glyphosate residues, non-extractable residues and the mineralized glyphosate were compared at the end of the biodegradation experiments and several significant correlations could be discovered.

Figure 7.1.2.1.1-26 Correlation between cumulative mineralization of glyphosate and glyphosate residues (from extractable residues) (bars indicate standard deviation of 4 samples).



Relationship between mineralized glyphosate and extractable acidity (extractable H^+ cations)
According to univariate correlation analysis there was highly significant and negative correlation between the cumulative mineralization glyphosate and extractable H+ cations (p = 0.000). This illustrates that the extractable H+ cations interfered the mineralization process in soils. Therefore, the assessment of extractable H+ cations in soils appears suitable for ranking of soil according to the mineralization of the compound.

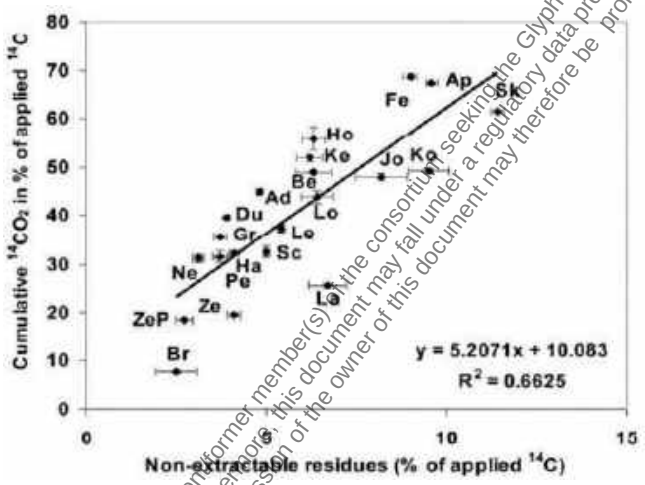
Relationship between mineralized glyphosate and NaOH extractable residues
NaOH extractable residues of the 21 investigated soils were performed after 32 days. The results show that the NaOH extractable fraction in all soils was relatively high and very various. Approximately between 23 and 91 % of initial glyphosate after 32 days incubation was extracted with NaOH 0.1 M (Table 3). Soils with higher mineralization had lower NaOH extractable fraction. A correlation was performed to check the

relationship between mineralized glyphosate and NaOH extractable residues. There was a negative correlation between mineralized glyphosate within 32 days and NaOH extractable residues (p = 0.0000). This shows that NaOH extractable residues were non-available for microorganisms to be degraded.

Relationship between mineralized glyphosate and ^{14}C-glyphosate residues from extractable pool

^{14}C-glyphosate is the major component in the NaOH extract as compared to AMPA and unknown metabolites. To test whether there is any relationship between the mineralized glyphosate and NaOH extractable residues, we calculated correlation between both values. There is exist significantly negative correlation between ^{14}C-glyphosate residues from extractable pool and mineralized glyphosate (p = 0.0000). This indicates that in soils with low mineralization glyphosate is present in a high amount and that this glyphosate could not be degraded/mineralized because it was adsorbed to Al- or Fe-oxides.

Figure 7.1.2.1.1-27: Correlation between cumulative mineralization and non-extractable residues (bars indicate standard deviation of 4 samples)



Relationship between mineralized glyphosate and non-extractable residues

The amount of non-extractable residues was relatively low. It varied between 2.5 % and 11.4 % of the initial glyphosate. The non-extractable residues and mineralized glyphosate were compared together to see whether there is any relationship between both parameters. A significant and positive correlation between mineralized glyphosate and non-extractable residues (p = 0.0000) was found. The high mineralization of glyphosate in soils coincided with non-extractable residues at the end of the experiment.

Relationship between mineralized glyphosate and bacterial cell counts

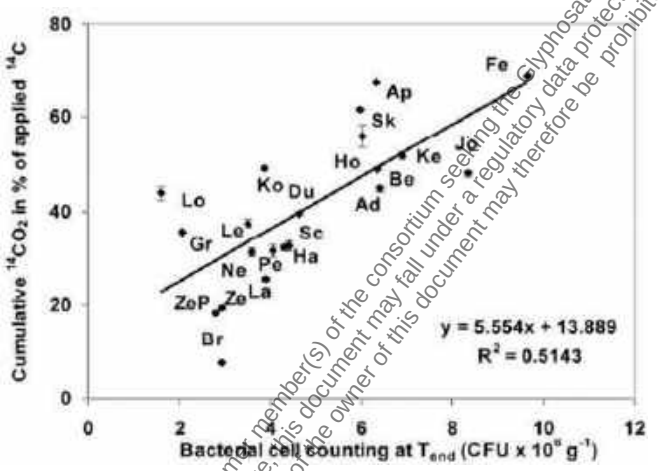
There was a significantly positive correlation between mineralization of glyphosate and bacterial cell counts (p = 0.003). This shows that the mineralization of glyphosate in soils is limited not only by availability of glyphosate and its degradation products, but also by the bacterial activity. Therefore, it can be assumed that the bacterial cell numbers at the end of the experiment seemed to be the degrading microorganisms for glyphosate in soils and it was likely that microbes capable of degrading glyphosate aerobically exist in soils.

The interacting junctions of the different soil parameters on mineralized glyphosate

In order to investigate the interacting functions of the different soil parameters on cumulative glyphosate mineralization, a multiple regression analysis was used. The input parameters were extractable H^+ cations, silt, clay, soil organic matter, C, N, C/N, plant available P, Cu^{2+}, oxalate extractable Al^{3+}, oxalate extractable Fe^{3+}, plant available K, Ca^{2+}, Mg^{2+}, K^+, Na^+, CEC, pH , CFUs at beginning and CFUs at the end of the experiments. The result of multiple regression analysis reveals extractable H^+ cations, Ca^{2+} and plant available K as key parameters governing glyphosate mineralization in the 21 tested soils and Ca^{2+} and plant available K contributes additionally to extractable H^+ cations to the mineralization of glyphosate. In this multiple regress ion, extractable H+ cations has a negative correlation with mineralization of glyphosate,

whereas exchangeable Ca^{2+} and plant available K have a positive correlation with cumulative mineralization of glyphosate. Once again, this result indicates that extractable H^+ cations is an important factor which reduces the bioavailability of glyphosate in soils, and as a consequence the mineralization of glyphosate is reduced. Regarding Ca^{2+} and plant available K, cumulative mineralization was found to be positively correlated with exchangeable Ca^{2+} and plant available K, respectively. Therefore, it is proposed in this study that a complexation between glyphosate with exchangeable Ca^{2+}/plant available K will not reduce the bioavailability and mineralization of glyphosate. In the contrary, Ca^{2+}-glyphosate complexes may be transported more efficiently across microbial cell walls than sole glyphosate compound as it has already been argued for Cu^{2+} complexes in literature (Kools *et al.*, 2005). However, these mechanisms have not been documented and should be clarified.

Figure 7.1.2.1.1-28: Correlation between cumulative mineralization of glyphosate and bacterial cell counting (bars indicate standard deviation of 4 sample)



Conclusions

Degradation of glyphosate in soils greatly varies depending on soil properties. Mineralized glyphosate is affected by extractable acidity (H^+ cations) and bacterial cell counts. Sorption behavior and bioavailability of glyphosate in soil are important to regulate mineralization. Extractable H^+ cations. Ca^{2+} ions and plant available K have been identified as important soil parameters that collectively control the mineralization of glyphosate in soil. Glyphosate that is absorbed by AI/Fe-oxides and extractable H^+ cations can be extractable with NaOH 0.1 M, but it is not available for degradation by soil microorganisms. Non-extractable residues of glyphosate which have been identified as a result of microbial activity.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the dissipation of glyphosate in agricultural soils in Europe. While a lot of experimental details are reported, the data are insufficient for kinetic evaluation since tests were run for 32 days in maximum only and determination of mineralization only, i.e. no detailed analysis for active substance and metabolites.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/017
Report author	Bergström, L. *et al.*
Report year	2011
Report title	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil
Document No	DOI 10.2134/jeq2010.0179 E-ISSN 1537-2537
Guidelines followed in study	Degradation experiment: none Adsorption experiment: OECD 106 Guideline Lysimeter experiment: none
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Due to the increasing concern about the appearance of glyphosate [N– (phosphonomethyl) glycine] and its major metabolite aminomethylphosphonic acid (AMPA) in natural waters, batch laboratory and lysimeter transport studies were performed to assess the potential for leaching of the compounds in two agricultural soils. Unlabelled and ^{14}C–labelled glyphosate were added at a rate corresponding to 1.54 kg a.s./ha on undisturbed sand and clay columns. Leachate was sampled weekly during a period of 748 d for analyses of glyphosate, AMPA, total ^{14}C, and particle–bound residues. Topsoil and subsoil samples were used for determination of glyphosate adsorption, glyphosate degradation, and formation of AMPA and its degradation. The influence of adsorption on glyphosate degradation was confirmed, giving very slow degradation rate in the clay soil (half–life 110–151 d). The kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation rate can be faster than that of glyphosate. The kinetics also suggest that apart from glyphosate being transformed to AMPA, the sarcosine pathway can be just as significant. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59 % of the initial amount of glyphosate added to the clay soil 748 d after application. Despite large amounts of precipitation in the autumn and winter after application, however, these residues were mainly located in the topsoil, and only 0.009 and 0.019 % of the initial amount of glyphosate added leached during the whole study period in the sand and clay, respectively. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil.

Materials and methods

Lysimeter Experiment

Soil Characteristics, Lysimeter Collection, and Management

Three undisturbed soil columns of a sandy soil and four of a clay soil were used. The smaller number of sand columns was based on the fact that sandy soils are usually more homogeneous and therefore show less variability in flow processes (Bergström & Shirmohammadi, 1999). Some physical and chemical properties of the two soils used are listed in Table 7.1.2.1.1-103. The soil columns were collected using coring equipment in which a polyvinyl chloride pipe (1.18–m long and 0.295–m inner diam.) is gently pushed into the soil by a steel cylinder with cutting teeth, which rotates around the pipe as it penetrates the soil (Persson and Bergström, 1991). After collection at the two field sites, Lanna in southwest Sweden (58°21′ N, 13°08′ E) and Nåntuna close to Uppsala (59°49′ N, 17°39′ E), the columns were prepared for gravity drainage by removing about 0.07 m of soil at the base, which was replaced by gravel, two stainless steel meshes, and a fiberglass lid, giving a final length of the soil columns of ~1.05 m. The lysimeters were then placed in vertical pipes permanently installed below ground at a lysimeter station located at the Swedish University of Agricultural Sciences in Uppsala, Sweden (Bergström, 1992).

Table 7.1.2.1.1-103: Selected soil characteristics of the Lanna clay and the Nåntuna sand. Standard laboratory methods were used throughout (Bergström *et al.*, 1994)

Layer	Soil texture (Gr/Sa/Si/Cl)†	Organic matter	Cation exchange capacity	Bulk density	pH‡	Water content at tensions (cm)		
						0§	100	15,000
cm	%		cmol$_c$ kg^{-1}	g cm^{-3}		m^3 m^{-3}		
Clay								
0–30	1.3/6.0/46.2/46.5	4.4	28.4	1.24	7.2	0.524	0.359	0.193
30–60	0.6/2.7/40.6/56.1	0	33.6	1.43	7.4	0.477	–	0.249
60–90	0.2/1.8/37.4/60.6	0	–	1.46	7.4	0.468	–	0.297
Sand								
0–30	0/87.8/4.5/7.7	2.0	4.7	1.43	7.4	0.448	0.180	0.034
30–60	0/95.4/4.6/0	1.0	1.8	1.47	6.4	0.427	0.185	0.019
60–90	–	1.0	1.4	1.46	7.0	0.452	0.065	0.016

† Gr = gravel, >2 mm; Sa = sand, 0.06–2 mm; Si = silt, 0.002–0.06 mm; Cl = clay, <0.002 mm.
‡ Determined in water.
§ Equivalent to porosity.

All management practices performed on the lysimeters were intended to reproduce field conditions as closely as possible. Just before sowing in each year, the soil in each lysimeter was hand–tilled to simulate light harrowing. Spring barley (Hordeum distichum L.) was sown at a rate of 2 g per lysimeter on 21 May 2006, 26 May 2007, and 30 May 2008. On each occasion, mineral fertilizers were applied at rates of 100 kg N/ha, 22 kg P/ha, and 56 kg K/ha. The barley was harvested on 1 Sept. 2006, 28 Sept. 2007, and 16 Sept. 2008 by cutting the aboveground plant parts at ground level.

In addition to natural precipitation, all lysimeters received supplemental irrigation on two occasions during the 2–yr experimental period (in total, 22 mm). On each occasion, water was added with spray bottles over a few hours at rates typical of heavy rain storms, but not exceeding the infiltration capacity of the soil.

Chemical Application
Glyphosate was applied to two lysimeters of the sand soil and to three lysimeters of the clay soil on 18 Sept. 2006 at a rate corresponding to 1.54 kg a.s./ha, which represents a normal dose in Swedish cereal production systems. Radiolabeled [^{14}C] glyphosate (ARC 1313 glyphosate-[phosphonomethyl–^{14}C], 50 mCi/mmol, American Radiolabeled Chemicals, Inc., St. Louis, MO) was used to obtain fast screening of the leachate samples using scintillation counting analysis. The radiolabeled portion (5.32 MBq) was mixed with formulated (Roundup BIO, contains 486 g glyphosate/L as isopropylamin salt, Monsanto Crop Sciences), unlabeled glyphosate (in total 10.5 mg/lysimeter), which was dissolved in 11 mL (0.16 mm) of water. This solution was applied to the lysimeters by dripping it on the soil surface using a syringe. After the solution had been applied, 5 mL (0.07 mm) of water was drawn up into the syringe and also applied to each lysimeter. In addition to glyphosate, KBr at a rate of 0.268 g Br^- per lysimeter (~40 kg Br^-/ha) was applied to provide information on the movement of water through the soil columns. The KBr was dissolved in water (0.4 g KBr in 5 mL), which was applied separately to the lysimeters, also using a syringe.

Soil and Water Sampling
On 17 Oct. 2007, samples of the topsoil (0–30 cm) and subsoil (30–80 cm) of each soil were collected for determination of adsorption and degradation characteristics. These samples were taken from the lysimeter of each soil used as control (i.e., no glyphosate added). Three soil cores from each lysimeter were collected with a tube drill. The individual samples were then mixed by layers into a topsoil and a subsoil sample for each lysimeter. On 5 Oct. 2008, after leaching measurements were terminated, soil samples were collected from the lysimeters to which glyphosate had been applied to determine the residual amounts of glyphosate and AMPA about 2 yr after application. Three cores from each lysimeter were taken with a tube drill and divided into three layers (0–30, 30–60, and 60–90 cm), which were pooled to one sample for each lysimeter and layer. After collection, all soil samples were stored in a freezer (–20 °C) until analyzed.

Leachate from the lysimeters was collected and weighed each week during the 2–yr period when drainage water was available. After collection, all leachate samples were stored in a freezer (–20 °C) until analyzed.

The amount of ^{14}C was measured in 10 mL of the leachate using a Beckman LS 6000TA liquid scintillation counter (Beckman Coulter Inc, Fullerton, CA) after addition of 10 mL of Insta–Gel Plus (PerkinElmer, Waltham, MA).

Adsorption Study

The adsorption study was performed according to the OECD 106 guideline (OECD, 2001). Adsorption data were obtained at five different concentrations in two replicate samples. Four grams dry weight (DW) of field–moist soil were shaken at 200 rpm on a shaker for pre–equilibration with 39 mL of 0.01 M $CaCl_2$ for 24 h at 20°C in 50–mL plastic tubes. Thereafter, the soil slurry was spiked with 1 mL of a mixture of labeled (1.98 kBq) and unlabeled glyphosate in 0.01 M $CaCl_2$ to give five initial concentrations in the range 0.1 to 10 µg/g dw of soil. After shaking for 24 h, the tubes were centrifuged for 20 min at 4000 rpm and then the radioactivity was measured in 10 mL of the supernatant. Tubes without soil and ^{14}C–labeled glyphosate were included for subtraction of background radiation, and tubes without soil were used to give the initial amount of ^{14}C activity added. No significant adsorption of glyphosate occurred on the plastic tubes. A pre–study showed that adsorption equilibrium was obtained after 24 h of contact time between soil and solution, which also indicates that negligible amounts of AMPA had been formed.

Adsorption data were fitted by nonlinear regression to the Freundlich adsorption isotherm:

[1]

$$c_{soil} = K_f c_{aq}^{1/n}$$

where c_{soil} (µg/g) is the adsorbed amount, c_{aq} (µg/mL) is the concentration in the aqueous phase, K_f[$µg^{1-1/n}$ $(mL)^{1/n}$/g] is the Freundlich adsorption coefficient, and $1/n$ (–) the measure of nonlinearity.

Degradation Study

Glyphosate dissolved in water (1.4 mg/mL) was applied dropwise (1.0 mL) to 15 g of fresh soil. The soil was dried and mixed, after which an additional amount of fresh soil (to give 140 g DW in total) was thoroughly mixed into the spiked soil to give an initial concentration of 10 µg glyphosate per g DW of soil. Portions corresponding to 10 g of dry soil were transferred to 50–mL plastic tubes. The water content was adjusted to 60 % of the water–holding capacity. The tubes were sealed with plastic caps that allow gas exchange and incubated at 20°C in the dark. After 2, 4, 8, 16, 32, and 64 d, two tubes were put in the freezer (−20 °C) until analysis for residual concentrations of glyphosate and the metabolite AMPA. The weight of the tubes was measured once a week during the incubation, and when necessary, the moisture content was adjusted to 60 % of the water–holding capacity.

Residual values of glyphosate were used for a least squares fitting procedure to determine values of the parameters of the function for first order exponential decay:

[2]

$$c_G(t) = c_{G0} e^{-kt}$$

where c_G (mg/kg) is the residual concentration of glyphosate at time t days after application, c_{G0} (mg/kg) is the initial concentration of glyphosate, and k (d^{-1}) is the first–order rate coefficient for degradation.

A branched reaction scheme was applied to describe the degradation of glyphosate to AMPA and sarcosine (Karpouzas and Singh, 2006; Borggaard and Gimsing, 2008) and the degradation of AMPA (Figure 7.1.2.1.1-29). According to this scheme and assuming first–order kinetics, the rate of AMPA formation and degradation is then

[3]

$$\frac{dc_A}{dt} = 0.66 k_1 c_G - k_2 c_A$$

where c_A (mg/kg) is the concentration of AMPA at the time t. Because the concentrations of glyphosate and AMPA were expressed in units mg/kg, the value of c_{G0} obtained from Eq. [2] was multiplied by the stoichiometric factor 0.66 (i.e., the ratio of the molecular weights of the dominant species of AMPA and glyphosate at pH 7) in these calculations. The equation describing the concentration of AMPA was obtained by combining Eq. [2] and [3], and integrating:

[4]

$$c_A = \frac{0.66 k_1 c_{G0}}{k_2 - k}(e^{-kt} - e^{-k_2 t})$$

In this branched pathway, k for glyphosate degradation in Eq. [2] equals the sum of k_1 for AMPA formation and k_3 for sarcosine formation. Then $k_3 = k - k_1$ and the fractions of glyphosate transformed into AMPA and sarcosine are k_1/k and k_3/k, respectively. Since no more than 100 % of the glyphosate can be transformed into AMPA, the upper limit for k_1 is k, in which case $k_3 = 0$. The maximum concentration of AMPA, c_{Amax}, occurs at time t_{Amax} when $dC_A/dt = 0$. Inserting this value into Eq. [3], replacing c_G and c_A in Eq. [3] by their expressions in Eq. [2] and [4], respectively, and rearranging gives the following:

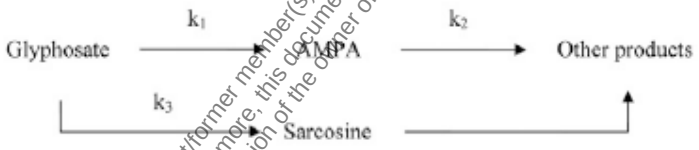
[5]

$$t_{A\max} = \frac{\ln(k) - \ln(k_2)}{k - k_2}$$

Nonlinear Regression

Least squares fits of data on adsorption and on residual values of glyphosate and AMPA were fitted to their respective equations by nonlinear regression. Residual values of AMPA were fitted using the values of c_{GO} and k for glyphosate degradation as obtained from Eq. [2]. The calculations were performed on a PC with the application SigmaPlot for Windows version 10.0 (Systat Software, Inc., San Jose, CA); the nonlinear regression method is based on the Levenberg and Marquardt method.

Figure 7.1.2.1.1-29: Branched reaction scheme with the first-order rate coefficients k_1 and k_3 for the degradation of glyphosate to aminomethylphosphonic acid (AMPA) and sarcosine, respectively, and k_2 for the degradation of AMPA



Glyphosate and AMPA Analyses

Reagents

Analytical standards used for calibration were (trivial names in italics): N−(phosphonomethyl) glycine, *glyphosate*, (Riedel−de−Haën, Sigma−Aldrich, Sweden AB) and (aminomethyl) phosphonic acid, *AMPA*, (Dr. Ehrenstorfer GmbH, Augsburg, Germany). Internal standards were ^{13}C; ^{15}N; ^{2}D−labeled AMPA and ^{13}C; and ^{15}N−labeled glyphosate (LCG Standards AB, Borås, Sweden). Concentrated HCl, ethyl acetate, and NaOH (analytical reagent grade from VWR, Stockholm, Sweden), were used for extraction and solvation. The AG1−X8, 100−200, formate form (Bio Rad Laboratories, Sundbyberg, Sweden) and Isolute C18 EC 200 mg (Sorbent AB, Gothenburg, Sweden) were used for ion exchange and clean−up. Trifl uoroacetic anhydride (TFAA) and trifluoroethanol (TFE), both analytical reagent grade from Sigma Aldrich Sweden AB (Stockholm, Sweden), were used for the derivatization. The 0.22−µm glass fiber filters # GSWP04700 were from Millipore VWR (Stockholm, Sweden).

Calibration

Stock solutions of glyphosate and AMPA were diluted in water to concentrations of 100 µg/mL and stored at +4°C. A solution containing 1 µg/mL of glyphosate and AMPA was prepared daily as a working standard. The labeled glyphosate and AMPA were diluted in deionized water to a concentration of 1 µg/mL and stored at −20 °C in 2−mL portions.

Clean−Up and Derivatization: Water Samples

A 50−mL volume of a water sample and 0.1 µg each of glyphosate and AMPA internal standard were adjusted to pH 2 with 6 M HCl in a plastic tube. The sample was left to precipitate for 1 h and centrifuged at 5000 rpm for 10 min. The upper, clear phase was adjusted to pH 7 to 8. Ag1−X8 (2.3 g) was weighed into an empty 6 mL−plastic column equipped with a piece of cotton at the bottom, and the column was

wetted with deionized water. A 3−mL (200 mg) C18 SPE column was activated with 3 mL of methanol and 3 mL of water and connected on top of the AG1−X8 column. An empty 75−mL plastic column was connected on top of the C18 and Ag1−X8 columns, and the sample was applied at a rate of 2 mL/min. The two upper columns were removed and the analytes were eluted with 3×4 mL of 0.6 M HCl at a rate of 1 mL/min and collected in a 100−mL pear−shaped fl ask. The sample was evaporated to approximately 2 mL under vacuum, quantitatively transferred to an 8−mL glass tube and evaporated to dryness under an air stream at 50°C. The derivatization was performed by adding 1 mL of trifluoroethanol and 2 mL of trifluoroacetic anhydride, and the sample was held at 100°C for 1 h. After being cooled to room temperature, the sample was evaporated under nitrogen and redissolved in 1.00 mL of ethyl acetate before analysis.

Clean−Up and Derivatization: Particle−Bound Glyphosate and AMPA in Leachate
Leachate samples from three lysimeters of each soil were analyzed for particle−bound glyphosate and AMPA. These samples comprised two samples from the untreated lysimeters and four samples from the glyphosate−treated lysimeters on sampling occasions when the highest concentrations of glyphosate and AMPA were detected in the leachate. A 300−mL portion of each sample was filtered through a 0.22−µm glass fiber filter. The filter was weighed before and after filtration, dried at 105°C, and the dry weight of the particles was calculated. The dry filter and the particles were analyzed for glyphosate and AMPA by extraction with 7 mL of 0.1 M NaOH following the same procedure as for soil samples (see below).

Clean−Up and Derivatization: Soil Sample
Ten grams of soil were extracted with 40 mL (for the degradation study) or 75 mL (for the lysimeter soil residue analysis) of 0.1 M NaOH by shaking for 30 min at 200 rpm, sonicated for 10 min and centrifuged for 10 min at 5000 rpm. The internal standards (0.1 µg each of glyphosate and AMPA) were added to a portion (40 µL and 4 mL for the degradation and the lysimeter studies, respectively) of the clear upper part of the sample, which was then analyzed according to the procedures described for the water samples. The portion from the degradation study was evaporated and derivatized directly after precipitation of the extract, since no column clean−up was needed due to the high residual concentrations in these samples.

Instrumentation
The gas chomatography−mass spectrometry (GC−MS) analyses were performed with a Hewlett−Packard 6890 GC (Agilent Technologies Sweden AB), equipped with a 30 m by 0.25 mm i.d. (0.25−µm fi lm thickness) fused silica capillary column (HP−5 for GC–MS), a mass spectrometer 5973, a split/splitless injector, and the software Chemstation, all from Agilent Technologies (Kista, Sweden). One microliter of the samples was injected (in the splitless mode at 270°C, oven temperature 70°C). After 2 min, the oven temperature was raised to 170°C at 30°C/min and then from 170 to 250°C at 120°C/min. Helium (N47 grade, 99.997 %) was used as the carrier gas and the flow rate was 1.2 mL/min. The mass spectrometer was operated in the electron impact (EI) mode; the transfer line and manifold temperatures were 270 and 230 °C, respectively. Fragment ions were detected by selected ion monitoring (SIM) and used for identification of the AMPA and glyphosate derivates as shown in Table 7.1.2.1.1-104.

Table 7.1.2.1.1-104: Molecular weights, retention times (RT) and specific selected ions for compound derivatives.

Molecule	Molecular weight	RT (min)	Quantification ion (m/z, % relative abundance)	Qualification ion (m/z, % relative abundance)
AMPA†	371	4.49	126 (100)	302 (23)
AMPA‡	375	4.49	130 (100)	306 (22)
Glyphosate†	511	5.35	411 (100)	384 (50)
Glyphosate‡	513	5.35	413 (100)	386 (48)

† AMPA = aminomethylphosphonic acid.
‡ internal standard.

Verification of compound identification was based on comparison of the areas of the selected ions in the samples with those of the standards. For quantification, the response areas for AMPA and glyphosate target ions were calculated in relation to those of the internal standards. The response was found to be linear in

the practical concentration range (2.5−100 pg) of individual components injected.
The quantification levels for glyphosate and AMPA were 0.1 µg/L in water and 0.01 µg/g in soil. In some samples, however, the quantification level was higher due to the specific background.

Results

Adsorption of Glyphosate

The high correlation coefficients (R^2 ≥0.997; Table 7.1.2.1.1-105) obtained when sorption data for both soils and soil layers were fitted to the Freundlich adsorption isotherm show that they could be accurately described by this model. The values of the K_f parameter obtained were considerably higher in the clay soil than in the sand and are similar to values previously reported for glyphosate sorption to soils of similar textures (Vereecken, 2005). In the sand, K_f was higher in the topsoil than in the sub−soil, whereas the opposite was true for the clay. The correlation between K_f and the amount of clay in the different soils was 0.987. Although based on only four soils (topsoil and subsoil in the respective soils), this result supports the generally held view that glyphosate is primarily sorbed to clay particles and their associated iron oxides (Vereecken, 2005). Normalisation of the distribution coefficients for glyphosate should therefore also account for the amount of clay and oxides present in soil and not organic carbon only, which is used to calculate K_{oc}. The 1/*n* parameter, which expresses the degree of linear relationship between c_{soil} and c_{aq}, was close to 1 for both layers of the clay soil and the sand topsoil, showing an almost constant distribution coefficient between sorbed and dissolved glyphosate in these soil layers in the range of concentrations studied. In the subsoil of the sand, the parameter 1/*n* was 0.82, indicating that the availability of sites for sorption in this layer becomes limiting at high glyphosate concentrations.

Table 7.1.2.1.1-105: Freundlich coefficients (K_f) (± SE, *n* = 10) for adsorption of glyphosate obtained by nonlinear regression according to Eq. [1]

Soil	K_f	1/*n*	R^2
	$\mu g^{1-n}(mL)^n g^{-1}$	–	–
Sand topsoil	40.0 ± 2.9	0.92 ± 0.04	0.997
Sand subsoil	28.7 ± 1.2	0.82 ± 0.02	0.998
Clay topsoil	178 ± 4.4	0.95 ± 0.01	1.000
Clay subsoil	165 ± 10.7	1.03 ± 0.02	0.999

Degradation of Glyphosate and AMPA

Best fits of glyphosate and AMPA residue data to Eq. [2] and [4], respectively, are shown in Figure 7.1.2.1.1-30 and the parameter values obtained in Table 7.1.2.1.1-106 and Table 7.1.2.1.1-107 5, respectively. Initial extraction efficiencies of glyphosate were 112 to 123 % as shown by comparing the initial concentrations obtained (Table 7.1.2.1.1-106) with the nominal value of 10 µg/g DW. All parameter values were significantly different from zero ($p < 0.05$, $n = 12$). The models gave good fits of the data for all soils ($R^2 \geq 0.90$), except for glyphosate in the clay subsoil ($R^2 = 0.56$). This poor fit could be due to difficulties in getting glyphosate homogeneously distributed in this clay−rich (56.1 %) subsoil with no organic matter (0 %). Another explanation could be that the R^2 values obtained by nonlinear and linear regression are not comparable. In nonlinear regression, R^2 refers to the fraction of the variance explained and is the model efficiency (EF). A disadvantage of EF is its dependency on the slope of the curve, as it is always relatively small for relatively flat decline patterns, or can even be negative for curves describing for instance formation and degradation of metabolites, irrespective of the scatter of measured data around the calculated curve (FOCUS, 2005). Therefore, from visual inspection of the fits to the data (Figure 7.1.2.1.1-30) and from the generally small standard errors in the parameters determined (Table 7.1.2.1.1-106 and Table 7.1.2.1.1-107), we concluded that the equations provide relevant quantitative information.

Figure 7.1.2.1.1-30: Best fits (A) to Eq. [2] of data on glyphosate and (B) to Eq. [4] of data on aminomethylphosphonic acid (AMPA) concentrations for sand topsoil (•), sand subsoil (○), clay topsoil (▼), and clay subsoil (^) (mean ± SE, n = 2). dw = dry weight

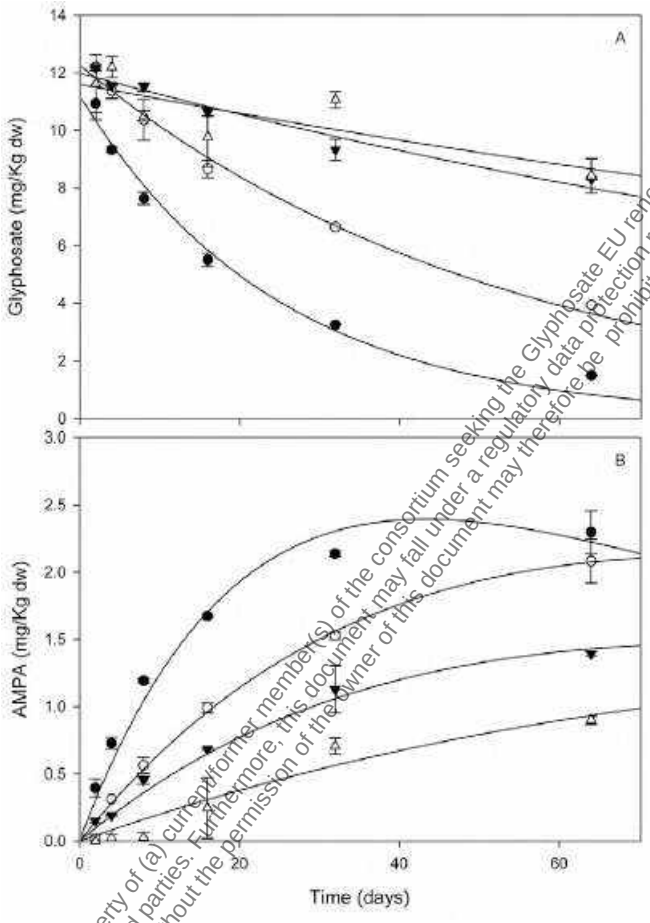


Table 7.1.2.1.1-106: Coefficients (± SE, n = 12) obtained by nonlinear regression for degradation of glyphosate according to first−order kinetics (Eq. [2]).

Soil	$c_{(0)}$†	k	R^2	$t_{1/2}$‡	DT_{90}§
	mg kg^{-1}	d^{-1}	–	d	d
Sand topsoil	14.21 ± 0.33	0.041 ± 0.003	0.978	16.9	56.2
Sand subsoil	12.27 ± 0.19	0.019 ± 0.001	0.985	36.5	121
Clay topsoil	11.99 ± 0.15	0.0063 ± 0.0005	0.948	110	365
Clay subsoil	11.61 ± 0.41	0.0046 ± 0.0014	0.562	151	501

† $c_{(0)}$ = initial concentration of glyphosate; k = first-order rate coefficient for degradation; $t1/2$ = half-life; DT_{90} = time for 90% degradation.
‡ Calculated as ln(2)/k.
§ Calculated as ln(10)/k.

Table 7.1.2.1.1-107: Coefficients (± SE, n = 12) obtained by nonlinear regression for formation and degradation of aminomethylphosphonic acid (AMPA) according to Eq. [4].

Soil	k_1†	k_2	$t_{1/2}$‡	R^2
	d^{-1}		d	–
Sand topsoil	0.0216 ± 0.0011	0.0115 ± 0.0019	60.4	0.965
Sand subsoil	0.0092 ± 0.0005	0.0076 ± 0.0018	91.3	0.988
Clay topsoil	0.0063§ ± 0.0005	0.0199 ± 0.0013	34.9	0.973
Clay subsoil	0.0028 ± 0.0006	0.0071 ± 0.0087	97.6	0.901

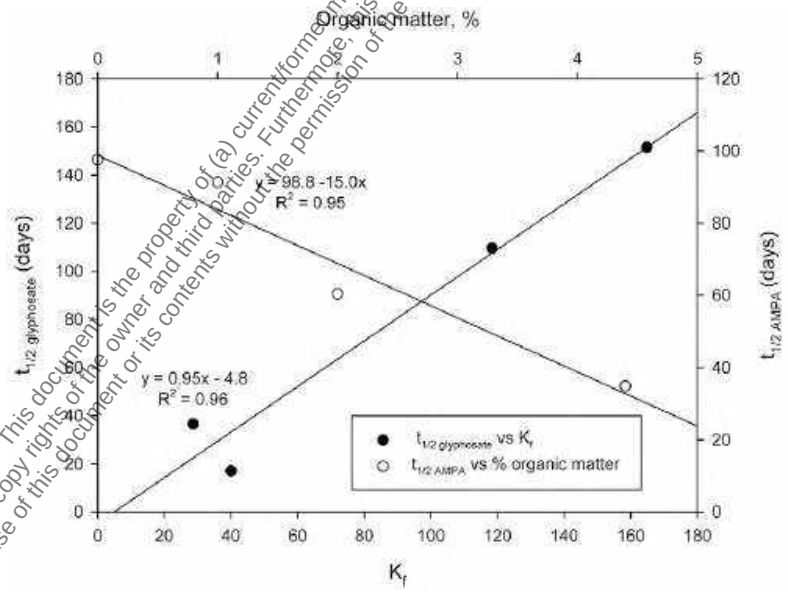
† k_1 = first-order rate coefficient for degradation of glyphosate to AMPA; k_2 = first-order rate coefficient for degradation of AMPA; $t_{1/2}$ = half-life.

‡ Calculated as $\ln(2)/k_2$.

§ In the clay topsoil, k_1 became 0.0073, i.e., larger than k (first-order rate coefficient for degradation; Table 4), leading to a formation fraction > 1, and was therefore set equal to k.

Glyphosate degraded relatively rapidly in the sand, with a half−life of 16.9 and 36.5 d in the topsoil and subsoil, respectively (Table 7.1.2.1.1-106). In the clay, very long half−life values of 110 and 151 d were obtained, and remarkable values of 365 and 500 d for 90 % degradation (DT_{90}). These half−life values are within the range previously reported for glyphosate degradation in agricultural soils (Giesy *et al.*, 2000). There was a high correlation between half−life and K_f (Figure 7.1.2.1.1-31), suggesting that adsorption is important for the amount of glyphosate available in the soil water for degradation.

Figure 7.1.2.1.1-31: Relationship between half−life ($t_{1/2}$) for glyphosate and Freundlich adsorption coefficient (K_f), and between $t_{1/2}$ for aminomethylphosphonic acid (AMPA) and % organic matter



The concentration of AMPA steadily increased during the incubation period of 64 d in all soils except the sand topsoil (Figure 7.1.2.1.1-30 B), where it peaked after 43.4 d at 2.4 mg of AMPA/kg, representing 32.4 % of the initial amount of glyphosate added (Table 7.1.2.1.1-108). The degradation rate of AMPA, as quantified by k_2, gave a half−life of 35 to 98 d, with slower rates in the subsoil (Table 7.1.2.1.1-107). The correlation between these half−life values and the amount of organic matter was −0.973 (Figure 7.1.2.1.1-31), suggesting that increasing amounts of organic matter, or perhaps AMPA−degrading microorganisms dwelling there, increase degradation rates.

Table 7.1.2.1.1-108: Derived parameter values on fraction of glyphosate degraded to aminomethylphosphonic acid (AMPA) (*k1/k*), rate constant for formation of sarcosine (*k₃*), incubation time (t_{Amax}) at which the AMPA−concentration peaks (c_{Amax}), and c_{Amax} as fraction of initially added glyphosate

Soil	k_1/k†	k_3‡	t_{Amax}§	c_{Amax}¶	c_{Amax} fraction#
	–	d^{-1}	d	mg kg^{-1}	%
Sand topsoil	0.53	0.0190	43.4	2.40	32.4
Sand subsoil	0.48	0.0098	80.4	2.12	26.2
Clay topsoil	1	0	84.7	1.47	18.6
Clay subsoil	0.61	0.0018	174	1.34	17.5

† Values of k (first-order rate coefficient for glyphosate degradation) from Eq. [2] (Table 4) and of k_1 (first-order rate coefficient for degradation of glyphosate to AMPA) from Eq. [4] (Table 5).

‡ $k_3 = k - k_1$.

§ Calculated according to Eq. [5].

¶ Obtained by inserting derived values of k, k_1, k_2, c_{G0} and t_{Amax} into Eq. [4].

Calculated as $(c_{Amax} \times 1.52 \times 100)/c_{G0}$, where 1.52 is the stoichiometric factor for conversion of AMPA to glyphosate concentration.

The degradation of AMPA is reported to be slower than that of glyphosate (Giesy *et al.*, 2000). In the Footprint database, AMPA is classified as persistent, with a typical half−life of 151 d, compared with 12 d for glyphosate (Footprint, 2009). The fact that AMPA is formed when glyphosate is degraded clearly means that the persistence of AMPA has to be equal to or longer than that of glyphosate. However, we did not find any previous study in which the degradation of AMPA was studied and compared with that of glyphosate in the same soil. In a study where glyphosate degraded with a half−life of 9 d, Simonsen *et al.* (2008) estimated a half−life of 32 d for AMPA from the descending part of data on AMPA residues. However, this is a worst−case scenario as these data represent the sum of AMPA formation from degradation of the glyphosate still present and AMPA degradation. This does not reveal how fast the AMPA molecule per se is degraded. Our data suggest that the AMPA degradation rate can be faster than that for glyphosate, for instance in soils with high clay content, which slows down glyphosate degradation, and high organic matter content, which stimulates AMPA degradation (Figure 7.1.2.1.1-31).

Microbial degradation is the main process controlling the disappearance of glyphosate in soil, and there are two well−described biological pathways for such degradation that give AMPA and sarcosine as the respective metabolites (Karpouzas and Singh, 2006; Borggaard and Gimsing, 2008). It has recently been shown that ligninolytic enzymes can also transform glyphosate into AMPA (Pizzul *et al.*, 2009). Because AMPA is the only significant soil metabolite found in soil degradation studies, it is frequently suggested that metabolism of glyphosate in soil usually proceeds via the AMPA pathway (Giesy *et al.*, 2000; Karpouzas and Singh, 2006). However, the fractions of AMPA formed in our study (48−100 %, Table 7.1.2.1.1-108) suggest that both pathways can be active in soil, with up to 52 % not following the AMPA pathway. Reasons for the sarcosine pathway not being considered significant in soil could be that soil residues of sarcosine are not determined in most studies and that sarcosine rapidly degrades to glycine (Karpouzas and Singh, 2006) in biologically active soil.

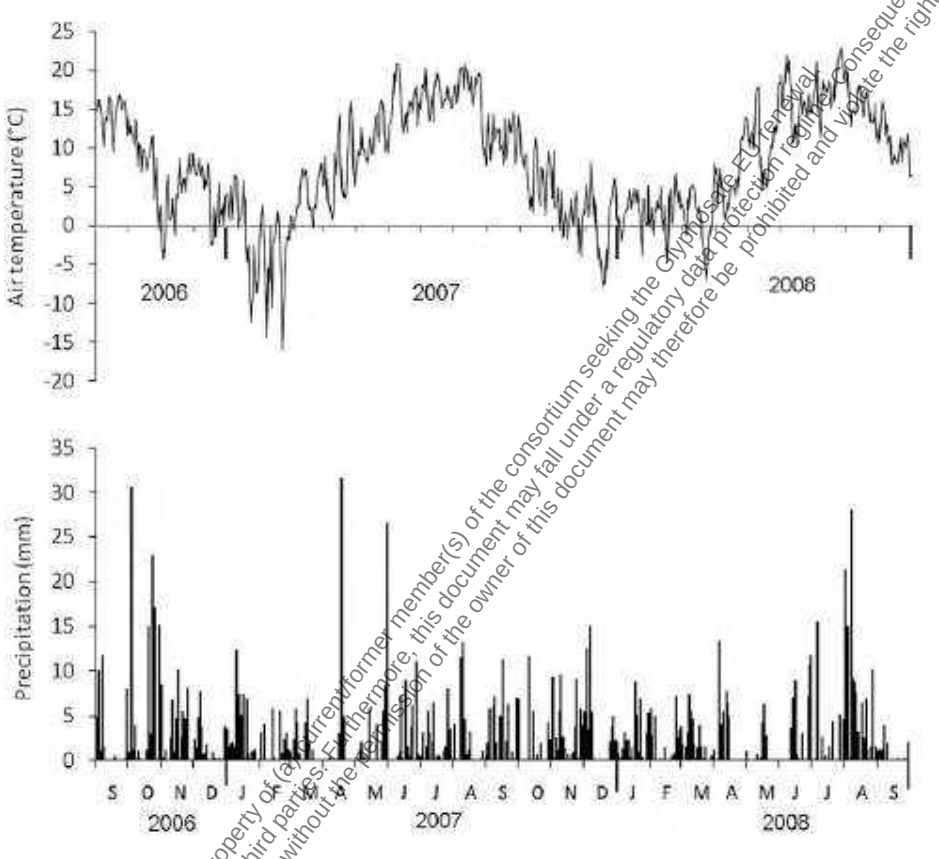
Precipitation and Drainage Conditions

Daily precipitation and average air temperatures at the lysimeter station are shown in Figure 7.1.2.1.1-32. Over the 2−yr study period (15 Sept. 2006−15 Sept. 2008), cumulative precipitation was 1192 mm, which, in combination with supplemental irrigation, resulted in a total water input to the lysimeters of 1214 mm. This total water input is slightly higher (10 %) than the long−term average precipitation for the Uppsala region (554 mm/yr). Average air temperature during the experimental period (7.8°C) was also higher than the long−term average at Uppsala (5.3°C).

A few weeks after glyphosate was applied, from 30 September onward, rain events were quite frequent (Figure 7.1.2.1.1-32), and precipitation totaled 232 mm by the end of 2006. This clearly created worst−case conditions for leaching of the herbicide, and the average amounts of leachate were 169 and 156 mm from the sand and clay soil, respectively, during this period. Peak weekly amounts of leachate, reaching 42 (sand) and 33 (clay) mm, occurred 8 wk after herbicide application. During 2007, precipitation was close to the

normal for the area, although quite unevenly distributed. During periods with low evaporation (November, December, and January), monthly precipitation was about 60 mm, which was clearly above the average and increased the risk of leaching. In 2008, precipitation was again above normal, causing large amounts of leachate.

Figure 7.1.2.1.1-32: Average daily temperature (upper graph) and daily precipitation (lower graph) at the lysimeter site during the experimental period



The cumulative amounts of leachate from the lysimeters each year are shown in Table 7.1.2.1.1-109. In total over the 2−yr period, the amount of leachate was 572 (± 17) mm from the sand and 461 (± 15) from the clay soil. In relation to water input, these amounts constituted 47 and 38 % of precipitation plus irrigation, which is considerably higher than in other similar leaching studies performed in Sweden (Bergström & Jokela, 2001).

Table 7.1.2.1.1-109: Water inputs to the lysimeters and mean annual amounts of leachate from the lysimeters to which glyphosate was applied (± SD; n = 2 for sand, n = 3 for clay)

Year	No. of days	Precipitation + irrigation	Leachate	
			Sand lysimeters	Clay lysimeters
		mm		
2006†	104	232	169 (± 1.4)	156 (± 4.7)
2007	365	550	229 (± 3.2)	158 (± 9.1)
2008‡	261	432§	174 (± 13)	148 (± 4.0)
Total	730	1214	572 (± 17)	461 (± 15)

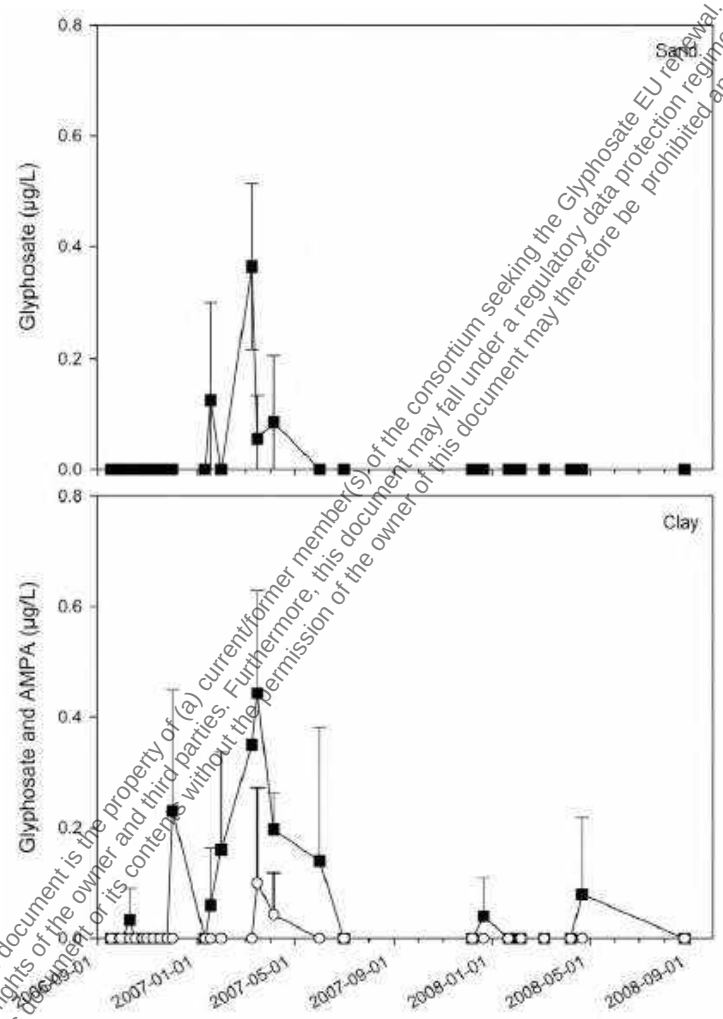
† Measurements made during the period 18 September to 31 December.
‡ Measurements made during the period 1 January to 18 September.
§ Includes 22 mm irrigation.

Leaching of Glyphosate and AMPA

Average concentrations of glyphosate and AMPA in leachate are shown in Figure 7.1.2.1.1-33. In the sand, the average peak concentration of glyphosate reached 0.36 µg/L in the beginning of March 2007, when temperatures were consistently above freezing, about 25 wk after pesticide application. During this period, the amount of leachate was about 250 mm (i.e., equivalent to 1.5 effective pore volumes). Thereafter the glyphosate concentration decreased and the average concentration was below 0.1 µg/L from 16 March 2007 onward. This leaching pattern indicates limited preferential transport of the herbicide through the sand profile, although some preferential transport must have occurred considering the strong sorption of glyphosate (Table 7.1.2.1.1-104) and thereby expected large retardation. This is a flow behavior reported in several other leaching studies in sandy soils (e.g., Bergström & Shirmohammadi, 1999). The fact that the glyphosate peak occurred about 15 wk later than the corresponding bromide peak is a reflection of bromide being a nonreactive tracer. In the clay soil, the initial glyphosate peak occurred in the beginning of December and reached 0.23 µg/L after about 150 mm of water (i.e., equivalent to 0.8 effective pore volumes) had leached out of the soil columns. This considerably smaller amount of leachate suggests that glyphosate was partly transported through preferential flow paths in the clay profile, as was the case for bromide. This flow pattern has been documented earlier in this clay soil for reactive solutes (Djodjic *et al.*, 1999; Bergström, 1995) and for nonreactive tracers (Bergström & Jarvis, 1993; Bergström & Shirmohammadi, 1999). However, the highest glyphosate peak (0.44 µg/L) in leachate from the clay soil coincided with that in the sand, i.e., in the beginning of March 2007. This glyphosate peak was washed out of the columns slightly earlier than the corresponding bromide peak, which was rather unexpected. Apart from preferential flow, another explanation could be that the highly water−soluble bromide diffused into micropores in the clay soil relatively soon after application and once in these pores it was largely protected from percolating water (Bergström & Stenström, 1998). From July 2007 onward, the average glyphosate concentration in clay soil leachate was <0.1 µg/L, although single samples had concentrations slightly exceeding the detection limit (0.1 µg/L). Average concentrations of AMPA in leachate were at or below 0.1 µg/L in both soils (Figure 7.1.2.1.1-33), with the highest concentration (0.30 µg/L) in a sample from one of the clay lysimeters. The average total amount of glyphosate that leached from the sand was 0.13 (± 0.03) g/ha and from the clay soil 0.28 (± 0.08) g/ha. These amounts correspond to 0.009 and 0.019 % of the amount of glyphosate applied to the soils. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil. Total leaching of the ^{14}C applied in September 2006 was on average 0.31 % from the sand soil and 0.25 % from the clay. This shows that constituents other than glyphosate and AMPA that were not positively identified formed the major proportion of the total radioactivity in leachate. The leaching rates determined in this study are quite small compared with those in many other studies. For example, in a study performed by Al−Rajab *et al.* (2008), which included microlysimeters of three soils (clay loam, silty clay loam, and sandy loam), the amounts of glyphosate leached during 11 mo ranged between 0.11 and 0.28 % of the amount applied. However, there are also studies showing similar results to those obtained in the present experiment. In a study in France performed using lysimeters filled with calcareous soil (Landry *et al.*, 2005), leaching of glyphosate was between 0.02 and 0.06 % of that applied after 680 mm of rainfall. Similarly, Cheah *et al.* (1997) recovered 0.04 to 0.07 %

of applied glyphosate in lysimeter leachate after 200 mm of simulated rainfall. However, the conditions in all the above−mentioned studies were quite different from those in this study; the lysimeters were only 9.8 to 25 cm long, the experimental periods were considerably shorter (a few days to 1 yr), and the amounts of rainfall were much smaller (200 to 869 mm). These differences certainly have to be taken into account in a comparison of results.

Figure 7.1.2.1.1-33: Average concentrations of glyphosate (■) and aminomethylphosphonic acid (AMPA) (○) in the leachate (mean + SD, n = 2 for sand and n = 3 for clay). No AMPA was found in the leachate from sand

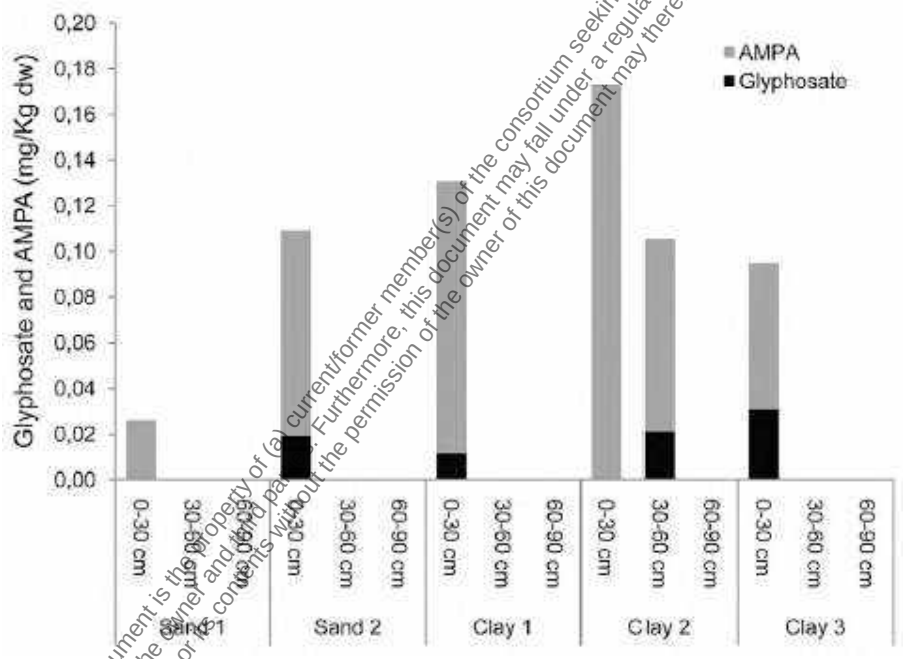


No glyphosate or AMPA was determined to be particle−bound, even though large quantities of particles were present in leachate from the clay soil. It is noteworthy that the particles were operationally defined as those being retained on a 0.22−µm glass−fiber filter. Some studies have shown that colloid−facilitated transport of glyphosate can occur. For example, de Jonge *et al.* (2000) showed in a study on lysimeters filled with undisturbed topsoil of a sandy loam that 1 to 27 % of leached glyphosate was particle−bound. Considering the overall low total concentrations of glyphosate in the present study (Figure 7.1.2.1.1-33), the particle−bound proportion would be below the detection limit (0.1 µg/L) if it constituted less than 25 % of what was leached. It is also important to bear in mind that topsoil lysimeters only include about 30 % of the profiles used in this study and may in fact, as indicated above, generate results that are quite atypical of results obtained in full−length lysimeters, such as those used here. The underlying subsoil can act as a sink or source for particles leaching through the soil profile.

The trend for glyphosate to leach in larger amounts from clay soils than from sandy soils is relatively well documented. In a Danish study, this was attributed to periods of high intensity rainfall shortly after application, when glyphosate was located on the soil surface and thereby exposed to rapid water transport in clay macropores extending up to the surface (Kjaer *et al.*, 2003).

Residues of Glyphosate and AMPA in Soil
Residues of glyphosate and AMPA in the 0− to 30−, 30− to 60−, and 60− to 90−cm soil layers 748 d after application are shown in Figure 7.1.2.1.1-34. Residues were found in the 0− to 30−cm layer in all lysimeters and also in the 30− to 60−cm layer in one of the lysimeters with clay soil, possibly due to preferential flow in clay macropores and translocation in plant roots (Laitinen *et al.*, 2007). No residues were found in the 60− to 90−cm layer in any of the lysimeters. Considering the worst−case conditions prevailing for leaching after application of glyphosate in the autumn of 2006, these results confirm the generally low mobility found for these compounds (Giesy *et al.*, 2000; Vereecken, 2005).

Figure 7.1.2.1.1-34: Residues of glyphosate and aminomethylphosphonic acid (AMPA) in the 0− to 30−, 30− to 60−, and 60− to 90−cm soil layers 748 d after application. dw = dry weight



No glyphosate was detected in one of the sand lysimeters, and 0.019 mg/kg remained at 0 to 30 cm in the other one. Low concentrations could be expected from the fast degradation in the sand topsoil (laboratory half−life 16.9 d). The concentrations of AMPA (0.026 and 0.090 mg/kg) remaining can be due to a combination of slow degradation (laboratory half-life 60.4 d) and continuous supply from degradation of remaining glyphosate. Related to the initial amount of glyphosate added, the remaining glyphosate residues represented 2.7 % and total residues of glyphosate + AMPA, calculated as glyphosate equivalents, represented 27 %.

In the clay soil, glyphosate and AMPA were found in all three lysimeters, probably due to very slow degradation of glyphosate in the topsoil and subsoil (Table 7.1.2.1.1-106), and thereby a long−term supply of AMPA, slow degradation of AMPA in the clay subsoil, and 100 % formation of AMPA from glyphosate degradation in the topsoil. Glyphosate residues represented 5.1 % and total residues 59 % of the initial amount of glyphosate added. Similar field persistence of glyphosate and AMPA residues was found in a sandy soil in Finland, where total residues in the 0− to 60−cm layer accounted for 72 % of the amount applied 20 mo after application (Laitinen *et al.*, 2009).

Conclusion

The influence of adsorption on glyphosate degradation was confirmed, giving very slow degradation in the clay soil. The kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation can be faster, for instance in soils with a high clay content, which slows down glyphosate degradation, and a high organic matter content, which stimulates AMPA degradation. The kinetics also suggest that apart from glyphosate being transformed to AMPA, the sarcosine pathway can be just as significant. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59 % of the initial amount of glyphosate added to the clay soil 748 d after application. However, despite quite frequent rain events and large amounts of precipitation in the autumn and winter after application, these residues were mainly located in the topsoil, confirming the generally low mobility reported for these compounds. This conclusion is also supported by the small amounts of glyphosate and AMPA leached during the whole study period. Possible residues of glyphosate and AMPA due to transport on particles >0.22 µm were below the limit of detection (0.1 µg/L), and this does not appear to be an important transport mechanism in the soils included in this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article investigates the behaviour of glyphosate and AMPA under conditions of outdoor lysimeters including the determination of sorption parameters and degradation data for two Swedish soils. The invesigations were performed with non-labelled test substances for which further information such as purity was not reported. The use of non-labelled test material does not allow for determination of mass balances. No detailed tabulated results per sample point are provided.

Lysimeter experiment: Not all required information is reported to allow for a check of the overall quality of the study.

Degradation and sorption tests: The tests for glyphosate were claimed to follow OECD 106 guideline while being unclear for soil degradation. Due to a lack of detail in reporting, information is insufficient to check the quality of data. In addition and for example, the LOD of the analytical methods used seem inappropriate to fulfill the requirements for EU data generation methods.

The article is therefore classified as reliable with restrictions for the three experiments.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/018
Report author	Ghafoor, A. *et al.*
Report year	2011
Report title	Measurements and modeling of pesticide persistence in soil at the catchment scale
Document No	DOI 10.1016/j.scitotenv.2011.01.049 E-ISSN 1879-1026
Guidelines followed in study	Degradation experiment: None Adsorption experiment: OECD 106 Guidance
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Degradation of pesticides in soils is both spatially variable and also one of the most sensitive factors determining losses to surface water and groundwater. To date, no general guidance is available on suitable approaches for dealing with spatial variation in pesticide degradation in catchment or regional scale modeling applications. The purpose of the study was therefore to study the influence of various soil physical, chemical and microbiological characteristics on pesticide persistence in the contrasting cultivated soils found in a small (13 km^2) agricultural catchment in Sweden and to develop and test a simple model approach that could support catchment scale modeling. Persistence of bentazone, glyphosate and isoproturon was investigated in laboratory incubation experiments. Degradation rate constants were highly variable with coefficients of variation ranging between 42 and 64 % for the three herbicides. Multiple linear regression analysis and Mallows Cp statistic were employed to select the best set of independent parameters accounting for the variation in degradation. Soil pH and the proportion of active microorganisms (r) together explained 69 % of the variation in the bentazone degradation rate constant; the Freundlich sorption co–efficient (K_f) and soil laccase activity together explained 88 % of the variation in degradation rate of glyphosate, while soil pH was a significant predictor ($p < 0.05$) for isoproturon persistence. However, correlations between many potential predictor variables made clear interpretations of the statistical analysis difficult. Multiplicative models based on two predictors chosen 'a priori', one accounting for microbial activity (e.g. microbial respiration, laccase activity or the surrogate variable soil organic carbon, SOC) and one accounting for the effects of sorption on bioavailability, showed promise to support predictions of degradation for large scale modeling applications, explaining up to 50 % of the variation in herbicide persistence.

Materials and methods

Study site and soils

The study was carried out in the E21 monitoring catchment in Östergötland, southern Sweden. The total catchment area of 13 km^2 consists of 95 % agricultural land, with main crops of winter and spring sown cereals, rape, potatoes and peas. The soils, which are derived from glacial and post–glacial fluvial sediments and glacial till (moraine), have a wide range of texture, from loamy sand to clay. Soil samples were collected from 60 locations in the catchment (1 location every 20 ha) on a grid pattern. Five soil samples from each location were taken in the surface 20 cm, bulked, homogenized by passing through a 2 mm sieve, put into plastic bags and stored at 4°C until use (within 48 days). Sixteen of these sampled locations were selected for further study to cover the range of measured textures, organic matter contents and pH values.

Soil pH was measured on fresh samples after shaking the samples in de–ionised water (1:2.5) at room temperature (Swedish Standard Institute, 1994). Particle size distributions were evaluated using the standard pipette method (Day, 1965). The contents of clay, sand, and silt are usually correlated with one another (Iqbal *et al.*, 2005). Thus the geometric mean particle diameter, d_g, was derived from the fundamental particle size classes as (Shirazi & Boersma, 1984):

[1]

$$d_g = exp(\sum_i m_i ln(X_i))$$

where *m* is the mass fraction of particle size class i and X is the mean diameter of that class. For the Swedish system, x–values are 0.001, 0.03 and 1.03 mm for clay, silt, and sand, respectively. Total organic C and N were measured using a Leco CN 2000 (LECO Corp., St. Joseph, MI, USA). Water contents at a pressure potential of –100 cm (pF 2) were measured on a sand table (Jamison, 1958). Ammonium–lactate extractable phosphorus and potassium were measured according to the method described by Egner *et al.* (1960).

The physical and chemical characteristics of the 16 soils are given in Table 7.1.2.1.1-110. There was a relatively wide range of SOC contents, ranging from 0.9 to 10.2 %. Soil pH ranged from 6.0 to 7.6. Soil texture is very variable for such a small catchment: clay, sand and silt contents ranged from 4–45 %, 12–87 %, and 8–54 % respectively, and 8 of the 11 USDA texture classes are represented. Ammonium–lactate extractable phosphorus and potassium ranged from 56–148 mg/kg and 54–209 mg/kg, respectively.

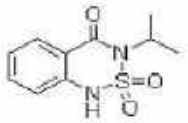
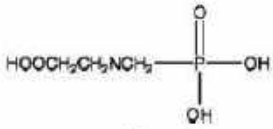
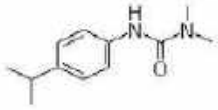
Table 7.1.2.1.1-110: Physico–chemical properties of soils

Soil N	pH	Texture			Textural class	SOC	$CaCO_3$	Total N	Water content at pF 2 (–100 cm)	Geometric mean particle diameter (d_g)	Available P	Available K
		Sand	Silt	Clay								
		%	%	%	International	%	%	%	g/g	mm	mg kg^{-1}	mg kg^{-1}
1	7.6	49	32	18	Loam	1.6	9.2	0.19	0.253	0.095	58	68
2	6.2	87	8	4	Sand	1.2	0.1	0.08	0.115	0.588	63	69
3	7.0	43	27	30	Clay Loam	2.3	0.1	0.22	0.363	0.049	99	116
4	7.1	58	25	17	Sandy Loam	2.1	0.4	0.22	0.3	0.131	89	84
5	6.9	68	17	15	Sandy Loam	2.1	0.2	0.23	0.285	0.199	111	114
6	6.5	70	21	9	Sandy Loam	1.1	0.1	0.09	0.175	0.268	90	120
7	6.5	85	9	6	Loamy Sand	1.6	0.1	0.15	0.169	0.494	159	70
8	7.6	55	28	17	Sandy Loam	2.6	0.2	0.25	0.265	0.118	56	68
9	6.4	12	45	44	Silty Clay	6.2	0.1	0.54	0.643	0.010	57	205
10	6.9	17	54	29	Silty Clay Loam	10.2	0.5	0.87	0.540	0.020	73	170
11	6.9	22	33	45	Clay	5.5	0.1	0.25	0.383	0.014	134	162
12	7.3	56	24	20	Sandy Loam	3.4	0.4	0.53	0.477	0.110	142	209
13	6.0	63	27	10	Sandy Loam	0.9	0.1	0.08	0.240	0.198	132	126
14	6.1	83	11	6	Loamy Sand	1.3	0.1	0.13	0.227	0.460	148	54
15	7.5	33	39	25	Clay Loam	3	0.1	0.28	0.410	0.048	101	97
16	7.1	31	40	29	Clay Loam	1.9	0.2	0.19	0.347	0.033	89	164

Chemicals

Unlabelled isoproturon (N,N–dimethyl–N′–[4–(1–methylethyl) phenyl]urea; 99 % purity), bentazone (3–(1–methylethyl)–1–H–2,1,3–benzothiadiazin–4(3 H)–one 2,2–dioxide; 97 % purity) and glyphosate (N–(phosphonomethyl)glycine, 98 % purity) were purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Ring (^{14}C) isoproturon (4.044 MBq/mg; purity >95 %) and [P–methylene–^{14}C]glyphosate (5.155 MBq/mg, purity >99 %) were purchased from Izotop, Institute of Isotopes, Budapest, Hungary. ^{14}C–labelled bentazone (3–(1–methylethyl–1 H–2,1,3–benzothiadiazin–4(3 H)–one 2,2–dioxide–[phenyl–U–^{14}C]; 5.211 MBq/mg; 100 % purity) was a gift from BASF, Limburgerhof, Germany. Table 7.1.2.1.1-111 gives the structural formulae and some physical and chemical properties of the three compounds. The 3–methyl–2–benzothiazolinone hydrazone (MBTH), 3–(dimethylamino) benzoic acid (DMAB) and 2,2′–azinobis(3–ethyl–benzthiazoline–6–sulfonic acid) (ABTS) were supplied by Sigma–Aldrich Sweden AB.

Table 7.1.2.1.1-111: Selected pesticides and their properties (data from the e–Pesticide Manual (3.0), British Crop Protection Council, 2003)

Herbicides	Structural formulae	pKa	mol wt	Solubility in water (mg L^{-1})
Bentazone		3.3	240.3	570
Glyphosate		2.3, 5.7, 10.2	169.1	10,500
Isoproturon		n.a	206.3	65

Degradation
Incubation experiments for each soil/pesticide combination were carried out on two replicate samples. A sub–sample of each soil (7 g) was spiked with glyphosate dissolved in water (0.7 mg herbicide/mL water) or isoproturon or bentazone dissolved in methanol (0.7 mg herbicide/mL methanol). The soil was dried, after which an additional amount of fresh soil (63 g) was thoroughly mixed into the spiked soil to give an initial concentration of 10 µg/g dry weight (d.w.) of soil (procedure adopted from Brinch *et al.* (2002)). Water contents were adjusted to and maintained at pF 2 throughout the experiment by the addition of de–ionized water as necessary. The samples were incubated in aerated glass tubes in the dark at 20°C for 64 days. Duplicate samples (5 g) were taken after 0, 2, 4, 8, 16, 32 and 64 days of incubation for measurement of the residual concentrations of glyphosate, isoproturon, and bentazone.

Analyses of bentazone and isoproturon in soil samples were carried out by HPLC as described by Larsbo *et al.* (2009), while for glyphosate, the GC–MS method developed by Börjesson & Torstensson (2000) was employed. The data from the incubation study were fitted to first–order degradation kinetics using non–linear regression:
[2]

$$c = c_0 e^{-kt}$$

where c is the mass of compound in the soil (µg/g) at a given time t (days), c_0 is the original mass of compound added to the soil (µg/g), and k (day^{-1}) is the first–order degradation rate coefficient. Degradation half–lives (DT_{50}, days) were calculated as ln(2)/k.

Adsorption
The adsorption experiments were carried out according to the OECD 106 guideline (Organization for Economic Cooperation and Development, 2000) on two replicates. Soil (four grams d.w. for glyphosate, two grams d.w. for isoproturon and bentazone) was shaken to pre–equilibrate with 0.01 M $CaCl_2$ (39 mL for glyphosate, 1.5 mL for bentazone and isoproturon) for 24 h at 20°C in test tubes (50 mL plastic tubes (Sarstedt) for glyphosate and 10 mL glass tubes for bentazone and isoproturon). Thereafter, the soil slurry was spiked with a 1 mL mixture of labeled (ca 7000–11000 dpm) and unlabeled pesticides in 0.01 M $CaCl_2$ to give 5 initial concentrations in the range of 0.1–10 µg/g soil. The tubes were shaken for 24 h and then centrifuged for 20 min at 4000 rpm. After mixing with 10 mL of Insta–Gel Plus (glyphosate) or 6 mL of Ultima Gold emulsifying cocktail (bentazone and isoproturon) (PerkinElmer, Waltham, MA, USA), the radioactivity was measured in the supernatant (10 mL for glyphosate, 1 mL for bentazone and isoproturon) using a LS 6000TA liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA). Tubes without soil and ^{14}C–labelled substances were included for subtraction of background radiation and tubes without soil were used to give the initial amount of ^{14}C activity. No significant adsorption of the tested substances occurred on the tubes.

The sorption measurements for each pesticide in the 16 soils were fitted to the Freundlich equation using non–linear regression:

[3]

$$S = K_f C_e^n$$

where S is the adsorbed amount (μg/g), c_e is the equilibrium concentration (μg/mL), K_f is the Freundlich constant ($\mu g^{1-n}\, mL^n/g$), and n (–) is an exponent that expresses the degree of isotherm nonlinearity.

Manganese peroxidase and laccase enzyme activities

Manganese peroxidase (MnP, EC 1.11.1.13) activity

Ten g of soil was mixed with 20 mL of a 1:1 mixture of 500 mM lactic acid/sodium succinate buffer (pH 4.5) in a Waring blender and homogenized for 3×30 seconds at high speed. The aliquots were centrifuged in 50 mL centrifuge tubes at 4000 rpm for 15 min at 4°C. The supernatant was filtered through 0.45 μm filter paper. Manganese peroxidase (MnP) activity was measured according to the method described by Castillo *et al.* (1994). Briefly, the assay is based on the oxidative coupling of MBTH and DMAB in the presence of H_2O_2, Mn^{+2} and MnP. This reaction gives a deep purple–blue color with a broad absorption band with a peak at 590 nm. The reaction mixture contained 300 μL 6.6 mM DMAB, 100 μL 1.4 mM MBTH, 30 μL 30 mM$MnSO_4$, 10 μL 10 mM H_2O_2 and 1.56 mL of sample extract in a total volume of 2 mL. A reagent blank without any sample extract was also run. Time zero was registered at the moment of addition of H_2O_2 and the increase in absorbance was then followed at 590 nm for 5 min by using a Shimadzu UV 1800–A spectrophotometer fitted with a time scan function. The initial rates were calculated by using linear regression. MnP activity (mU/min/g soil) in soil was calculated as:

[4]

$$\text{Unit of enzyme } g^{-1}\text{soil} = \frac{\text{Abs}/min \times 0.002}{E_m \times \text{mL of sample} \times \text{Dry weight of soil} \times \text{mL of buffer added}}$$

where E_m is the molar extinction coefficient (0.053 μM^{-1}/cm). One unit is defined as the amount of enzyme needed to form 1 μmol of product in 1 min.

Laccase (EC 1.10.3.2) activity

Laccase activity was measured by monitoring the oxidation of ABTS (Wolfenden & Willson, 1982) in a citrate/phosphate (100 mM citrate, 200 mM phosphate) buffer (pH 4.5) at 420 nm. Briefly, five g of soil was extracted with 20 mL 100 mM citrate/phosphate buffer (pH 4.5) for 1 h and then centrifuged for 15 min at 4000 rpm. The supernatant was filtered through a 0.45 μm filter. The reaction mixture contained 900 μL soil extract and 100 μL 30 m MABTS solution. The absorbance was measured at 420 nm at 25°C for 1 min with Shimadzu UV 1800–A spectrophotometer. Absorbance per minute (Abs/min) was calculated from the linear range of the curve and laccase activity was calculated as:

Unit of enzyme g^{-1}soil

[5]

$$= \frac{\text{Abs}/min \times \text{mL of buffer added} \times 1000 \times \text{Volume}_{\text{reaction mixture}(\mu L)}}{E_m \times \text{Volume}_{\text{enzyme solution}(\mu L)} \times \text{Dry weight of soil}}$$

where Em is 0.036 μM^{-1}/cm.

Respiration

Respiration was measured as described previously (Stenström *et al.*, 2001) with some modifications. Two replicates of soil (10 g d.w.) were weighed into 250 mL respirometric jars. The jars were installed inside a respirometer, and the accumulation of CO_2 trapped in KOH solution (0.2 M; 10 mL) was determined automatically twice every hour for each jar by measuring the electrical conductivity. The soil samples were incubated until a constant basal respiration rate (BR) was established (after about 3 days) at a constant temperature of 22 °C and with a moisture content adjusted to pF 2. A substrate was prepared, consisting of glucose (7.5 g), $(NH_4)_2SO_4$ (1.13 g), KH_2PO_4 (0.35 g) and talcum powder (10 g), and 0.19 g of this mixture was thoroughly mixed into each jar. Empty jars were incubated as controls. The BR was calculated by

linear regression of accumulated CO_2 produced versus time. The instantaneous rate of CO_2 formation after addition of the substrate (substrate–induced respiration, SIR) was calculated using non–linear regression. The SIR was divided into the CO_2 production rate of active, exponentiallly growing (r) and dormant, non–growing (*K*) microorganisms as described by Stenström *et al.* (2001).

^{14}C–DHP mineralization

Synthetic ^{14}C–ring–labeled dehydrogenated polymerizate (^{14}C–DHP) of coniferyl alcohol (gift from Paul Ander, Department of Forest Products, SLU) with a molecular weight of 4–10 kDa and a specific activity of 0.16 MBq/mg was used to quantify lignin degrading activity in situ. The ^{14}C–DHP was added as a DMF–water suspension to 10 g dw of soil in 20 mL plastic jars. The final radioactivity was approximately 13,000 dpm per sample. The water contents were adjusted to pF 2. The plastic jars were each installed into air–tight glass jars together with scintillation vials containing NaOH (0.2 M; 4 mL) to trap carbon dioxide. The glass jars were incubated in the dark at 20°C and the base traps were changed regularly. The amount of ^{14}C in the base traps was measured on an LS 6000TA liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA) after mixing with 4 mL of Insta–gel Plus and incubated in the dark overnight. The ^{14}C liberated was corrected for the background radiation in controls without soil. Kinetic parameters describing ^{14}C–DHP mineralization were determined by non–linear regression according to first–order kinetics:

[6]

$$P = P_{max}\left(1-e^{-kt}\right)$$

where P is the accumulated ^{14}C–CO_2 released (% of the added ^{14}C) at time t, P_{max} is the maximum ^{14}C mineralized (% of applied) and k is the mineralization rate constant (day^{-1}).

Statistical analysis

General regression models (GRM) for best–subset–regression were fitted to the data, where replicate 1 was cross–validated with replicate 2 under the assumption of homogeneous variance. Hence, the two replicates were pooled for variance estimation, and all possible combinations of regressors examined with respect to explanatory power of the response variable (k). When the best–subset–regression models were built, our objective was to identify the subset of explanatory variables that combine optimal orthogonality with maximum explanatory power, in order to explain the variance of the response variable across soil samples. Orthogonality is synonymous with independence across regressor variables, and many methods have been suggested for estimating the ideal subset. Mallow's Cp statistic (Mallows, 1973) is an effective way to punish a linear combination of potential regressors with respect to multi–collinearity and accumulated error (Ryan, 1997). Mallow's Cp is identical to Akaike's information criteria when the generic variance σ^2 is known 'a priori'. Since we estimate σ^2 from our data, Mallow's Cp is a better choice. Statistical analyses were performed with STATISTICA™ (StatSoft, 1995).

Results and discussion

Degradation

The results from the degradation experiments are presented in Table 7.1.2.1.1-112. Bentazone, glyphosate and isoproturon degradation in soils generally followed first–order kinetics (all $R^2 > 0.91$ and statistically significant at $p < 0.001$). The degradation rate constants listed in Table 7.1.2.1.1-112 show considerable differences between soils with coefficients of variation ranging from 42 to 64 % for the three compounds. Degradation rate constants for bentazone were in the range 0.005–0.034/day which corresponds to half–lives of 20 to 139 days. Our data are consistent with those (8–133 days) reported by others (Rodriguez–Cruz *et al.*, 2006; Thorstensen & Lode, 2001). Degradation rate constants for isoproturon were in the range 0.011–0.104/day which corresponds to half–lives of 7–63 days. Again, this degree of variation is similar to that reported in the literature for isoproturon, with values ranging from 1.4 to 40 days (Larsbo *et al.*, 2009; Rodriguez–Cruz *et al.*, 2006; Walker *et al.*, 2001). The degradation rates of glyphosate (0.006–0.05/day, which correspond to half–lives of 14–116 days) are also consistent with other studies where the DT_{50} values in a variety of different soil types have been reported in the range of 1.7 to 197.3 days (Giesy *et al.*, 2000; Sorensen *et al.*, 2006).

Table 7.1.2.1.1-112: Degradation rate constant of bentazone, isoproturon, and glyphosate in different soils

Soils	Bentazone		Isoproturon		Glyphosate	
	k (day^{-1})	R^2	k (day^{-1})	R^2	k (day^{-1})	R^2
1	0.024 ± 0.002*	0.963	0.045 ± 0.002	0.981	0.044 ± 0.006	0.93
2	0.013 ± 0.001	0.984	0.031 ± 0.000	0.977	0.018 ± 0.001	0.95
3	0.019 ± 0.001	0.980	0.024 ± 0.001	0.986	0.032 ± 0.002	0.86
4	0.021 ± 0.001	0.986	0.044 ± 0.003	0.940	0.046 ± 0.002	0.88
5	0.015 ± 0.001	0.959	0.016 ± 0.001	0.980	0.031 ± 0.001	0.87
6	0.015 ± 0.002	0.972	0.041 ± 0.001	0.968	0.033 ± 0.003	0.97
7	0.010 ± 0.000	0.983	0.032 ± 0.005	0.990	0.024 ± 0.000	0.95
8	0.018 ± 0.002	0.976	0.062 ± 0.006	0.968	0.050 ± 0.001	0.89
9	0.032 ± 0.001	0.984	0.027 ± 0.000	0.941	0.027 ± 0.004	0.98
10	0.034 ± 0.001	0.986	0.015 ± 0.001	0.960	0.009 ± 0.001	0.95
11	0.014 ± 0.000	0.936	0.027 ± 0.003	0.983	0.038 ± 0.001	0.96
12	0.015 ± 0.002	0.961	0.034 ± 0.001	0.967	0.029 ± 0.001	0.96
13	0.006 ± 0.000	0.987	0.023 ± 0.003	0.964	0.042 ± 0.000	0.95
14	0.005 ± 0.000	0.910	0.011 ± 0.001	0.933	0.027 ± 0.000	0.97
15	0.017 ± 0.002	0.985	0.104 ± 0.002	0.937	0.028 ± 0.001	0.96
16	0.023 ± 0.002	0.968	0.077 ± 0.007	0.939	0.032 ± 0.004	0.98
Mean	0.018		0.039		0.028	
CV %	46		64		42	

* indicates the ± standard deviation of two replicates.

Correlations between variables

Soil physical, chemical and microbial parameters

Correlations between basic soil properties, microbiological parameters, sorption strength and the degradation rate of pesticides are reported in Table 7.1.2.1.1-113. As is quite typical, the sandy soils in our catchment (large dg values) generally had lower pH and SOC contents than the finer–textured loamy and clayey soils (Table 7.1.2.1.1-113). Activities of lignimolytic enzymes (MnP and laccase) were highly variable in our soils. Sinsabaugh *et al.* (2008) found a coefficient of variation for phenol oxidase (e.g., laccase) and peroxidase (e.g., MnP) activities among ecosystems of nearly 300 %. This variability can be attributed to differences in both the enzymology of various enzyme–producing white–rot species and differences in growth and enzyme production responses of the fungi to different soil and environmental factors (Sinsabaugh, 2010). Correlation analysis suggested that significantly higher enzyme activities in our soils were associated with higher soil organic carbon and soil pH (Table 7.1.2.1.1-113). MnP was positively correlated with SOC ($r = 0.78$; $p < 0.0001$). For peat soils, Sinsabaugh *et al.* (2008) also found that peroxidase activity increased with SOC. The activity of laccase was positively correlated with soil pH ($r = 0.55$; $p < 0.001$). This has also been found in other studies (Sinsabaugh, 2010; Sinsabaugh *et al.*, 2008). Laccases deprotonate at high soil pH, which reduces their redox potential and increases their solubility, both of which may enhance their reaction potential (Sinsabaugh, 2010). Because laccases are widely produced for varied purposes, it is arguable that the diversity of the soil enzyme pool and potentially its range of action may also increase with soil pH (Sinsabaugh *et al.*, 2008). Soil pH is also known to be an important predictor of microbial diversity (Sinsabaugh, 2010). Thus, SIR was positively correlated with soil pH, as well as SOC and available potassium, whereas it was negatively correlated with d_g and available P. The proportion of active microorganisms (r) was positively correlated with SOC, available K, and MnP whereas it was negatively correlated with d_g.

Table 7.1.2.1.1-113: Linear correlation coefficients

	pH	q_e	SOC	P	K (Potassium)	MnP
q_e	0.621**					
SOC	0.186	−0.479*				
P	−0.32	0.299	−0.254			
K (potassium)	0.057	−0.605**	0.645***	0.018		
MnP	−0.132	−0.172	0.777***	0.041	0.700**	
Laccase	0.546**	−0.305	−0.274	−0.432*	−0.26	−0.588**
SIR	0.458*	−0.699***	0.494*	−0.489*	0.516**	0.323
r (active)	−0.015	−0.455*	0.834***	−0.448*	0.578**	0.548**
DHP k	0.099	−0.032	0.509**	0.229	0.253	0.648**
Bentazone k	0.448*	−0.655**	0.736***	−0.706***	0.477*	0.701
Isoproturon k	0.592**	−0.295	−0.169	−0.268	−0.115	0.1
Glyphosate k	0.516**	−0.053	−0.503**	−0.223	−0.492*	0.5
Bentazone K_f	−0.286	−0.178	0.551**	0.035	0.610**	0.803***
Isoproturon K_f	0.025	−0.496*	0.814***	−0.221	0.697**	0.719***
Glyphosate K_f	−0.523**	0.059	0.428*	0.054	0.444*	0.363

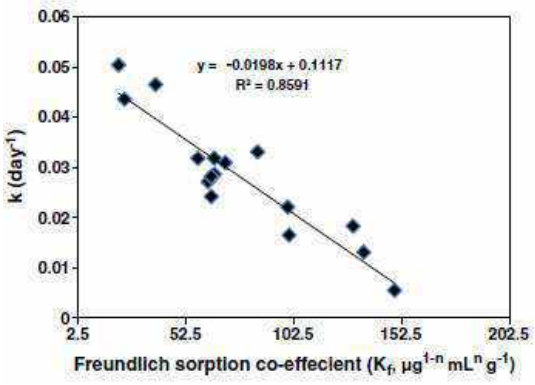
*, **, and *** indicate significance at p < 0.1, 0.05 and 0.01, respectively.

Laccase	SIR	r	DHP k	Bentazone k	Isoproturon k	Glyphosate k
0.324						
−0.084	0.646***					
−0.504**	−0.019	0.13				
0.204	0.761***	0.787***	0.077			
0.546**	0.429*	−0.054	−0.274	[illegible]		
0.588**	−0.026	−0.536**	−0.03	0.10	0.413	
−0.423	0.525**	0.555**	0.[illegible]	0.274	−0.14	−0.538**
−0.225	0.629***	0.761***	[illegible]	0.694	−0.23	0.413
−0.404	−0.078	0.498**	[illegible]	0.112	0.11	−0.924***

Pesticide degradation

Glyphosate degradation was significantly positively correlated to soil pH and laccase activity and negatively correlated with SOC and K_f. The strong relationship between glyphosate degradation and the Freundlich sorption coefficient is illustrated in Figure 7.1.2.1.1-35. A negative correlation between glyphosate adsorption and degradation in soil has also been reported by others (Zablotowicz *et al.*, 2009).

Figure 7.1.2.1.1-35: Relationship between the degradation rate constant k (day^{-1}) for glyphosate and the Freundlich sorption coefficient (K_f, μg^{1-n} mL^n g^{-1}).

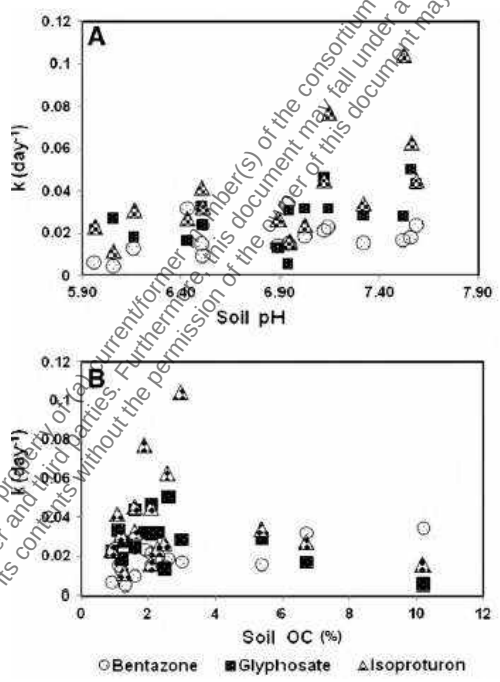


In this catchment, the finer-textured loamy and clay soils of higher pH showed faster degradation than sandy soils of low pH (Figure 7.1.2.1.1-36 A and Table 7.1.2.1.1-112). Soil bacterial diversity and richness decline as pH decreases (Sinsabaugh *et al.*, 2008) and other studies have also found pesticide persistence

to increase as soil pH decreases (Walker *et al.*, 2001). However, caution should be exercised in interpreting our data, since pH and SOC are strongly (positively) correlated if the three locations with highly organic (peaty) topsoils are excluded.

Figure 7.1.2.1.1-36 B shows that the influence of SOC on pesticide degradation was rather complicated. An increase in soil organic matter increases biological activity and pesticide degradation rates in soil by providing conditions favorable to microbial growth (Thorstensen & Lode, 2001). On the other hand, pesticide sorption in soil, which is often positively related to SOC (Kah *et al.*, 2007), may reduce the bioavailability of pesticides (Boivin *et al.*, 2005; Kah *et al.*, 2007; Sorensen *et al.*, 2006; Thorstensen and Lode, 2001). Many studies have therefore demonstrated strong negative relationships between pesticide sorption and degradation in soils (Bolan & Baskaran, 1996; Dyson *et al.*, 2002; Lehmann *et al.*, 1992). In our study we observed a strong negative relationship between glyphosate degradation and its Freundlich sorption coefficient (Figure 7.1.2.1.1-35). The competing effects of organic matter content on microbial activity and sorption (bio–availability) mean that both positive and negative relationships between sorption and degradation have been reported, as well as non–monotonic relation–ships which display an optimum (Bolan & Baskaran, 1996), as in Figure 7.1.2.1.1-36 B for isoproturon.

Figure 7.1.2.1.1-36: Relationships between soil pH (A) and organic carbon content (B) and the degradation rate constants of all three pesticides



Regression analysis

Table 7.1.2.1.1-114 shows the results of best-subset regression analysis. The use of Mallows Cp led to the selection of five out of 12 potential regressor variables: soil pH, SOC, r, laccase, and K_f. Soil pH and r together explained 69 % of the total variation in the bentazone degradation rate constant. There was, however, a problem with this model, arising from the skewed distribution of r, which resulted in heteroscedasticity. After applying Box–Cox transformation to the original r variable (rBC), we obtained a regression equation for which the residual distributions were approximately normal homoscedastic:

[7]

$$k = 0.006(\mathrm{pH}) + 0.004(\mathrm{rBC}) - 0.012$$
$$R^2 = 0.57, \mathrm{Adj}\, R^2 = 0.51, F(2,14) = 8.75, p < 0.01$$

As an alternative model, pH and SOC explained 56 % of the variation in bentazone degradation, with an

acceptable behaviour of the residuals (Table 7.1.2.1.1-114). This is because SOC and *r* are strongly correlated (Table 7.1.2.1.1-113). For glyphosate, 88 % of the variation in degradation rate coefficient could be explained by the Freundlich co–efficient K_f and soil laccase activity. Soil pH was the most significant predictor (pb 0.05) for isoproturon degradation and the inclusion of two more terms (SOC and r) significantly increased R^2 from 0.29 to 0.42 (Table 7.1.2.1.1-114).

Table 7.1.2.1.1-114: Best subset regression models relating first–order degradation rate constants for bentazone, glyphosate and isoproturon to soil parameters (β is the unscaled regression coefficient)

Pesticide	Intercept	Soil pH	SOC	r	Laccase	K_f	Overall performance
Bentazone	−0.030	β = 0.006 p <0.05 F (1, 15) = 8.25	–	β = 0.007 p<0.001 F (1, 15) = 25.2	–	–	Adj R^2 = 0.69 F (2, 14) = 17.8 p <0.001
	−0.024	β = 0.005 p = 0.07 F (1, 15) = 3.85	β = 0.002 p<0.01 F (1, 15) = 13.9	–	–		Adj R^2 = 0.56 F (2, 14) = 10.6 p<0.01
Glyphosate	0.04	–	–	–	β = 0.00001 p <0.05 F (1, 15) = 7.5	β = 0.0002 p<0.0001 F (1, 15) = 60.7	Adj R^2 = 0.88 F (2, 14) = 56.5 p<0.000
Isoproturon	−0.14	β = 0.030 p<0.05 F (1, 15) = 7.08	–	–	–	–	Adj R^2 = 0.29 F (1, 15) = 7.08 p<0.05
	−0.17	β = 0.032 p<0.01 F (1, 15) = 11.6	β = −0.008 p<0.05 F (1, 15) = 4.9	β = 0.02 p = 0.13 F (1, 15) = 2.5	–	–	Adj R^2 = 0.42 F (3, 13) = 4.57 p<0.05

As discussed above, these predictor variables are more or less strongly correlated, both with each other and with other potential predictors (Table 7.1.2.1.1-113). Furthermore, multiple linear regression models comprising linear additive terms (e.g. for SOC and K_f) cannot reproduce observed non–monotonic relationships between degradation rate coefficients and either sorption constants or soil organic carbon content (see Figure 7.1.2.1.1-36 B). It may therefore be more fruitful to develop models based on a mechanistic understanding of the processes controlling degradation. For microbial degradation, Allen and Walker (1987) suggested that degradation rates should be controlled by some measure of microbial activity multiplied by a factor related to the bio–availability of the compound. We can write:

[8]

$$k = k_{ref} \cdot (B)^m \cdot (A)^n$$

where *k* is the degradation rate constant, k_{ref} is a pesticide–specific reference rate coefficient which, in addition to the influence of variables not included in the model, should be related to the inherent degradability of the compound as determined by its molecular structure, *m* and *n* are constants, *A* is some measure of microbial activity and B is some measure of bioavailability. We tested six different forms of Eq. 8, combining three potential descriptors of microbial activity (laccase activity, SIR, and SOC) with two for bioavailability, K_f or the calculated fraction of pesticide in soil solution, Fs, given by:

[9]

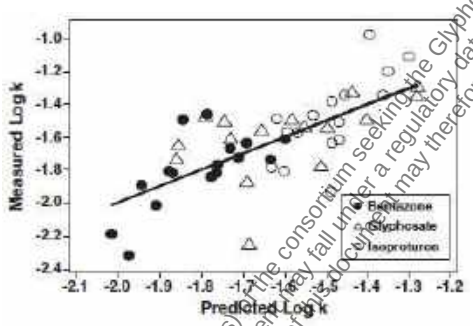
$$F_s = \frac{m_g}{m_g + K_f c_e^{n-1}}$$

where m_g is the gravimetric water content at pF 2 and c_e is the equilibrium concentration of the pesticide in solution at the start of the incubation experiment, which was iteratively calculated from the applied amount, the gravimetric moisture content and the parameters of the Freundlich equation. Although pH could also have been considered, we chose SOC as a surrogate variable for microbial activity, since it was strongly correlated to the microbial parameters MnP, SIR and r.

The data for all three pesticides were fitted to the logarithmic form of Eq. (8). The parameter values were estimated by introducing three 'class' variables into the data set (B, G, and I for bentazone, glyphosate and isoproturon, respectively, which take values of either 1 or zero) and k_{ref} values were obtained as regression

coefficients for G, I and B. Table 7.1.2.1.1-115 shows that several of the models fitted the data well, especially models 1, 3 and 5, which had R^2 values ranging from 39 to 50 % and regression co–efficients that were all significant ($p < 0.1$). As an example, Figure 7.1.2.1.1-37 shows a comparison of measured k with predictions using model 1 (i.e. Eq. (8) based on K_f and SIR). In contrast, model 6 (using Fs with SOC as a surrogate measure of microbial activity) gave the poorest results, with no significant effect of SOC and clear bias in the residuals (not shown). However, after excluding glyphosate from the model, the overall regression became highly significant ($p < 0.0001$) and the distribution of residuals was unbiased. It is interesting that bioavailability, as reflected in the parameter F_s, emerges here as a significant factor controlling degradation rates of bentazone and isoproturon, something which was not readily apparent from the classical correlation and regression analysis. Furthermore, although K_f is a very good predictor of glyphosate degradation (Figure 7.1.2.1.1-35), F_s is not. The reason for this is not clear.

Figure 7.1.2.1.1-37: Comparison of measured degradation rate constants with those predicted using model 1 (see Table 7.1.2.1.1-115)



No single model of pesticide degradation can be generally valid (Kah *et al.*, 2007) as the mode of degradation varies considerably between compounds (e.g. chemical hydrolysis, co–metabolic or metabolic microbial degradation). However, although further testing is required, these results suggest that at least for some particular classes of pesticides, a multiplicative model based on soil organic carbon content and the sorption co–efficient (e.g. models 3 and 6, Table 7.1.2.1.1-115) may be an effective and practical way to account for the effects of microbial activity and bio-availability on pesticide degradation in the context of modeling applications at catchment or regional scales.

Table 7.1.2.1.1-115: Parameter values and their significance for different models developed to predict degradation rate for all three pesticides together

With K_f in the models					With F_s in models				
Model	Parameters	Values	P value	Centered R^2	Model	Parameters	Values	P value	Centered R^2
1	Log ($k_{ref(B)}$)	2.264	<.0001	0.50	4	Log ($k_{ref(B)}$)	−1.797	<.0001	0.42
	Log ($k_{ref(G)}$)	−0.893	0.0006			Log ($k_{ref(G)}$)	−0.659	0.1515	
	Log ($k_{ref(I)}$)	−1.472	<.0001			Log ($k_{ref(I)}$)	−1.182	<.0001	
	Log K_f	−0.500	0.0005			Log F_s	0.433	0.0179	
	Log SIR	0.366	0.0006			Log SIR	0.176	0.1134	
2	Log ($k_{ref(B)}$)	−2.825	<.0001	0.45	5	Log ($k_{ref(B)}$)	−2.586	<.0001	0.49
	Log ($k_{ref(G)}$)	−2.106	<.0001			Log ($k_{ref(G)}$)	−1.510	0.0076	
	Log ($k_{ref(I)}$)	−2.296	<.0001			Log ($k_{ref(I)}$)	−1.991	<.0001	
	Log K_f	−0.218	0.1408			Log F_s	0.804	0.0149	
	Log Laccase	0.353	0.0057			Log Laccase	0.344	0.0038	
3	Log ($k_{ref(B)}$)	−2.166	<.0001	0.39	6	Log ($k_{ref(B)}$)	−1.659	<.0001	0.38
	Log ($k_{ref(G)}$)	−0.715	0.0179			Log ($k_{ref(G)}$)	−0.295	0.4889	
	Log ($k_{ref(I)}$)	−1.336	<.0001			Log ($k_{ref(I)}$)	−0.972	<.0001	
	Log K_f	−0.530	0.0029			Log F_s	0.538	0.0030	
	Log SOC	0.242	0.0773			Log SOC	−0.029	0.8036	

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article investigates data of degradation and sorption tests performed for glyphosate on several Swedish agricultural soils. The analytical methods are not provided in detail, thus not allowing to check whether analytical methods could fulfill the requiremnts as set out for EU data generating methods including the appropriateness of LOD or LOQ. For the sorption experiment, no results are provided. No mass balances and measurement per sample date are provided for both experiments.
The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/019
Report author	Alexa, E. *et al.*
Report year	2010
Report title	Studies on the biodegradation capacity of ^{14}C–labelled glyphosate in vine plantation soils
Document No	ISSN 1459-0225
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Glyphosate is among the most widely used broad spectrum herbicides in the world because they are highly efficacious, cost effective, practically non–toxic and degrade readily in the environment. The herbicide is inactivated and biodegraded by soil microbes, degradation rate depends on soil microbial activity and factors that affect this activity. Glyphosate degradation rates vary considerably across a wide variety of soil types and microflora population types. The aim of this paper was to study the biodegradation capacity of glyphosate in soil samples collected from vine plantation from Timis county, Romania, belonging to Banat's University of Agricultural Science, Timisoara, in presence of organic and inorganic supplement, at different concentration levels. After addition of glyphosate–phosphonomethyl–^{14}C–labeled, the accumulated $^{14}CO_2$ (as % of total ^{14}C) was monitored during 44 days. Investigated soil shows a high degradation capacity of over 85 % of total radioactivity after 44 days from the treatment application. Addition of inorganic supplement causes a decrease of glyphosate biodegradation capacity to 10.77–12.87 % of total radioactivity, while in presence of straw the accumulated $^{14}CO_2$ (as % of total ^{14}C) during the 44 days ranged between 59.97 and 87.58 %. The amount of $^{14}CO_2$ released reached the highest level in the first 4 days after herbicide application, both in control and experimental variants with organic and inorganic supplement (from 2.61 to 30.27 % of total radioactivity). By glyphosate addition the growth and multiplication of soil microorganisms, whose biomass is digested in the range of 9–12 days of treatment, according to the daily mineralization rate (DMR) values, is stimulated. Our results on the activity of microorganisms showed that glyphosate degradation in soil is mainly performed by micromyces.

Materials and methods

Chemicals and soil samples

Glyphosate-phosphonomethyl–^{14}C– labeled (Sigma) lot number 012K9428/29, specific activity 2.2 mCi mmol^{-1} and commercially formulated glyphosate of isopropylamine ammonium salt (Roundup) were purchased from Monsanto, Romania. Liquid Scintillation Cocktail (Quicksafe A cocktail) was used in Triathler Liquid Scintillation Counting. All other reagents were of analytical reagent grade.

The soil characterized as cambic moderately gleyed chernozem were sampled in March 2010 from the vine plantation (Burgundy grape variety) of Banat's University of Agricultural Science in Timisoara (Western part of Romania). Sampling depth was between 0 and 10 cm. The glyphosate treatments and both organic and inorganic fertilizers are usually applied in grape–vine plantation. The soil samples were dried at room temperature for 48 h and crushed to pass a 2 mm sieve.

The basic physico–chemical soil characteristics and chemical composition of added inorganic and organic supplements were as follows:

- soil: clay 42.1 %; sand 29.2 %; silt 28.7 %; pH in H_2O 7.93; organic matter 3.95 %; N total 0.266 %; P 30 ppm; Fe 20,340 ppm; Cu 10 ppm; Mn 300 ppm; Zn 8 ppm

- organic supplement: pH 6.5; N_{total} 14 %; organic matter 7.5 %; P 30 ppm; Zn 35.89 ppm; Cr 42.60 ppm; Ni 25.61 ppm; Cu: 31.51 ppm; Cd 2.01 ppm; Fe 487.7 ppm

- inorganic supplement: N_{total} 15 %; P_2O_5 5 %; K_2O 20 %; CaO 2 %; MgO 1 %; S 9 %; Cu 0.1 %; Fe 0.1 %; Mn 0.5 %; Zn 0.1 %

- wheat straw: cellulose 35 %; lignin 18 %; ash 8 %; hemicellulose 35 %

^{14}C–labelled glyphosate biodegradation radio-assay

Evaluation of ^{14}C–labelled glyphosate biodegradation was done according to Getenga *et al.* using liquid scintillation counter Triathler (Finland) for radio–assay. In the incubation experiment, 25 g soil samples in duplicates were placed in biometer flasks. The soil was conditioned by being moistened to 85 % of the field water capacity. The biometer flask content is a plastic vial with soil treated with glyphosate, a vial containing 10 ml distillated water, which assures atmosphere saturation with water vapor and a plastic vial filled with 10 ml 0.2 M NaOH, to trap the $^{14}CO_2$ released during mineralization by soil microorganisms. Non–labelled glyphosate solution in distilled water in concentration of 1.5 ppm was added to each soil sample and the initial radioactivity was done by glyphosate–phosphonomethyl–^{14}C–labeled with specific activity 0.5 mCi. The soils were incubated at 20°C, in the dark for 44 days. In order to evaluate the biodegradation of ^{14}C–labeled glyphosate during the incubation period, samples were taken every 4 days. The NaOH solution was mixed with 5 ml of Quicksafe A cocktail in a 20 ml scintillation vial before it was radio–assayed. After every sampling the vial was refilled with fresh 0.2 M NaOH. The amount of $^{14}CO_2$ released during mineralization was quantified on the base of ^{14}C disintegration number.

By adding the percentages at each sampling, the total amount of mineralized glyphosate depending on time is obtained. The mineralization curves of $^{14}CO_2$ accumulated were compared during 44 days.

The experimental treatments were: Control – soil with glyphosate in concentration of 1.5 ppm; OSI – soil with glyphosate and addition of organic supplement 3.2 %; OSII - soil with glyphosate and addition of organic supplement 6.4 %; ISI – soil with glyphosate and addition of inorganic supplement 8 %; ISII – soil with glyphosate and addition of inorganic supplement 16 %; WSI – soil with glyphosate and addition of wheat straw 1 %; WSII – soil with glyphosate and addition of wheat straw 2 %.

Evaluation of microbial response parameters

Microbial communities in soils treated with glyphosate were evaluated using the method described by Seeley *et al.* 20. A soil sample (about 20 g) was treated with 1.5 ppm glyphosate unlabeled solution (Roundup) and incubated at 22 ± 3°C in an Erlenmeyer flask. Daily humidity was corrected so that it does

not to fall below 75–80 % of the wet field capacity. After 3 and 10 days we determined the number of culturable microorganisms using the count plate method. For the quantitative determination of eubacterias we used Topping medium: yeast extract 0.25 %, peptone powder 0.25 %, agar 1.8 % and distillated water, pH 7.6. To quantify the number of actinomycetes we used Gause medium: KNO_3 0.1 %, K_2HPO_4 0.05 %, $MgSO_4$ 0.05 %, NaCl 0.05 %, $FeSO_4$ 1 %, corn starch 2 %, agar 2 %, distillated water, pH 7, and for estimation of the micromycetes number we used Czapek Dox medium: $NaNO_3$ 0.3 %, K_2HPO_4 0.1 %, $MgSO_4$ 0.05 %, KCl 0.05 %, $FeSO_4$ 0.001 %, sucrose 0.3 %, agar 1.5 %, pH 5.5. To secure a microbial count the samples were diluted (in 0.1 % sodium pyrophosphate) and plated, and after incubation the colonies that develop were counted. The microbial count of the original samples was then determined by multiplying the average number of colonies that develop by the degree of dilution (dilution factor of the samples in the plate). Dilutions, expressed as negative exponents, were 10^{-5} for micromycetes and 10^{-7} for eubacterias and actinomycetes determinations. The results were expressed in colony forming units (CFU) per g soil (dry matter).

Results

^{14}C–glyphosate calibration

^{14}C–glyphosate calibration was done on the basis of quench curve method. The curve establishes the relationship between a quench parameter (QP) and the counting efficiency. Quench parameter indicates a relative light production from the sample. In the Triathler the quench parameter (QP) is, in mathematical terms, the center of spectrum gravity in the counting window. The collective effect of quench is a reduction in the number of photons produced and, therefore, detected CPM (counts per minute). The Triathler uses parabolic regression to form the curve. First the quench curve was made by counting a set of standard samples with the same activity but variable quench (Table 7.1.2.1.1-116). The Triathler prints the quench parameters and the corresponding efficiencies of the standards. When unknown samples are counted, the quench parameter is measured for each sample. Corresponding efficiency for the measured quench parameter is obtained from the curve and the DPM (desintegrations per minute or absolute radioactivity) corresponding value is calculated (DPM = CPM·Eff^{-1}). The efficiency taken from the curve and an error percentage (err %), which is the difference of efficiencies (difference between measured eff. and the one taken from the quench curve, are indicated in Table 7.1.2.1.1-117.

Table 7.1.2.1.1-116: The data recorded for Triathler calibration

Sample	Time (s)	Counts	CPM	QP
1	300	1,094,490	218,838	44.144
2	300	550,900	110,180	42.995
3	300	489,535	97,907	34.861
4	300	108,650	21,730	28.142
5	300	27,095	5,419	23.558

Std DPM: 220,000.
Eff.= - 0.0006·qp2 + 0.0743·qp - 1.2895.

Table 7.1.2.1.1-117: The efficiencies obtained on the basis of data analysis

Eff	QP	Eff	err (%)
0.99472	44.14	0.99472	0.00
0.50082	43.00	0.50082	0.00
0.44503	34.86	0.44503	0.00
0.09877	28.14	0.09877	0.00
0.02463	23.56	0.02463	0.00

Results regarding ^{14}C–glyphosate biodegradation

Experimental results regarding the amount of $^{14}CO_2$ (%) released reported to total initial radioactivity, in accordance with prelevation chart are represented in Table 7.1.2.1.1-118. The biodegradation degree of

glyphosate in soil was estimated as ratio between the number of ^{14}C–glyphosate disintegration in the sample and the number of disintegration in the standard. From these data it can be observed that in both control and experimental variants with organic and inorganic supplement addition, the amount of $^{14}CO_2$ released recorded the maximum value in the first 4 days after herbicide application, ranging from 2.61 % to 30.27 % of total radioactivity. The biodegraded glyphosate amount decreases for all analyzed samples, being less than 1 % after 44 days. The experimental results are in accordance with previous data obtained, which show that the glyphosate biodegradation has only two phases, the initial rapid phase for about 20 days due to microorganisms action on free glyphosate in soil followed by a slow final phase when the microorganisms act on glyphosate adsorbed on the soil compounds. From Table 7.1.2.1.1-118 it can be observed that, for control, $^{14}CO_2$ resulting from the glyphosate decomposition reached maximum value after 4 days (30.27 %) and decreases with time advancing: 20.27 % after 8 days, 11.86 % after 12 days, 10.94 % after 16 days and only 3.94 after 20 days reaching 0.35 mg $^{14}CO_2$ after 44 days. In Figure 7.1.2.1.1-38 a–c the glyphosate mineralization curves expressed as accumulated $^{14}CO_2$ as % from total radioactivity are represented. Accumulated $^{14}CO_2$ in the case of control sample, without fertilizers, increased from 30.24 % after 4 days, to 50.54 % after 8 days from herbicide application, respectively, 80 % after first 24 days and slow growing to 85.96 % of total radioactivity after 44 days (Figure 7.1.2.1.1-38 a). The soil characteristics influence the degradation capacity of glyphosate in the presence of microorganisms. In the literature there are several papers describing that the adsorption of glyphosate by soils depends on cationic exchange capacity, clay content, pH and organic matter. Studies regarding the effect of pH on the adsorption of glyphosate by soils and clays agreed that an increase of pH decreased the adsorption of glyphosate. It was due to an increase in negative charge of glyphosate and mineral surface with an increase in pH value resulting in a decrease in the adsorption. The analyzed soil has a high content of clay (42.1 %), iron and pH in H_2O (7.93). The experimental results show a high glyphosate biodegradation capacity in control sample (85.96 % after 44 days) and availability of glyphosate to microorganisms, due to low level of glyphosate adsorption on soil particles, according to other studies.

Table 7.1.2.1.1-118: Impact of added supplement on $^{14}CO_2$ release (% of total radioactivity)

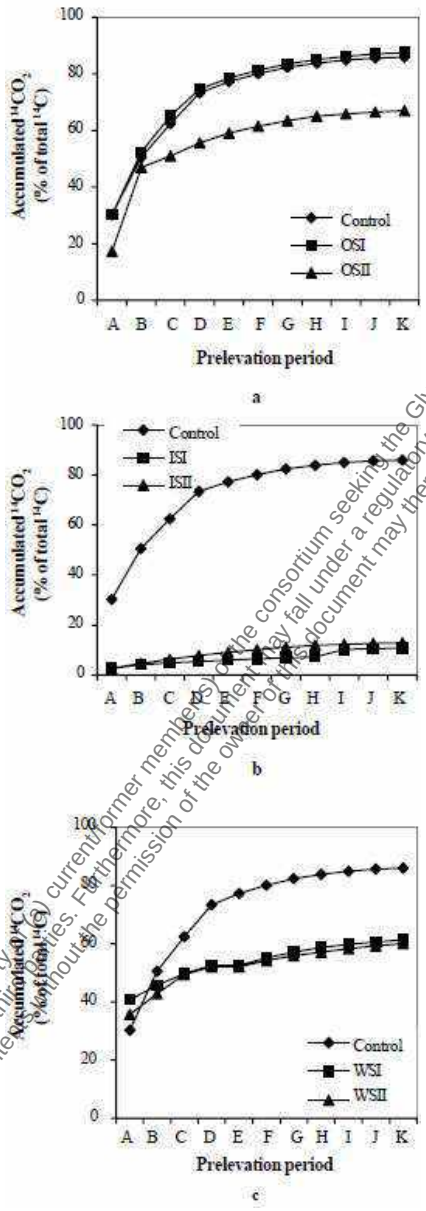
Variant	Accumulated $^{14}CO_2$ (% of total ^{14}C) in the prelevation time (A-K)										
	A	B	C	D	E	F	G	H	I	J	K
Control	30.27	20.27	11.86	10.94	3.9	2.82	2.27	1.47	1.12	0.69	0.35
OSI	30.23	19.13	12.76	9.54	3.86	2.8	2.27	1.47	1.12	0.91	0.49
OSII	33.66	16.38	13.24	4.13	3.9	2.53	1.9	1.62	0.7	0.7	0.49
ISI	2.61	1.5	0.69	0.62	0.54	0.48	0.46	0.36	0.3	0.26	0.25
ISII	2.73	1.98	1.03	1.49	1.28	1.1	0.91	0.76	0.41	0.42	0.2
WSI	25.51	15.27	4.86	4.08	2.78	2.62	1.94	1.58	1.13	0.81	0.73
WSII	21.96	13.71	7.13	6.58	2.64	2.17	1.65	1.24	1.19	0.96	0.74

In Figure 7.1.2.1.1-38 a–c the glyphosate mineralization curves expressed as accumulated $^{14}CO_2$ as % from total radioactivity, in the case of organic and inorganic supplement addition are represented. The experimental results obtained show significant differences between the amount of biodegradable glyphosate according to the type and amount of organic or inorganic fertilizer added. Addition of organic fertilizer at a rate of 3.2 % does not lead to significant changes in curve shape of glyphosate mineralization (Figure 7.1.2.1.1-38 b). Increasing the amount of organic fertilizer to 6.4 % leads to decrease in the amount of released $^{14}CO_2$. Total accumulated $^{14}CO_2$ after 44 days from the glyphosate application was 87.58 % for organic substrate addition OSI, respectively, 67 % in case of organic substrate addition of OSII. Our results are in accordance with those of Getenga and Kengara, showing that compost addition does not stimulate intense mineralization of glyphosate by microbes.

Mineralization curves in Figure 7.1.2.1.1-38 b show the reduced availability of glyphosate to biodegradation in the presence of inorganic fertilizers. In case of mineral fertilizers addition in a proportion of 8 % of inorganic supplement, the amount of $^{14}CO_2$ 4 days after the herbicide administration was 2.61 % of the total radioactivity and decreased slowly reaching 0.25 % between 40 and 44 days. Biodegradation capacity of glyphosate in the presence of mineral fertilizers was much reduced compared to the control sample (Table 7.1.2.1.1-118, Figure 7.1.2.1.1-38 b). The total amount of $^{14}CO_2$ released after 44 days was

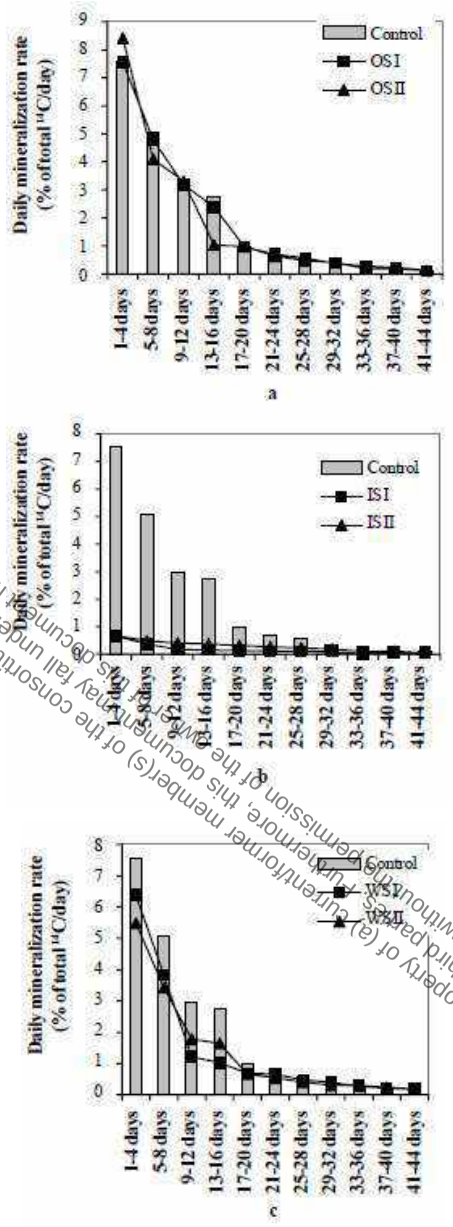
only 10.77 % in case of ISI and 12.87 % in case of ISII.
From Table 7.1.2.1.1-118 it can be observed that the biodegraded glyphosate percentage was between 2.61 to 2.73 % after the first 4 days and decreased to 0.2 % after 44 days of experimentation. Glyphosate contains functional groups of amine, carboxylate and phosphonate that can form strong coordination bonds with metal ions to give bidentate and tridentate complexes. Addition of inorganic fertilizers rich in metal ions leads to decrease in glyphosate biodegradation ability and reduces the amount of $^{14}CO_2$ released. Cruz *et al.* studied the competitive adsorption between glyphosate and phosphate in different Brazilian soils. The results showed that on the clays glyphosate was not easily displaced by phosphate even in the ratio of 10.0 of phosphate/glyphosate. Our results are in accordance with those because in analyzed soil with high content of clays (42.1 %) the glyphosate is not displaced by phosphate ions. On the other hand, the addition of inorganic fertilizer rich in phosphate and nitrate led to micro–organisms orientation on nitrogen and phosphate source easily accessible, respectively, reduced availability of glyphosate for biodegradation. Thus, the amount of $^{14}CO_2$ released is 10 times lower in variants fertilized with inorganic supplement (ISI, ISII). The increased content of mineral fertilizer, in the case of ISII, did not lead to significant changes regarding the release of $^{14}CO_2$ from the glyphosate biodegradation. In WSI and WSII where wheat straw at a rate of 1 % and 2 % was added, there was a noticeable decrease in the amount of $^{14}CO_2$ pursued as a result of glyphosate biodegradation compared with the control. Thus, after 4 days the percentage of released $^{14}CO_2$ was 25.51 % in WSI and 21.96 % in WSII (Table 7.1.2.1.1-118). After 8 days from the glyphosate application, the biodegradation capacity decreased to 4.86 and 7.13 %. $^{14}CO_2$ total amount accumulated as a result of glyphosate biodegradation was 61.31 %, in the case of 1 % straw addition and 59.97 % to 2 % straw addition (Figure 7.1.2.1.1-38 c).

Figure 7.1.2.1.1-38: Mineralisation of ^{14}C–glyphosate in soil with different supplements (a– control versus OS, b– control versus IS, c– control versus WS)



The daily mineralization rate (DMR) of glyphosate (Figure 7.1.2.1.1-39 a–c) in the case of different supplement addition was highest for all variants in the first 4 days of experiment, decreasing during incubation. If mineral fertilizers were added (ISI, ISII), the DMR value was much lower than in other cases. The explication is due to existing mineral compounds intake in inorganic fertilizers, compounds with which glyphosate forms complexes hard accessible for microbial metabolism but also, due to lack of energy substrate supporting the respiratory activity of microorganisms.

Figure 7.1.2.1.1-39: Daily mineralization rate of glyphosate (a– control versus OS, b– control versus IS, c– control versus WS)



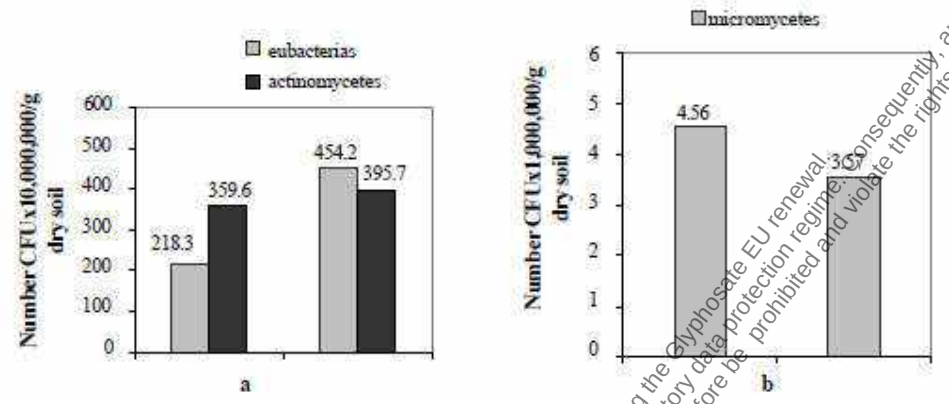
Besides, in the organic material addition, OSI, OSII, WSI and WSII, DMR value was highest in the first 4 days of experimentation. The highest values of $^{14}CO_2$, corresponding to DMR, were determined in OS even exceeding the control (7.567 mg $^{14}CO_2$), DMR value was higher than control value also for range of 9–12 days. This could be due to labelled carbon release from the microbial protoplasm which assimilates the labelled glyphosate, respectively of fungal biomass.

Results regarding microorganism activity in soil

Glyphosate remains unchanged in the soil for varying lengths of time, depending on soil texture and organic matter content. Soil microorganisms break down glyphosate and many can use glyphosate as a sole source of phosphorus. On the base of results regarding the number of culturable microorganisms existing in the soil with glyphosate (Figure 7.1.2.1.1-40 a, b) it can be observed that at 10 days after the treatment

application, the eubacteria number increases from $218.3x10^5$ to $454.2x10^5$ CFU g^{-1} dry soil.

Figure 7.1.2.1.1-40: The variations of microorganisms number after 3 and 10 days since glyphosate addition in control a) eubacterias and actinomycetes, b) micromycetes



Conclusion

The soil characteristics influence the degradation capacity of glyphosate in the presence of microorganisms. The soil sampled from the vine plantation (Burgundy grape variety) of Banat's University of Agricultural Science in Timisoara shows a high degradation capacity, over 85 % of total radioactivity after 44 days from the treatment application. Addition of inorganic substratum causes a decrease in glyphosate biodegradation capacity to 10.77 – 12.87 % of total radioactivity, while in presence of straw the accumulated $^{14}CO_2$ (as % of total ^{14}C) during the 44 days ranged between 59.97 – 87.58 %. The amount of $^{14}CO_2$ released reached the highest level in the first 4 days after herbicide application both in the control and experimental variants with organic and inorganic substratum (from 2.61 to 30.27 % of total radioactivity). The growth and multiplication of soil microorganisms whose biomass is digested in the range of 9 – 12 days of treatment, according to the daily mineralization rate (DMR) values is stimulated by glyphosate addition. Our results on the activity of microorganisms have shown that glyphosate degradation in soil is mainly performed by micromyces.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article investigates the degradation of glyphosate in a European agricultural soil originating from vine in the laboratory. Only data on mineralisation are reported. Further data like mass balances, residues in soil and a half-life are not reported. The validity of the study cannot be evaluated due to missing information.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.1.1/020
Report author	Al–Rajab, A., Schiavon, M.
Report year	2010
Report title	Degradation of ^{14}C–glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils
Document No	DOI 10.1016/S1001-0742(09)60264-3 ISSN 1001-0742
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Glyphosate (N–phosphonomethyl glycine) is the most used herbicide worldwide. The degradation of ^{14}C–labeled glyphosate was studied under controlled laboratory conditions in three french agricultural soils: a silt clay loam, a clay loam and a sandy loam soil. The kinetic and intensity of glyphosate degradation varied considerably over time within the same soil and among different types of soil. Our results demonstrated that the mineralization rate of glyphosate was high at the beginning of incubation and then decreased with time until the end of the experiment. The same kinetic was observed for the water extractable residues. The degradation of glyphosate was rapid in the soil with low adsorption capacity (clay loam soil) with a short half–life of 4 days. However, the persistence of glyphosate in high adsorption capacity soils increased, with half-live of 19 days for silt clay loam soil and 14.5 days for sandy loam soil. HPLC analyses showed that the main metabolite of glyphosate, aminomethylphosphonic acid (AMPA) was detected after three days of incubation in the extracts of all three soils. Our results suggested that the possibility of contamination of groundwater by glyphosate was high on a long–term period in soils with high adsorption capacity and low degrading activities and/or acid similar to sandy loam soil. This risk might be faster but less sustainable in soil with low adsorption capacity and high degrading activity like the clay loam soil. However, the release of non–extractable residues may increase the risk of contamination of groundwater regardless of the type of soil.

Materials and methods

Chemicals

[Phosphonomethyl–^{14}C]–glyphosate was obtained from ARC–ISOBIO (Belgium) diluted in water. Its specific radioactivity was 385 GBq/mmol and its radiochemical purity 99 %. Non–radioactive glyphosate (purity 98.5 %) was obtained from CIL Cluzeau (France). AMPA, 10 ng/µL in water, was obtained from Dr. Ehrenstorfer GmbH (Germany). Sarcosine (N–methylglycine) $C_3H_7NO_2$, purity 99 %, was obtained from Fluka (Germany). FMOC–chloride (purity 99 %), sodium tetraborate decahydrate (purity 99.5 %), potassium hydroxyde (purity 86 %), potassium dihydrogen phosphate (purity 99.5 %) were also obtained from Fluka (Germany). Acetonitrile was obtained from (SDS, France). All solvents were of high performance liquid chromatography (HPLC) grade.

Selected soils and treatments

Three cultivated soils from the Lorraine region in eastern France were selected on the basis of their texture and pH (Table 7.1.2.1.1-119). None of these soils had ever been exposed to glyphosate. Soil types were classified as rendzic leptosol, fluvic cambisol, and stagnic luvisol, hereafter referred to as: clay loam soil, sandy loam soil and silt clay loam soil, respectively. The surface layers (0–25 cm) of all three soils were sampled on the same day.

Soils were air dried and sieved to 2 mm maximum particle size. Soil samples (25 g) were placed in glass jars of 60 mm diameter by 40 mm high. Samples were prepared in triplicates for each soil and each sampling time. An aqueous solution of 0.51 mg glyphosate and 45.1 kBq (equivalent to 1800 g/ha) was added to each soil sample. The volume of aqueous solution was calculated for each soil to obtain samples with moisture content of 80 % of soil retention capacity.

Table 7.1.2.1.1-119: Principal characteristics of the soils (surface layers, 0-25 cm) used in this study

Soil	Clay (%)	pH (water)	OC[a] (%)	K_f[b]	Fe oxides[c] (g/kg)	Fe amorphous[d] (g/kg)	Total Cu[e] (mg/kg)	Total P_2O_5 (g/kg)
Sandy loam	10.5	5.1	0.82	34.5	9.73	2.89	7.49	1.24
Silt clay loam	30.6	6.3	1.45	33.6	40.05	8.52	19.81	3.24
Clay loam	34.9	7.9	1.91	16.6	33.16	2.51	18.7	2.74

[a] Organic carbon content; [b] K_f values obtained from Al-Rajab et al., 2008; [c] subtraction of extracted iron by sodium dithionite-citrate and by acid ammonium oxalate; [d] extracted iron by acid ammonium oxalate in darkness; [e] dissolved by HF.

Laboratory degradation studies

Each soil sample was placed in an individual airtight jar (1.5 L). A scintillation vial containing 10 mL water was placed in each jar to maintain a humid atmosphere and prevent desiccation of the soil. A second scintillation vial with 10 mL of 0.5 mol/L NaOH solution was also placed into each jar to trap any CO_2, which evolved from the soil due to mineralization of organic matter and $^{14}CO_2$. The jars were incubated in the dark at 20°C for 80 days. Analyses were performed in triplicates and one control of unspiked soil per type of soil was considered.

Evaluation of soil micro-organism activity

The total CO_2 fixed by the NaOH was evaluated by titrating an aliquot (8 mL) with 0.2 mol/L HCl, in the presence of 3 mL of 20 % $BaCl_2$ and thymolphtalein at 4 % in ethanol, on day 0, 1, 2, 3, 5, 8, 12, 17, 22, 30, 40, 65, and 80. On each sampling date, the replacement of the CO_2 trapping solution by fresh solution allowed air renewal in the jars.

Estimation of mineralization of glyphosate

The amount of $^{14}CO_2$ trapped by NaOH as a result of the mineralization of ^{14}C–glyphosate was determined by liquid scintillation counting. NaOH (1 mL, in duplicates) of each sample received 10 mL Ultima Gold scintillation cocktail (LSC–cocktail) from Packard (USA) in a plastic scintillation vial. Radioactivity was measured during 10 min using a Packard Tri–Carb 1900 CA liquid scintillation counter (Packard, USA).

Residues in soil

Extractable residues of glyphosate were evaluated and analysis as follow. Soils samples in triplicates were removed from incubation for each soil on day 0, 1, 2, 3, 5, 8, 12, 17, 22, 30, 40, 65 and 80 after treatment. The soil of each sample (25 g) was transferred into a 250–mL PPCO (Nalgene, VWR, USA) centrifuge flask. The soil was extracted thrice with 100 mL distilled water (easily available residues) then 3 times with 100 mL of 0.1 mol/L KH_2PO_4. The samples were rotary shaken at (20 ± 2)°C for 2 hr, and then centrifuged at 5000 ×g for 20 min. The supernatants were combined, the volumes adjusted and radioactivity was determined using liquid scintillation as described above. The supernatants of each sample were filtered through Whatman 40 filter papers, and transferred into a round bottom glass bottle (1000 mL), and then frozen at –30°C for 48 hr before being freeze dried (Edwards–Modulyo–RUA). The freeze–dried extracts were dissolved in 7 mL distilled water and filtered through 0.2 µm using Minisart RC–25 filters (Sartorius, France), then the extracts were stored in freezer at –30°C till derivatization and analysis by HPLC.

Analysis

Derivatization of residues

This analysis was carried out only on the aqueous soil extracts. A 0.5 mL of 0.05 mol/L buffer borate was added to 3 mL of the aqueous solution to be analysed, then left to settle for 15 min. Then 3 mL ethyl ether were added and the solution was agitated vigorously for 2 min. The mixture was left to settle. After 15 min, 1.5 mL of the aqueous phase was removed and 0.25 mL acetonitrile added, followed by 0.25 mL of a

solution of FMOC–Chloride in acetonitrile (1 g/L). The mixture was left to react for 60 min at ambient temperature. Two milliliter of ether ethyl was added and the solution was agitated vigorously for 2 min. The solution was left to settle for 1 hr and then the aqueous phase was recovered in a 2–mL vial for high performance liquid chromatography (HPLC) analysis.

Analysis of residues
The residues were analyzed by HPLC in a Varian chromatograph equipped with a fluorescence detector and a β–radioactivity detector (Flo–one β, Packard, USA) in the following operating conditions: Lichrosorb–NH2 column (5 µm, 4 mm × 250 mm) (CIL–Cluzeau, France) thermostated at 30 °C, injection volume 50 µL, analysis time 22 min, flow rate 0.8 mL/min, elution KH_2PO_4 0.05 mol/L, pH 5.7, acetonitrile (70/30) (V/V). Detection was performed in the following conditions: (1) β–radioactivity detector: Scintillator Ultima–Flo, flow rate 1.2 mL/min, counting cell 500 µL, and (2) fluorescence detector: λ excitation 260 nm; λ emission 310 nm. Standards of the glyphosate (purity >98.5 %), AMPA (purity >98.5 %, CIL–Cluzeau, France) and sarcosine (N–methylglycine, purity >99 %, Fluka) were used for calibration (0, 10, 20, 50 and 100 µg/L). The retention time was 4.2 min for sarcosine, 6.6 min for AMPA, and 13.3 min for glyphosate.

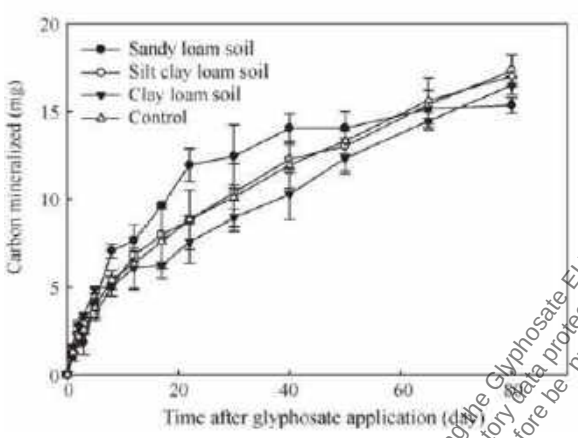
Non–extractable radioactivity
After extraction by water and KH_2PO_4, all soil samples were air dried. Remaining non–extractable ^{14}C–radioactivity was determined by combustion. An aliquot of 0.3 g was mixed with 0.15 mg cellulose powder and the sample was burnt at 900°C with a 307 Packard Oxidizer (Packard, USA). The released $^{14}CO_2$ was trapped with 10 mL Carbosorb (Packard, USA) and the radioactivity was counted after the addition of 10 mL of Permafluor (Packard, USA).

Statistics
Statistical analyses were performed using Stat Box computer software (Grimmer Software version 6.4). Comparison of the means was done using the Newman–Keuls test at levels of 0.05, 0.01 and 0.001. Curves were plotted using SigmaPlot (Version 10, Systat Software Inc., USA). Data in figures represent the mean and standard deviation of triplicate samples.

Results
Microbial activity
Total carbon mineralization of treated or untreated soils during the incubation was used as an indicator of the total microbial activity in the soils (Figure 7.1.2.1.1-41). Endogenous carbon was steadily mineralized in each soil during incubation and the intensity of mineralization differed slightly among soils between day 5 and day 50. During this period, mineralization was slightly faster in the sandy loam soil (14.4 mg carbon) than in the other two soils (13.73 mg for silt clay loam soil and 11.8 mg for clay loam soil). After 50 days, the slowdown in mineralization activity was more rapid for sandy loam soil than for the other two soils.

At the end of experiment (after 80 days of incubation), the total amount of carbon mineralized was similar for all three soils indicating that each soil presented significant microbial activity and that glyphosate had no toxic effect on soil micro–organisms.

Figure 7.1.2.1.1-41: Mineralization activity of microflora of three soils (clay loam, sandy loam and silt clay loam soils). The control is the average of mineralization activity for the three untreated soils



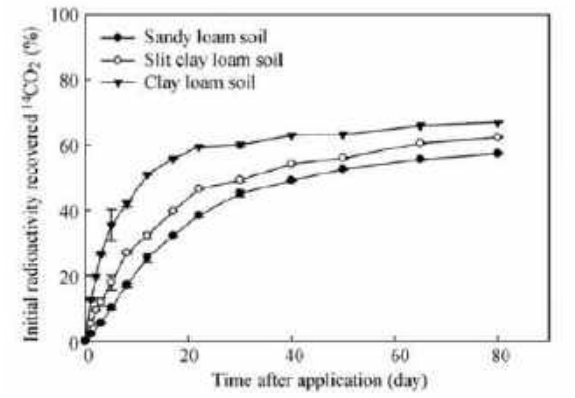
Mineralization of glyphosate

We observed an immediate and high rate of glyphosate degradation after its application on soil (Figure 7.1.2.1.1-42). The absence of lag phase indicates that the microflora of soil already had an enzymatic system capable of degrading glyphosate and as such did not need an adaptation period.

Mineralization of glyphosate after 17 days of incubation reached 32.2 % to 39.7 % of the initial amount applied to the two soils (sandy loam (pH 5.1) or silt clay loam (pH 6.3)). However, the mineralization rate was more rapid and intense for the clay loam soil (pH 7.9) with 48.4 % reached by 12 days of incubation. Thereafter, the mineralization of glyphosate declined gradually for all three soils. The endogenous activity of mineralization was comparable for the three investigated soils. The fast mineralization of glyphosate in clay loam soil appears due exclusively to a bioavailability more important than in other two soils.

We have previously shown that the adsorption of glyphosate in clay loam soil (K_f = 17) is lower than the other two soils (K_f = 34) (Al–Rajab *et al.*, 2008). The half–lives of glyphosate derived from the mineralization rates were significantly different for the three soils, and were 42, 31, and 12 days for sandy loam, silt clay loam, and clay loam soils respectively. These results show that the degradation of glyphosate in biologically active agricultural soils could be influenced by the adsorption of glyphosate. Otherwise, the effect of organic matter content in the soil on mineralization of glyphosate was not clear under the conditions of this study.

Figure 7.1.2.1.1-42: Mineralization of ^{14}C–glyphosate to $^{14}CO_2$ in three soils incubated at 20 °C



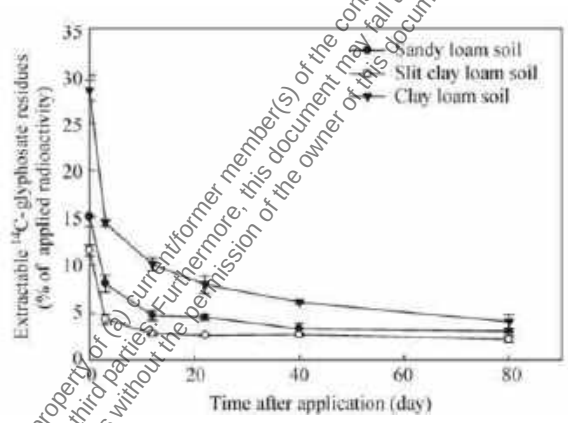
Glyphosate degradation products – Extractable residues

The soil was extracted separately three times with distilled water, then three times with 0.1 mol/L KH_2PO_4. The extraction rate of glyphosate residues with H_2O is influenced by: (1) the degradation, which produces new products (metabolites) that differ in their water solubility and their reactivity with soil constituents; (2) by the process of adsorption–desorption, and (3) the formation of non–extractable residues over time; these sequestered residues are not available to be extracted by H_2O.

The extraction rate of glyphosate with water is an indication of the accessibility of the residues for microbial degradation and/or their transfer to groundwater under natural conditions. The extraction of glyphosate residues with water is directly related to the K_f measured for these soils (Figure 7.1.2.1.1-43). The observed difference of glyphosate extractable residues with water between the sandy loam soil and silt clay loam soil (which have the same K_f value) is certainly related to their texture. For the sandy loam soil, the sandy texture and unstable structure results in a better accessibility to the extraction solution, which in turn leads to a greater extraction efficiency when compared to clay loam soil.

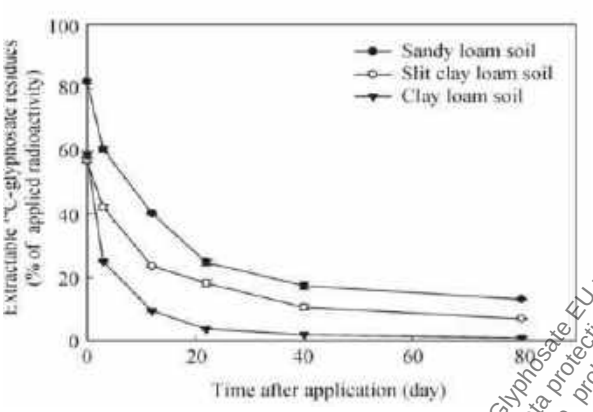
The extraction curves are opposite to those of the mineralization, with the same ranking of soils. These results indicate that the degrading activity of the microflora of soil is linked to the rate of glyphosate available for passage in the aqueous phase.

Figure 7.1.2.1.1-43: Evolution of extractable ^{14}C–glyphosate residues with H_2O from the three soils during incubation at 20 °C.



On the other hand, the extraction of glyphosate from soil with 0.1 mol/L KH_2PO_4 was more efficient than extraction with H_2O. It did not seem affected by the level of bonds energy between the soil and residues of herbicide (Figure 7.1.2.1.1-44). In fact, in the sandy loam soil of Kf = 34, the percentage of glyphosate ^{14}C–phosphonomethyl extracted at T0, immediately after treatment, was (81.9 ± 0.55) % of the initial amount applied (Figure 7.1.2.1.1-44). Thereafter, this value decreased slowly to reach (13.0 ± 0.41) % of the initial amount applied at the end of incubation. In contrast, in the silt clay loam soil, with similar value of Kf = 34, the percentage of extracted residues at day 0 was only (56.9 ± 0.7) %, which is similar to that obtained for the clay loam soil which has a different Kf value of 17. This difference may be due to the high clay content in these two soils (silt clay loam and clay loam) and their structures, which reduces the performance of extraction of KH_2PO_4. We can assume that the treatment in a dry soil may cause an entry of glyphosate into the microporisity of aggregates during the capillary invasion by the aqueous solution of treatment (Guimont [redacted] 2005). The size of this compartment would be defined at the time of treatment and may depend on the physicochemical and physical properties (size of microporal compartment), and the moisture rate of soil at application time. This availability to extraction decreased overtime, more quickly in the sandy loam soil than in the other two brown soils, and at the end of experiment it reached 13.0 %, 6.9 %, and 0.8 % of the initial amount for sandy loam, silt clay loam, and clay loam soils, respectively. The evolution of extraction rate with KH_2PO_4 over time in the three soils is related to the mineralization of residues and the rate that non–extractable residues become available for mineralization and extraction.

Figure 7.1.2.1.1-44: Evolution of extractable ^{14}C–glyphosate residues with KH_2PO_4 from the three soils during incubation at 20 °C



Glyphosate degradation products – Degradation products

The analysis of water extracts by HPLC showed the appearance of two degradation products of glyphosate AMPA and/or sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co–eluted and unlabelled organic compounds. This analysis showed only the very rapid onset of AMPA in the extracts and its predominance compared to glyphosate as of the day 12 of application for the clay loam soil.

The appearance of AMPA during incubation varied significantly depending on the speed of mineralization of glyphosate in each soil (Table 7.1.2.1.1-120). In sandy loam soil, there was only 12.7 % of AMPA present on day 3 after treatment, whereas 87.3 % of the initial radioactive glyphosate was present on the same day. Thereafter, the percentage of AMPA increased gradually overtime, reaching 58.9 % of residues after 22 days of incubation, and 91.1 % at the end of the experiment.

Table 7.1.2.1.1-120: Mass balance of glyphosate and AMPA in extracted residues during incubation over 80 days (%)

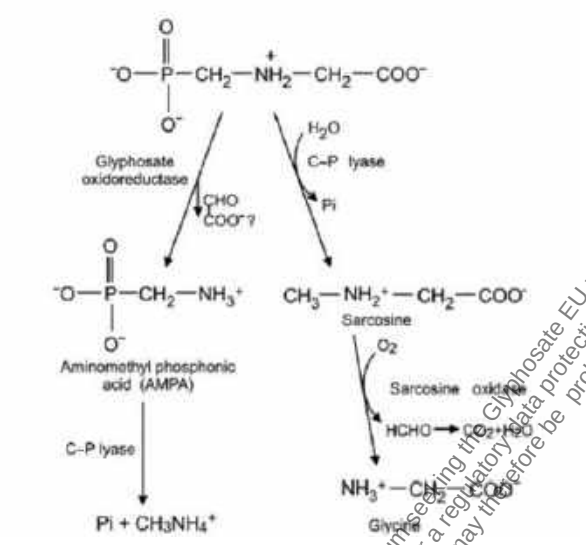
Incubation time (day)	Sandy loam soil		Silt clay loam soil		Clay loam soil	
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
0	100	nd	100	nd	100	nd
3	87.3	12.7	79.7	20.3	51.5	48.5
12	71.0	29.0	58.5	41.5	40.2	59.8
22	41.1	58.9	25.6	74.4	12.0	88.0
40	22.3	77.7	22.5	77.5	5.6	94.4
80	8.9	91.1	14.9	85.1	0.9	99.1

AMPA: aminomethylphosphonic acid, nd: not detected.

The extractable residues of glyphosate with water are easily available to the degradation or transfer by water in soil. The half–life of glyphosate extractable with water was estimated and was found to vary depending on the biological activity of soil. It was 19 days for the sandy loam soil, 14.5 days for the silt clay loam soil and 4 days for the clay loam soil.

Together, our results suggest that the rupture of the $–CH_2–NH–$ bond giving rise to AMPA is easier than breaking the $–CH_2–PO_3H_2$ bond that results in either sarcosine and phosphorus, or methylamine and phosphorus (Figure 7.1.2.1.1-45). The break of the $–CH_2–NH–$ bond may depend on the overall activity of the microflora and the retention of glyphosate by the soil; while the rupture of the $–CH_2–PO_3H_2$ bond could be related to a more specific bacterial population. This difference in the rupture speed of these two links leads to some accumulation of AMPA in the soil (Figure 7.1.2.1.1-45).

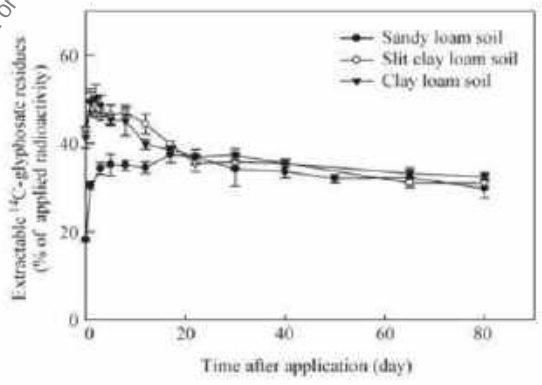
Figure 7.1.2.1.1-45: Microbial degradation of glyphosate in soil through sarcosine or AMPA (Liu *et al.*, 1991)



Non–extractable glyphosate residues

The non–extractable residues represent the fraction, which cannot be extracted from the soil by the series of KH_2PO_4 extractions (exhaustive extraction) (Figure 7.1.2.1.1-46). Upon application of glyphosate on a sandy loam soil, we observed the formation of non–extractable residues at 18.1 % of the initial applied amount of herbicide. Subsequently, it progressed during 3 days to 35 %, staying stable until day 22, and then decreased very gradually over time until 30 % of initial applied amount of glyphosate was present at the end of experiment. In contrast, the formation of non–extractable residues for the clay loam and the silt clay loam soils was more intense and rapid than in the sandy loam soil. It reached 41.3 % and 43 % of the initial applied amount for the clay loam and silt clay loam soils respectively at day 0, and 49.4 % for both soils at day 1. For both soils, the rate stayed stable after day 2 until which decreased to 32.4 % and 30.9 %, respectively by the end of experiment. The rates of non–extractable residues seems specific for each soil, but are defined by day 3 after treatment.

Figure 7.1.2.1.1-46: Evolution of non–extractable residues in three soils during incubation time at 20 °C



The rate of non–extractable residues is probably dependent on the physico–chemical properties and physical aspects of the soils including the size of the microporal compartment. This rapid formation of non–extractable residues immediately after treatment with a maximum reached within 2 to 8 days after application is very specific for glyphosate and could probably be due to: (1) the high solubility of glyphosate

in water (10.5 g/L) (Agri–tox, 2009), (2) the physico–chemical properties that allow glyphosate to immediately establish high energy bonds with the constituents of soil, (3) the physico–chemical properties of soils (texture, meso and microporisity), and/or (4) the treatment conditions.

The treatment of herbicide on a dry soil promotes the capillary invasion and the rapid transport of the solution of treatment in the microporisity intra aggregate (Guimont *et al.*, 2005) subsequently making the glyphosate inaccessible to KH_2PO_4. Furthermore, the clayey texture promotes the importance of the microporosity. This explains the similar behaviour of clay loam and silt clay loam soils in the formation of non–extractable residues of glyphosate. In fact, these two soils have very different K_f values (17 and 34 respectively) but they have the same texture. These two soils, particularly the silt clay loam soil, differs strongly from the sandy loam soil which forms relatively a low rate of non–extractable residues and whose texture is sandy although having the same K_f (34) as the silt clay loam soil. We also noted that the initiation of the degradation of glyphosate did not affect the evolution of extractable residues rate. This implies that AMPA was not playing different role comparing to glyphosate. The very slow decrease of non–extractable residues showed that these residues can return by diffusion, and under the effect of a concentration gradient, to areas accessible to microorganisms to subsequently undergo mineralization. We note that from day 22 until the end of incubation the rates of non–extractable residues of glyphosate were similar for the three soils. The mineralization of glyphosate in three soils affects only the extractable fractions with water and KH_2PO_4 influenced by the forces adsorption defined by K_f.

The ^{14}C mass balance for each sample revealed a deficit (loss) that fluctuated from (4 ± 2) % at day 0 (application of glyphosate) to (6.0 ± 3.4) % after 80 days of incubation independent of soil type and different sampling dates over time. These losses were probably partially caused by the handling of samples during analyses (extraction and concentration). Because of these low losses, results were corrected and returned to 100 % by distributing the deficit on the various compartments assessed in proportion to their respective importance.

Conclusion

We simultaneously monitored in controlled conditions the principal processes involved in ^{14}C–glyphosate dissipation and their interactions in three agricultural soils over a period of 80 days. The results of this experiment showed that for agricultural soils with a significant and comparable biological activity, the fate of glyphosate and its potential in polluting water is closely related to the adsorption and the formation of non–extractable residues, which are dependent on soil texture and its moisture condition at the time of treatment. Our results showed that for a clay soil at basic pH, the glyphosate could be available to reach the groundwater in few days after treatment if the conditions are favourable for precipitation. Conversely, in the case of an acid sandy soil, the potential pollution of groundwater by glyphosate is greatly reduced by the strong adsorption of its residues in the soil. In case of rain following treatment, the risk of groundwater pollution by glyphosate will be low but may continue to be present for long time since the mineralization is slow. In this system, the silt clay loam soil is apparently less favourable for water pollution since it showed a strong adsorption of glyphosate and the formation of large amount of non–extractable residues. In the three investigated soils, a low level of water pollution (background) could be occurred over a long time by the sequestered residues of glyphosate, which are either gradually released into the soil solution, or circulated by the water through the soil.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:
The article investigates the soil degradation of glyphosate in three agricultural soils from the EU. The test was performed with radio-labelled and non-radiolabelled test substance. For the part dealing with radiolabeled test substance, only mineralisation was followed after application to soil. Deviations in conduct were the use of air–tight test vessels not allowing for air exchange; no information whether the applied test solution was mixed with the soil; $^{14}CO_2$ was passively (and potentially not quantitatively) collected; soil moisture was rather high (80 % of soil retention capacity). For the tests with non-radiolabelld test substance the details do not allow to assess the quality of the analytical method as EU data generating method including no LoD/LoQ provided. Only few results are reported quantitatively, mainly graphical plots; calculation method of DT_{50} not reported. The article is therefore classified as reliable with restrictions.

<u>Assessment and conclusion by RMS:</u>

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

Information on the rate of degradation of aminomethylphosphonic acid (AMPA) in soil under aerobic conditions can be either generated from studies with the active substance (see CA 7.1.2.1.1) or from tests performed separately with the metabolite.

Two new AMPA applied studies investigated the rate of degradation of AMPA in aerobic soil and are considered as valid to address the data point (Simmonds, 2020, CA 7.1.2.1.2/002 incl. addendum CA 7.1.2.1.2/004 and Göcer, 2017, CA 7.1.2.1.2/003). Göcer (2017) includes a kinetic evaluation according to FOCUS. For the 120 d degradation study by Simmonds (2020, CA 7.1.2.1.2/002, CA 7.1.2.1.2/004), ~~an interim report was available at the time of dossier preparation (including data up to 92 DAT). T~~the data ~~are considered as preliminary and~~ were not used as endpoints for the actual risk assessment due to the late finalization time. However, no significant changes to the conclusions ~~in the given dossier~~ of risk assessment are expected from these ~~additional~~ endpoints.

The existing parent applied studies were kinetically evaluated according to the current kinetic guidances (FOCUS, 2006, 2014, [redacted] 2020, CA 7.1.2.1.2/001).

For comparison with EU triggers, DT_{50}- and DT_{90}–values of AMPA range from 29.4 to >10,000~~497~~ days and from 97.7 to 1040~~>1000~~ days, respectively (Table 7.1.2-2). For input as modelling endpoints, normalised values of the DT_{50} values range from 14.2 to 1040~~497~~ days (Table 7.1.2-2).

In the scientific literature review for glyphosate (2010-2019), no article was identified to provide further information relevant to the data point.

Table 7.1.2.1.2-1: Studies on aerobic degradation of AMPA

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.2.1.2/001	[redacted] 2020	Kinetics evaluation	Glyphosate, AMPA	Valid	Summary under CA 7.1.2.1.1/001
CA 7.1.2.1.2/002	[redacted] 2020	Aerobic rate	AMPA	Valid	Interim report with preliminary results
CA 7.1.2.1.2/004	[redacted] 2020	Aerobic rate	AMPA	Valid	Addendum report with results from 120 day sampling
CA 7.1.2.1.2/003	[redacted] 2017	Aerobic rate	AMPA	Valid	

Rate of degradation studies with glyphosate

1. Information on the study

Data point:	CA 7.1.2.1.2/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from aerobic laboratory soil degradation studies
Report No	112148-001
Document No	
Guidelines followed in study	FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp. FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not applicable for this study type
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

The study provides information for multiple data points. The summary is provided under CA 7.1.2.1.1/001.

1. Information on the study

Data point:	CA 7.1.2.1.2/002
Report author	[redacted]
Report year	2020
Report title	AMPA – Rate of Degradation of Aminomethylphosphonic Acid (AMPA) in Aerobic Soil
Report No	3202599
Document No	
Guidelines followed in study	OECD 307 EPA 835.4100 Commission Regulation (EU) No. 283/2013 Regulation (EC) No. 1107/2009 (2009)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

Data point:	CA 7.1.2.1.2/004
Report author	[redacted]
Report year	2020
Report title	AMPA – Rate of Degradation of Aminomethylphosphonic Acid (AMPA) in Aerobic Soil Final Report Addendum
Report No	3202599
Document No	
Guidelines followed in study	OECD 307 EPA 835.4100 Commission Regulation (EU) No. 283/2013 Regulation (EC) No. 1107/2009 (2009)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The rate of degradation of Aminomethylphosphonic acid (AMPA) was investigated in two different soils with a pH range between 5 – 6 in water and calcium chloride: 18 Acres (sandy clay loam), Brierlow (silt loam). AMPA was applied at a rate of 2.99 mg/kg dry weight equivalent of soil. Results are presented as a percentage of the nominal amount applied (2.80 mg/kg) which is based on the proportions of AMPA

found from parent (glyphosate) degradation in previous studies. The soils were incubated under aerobic conditions at 20 ± 2°C in the dark and maintained at a soil moisture of *ca.* pF 2 for up to 120 ~~92~~ days. For each soil, duplicate samples were taken for analysis and extracted at 0, 2, 8, 13, 29, 43, 62, ~~and~~ 92 and 120 days after treatment (DAT). The amount of AMPA present in each extract was quantified by LC-MS/MS analysis using a calibration curve. ~~The 120 DAT data will become available after finalisation of the report and therefore an addendum to the report will be issued.~~

The specificity and efficiency of the analytical method was tested at each sampling interval by the inclusion of control (untreated) and recovery samples (fortified after sampling with a known amount of AMPA), which were processed in the same way as the test samples.

Control (untreated) soil extracts were free from components that interfered with the analysis of AMPA and therefore, the analytical procedure was considered specific for AMPA. The procedural recoveries in fortified samples for 18 Acres and Brierlow soil ranged from 76.8-105.0 % and 77.8-116.0 %, where the mean recoveries were within acceptable limits (70-110 %), thereby confirming the efficiency of the extraction method and the stability of the compound throughout the procedure.

AMPA concentration in treated samples of soil 18 Acres declined slowly over the course of the experiment from mean values of 97.8 to 87.3 % ~~and 104.5 %~~ of the nominal applied amount ~~at~~ between 0 DAT ~~to 91.9~~ and 120 DAT. In soil Brierlow mean values were 104.5 and 108.0 % at 0 DAT and 120 DAT ~~100.6 % by 92 DAT in 18 Acres and Brierlow soil~~, respectively. The ~~preliminary~~ rate of degradation of AMPA was estimated using single first order kinetics (SFO), see Table 7.1.2.1.2-5. The calculated DT_{50} value was ~~787~~ 1040 days in 18 Acres. No statistically reliable fit could be derived for Brierlow soil, therefore, endpoints could not be derived. ~~, however an indication for the trigger value of 1260 days is reported.~~

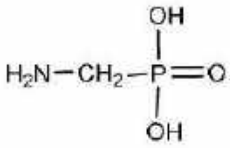
~~**Table 7.1.2.1.2-2: DT_{50} and DT_{90} values for AMPA in soil**~~

~~Soil~~	~~SFO~~					
	~~DT_{50} [days]~~	~~DT_{90} [days]~~	~~k_1~~	~~χ^2~~	~~R^2~~	~~Prob > t~~
~~18 Acres~~	~~787~~	~~2620~~	~~0.000880~~	~~3.12~~	~~0.2988~~	~~0.01394~~
~~Brierlow~~	~~1260~~	~~4170~~	~~0.000552~~	~~2.76~~	~~0.1212~~	~~0.09239~~

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: **Aminomethylphosphonic acid**



Description: White crystalline solid

Lot/Batch #: 107785

Purity: ≥ 99 2%

Stability of test compound: Stable, >99 days in 0.1% formic acid (aq) (refrigerated)
≥23 months in water (refrigerated)

Application vehicle: RO (reverse osmosis) Water

Soils: Two soils were used for the study. Soils were chosen based on having a soil pH in water of 5 - 6

Name	Soil 1	Soil 2

Sampling location	Jealotts Hill Farm, Nuptown Road, Bracknell, Berks, UK	Brierlow, Derbyshire, UK
GPS co-ordinates	51° 27'16.2828"N, 0° 42'14.9232"W	53°13' 9.4"N, 1° 50' 32.4"W
Date of collection	14 November 2019	18 September 2019 (source site) 21 November 2019 (soil nursery)
Batch reference	S19/18A/042	S19/BRI/058
Pesticide History	No pesticide use in last 5 years	No pesticide use in last 5 years
Sampling depth (cm)	5 cm to 15-20 cm	10 cm to 20 cm
Collection procedures	Excavator	In accordance with ISO 18400-206
Storage conditions	4 ± 2ºC, in loosely tied plastic bags	4 ± 2ºC, in loosely tied plastic bags
Duration of storage	48 days	48 days
Duration of acclimation	7 days	7 days
Soil preparation	Soils were thoroughly mixed and passed through a 2 mm mesh sieve, with the minimum of air drying. Soils were adjusted to just below the water holding capacity at pF 2.0 by the addition of reverse osmosis water.	
Particle size (% w/w):		-
Sand (2000-50 µm)	48	30
Silt (50-2 µm)	27	60
Clay (<2 µm)	25	10
Texture (USDA)	Sandy clay loam	Silt loam
USA Taxonomy (order and sub-order)		
pH (1:1 w/v soil:water)	5.5	5.7
pH (1:2 w/v soil:0.01M $CaCl_2$)	5.3	5.4
Organic matter[1] (%)	3.3	7.3
Organic carbon[1] (%)	1.9	4.3
CEC[2] (meq/100 g soil)	15.7	12.9
Moisture content at pF 2.0[3] (0.1 bar, % w/w)	23.2	34.7
Moisture content at pF 2.5[3] (0.33 bar, % w/w)	17.5	24.4
Moisture content at 15 bar[3] (% w/w)	11.1	16.0
Moisture content on arrival (% w/w)	20.47	27.94
Moisture content used in study (% w/w) [4]	23.03	34.47
Initial biomass (start of study)	574 µg C/g dry soil 3 % OC	633 µg C/g dry soil 1.5 % OC
Final biomass (end of study)	615 µg C/g dry soil 3.2 % OC ~~Data available at 120DAT~~	551 µg C/g dry soil 1.3 % OC ~~Data available at 120DAT~~

[1] Organic carbon (OC) % = organic matter (OM) %/1.724
[2] CEC = cation exchange capacity
[3] Measured in 2 mm sieved soil
[4] Representative soil moisture content determined from biomass samples at 0 DAT

B. STUDY DESIGN

1. Experimental design

Parameter		Description
Duration of the test		120 ~~92~~ days
Soil condition		Fresh soil, passed through 2 mm sieve prior to use
Soil sample weight		100 g (dry weight) per replicate
Test concentration		(Nominal) 2.80 (mg/kg soil dry weight) (Achieved) 2.99 (mg/kg soil dry weight)
Number of replicates	Treated soil samples	2
	Control soil samples	1
	Recovery soil samples	1
Test apparatus		250 mL Duran borosilicate glass incubation vessels (*ca.* 7 cm diameter) plugged with polyurethane bungs to allow continuous air exchange
Test material application	Identity of solvent	Treated in RO water
	Volume of test solution used/treatment	0.56 mL
	Application method	Positive displacement pipette
	Evaporation of application solvent	No
Experimental conditions	Temperature (°C)	20±2
	Moisture content	At 0 DAT, soil adjusted to just below pF2 moisture tension (to allow for water to be added in the treatment solution)
	Moisture maintenance method	The polyurethane bungs were moistened with RO water, incubation vessels weighed periodically and any weight loss relative to 0 DAT attributed to water loss. Water added to restore original system
	Continuous darkness	Yes

2. Sampling

Parameter		Description
Sampling intervals	Treated soil samples	Duplicate samples from 18 Acres and Brierlow: 0, 2, 8, 13, 29, 43, 62, ~~and~~ 92 and 120 DAT
	Control soil samples	Single samples from 18 Acres and Brierlow: 0, 2, 8, 13, 29, 43, 62, ~~and~~ 92 and 120 DAT
	Recovery soil samples	Single samples from 18 Acres and Brierlow: 2, 8, 13, 29, 43, 62, ~~and~~ 92 and 120 DAT
	Untreated soils for biomass	At 0 DAT and 120 DAT
Soil sampling procedures		Treated test, blank control and recovery vessels were removed at each sampling interval. At the time of sampling, recovery soil samples were fortified with AMPA and subjected to the same extraction procedures as the test samples and blank controls as detailed below
Sample storage before analysis		All samples were extracted on the day of sampling and analysed by LC-MS-MS within 8 days of refrigerated storage.

3. Description of analytical procedures

20 g (or 100 g for incubation vessels) dry weight equivalent of soil sample was transferred to plastic pots (recovery vessels fortified with known amounts of AMPA) and extracted with 200 mL (1000 mL for incubation vessels) 1M NaOH(aq) (minus the volume of water already present in the soil) for 20 minutes via mechanical agitation. A portion of extract was transferred into a centrifuge tube centrifuged at 1455 g for 5 minutes.

A portion of the resulting supernatant (3 mL) was cleaned-up via filtration (passed through a Macherery-Nagel™ Chromafil™ MV Cellulose Mixed Esters syringe filter; 2.5 mm diameter, 0.45 µm pore). The filtrate (1.7 mL) was acidified with ≥ 98 % formic acid (0.1 mL) and spiked with 0.5 µg/mL internal reference standard (0.2 mL). An aliquot (1 mL) was cleaned-up further by solid phase extraction, SPE (Strata-X 33u Polymeric RP 3 mL; 60 mg) prior to LC-MS/MS analysis.

Injected samples were quantified by peak area ratio with reference to a calibration curve. The latter was obtained by correlation of the peak area ratio of the calibration standards (made up in 0.1 % formic acid (v/v), non-matrix matched) with the corresponding concentrations of the test item.

At each sampling interval, control (untreated) samples and recovery samples (fortified after sampling with a known amount of AMPA) were processed in the same way as the treated soil samples to determine the specificity and efficiency of the analytical method.

The half-lives (DT_{50}) of AMPA in each soil were determined using a Single First Order (SFO) kinetic model.

II. RESULTS AND DISCUSSION

1. Specificity of the Analytical Method

Control (blank) soil extracts were free from components that interfered with the analysis of AMPA. Therefore, the analytical procedure was considered specific for AMPA.

2. Recovery of AMPA in Fortified Samples

The procedural recoveries in fortified samples for 18 Acres and Brierlow are shown in Table 7.1.2.1.2-2 and Table 7.1.2.1.2-3, respectively. Recoveries ranged from 76.8-105.0 % and 77.8-116.0 % in 18 Acres and Brierlow, respectively. Since the mean recoveries were within acceptable limits (70-110 %), no correction was made for procedural recoveries in the test samples.

3. Degradation of AMPA in Soils

The AMPA concentration in treated samples is shown in Table 7.1.2.1.2-4. AMPA slowly declined in soil 18 Acres over the course of the experiment from mean values of 97.8 to 87.3 % ~~and 104.5 %~~ of the nominal applied amount between ~~applied at~~ 0 DAT ~~to 91.9~~ and 120 DAT. ~~100.6 % by 92. in 18 Acres and~~ In soil Brierlow, mean values were 104.5 and 108.0 % at 0 DAT and 120 DAT, respectively.

The degradation rate (DT_{50}) of the parent was determined using non-linear regression and a single first order kinetic model (SFO, CAKE, version 2.0). SFO kinetics describes the degradation of AMPA well with Chi-square (χ^2) values of <15 ~~and r2 values of >0.7~~ in soil 18 Acres. Although visually acceptable, a statistically reliable fit could not be derived for Brierlow soil using SFO, where the t-test > 0.05 and k was not significantly different from zero, suggesting there was no significant degradation of AMPA under test conditions.~~, however no reliable fit could be derived for Brierlow soil (visually acceptable, however t-test of k >0.05 and k not significantly different from zero.~~ The results are presented in Table 7.1.2.1.2-5.

Kinetic evaluation using biphasic kinetic models as recommended by FOCUS (2014) was performed separately and is provided below this summary.

Table 7.1.2.1.2-2: Procedural Recoveries in Recovery Samples: 18 Acres

DAT	Fortification level[1] (%)	Fortified Concentration (µg/mL)	Measured conc. in soil (mg/kg)	Procedural recovery (%)
2	0.5	0.14	0.136	97.1
8	0.5	0.14	0.145	104.0
13	0.5	0.14	0.147	105.0
29	0.5	0.14	0.146	104.
43	0.5	0.14	0.142	102.0
62	0.5	0.14	0.137	97.0
92	0.5	0.14	0.143	102.0
120	0.5	0.14	0.141	101.0
2	110	3.10	3.040	98.1
8	110	3.10	2.550	82.4
13	110	3.10	2.990	96.5
29	110	3.10	2.730	88.0
43	110	3.10	2.590	83.6
62	110	3.10	2.380	76.8
92	110	3.10	2.900	93.5
120	110	3.10	2.960	95.5

[1] Based on a nominal application rate of 2.80 mg/kg

Table 7.1.2.1.2-3: Procedural Recoveries in Recovery Samples: Brierlow

DAT	Fortification level[1] (%)	Fortified Concentration (µg/mL)	Measured conc. in soil (mg/kg)	Procedural recovery (%)
2	0.5	0.14	0.148	106.0
8	0.5	0.14	0.155	111.0
13	0.5	0.14	0.161	115.0
29	0.5	0.14	0.163	116.0
43	0.5	0.14	0.155	111.0
62	0.5	0.14	0.139	99.2
92	0.5	0.14	0.151	108.0
120	0.5	0.14	0.144	103.0
2	110	3.10	3.090	99.7
8	110	3.10	2.770	89.4
13	110	3.10	3.090	99.6
29	110	3.10	2.840	91.5
43	110	3.10	2.930	94.5
62	110	3.10	2.380	77.8
92	110	3.10	3.02	97.4
120	110	3.10	2.940	95.0

[1] Based on a nominal application rate of 2.80 mg/kg

Table 7.1.2.1.2-4: Concentration of AMPA in soil (values as % of applied)

Soil	Replicate	% of applied at Time (DAT)[1]								
		0	2	8	13	29	43	62	92	120
18 Acres	A	98.4	91.2	92.3	91.6	90.1	85.4	83.1	92.6	87.4
	B	97.2	95.2	93.5	91.2	89.1	86.0	83.3	91.2	87.1
	Mean	**97.8**	**93.2**	**92.9**	**91.4**	**89.6**	**85.7**	**83.2**	**91.9**	**87.3**
Brierlow	A	106.0	100.0	98.2	99.6	93.4	98.4	87.5	102.0	101.0
	B	103.0	104.0	97.0	97.4	106.0	95.5	94.2	99.2	115.0
	Mean	**104.5**	**102.0**	**97.6**	**98.5**	**99.7**	**97.0**	**90.9**	**100.6**	**108.0**

[1] Based on a nominal application rate of 2.80 mg/kg

Table 7.1.2.1.2-5: DT_{50} and DT_{90} values for AMPA in soil

Soil	SFO					
	DT_{50} [days]	DT_{90} [days]	K	χ^2	R^2	Prob > t
18 Acres	1040	3450	0.000667	3.04	0.3114	0.008042
Brierlow	7.9×10^{11}	2.62×10^{12}	8.78×10^{-13}	3.68	0.02441	0.5

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was performed according to the guidelines that were in force at time of submission of this dossier. The rate of degradation of AMPA was investigated in two different aerobic soils incubated at a temperature of 20°C and a moisture content of *ca.* pF 2. AMPA was applied at a nominal rate of 2.80 mg/kg dry weight soil (based on the proportions of AMPA found from parent (glyphosate) degradation in previous studies).

The ~~preliminary~~ rate of degradation of AMPA was estimated using single first order kinetics (SFO) derived from 0 to ~~92~~ 120 DAT data set. The calculated DT_{50} value was ~~787~~ 1040 days in 18 Acres. No statistically reliable fit could be derived for Brierlow soil, suggesting there was no significant degradation of AMPA under test conditions. ~~however an indication for the trigger value of 1260 days is reported. Conclusions from 0 – 120 DAT incubation will be presented in an addendum to the report.~~

The study is considered valid, however not yet included in risk assessment due to timing of finalization. ~~The preliminary DT_{50} values were tested for their effect on the combination of laboratory and field data and the pH dependency assessment of AMPA with no changes to the currently presented conclusions.~~

Assessment and conclusion by RMS:

Kinetic evaluation including biphasic models

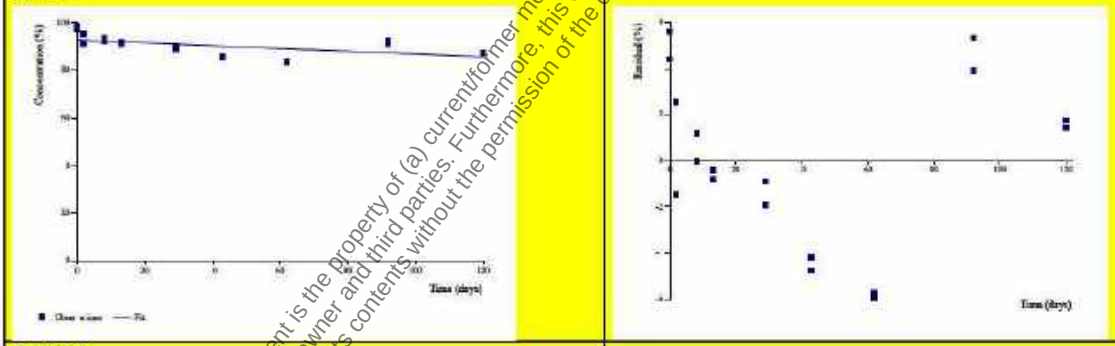
The biphasic models FOMC and DFOP were tested using the software CAKE version 3.3 and the results are shown below. For comparison, also the results of the SFO fit are repeated.

Table 7.1.2.1.2-6: Kinetic models and goodness-of-fit statistics of AMPA fits in soil 18 Arpes

Kinetic results									
Kinetic model	**Visual assessment**	**M_0**	**Kinetic parameters**	**χ^2 error (%)**	**Prob > t (5 % level)**	**Lower CI (95%)**	**Upper CI (95%)**	**DT_{50} (d)**	**DT_{90} (d)**
SFO	acceptable	92.8	k: 6.67×10^{-4}	3.04	k: <0.009	k: 1.42×10^{-4}	k: 0.001	1040	3450
FOMC	good	97.79	α: 0.01932 β: 0.2471	2.32	-[1]	β: -0.8892	β: 1.384	>10,000	>10,000
DFOP	good	96.54	k_1: 0.07364 k_2: 8.69×10^{-10} g: 0.09605	2.37	k_1: 0.1238 k_2: 0.5	k_1: -0.05524 k_2: -0.001016	k_1: 0.205 k_2: 0.001	>10,000	>10,000
HS	Not calculated								

SFO model does properly estimate the degradation (acceptable visual and reliable statistical assessment).
FOMC and DFOP models were tested to determine the best fit model, however both biphasic models do not provide reliable statistical results i.e. beta and k_2 not significantly different from zero (FOMC, DFOP), t-test not <0.05 (DFOP).
Conclusion: SFO to be used for trigger and modelling endpoints (at 20°C and pF2 due to study conditions).

SFO



FOMC

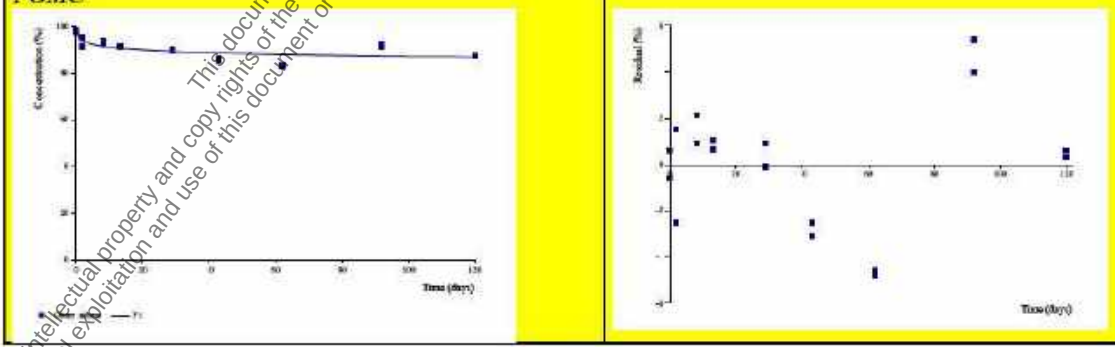
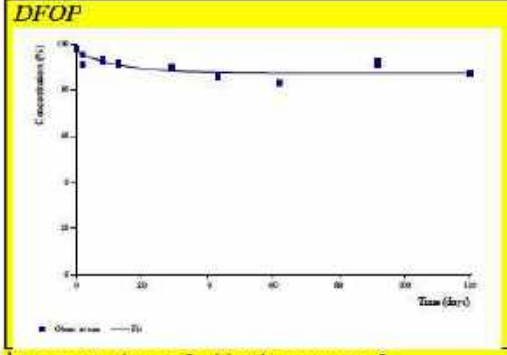
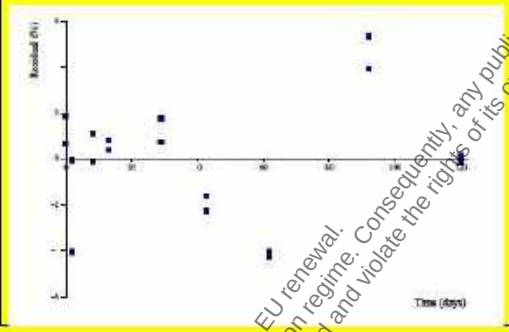


Table 7.1.2.1.2-6: Kinetic models and goodness-of-fit statistics of AMPA fits in soil 18 Acres

DFOP





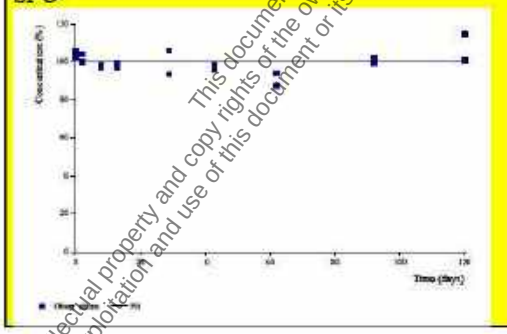
[1] t-test not relevant for kinetic parameter β

Table 7.1.2.1.2-7: Kinetic models and goodness-of-fit statistics of AMPA fits in soil Brierlow

Kinetic results									
Kinetic model	**Visual assess-ment**	**M_0**	**Kinetic parameters**	**χ^2 error (%)**	**Prob > t (5 % level)**	**Lower CI (95%)**	**Upper CI (95%)**	**DT_{50} (d)**	**DT_{90} (d)**
SFO	acceptable	99.86	k: 8.78×10^{-13}	3.68	k: 0.5	k: -0.000765	k: 0.001	>10,000	>10,000
FOMC	Data could not be fitted by the model, therefore no reliable fit achieved.								
DFOP	good	104.7	k_1: 0.4097 k_2: 1.56×10^{-18} g: 0.05521	3.78	k_1: 0.3471 k_2: 0.5	k_1: -1.78 k_2: -0.001001	k_1: 2.599 k_2: 0.001	>10,000	>10,000
HS	Not calculated								

SFO model does not properly estimate the degradation, as no statistically reliable fit (k not significantly different from zero, t-test not <0.05).
FOMC and DFOP models were tested to determine the best fit model, however no fit could be achieved for FOMC and no statistically reliable results were provided for DFOP.
Conclusion: No reliable endpoints can be derived. Use FOCUS default of 1000 d for modelling.

SFO



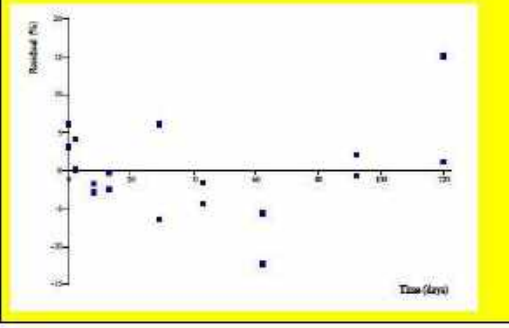
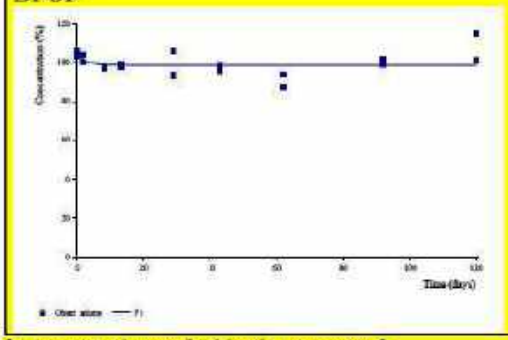
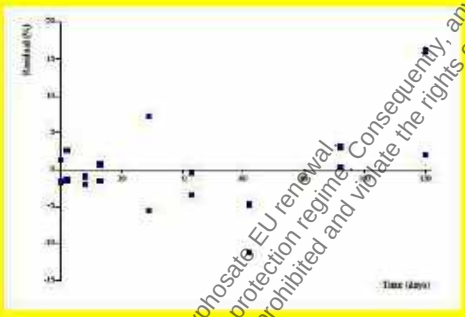


Table 7.1.2.1.2-7: Kinetic models and goodness-of-fit statistics of AMPA fits in soil Brierlow

FOMC
Data could not be fitted by the model, therefore no reliable fit achieved.

DFOP





[1] t-test not relevant for kinetic parameter β

1. Information on the study

Data point:	CA 7.1.2.1.2/003
Report author	[redacted]
Report year	2017
Report title	Aminomethylphosphonic Acid (AMPA): Rate of Degradation of AMPA in one Acidic Soil Incubated under Aerobic Conditions
Report No	S16-04460
Document No	-
Guidelines followed in study	OECD 307; SANCO/3029/99-rev.4
Deviations from current test guideline	From OECD 307: none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The degradation of aminomethylphosphonic Acid (AMPA) was investigated in one soil under aerobic conditions. The study was performed in the dark in the laboratory at 20 ± 2°C and 45 % of the maximum water holding capacity for 120 days.

A loamy sand soil (Warsop) was used in this study. The amount of organic carbon in the soil was 1.76 % and the pH in $CaCl_2$ was 3.90.

The test was performed in static test systems, consisting of 300 mL glass flasks filled with soil and closed by polyurethane plug.

The application rate of AMPA was 280 µg/100 g soil (dry weight).

Duplicate samples from each system were processed and analysed at 0, 2, 8, 13, 30, 62, 90, and 120 days after treatment (DAT).

The mean amount of AMPA in soil extracts decreased from 0 DAT to 120 DAT from 108.6 to 82.9 % AR in acidic soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Aminomethylphosphonic acid (AMPA)
Lot No.:	GLP-1508-24086-A
CAS number:	1066-51-9
Chemical purity:	98.8 %

2. Soil:

Soil was sieved to ≤2 mm. The soil was received and stored at 20°C. Characteristics of the test soil is presented in the table below.

Table 7.1.2.1.2-8: Characteristics of test soil

Parameter	Results
Soil	Warsop
Country	United Kingdom
Textural Class (USDA)	Loamy sand
Sand (50 µm – 2 mm) (%)	84.2
Silt (2 µm – 50 µm) (%)	11.2
Clay (< 2 µm) (%)	4.6
pH (water)	4.71
pH ($CaCl_2$)	3.90
Organic carbon (%)	1.76
Organic matter (Organic carbon x 1.72) (%)	3.03
Cation exchange capacity (meq/ 100 g soil)	7.1
Maximum Water Holding Capacity (%)	37.25
Bulk Density (disturbed) (g/L)	1400
Microbial biomass (mg C/ 100 g soil)	
After arrival	20.5
At the start (1 DAT)	21.1 treated / 21.3 untreated
59 DAT	20.4 treated / 17.6 untreated
Study end (120 DAT)	17.6 treated / 18.6 untreated

B. STUDY DESIGN

1. Experimental conditions

Static test systems were used, consisting of Erlenmeyer flasks filled with soil closed by polyurethane plug.

100 g of sieved soil (dry weight equivalents) were weighed into each test vessel, soil moisture was adjusted to 45 % of the maximum water holding capacity, and the test systems were acclimated for 11 days at test conditions.

The application rate of AMPA was 280 µg/100 g soil (dry weight). AMPA was dissolved in water and 560 µL of this solution were applied to each test system. The verification of application concentration was performed by determination of recoveries at levels of 110 % of the applied concentration and the LOQ of the method at each sampling date. Determined recoveries were in the range from 92.7 and 108.6 %, demonstrating the validity of the extraction and analysis.

Test systems were incubated under aerobic conditions in the dark for 120 days at 20 ± 2°C and 45 % of the maximum water holding capacity.

2. Sampling
Duplicate samples from each system were processed and analysed at 0, 2, 8, 13, 30, 62, 90, and 120 days after treatment (DAT). All soil samples were processed on the designated sampling day. At every sampling time point both flasks were extracted on the same day of collection, extracts were stored in a freezer at ≤ -18 °C and analysed by LC-MS/MS within 10 days of collection.

3. Analytical procedures
At each sampling interval, soil samples were extracted with 1000 mL of 1 N NaOH and agitated for 30 seconds by hand followed by agitation on a flatbed shaker for 20 minutes at ambient temperature. Extracts and soil were separated by centrifugation and decantation. 10 mL of the extract was filtrated through a single use syringe filter. 0.2 mL of a 500 ng/mL internal standard solution (^{13}C and ^{15}N isotope enriched AMPA) was mixed with 0.1 mL formic acid and 1.7 mL of the filtrated extract. About 1 mL of the mixed solution was cleaned-up through a SPE cartridge and transferred into a glass vial for LC-MS/MS analysis.

AMPA was identified by HPLC-MS/MS with multiple reaction monitoring (MRM) mode using AMPA standards in solvent for calibration.

II. RESULTS AND DISCUSSION

A. DATA

Degradation of AMPA in soil extracts are summarised in Table 7.1.2.1.2-9.

Table 7.1.2.1.2-9: Degradation of AMPA in Warsop soil under aerobic conditions (expressed as percent of applied analyte)

Compound			DAT							
		Replicate	0	2	8	13	30	62	90	120
AMPA	Soil	A	108.2	105.0	108.6	105.7	102.5	97.9	91.4	83.6
		B	108.6	107.5	108.6	106.1	97.5	97.9	88.2	81.8
		Mean[1]	108.6	106.4	108.6	106.1	100.0	97.9	89.8	82.9

DAT: days after treatment
[1] Mean was calculated from two replicates

B. DEGRADATION OF TEST ITEM
The mean residues of AMPA in soil extracts slowly decreased from 0 DAT to 120 DAT from 108.6 to 2.9 % AR.

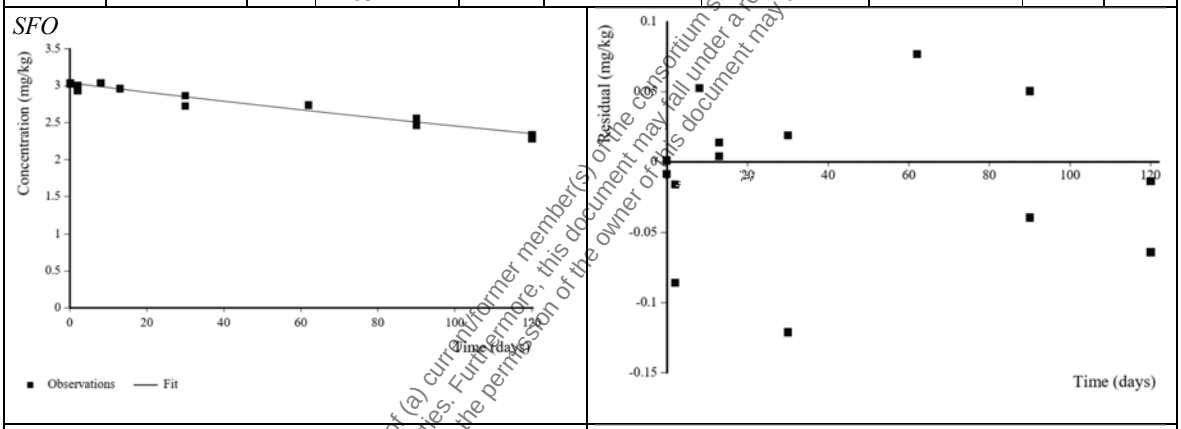
C. KINETICS
The analytical results were evaluated using CAKE 3.3 (2016) software according to FOCUS Guideline using four kinetic models (single first order (SFO), double first order in parallel (DFOP), first order multi compartment (FOMC) and hockey-stick (HS)) using replicate values. The degradation of AMPA was best described using Single First-Order kinetics (SFO) where the time for a decrease in the concentration of the test item is constant throughout the experiment and independent of its initial concentration. SFO results

were selected since this model yielded a low percent chi^2 error (1.25 %), acceptable statistical parameters and visually acceptable goodness-of-fit and hence the best fit. SFO will also be chosen as modelling endpoint. The DT_{50} value determined was 326 days and the DT_{90} value was 1080 days.

Table 7.1.2.1.2-10: Kinetic models and goodness-of-fit statistics

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error [%]	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} [d]	DT_{90} [d]
SFO	**good**	**3.039**	**0.002128**	**1.25**	**k: <0.001**	**0.00184**	**0.002**	**326**	**1080**
FOMC	good	3.039	α: 25.97 β: 0.000122	1.34	N/A	β: -84340	β: 109000	329	1130
DFOP	good	3.039	k_1: 0.002143 k_2: 0.00027 g: 0.9929	1.44	k_1: 0.3601 k_2: 0.4998	k_1: -0.01059 k_2: -1.148	k_1: 0.015 k_2: 1.149	326	1100
HS	good	3.023	k_1: 0.001739 k_2: 0.002763 tb: 63.22	1.19	k_1: <0.001 k_2: 0.002	k_1: 0.001017 k_2: 0.001101	k_1: 0.002 k_2: 0.004	274	857

SFO



FMOC

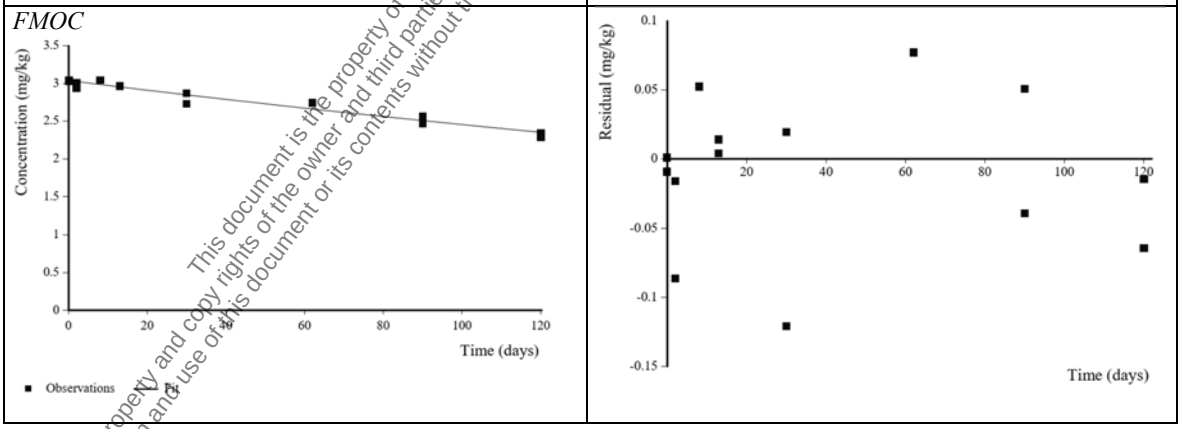
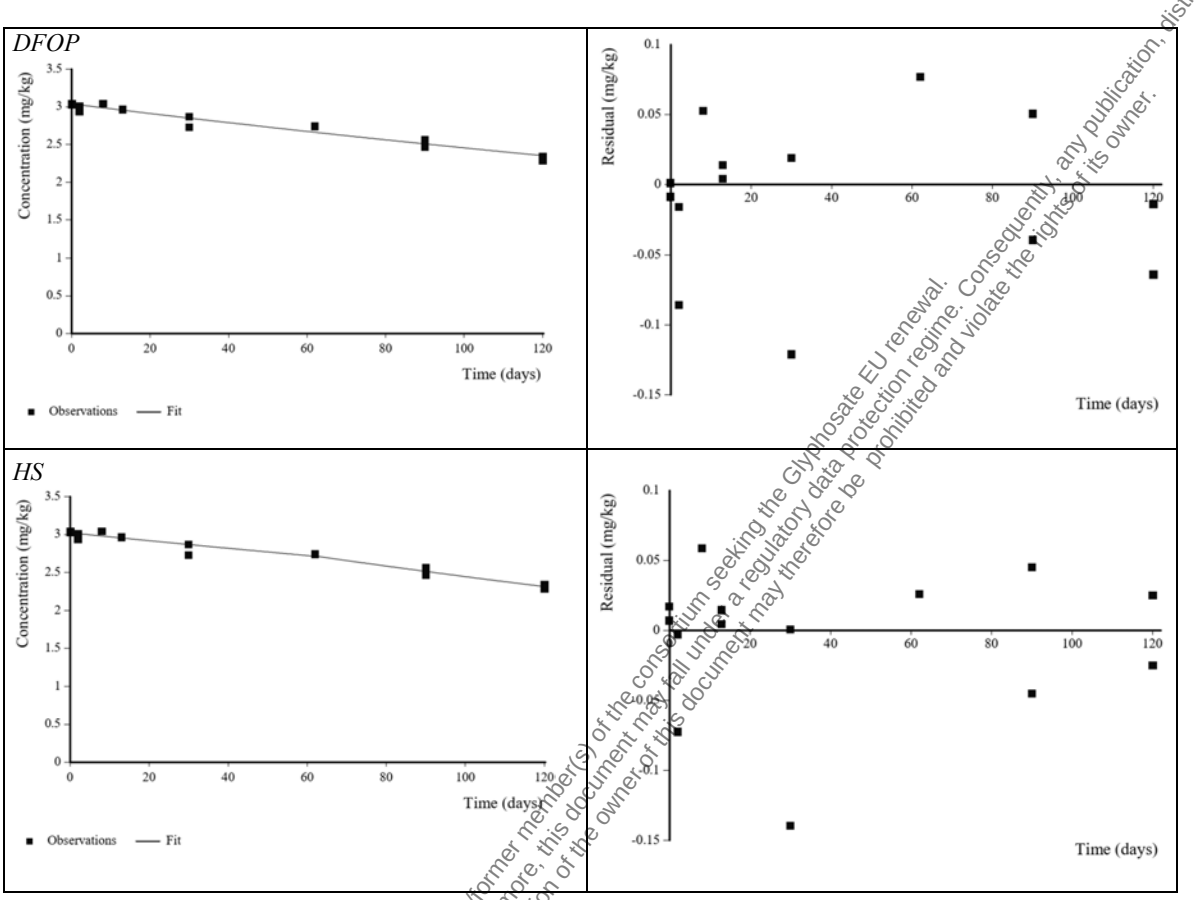


Table 7.1.2.1.2-10: Kinetic models and goodness-of-fit statistics



III. CONCLUSIONS

The aim of this study was to determine the degradation rate of the non-labelled test item AMPA in one microbially active highly acidic soil. The study was performed in the dark at 20 ºC under aerobic conditions over an incubation period of 120 days.

The test item degraded slowly under aerobic laboratory conditions with a half-life of 326 days and a DT_{90} value of 1080 days. The slow rate of degradation of AMPA in this soil is consistent in general with the strong ability of AMPA to tightly bind to most soils, which limits its availability to microorganisms for degradation.

The study suggests that degradation of AMPA follows a Single First-Order kinetic as best fit and hence also modelling endpoint indicating that the time for the decrease in the concentration of AMPA will be constant while present in soil and is independent of its initial concentration.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is conducted according to the current guidelines and is therefore considered valid.

Assessment and conclusion by RMS:

CA 7.1.2.1.3 Anaerobic degradation of the active substance

The fate of glyphosate was investigated in one anaerobic soil in the course of one study which is considered valid to address the data point ([redacted] 2003, CA 7.1.1.2/003). The results of this study were evaluated according to the current FOCUS kinetic guidances ([redacted] 2020, CA 7.1.2.1.3/001).

For glyphosate, estimated DT_{50} and DT_{90} are >1000 days (DFOP model). For AMPA, no reliable endpoints could be derived.

In the scientific literature review for glyphosate (2010-2019), one article was identified to provide further information relevant to the data point. The reliability of the article was assessed as "reliable with restrictions". Thus, no new endpoints were derived, and the article is considered as supportive information. The article of Kanissery *et al.* (2015, CA 7.1.2.1.3/002) showed in aerobic and anaerobic degradation experiments that degradation and mineralisation of glyphosate is slower under anoxic conditions. The addition of soil phosphate was found to stimulate degradation in anoxic soils only.

Table 7.1.2.1.3-1: Studies on anaerobic soil degradation with glyphosate (rate)

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.2.1.3/001	[redacted] 2020	Kinetic evaluation	Glyphosate	Valid	Updated kinetic evaluation of [redacted] 2003, CA 7.1.1.2/003

Table 7.1.2.1.3-2: Anaerobic rate of degradation - relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.2.1.3/002	Kanissery *et al.*, 2015	Soil anaerobic degradation rate	Glyphosate	Reliable with restrictions	

Updated kinetic evaluation of anaerobic soil degradation studies with glyphosate as test item

1. Information on the study

Data point	CA 7.1.2.1.3/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from an anaerobic laboratory soil degradation study
Report No	112148-004
Document No	
Guidelines followed in study	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: none
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities	No, not applicable for this study type
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
A kinetic evaluation of one anaerobic soil degradation study was performed in order to derive trigger (persistence) endpoints for glyphosate and its major soil metabolite AMPA. The evaluation was conducted according to FOCUS kinetics guidance (2006, 2014) using the fitting software CAKE v3.3.

For glyphosate, estimated DT_{50} and DT_{90} are >1000 days (DFOP model). For AMPA no reliable endpoints could be derived.

I. MATERIALS AND METHODS

The purpose of this assessment was to conduct a kinetic modelling evaluation for glyphosate and its major soil metabolite AMPA using results from an anaerobic laboratory soil degradation study ([redacted] 2003, CA 7.1.1.2/003). The aim of the evaluation was to derive trigger endpoints for glyphosate and AMPA.

Kinetic evaluation was performed with the data from the anaerobic phase, only.

1. Data pre-processing
The standard procedures recommended by FOCUS (2006, 2014) were followed for all residues to adjust the experimental data for kinetic modelling where necessary.

The residues used for the kinetic evaluation were based on the sum of results in the overlying water and soil extracts. Prior to day 28, glyphosate and AMPA were not quantified in the overlying water since there was overall less than 5 % of the total applied radioactivity found in the water samples. Only glyphosate was present in all subsequent water samples (with the exception of day 84 where AMPA residues of <1 % AR were found). Therefore, for the kinetic evaluation, it was assumed that the radioactivity in the water samples collected prior to day 28 only could be attributed to glyphosate, and the radioactivity in the water samples prior to day 28 was added to the amount of glyphosate in the respective soil extracts.

The initial amounts of glyphosate and AMPA were left at their originally measured values at day 0 since glyphosate was already applied in the aerobic phase.

Processed residue data for kinetic evaluation are presented in the following table.

Table 7.1.2.1.3-3: Processed residue data (% AR) of glyphosate and its metabolite AMPA in [redacted] (2003)

Time (d)	Glyphosate (% AR)	AMPA (% AR)
0	57.99[1,2]	19.46[1]
0	57.48[1,2]	21.19[1]
3	54.55[2]	19.41
3	56.32[2]	19.76
7	54.05[2]	18.28
7	53.01[2]	18.62
14	48.20[2]	20.92
14	48.50[2]	20.49
28	44.96[2]	26.19
28	46.73[2]	25.24
56	37.00	30.56
56	46.00	22.54
84	38.31	29.60
84	37.21	30.89
120	37.93	29.93
120	40.18	26.96

[1] Since the test item was applied in the aerobic phase, the measured values were used for M_0 and no corrections were made

[2] Total radioactivity in the overlying water for these samples accounted for <5 % applied activity. For the evaluation, it was assumed to be glyphosate and was included in total.

2. Kinetic models and analysis

Kinetic models

Three kinetic degradation models were considered to describe the degradation behaviour of the compounds in soil: single first-order (SFO), first-order multi-compartment (FOMC = Gustafson and Holden model) and the double-first-order-in-parallel (DFOP) (FOCUS; 2006, 2014).

For the parent compound, the best-fit model was accepted for deriving trigger endpoints.

For the metabolite, a pathway fit was conducted using the appropriate kinetic model for trigger endpoints for the parent determination and SFO for the metabolite.

The kinetic endpoints for parent and metabolite are normally derived from the pathway fit but since no reliable endpoints could be derived from the pathway fit, trigger endpoints for the parent were derived from the parent-only fit.

Optimisation

The kinetic analysis was conducted using the software CAKE v3.3.

The data were fitted with the complete dataset and unconstrained initial concentration (M_0) for glyphosate and AMPA. Iteratively Reweighted Least Square (IRLS) was used as the solver, as implemented in CAKE. Optimisations were carried out for the initial soil residue (M_0), degradation model parameters k, α, β or g, depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually, based on the observed degradation pattern and preliminary model runs. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration vs. time plots and residual plots) and statistically (Chi-square (χ^2) test). The visual inspection focused on the residuals which should not be distributed systematically around the zero line, but randomly. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered
- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered

A statistical measure of the quality of a fit is given by the χ^2-test. The χ^2-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements. The model with the smallest error percentage was defined as the most appropriate, because it described the measured data in the most robust way.

In general, for parent compounds, it is recommended that if the χ^2 error is <15 %, then the model has adequately reflected the measured data. However, this value should only be considered as guidance and not an absolute cut-off criterion. The guidance is less clear for metabolites due to the complexity of the curve fitting for multiple components, and so this criterion is a little more relaxed.

Significance of parameters

A single-sided t-test was performed to evaluate whether the optimised parameters were significantly different from zero at a chosen significance level of 5 %. In case of metabolite data, a significance level of 10 % or higher may still be acceptable due to the inherent variability that often occurs in these types of data. This is particularly relevant for the degradation rate constants (k) of the SFO and DFOP kinetic models. For the FOMC kinetic model, only the significance of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The CAKE software also reports a confidence interval on the optimised parameter estimates. The confidence interval should be relatively tight and not contain 0 to be considered statistically robust.

II. RESULTS AND DISCUSSION

The results of the kinetic evaluation of glyphosate and AMPA in anaerobic soil are presented in tables below.

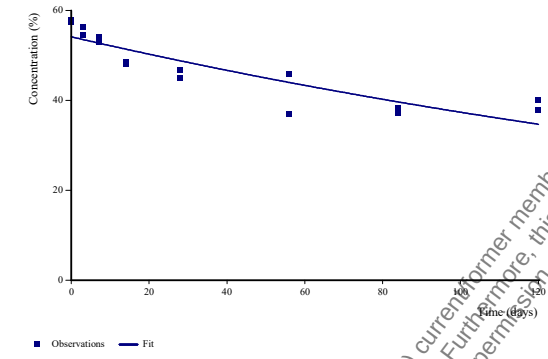
Table 7.1.2.1.3-4: Kinetic models and goodness-of-fit statistics of parent-only fits

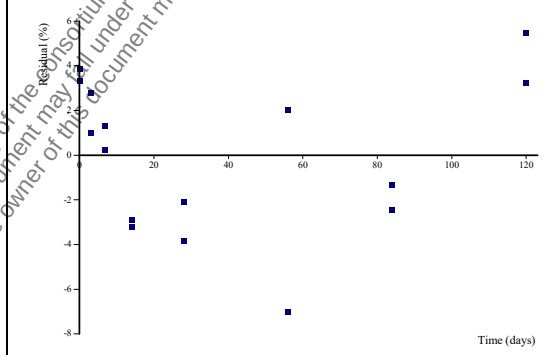
Kinetic model	Visual assessment	M_0[1]	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	54.2	k: 0.0037	4.8	k: <0.001	k: 0.0026	k: 0.005	187	621
FOMC	Good	58.2	α: 0.1536 β: 7.248	1.8	-[2]	β: -2.956	β: 17.45	654	>1000
DFOP	Good	57.5	k_1: 0.0373 k_2: 7.98×10^{-10} g: 0.3344	1.6	k_1: 0.059 k_2: 0.5	k_1: -0.0109 k_2: -0.0043	k_1: 0.085 k_2: 0.004	>1000	>1000

The anaerobic degradation of glyphosate in soil is best described by bi-phasic models.
Both bi-phasic models provide visually good fits. The estimate provided by the DFOP model for the slow phase degradation parameter (k_2) indicates that all visible degradation takes place during the fast phase. Hence k_2 is not significantly different from zero. Nonetheless, the DFOP model provides a slightly better statistical assessment than the FOMC model.

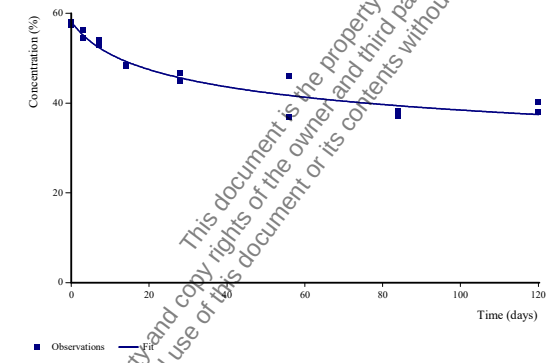
Conclusion: DFOP to be used in pathway fit for trigger endpoints

SFO





FOMC



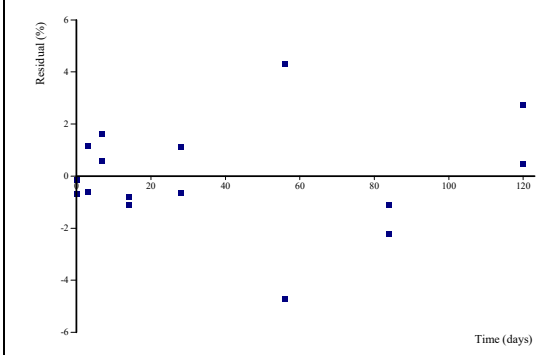
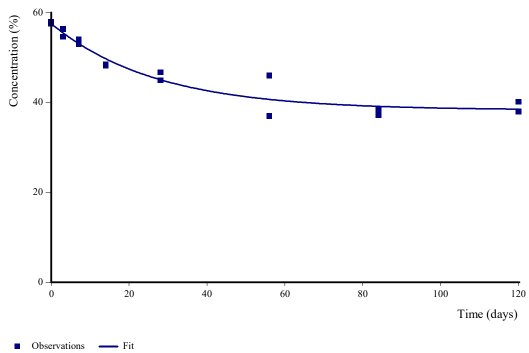
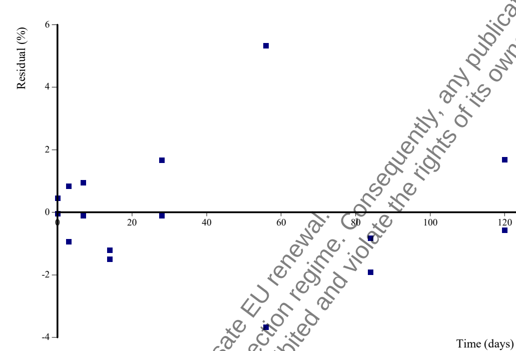


Table 7.1.2.1.3-4: Kinetic models and goodness-of-fit statistics of parent-only fits

DFOP





[1] Since glyphosate was applied in the aerobic phase, M_0 at day 0 were left at the measured values
[2] t-test not relevant for kinetic parameter β

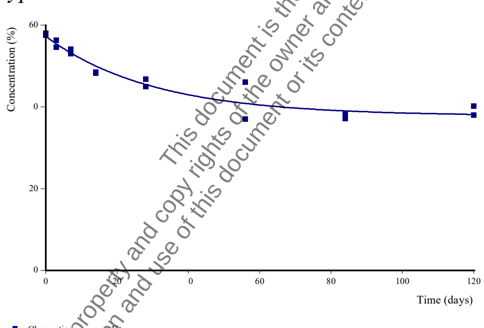
Table 7.1.2.1.3-5: Kinetic models and goodness-of-fit statistics of pathway fit

Kinetic model	Visual assess-ment	M_0[1]	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	57.2	k_1: 0.0331 k_2: 0.0000 g: 0.3396	1.7	k_1: 0.0554 k_2: 0.5	k_1:-0.0081 k_2:-0.0044	k_1: 0.074 k_2: 0.004	>1000	>1000	-
AMPA: SFO	Accep-table	18.3	k: 0.0000	5.1	k: 0.5	k: -0.0029	k: 0.003	>1000	>1000	0.559 (±0.216)

The measured data of glyphosate and AMPA are well described by the pathway fit. However, the degradation rate of AMPA is not significantly different from zero as no decline is observed. No reliable endpoints can thus be derived.

Conclusion: For glyphosate, estimated DT_{50} and DT_{90} are >1000 d each
For AMPA, no reliable endpoints could be derived

Glyphosate: DFOP



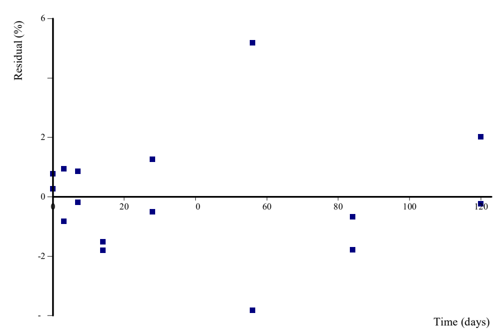
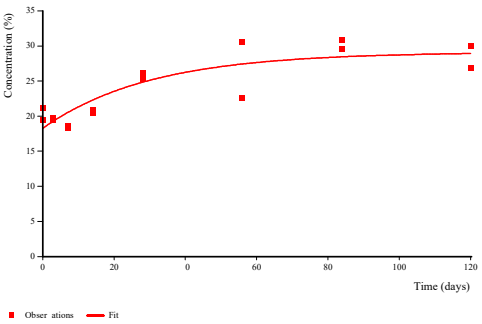
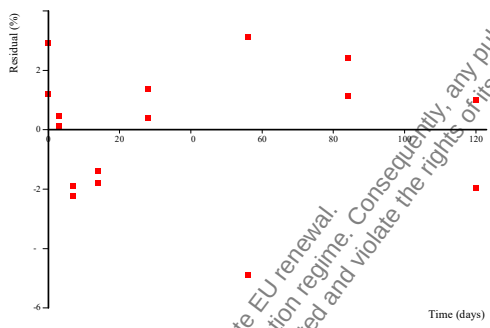


Table 7.1.2.1.3-5: Kinetic models and goodness-of-fit statistics of pathway fit

AMPA: SFO	 

[1] Since glyphosate was applied in the aerobic phase, M_0 at day 0 were left at the measured values.

For glyphosate, estimated DT_{50} and DT_{90} are >1000 days (DFOP model). For AMPA no reliable endpoints could be derived.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The kinetic evaluation was conducted according to current guidance and was therefore considered to be valid.

Assessment and conclusion by RMS:

Relevant articles from literature search

1. Information on the study

Data point:	CA 7.1.2.1.3/002
Report author	Kamissery, R. G. *et al.*
Report year	2015
Report title	Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate
Document No	DOI 10.2134/jeq2014.08.0331 E-ISSN 1537-2537
Guidelines followed in study	USEPA guidelines for adsorption studies (USEPA, 2008)
Deviations from current test guideline	Not applicable; insufficient details reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple data points. The summary is provided under CA 7.1.2.1.1/013.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

Information on the degradation of metabolites of glyphosate under anaerobic conditions is available in studies conducted with the parent (see CA 7.1.1.1.2 & CA 7.1.2.1.3).

CA 7.1.2.2 Field Studies

CA 7.1.2.2.1 Soil dissipation studies

Field dissipation studies were carried out either with glyphosate or its trimesium salt (glyphosate-trimesium) at locations representative of Central Europe (multiple field locations in Germany and Switzerland) and those where climate and soil characteristics were comparable with Southern Europe ecoregion (USA/ Tennessee, California, Georgia) and Northern Europe (Canada).

For studies performed with glyphosate-trimesium only the results for the glyphosate (PMG) anion are considered for evaluation and further assessment.

The field dissipation of glyphosate was investigated as reported in five studies at ten locations in the EU which are considered valid to address the data point ([redacted] 1992, CA 7.1.2.2.1/008, [redacted] 1992, CA 7.1.2.2.1/009, [redacted] 1992, CA 7.1.2.2.1/010, [redacted] 1992, CA 7.1.2.2.1/011 and [redacted] 1992, CA 7.1.2.2.1/013).

The residue data of glyphosate and, where applicable, AMPA of the field dissipation studies conducted in Europe were evaluated according to the current FOCUS kinetic guidance ([redacted] 2020, CA 7.1.2.2.1/001).

For field dissipation studies with glyphosate conducted in the US and Canada, an Ecoregion Crosswalk exercise was performed to evaluate the representativeness for European conditions ([redacted] 2020, CA 7.1.2.2.1/002).

A total of three studies performed at nine locations were identified to be representative for EU conditions ([redacted] 1993, CA 7.1.2.2.1/005, [redacted] 1993, CA 7.1.2.2.1/006 and [redacted] 1992, CA 7.1.2.2.1/014).

The residue data of glyphosate and, where applicable, AMPA for the US/Canadian trials representative for the EU was evaluated according to the current FOCUS kinetic guidances ([redacted] 2020, CA 7.1.2.2.1/003).

There are two field dissipation studies performed in the US that are considered as supportive since being identified as not representative for European conditions ([redacted] 1989, CA 7.1.2.2.1/017 and [redacted] 1989, CA 7.1.2.2.1/018).

In addition, three storage stability studies provide supportive information. These studies prove the storage stability of glyphosate and AMPA in soil for at least 404 days ([redacted] 1993, CA 7.1.2.2.1/007, [redacted] 1995, CA 7.1.2.2.1/012 and [redacted] 1986, CA 7.1.2.2.1/019).

Glyphosate degraded in soil under field conditions to form aminomethylphosphonic acid (AMPA), which was further degraded. The majority of glyphosate and AMPA residues was found in the top layer (ca 0-10 cm) of soil, suggesting that leaching is not a significant route of dissipation. The trigger DT_{50}- and DT_{90}-values for dissipation of glyphosate in the soil range from 2.1 to 147 days and from 35.3 to >1000 days, respectively (Table 7.1.2-3). Normalised DT_{50} values as used as modelling input endpoints range from 12.7 to 182 days (Table 7.1.2-4).

AMPA was found at a maximum of 20.2 to 63.0 % when being referred to glyphosate residues recovered at day 0 (Table 7.1.2-5). The trigger DT_{50}- and DT_{90}–values for AMPA in soil range from 65.0 to 634 days and from 216 to >1000 days, respectively (Table 7.1.2-6). Normalised modelling endpoints range from 90.7 and 471 days (Table 7.1.2-7).

The normalised field DT_{50} values for glyphosate and AMPA were assessed against soil pH to investigate the potential for pH-dependency. The assessment including laboratory data can be found in Section 7.1.2 (see Table 7.1.2-8 & Figure 7.1.2-3 for glyphosate and Table 7.1.2-9 & Figure 7.1.2-4 for AMPA).

The search for peer reviewed scientific literature (2010-2019) resulted for glyphosate in two publications to potentially provide further information relevant to the data point.

The reliability assessment resulted in a classification "reliable with restrictions" for the two publications. Consequently, the articles are considered as supportive information with no additional endpoints derived for risk assessment.

The publication by Passeport *et al.* (2014, CA 7.1.2.2.1/026) investigated wet forest buffer zones (soil and organic rich litter layer) to have a retarding effect on molecule transfer, thereby reducing concentrations and loads of chemicals like glyphosate and its main soil metabolite AMPA in the buffer water outflow and, consequently, adjacent surface water.

The publication by Todorovic *et al.* (2014, CA 7.1.2.2.1/027) investigated the runoff of residues from soils being susceptible to erosion under field conditions. The authors concluded that total residue loads from runoff and consequently, loads of glyphosate and AMPA in runoff water and sediment depend on soil type, soil structure and soil management practice (here: conventional tillage vs. no tillage). Further conclusions on implications under acgricultural practice conditions are limited due to the artificial character of the experimental conditions.

Table 7.1.2.2.1-1: Field soil dissipation studies

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.2.2.1/001	[redacted] 2020	Kinetic evaluation	Glyphosate and AMPA	Valid	Kinetic evaluation of European studies
CA 7.1.2.2.1/002	[redacted] 2020	Ecoregion Crosswalk	Glyphosate and AMPA	Valid	
CA 7.1.2.2.1/003	[redacted] 2020	Kinetic evaluation	Glyphosate and AMPA	Valid	Kinetic evaluation of US & Canadian studies
CA 7.1.2.2.1/004	[redacted] 1994	Terrestrial field dissipation	Glyphosate-Trimesium	Invalid	
CA 7.1.2.2.1/005	[redacted] 1993	Terrestrial field dissipation	Glyphosate	Valid	Kinetic evaluation in Sachers, 2020, CA 7.1.2.2.1/003
CA 7.1.2.2.1/006	[redacted] 1993	Terrestrial field dissipation	Glyphosate	Valid	Kinetic evaluation in Sachers, 2020, CA 7.1.2.2.1/003
CA 7.1.2.2.1/007	[redacted] 1993	Storage stability study	Glyphosate and AMPA	Supportive	
CA 7.1.2.2.1/008	[redacted] 1992	Terrestrial field dissipation	Glyphosate	Valid	Kinetic evaluation in Robinson, 2020, CA 7.1.2.2.1/001
CA 7.1.2.2.1/009	[redacted] 1992	Terrestrial field dissipation	Glyphosate	Valid	Kinetic evaluation in Robinson, 2020, CA 7.1.2.2.1/001
CA 7.1.2.2.1/010	[redacted] 1992	Terrestrial field dissipation	Glyphosate	Valid	Kinetic evaluation in Robinson, 2020, CA 7.1.2.2.1/001
CA 7.1.2.2.1/011	[redacted] 1992	Terrestrial field dissipation	Glyphosate	Valid	Kinetic evaluation in Robinson, 2020, CA 7.1.2.2.1/001

Table 7.1.2.2.1-1: Field soil dissipation studies

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.2.2.1/012	[redacted] 1995	Storage stability study	Glyphosate; AMPA	Supportive	
CA 7.1.2.2.1/013	[redacted] 1992	Terrestrial field dissipation	Glyphosate-Trimesium	Valid	Kinetic evaluation in [redacted] 2020, CA 7.1.2.2.1/001
CA 7.1.2.2.1/014	[redacted] 1992	Terrestrial field dissipation	Glyphosate-Trimesium	Valid	Kinetic evaluation in [redacted] 2020, CA 7.1.2.2.1/003
CA 7.1.2.2.1/015	[redacted] 1990	Terrestrial field dissipation	Glyphosate; AMPA	Invalid	
CA 7.1.2.2.1/016	[redacted] 1989	Terrestrial field dissipation	Glyphosate	Valid	Kinetic evaluation in [redacted] 2020, CA 7.1.2.2.1/003
CA 7.1.2.2.1/017	[redacted], 1989	Terrestrial field dissipation	Glyphosate	Supportive	Not representative for Europe according to [redacted] 2020, CA 7.1.2.2.1/002
CA 7.1.2.2.1/018	[redacted] 1989	Terrestrial field dissipation	Glyphosate	Supportive	Not representative for Europe according to [redacted] 2020, CA 7.1.2.2.1/002
CA 7.1.2.2.1/019	[redacted] 1986	Storage stability study	Glyphosate-Trimesium	Supportive	
CA 7.1.2.2.1/020	[redacted] 1984	Terrestrial field dissipation	Glyphosate	Invalid	
CA 7.1.2.2.1/021	[redacted] 1983	Terrestrial field dissipation	Glyphosate	Invalid	Addendum: [redacted] 1988, CA 7.1.2.2.1/022
CA 7.1.2.2.1/022	[redacted] 1988	Terrestrial field dissipation	Glyphosate	Invalid	Addendum to [redacted] 1983, CA 7.1.2.2.1/021
CA 7.1.2.2.1/023	[redacted] 1983	Terrestrial field dissipation	Glyphosate	Invalid	
CA 7.1.2.2.1/024	[redacted] 1982	Terrestrial field dissipation	Glyphosate	Invalid	
CA 7.1.2.2.1/025	[redacted] 1979	Terrestrial field dissipation	Glyphosate	Invalid	

Table 7.1.2.2.1-2: Field soil dissipation – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.2.2.1/026	Passeport *et al.*, 2014	Field studies - other	Glyphosate	Reliable with restrictions	
CA 7.1.2.2.1/027	Todorovic *et al.*, 2014	Field studies - other	Glyphosate and AMPA	Reliable with restrictions	

Field soil dissipation studies

1. Information on the study

Data point:	CA 7.1.2.2.1/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from terrestrial field dissipation studies in Europe
Report No	112148-003
Document No	
Guidelines followed in study	EFSA (2014): EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662 [37 pp.]. FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp. FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not applicable for this study type
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L-docs)	Category 1

2. Full summary

Executive Summary

A kinetic re-evaluation of five terrestrial field soil dissipation studies was performed for glyphosate and its major soil metabolite AMPA. The kinetic endpoints may be used for comparison against regulatory trigger values (trigger/ persistence endpoints), or for calculating predicted environmental concentrations (PECs) in fate and exposure models (modelling endpoints).

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics, and of the European Food Safety Authority (EFSA). Trigger endpoints were evaluated according to best-fit kinetics. For modelling endpoints, a time-step normalisation method was applied to the data (standard reference conditions of 20 °C and pF 2) and samples taken prior to 10 mm cumulative rainfall were excluded. Following normalisation, all datasets were checked for whether the field decline curve could be described well with a single first-order (SFO) model using procedures proposed by FOCUS.

The evaluation was based on soil residue data from five legacy field soil dissipation studies comprised of 10 trial sites in Germany and Switzerland. The evaluation was performed using the model fitting software CAKE 3.3.

I. MATERIALS AND METHODS

The FOrum for the Coordination of Pesticide Fate-Models and their USe (FOCUS) developed recommendations for the kinetic evaluation of soil degradation studies conducted in the laboratory or in the field (FOCUS, 2006, 2014). These recommendations intend to harmonise the derivation of degradation or dissipation parameters from soil studies. For modelling endpoints, further guidance has been published to help derive $DegT50_{matrix}$ values in soil (EFSA, 2014).

Glyphosate is a broadcast herbicide used widely in agricultural and non-agricultural practice. An assessment of the potential environmental impact in soil, groundwater and surface water requires an understanding of the key degradation/ dissipation pathways and rates in soil.

The purpose of this evaluation was to conduct a kinetic evaluation for glyphosate and its major soil metabolite aminomethylphosphonic acid (AMPA) using data from field soil dissipation studies in order to: i) derive DT_{50} and DT_{90} values for use in soil exposure calculations and for comparison with trigger values from guidelines, and ii) derive $DegT50_{matrix}$ values for use in environmental exposure models for groundwater and surface water.

Five legacy field dissipation studies, comprised of 10 field trials located in Germany and Switzerland ([redacted] 1992, CA 7.1.2.2.1/013; [redacted] 1992, CA 7.1.2.2.1/008-CA 7.1.2.2.1/011), were evaluated according to the most recent guidance (FOCUS, 2006, 2014; EFSA, 2014). The kinetic evaluation was performed using the model fitting software CAKE 3.3 (CAKE, 2016).

1. Description of the terrestrial field dissipation studies

The five field soil dissipation studies ([redacted] 1992, CA 7.1.2.2.1/013; [redacted] 1992, CA 7.1.2.2.1/008-CA 7.1.2.2.1/011) included for kinetic evaluation were conducted at 10 sites in Germany and Switzerland, representing soils and climate typical of Central Europe. Different amounts of glyphosate, formulated as either glyphosate-trimesium or the isopropylamine salt, were applied to bare soil. Soil samples from studies conducted with either formulation of glyphosate were analysed for glyphosate and its metabolite AMPA.

A summary of the trial locations and application data is given in the following table.

Table 7.1.2.2.1-3: Summary of trial locations and application data in field soil dissipation studies

Study	Trial/ location	Formulation	Crop	Date of Application	Target rate (kg a.s./ha)[1]	Actual rate (kg a.s./ha)[1]
[redacted] 1992, CA 7.1.2.2.1/013	Büchen, Germany	Glyphosate-trimesium	Bare soil	11/04/90	3.31	3.59
	Klein-Zecher, Germany	Glyphosate-trimesium	Bare soil	10/08/90	3.31	3.93
	Unzhurst, Germany	Glyphosate-trimesium	Bare soil	03/05/90	3.3.1	3.31
	Rohrbach, Germany	Glyphosate-trimesium	Bare soil	25/07/90	3.31	3.45
	Herrngiersdorf, Germany	Glyphosate-trimesium	Bare soil	08/05/90	3.31	3.17
	Wang-Inzkofen, Germany	Glyphosate-trimesium	Bare soil	02/07/90	3.31	3.31
[redacted] 1992, CA 7.1.2.2.1/008	Diegten, Switzerland	Isopropylamine salt	Bare soil	05/09/90	n.r.	3.53
[redacted], 1992, CA 7.1.2.2.1/009	Egerkingen, Switzerland	Isopropylamine salt	Bare soil	04/09/90	n.r.	3.87
[redacted] 1992, CA 7.1.2.2.1/010	Bad Krozingen, Germany	Isopropylamine salt	Bare soil	05/09/90	n.r.	3.67
[redacted] 1992, CA 7.1.2.2.1/011	Menslage, Germany	Isopropylamine salt	Bare soil	07/09/90	n.r.	3.67

n.r. = not reported
[1] Converted to glyphosate-equivalent where appropriate

In general, a single treated plot was considered at all trial sites. 20 cores were taken at each sampling time, dissected into soil horizons (up to 30 cm depth) and blended to give a composite sample for each horizon. The duration of sampling varied between 61 and 582 days across the trial sites.

2. Data pre-processing

The data from the legacy field trials required pre-processing in order to generate appropriate input datasets for the kinetic evaluation. The standard procedures recommended by FOCUS (2006, 2014) were applied. Single samples were available for all studies.

The time-zero concentration for the metabolite was set to zero and the initial metabolite amount was converted to parent-equivalent (accounting for the molar weight difference between the compounds) and added to the parent substance.

In all of the studies considered, the LOQ and LOD were indistinguishable; only the 'limit of determination' is reported. Hence, the LOQ and LOD were both assigned the same value and the FOCUS guidance was then applied as follows. Values below LOD were replaced by half the LOD. If the concentrations of the applied substance in soil declined to values below LOD, the curve was cut off after the first value below LOD, unless detections above LOQ were made later in the experiment (FOCUS, 2006, 2014). These corrections were performed along the time course, as well as with depth along the soil horizon, with the exception for 0 DAT where it was assumed that residues only resided in the upper most soil layer.

For each treated plot (trial site) the measured residues (mg/kg) in the different soil layers were converted into residues expressed in kg/ha (considering the layer depth and bulk density) and then summed up. They were then expressed as percentage values of the residue at 0 DAT (so the time zero value is 100 %). Thus, if the maximum concentration occurred after 0 DAT, the respective maximum percentage value was greater than 100 %. For the studies of [redacted] (1992, CA 7.1.2.2.1/008-CA 7.1.2.2.1/011), a default value of 1.5 g/cm^3 was assumed for the bulk density. For the study of [redacted] (1992, CA 7.1.2.2.1/013), the horizon-specific bulk density was calculated at each sampling time using the reported soil core surface area, depth and dry weight.

The input values of AMPA were expressed as percentage values of the parent (glyphosate) residue at 0 DAT (correcting for molar weight differences).

Processed residue data, adjusted as described above, are presented in the following tables and were used in the kinetic evaluation.

Table 7.1.2.2.1-4: Processed residue data (kg/ha and % of DAT 0) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1992, CA 7.1.2.2.1/013)

Time (DAT)	Sum of horizons (0 - 20 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Büchen				
0	3.25	0.00	100.00	0.00
7	3.15	0.23	96.94	10.61
14	2.76	0.33	84.73	15.51
28	2.17	0.37	66.71	17.37
61	1.12	0.48	34.42	22.26
91	0.89	0.77	27.45	35.83
121	0.39	0.32	12.12	14.93
182	0.44	0.60	13.59	28.24
240	0.31	0.49	9.39	23.09
322	0.26	0.32	8.04	14.76
475	0.26	0.51	7.89	23.81
Klein-Zecher				
0	2.93	0.00	100.00	0.00
7	2.94	0.43	100.10	22.16
14	2.22	0.48	75.69	25.03
28	1.55	0.47	52.74	24.64
61	1.30	0.67	44.16	34.94
91	0.74	0.53	25.28	27.42
119	0.92	0.59	31.26	30.83
201	0.75	0.73	25.55	38.06
244	0.65	0.70	22.15	36.15
298	0.30	0.58	10.36	29.89
479	0.18	0.68	6.09	35.51
567	0.04	0.52	1.42	26.80
Unzhurst				
0	3.47	0.00	100.00	0.00
7	2.59	0.24	74.61	10.63
13	2.47	0.31	71.12	13.72
27	1.92	0.27	55.36	11.91
57	0.71	0.55	20.52	23.92
90	0.53	0.61	15.22	26.91
117	0.35	0.54	9.98	23.63
187	0.25	0.53	7.22	23.04
251	0.24	0.62	7.05	26.98
314	0.22	0.55	6.30	24.21
418	0.15	0.43	4.26	18.74

Table 7.1.2.2.1-4: Processed residue data (kg/ha and % of DAT 0) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1992, CA 7.1.2.2.1/013)

Time (DAT)	Sum of horizons (0 - 20 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Rohrbach				
0	2.28	0.00	100.00	0.00
7	2.68	0.34	117.47	22.36
14	2.05	0.46	89.68	30.53
28	1.36	0.44	59.67	29.28
56	0.44	0.66	19.20	43.65
85	0.20	0.63	8.66	41.86
231	0.04	0.56	1.53	37.58
282	-[2]	0.52	-[2]	34.41
418	-[2]	0.28	-[2]	18.60
582	-[2]	0.23	-[2]	15.61
Herrngiersdorf				
0	2.05	0.00	100.00	0.00
6	1.96	0.36	95.89	26.44
13	1.48	0.29	72.53	21.64
28	1.46	0.41	71.60	30.46
58	0.47	0.41	23.08	30.44
90	0.30	0.41	14.61	30.47
125	0.18	0.38	8.81	27.93
168	0.04	0.27	1.96	20.11
330	-[2]	0.28	-[2]	20.91
464	-[2]	0.13	-[2]	9.64
541	-[2]	-[2]	-[2]	-[2]
Wang-Inzkofen				
0	2.97	0.00	100.00	0.00
7	2.11	0.57	71.03	29.03
15	1.44	0.66	48.50	33.71
29	1.37	0.80	46.22	41.12
58	0.69	0.64	23.10	32.67
94	0.40	0.65	13.60	33.39
114	0.36	0.66	12.24	33.96
275	0.24	0.59	7.93	30.06
414	0.14	0.46	4.88	23.55
549	0.04	0.35	1.32	17.82

[1] Expressed as Glyphosate equivalent = percentage of Glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite.
[2] Data omitted according to FOCUS (2006, 2014)

Table 7.1.2.2.1-5: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1992 CA 7.1.2.2.1/008, CA 7.1.2.2.1/011)

Time (DAT)	Sum of horizons (0 - 30 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Diegten				
0	3.61	0.00	100.00	0.00
7	1.66	0.56	45.95	23.51
15	0.88	0.33	24.41	13.97
30	0.45	0.29	12.37	12.03
62	0.49	0.54	13.61	22.94
194	0.36	0.57	10.05	24.21
282	0.13	0.39	3.57	16.31
Egerkingen				
0	2.19	0.00	100.00	0.00
7	0.97	0.19	44.22	13.01
15	0.97	0.37	44.22	25.50
30	0.72	0.47	32.94	32.47
62	0.68	0.51	30.76	35.17
202	0.15	0.34	6.90	23.62
Bad Krozingen				
0	4.26	0.00	100.00	0.00
7	1.42	0.36	33.40	13.02
15	1.23	0.41	28.93	14.79
30	0.67	0.47	15.70	16.61
61	0.60	0.65	14.08	23.31
Menslage				
0	4.20	0.00	100.00	0.00
7	1.99	0.35	47.43	12.71
15	0.89	0.48	21.06	17.50
30	1.03	0.58	24.55	20.87
60	0.77	0.79	18.41	28.53
192	0.36	0.64	8.46	23.15
271	0.44	1.29	10.38	46.89
315	0.20	0.64	4.71	23.20

[1] Expressed as Glyphosate equivalent – percentage of Glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite

3. Normalisation of field degradation half-life values to reference conditions

General approach

Time-step normalisation according to FOCUS (2006, 2014) and [redacted] (2003) was conducted in order to derive modelling endpoints at reference conditions (20 °C and pF 2). Daily correction factors for soil temperature (f_T) and moisture (f_θ) were calculated for a given reference soil temperature of 20 °C and a reference soil moisture of pF 2.

According to FOCUS, the exponent of the moisture response function was set to 0.7 and the temperature coefficient Q_{10} was set to 2.58, respectively.

The following constraints were applied to the normalisation procedure:

- no further increase of the degradation rate if soil moisture > reference moisture
- no degradation if soil temperature <0 °C (resulting in a transformed day length of zero)

The obtained correction factors resulted in normalised transformation rates by reducing or increasing day lengths. Processed residue data, in combination with the transformed time course (*i.e.* under constant

temperature and moisture conditions), were used for the evaluation of modelling endpoints according to recommendations for obtaining $DegT50_{matrix}$ values in soil from field dissipation studies for modelling purposes (FOCUS, 2006, 2014; EFSA, 2014). For the time between application and first sampling (0 DAT), no normalisation was considered and application was assumed to occur at time point zero.

Estimation of soil temperature and moisture

Soil temperature and moisture data were not directly available from the trial sites. Therefore, daily values of these variables (mean of top 10 cm) were calculated with the environmental fate model FOCUSPEARL 4.4.4. Site-specific weather and soil data were used as input parameters to the model.

Weather data

In order to estimate the daily soil temperature and moisture, the evapotranspiration process must be defined. The Penman-Monteith approach was selected in FOCUSPEARL v4.4.4 to calculate the potential evapotranspiration. The required meteorological data for this estimation method (maximum and minimum temperature, precipitation, global radiation, average vapour pressure and average wind speed) were obtained from local meteorological stations (where available) and/ or the Monitoring Agricultural ResourceS Unit (MARS) of the EC Joint Research Centre as shown in the following table.

Table 7.1.2.2.1-6: Availability of weather data

Study	Trial/ location	DWD[1] station	Distance from test site (km)	MARS grid number (25 km grid)
[redacted] 1992, CA 7.1.2.2.1/013	Büchen, Germany	Grambeck (1736): rain, min/ max temp, v.p. Boizenburg (591): wind speed	12.6 10.1	113111 (global radiation)
	Klein-Zecher, Germany	Grambeck (1736): rain, min/ max temp, v.p. Boizenburg (591): wind speed	11.3 22.6	113112 (global radiation)
	Unzhurst, Germany	Rheinau-Freistett (4169): rain, min/ max temp, v.p.	10.2	91104 (global radiation and wind speed)
	Rohrbach, Germany	Bad Bergzabern (377): rain, min/ max temp, v.p.	12.8	94104 (global radiation and wind speed)
	Herrngiersdorf, Germany	Mallersdorf (3147): rain, min/ max temp, v.p.	13.0	92115 (global radiation and wind speed)
	Wang-Inzkofen, Germany	Weihenstephan (5404): rain, min/ max temp, v.p.	17.7	91115 (global radiation and wind speed)
[redacted] 1992, CA 7.1.2.2.1/008	Diegten, Switzerland	n.a.	-	86103 (rain, min/ max temp, v.p., global radiation and wind speed)
[redacted] 1992, CA 7.1.2.2.1/009	Egerkingen, Switzerland	n.a.	-	86103 (rain, min/ max temp, v.p., global radiation and wind speed)
[redacted] 1992, CA 7.1.2.2.1/010	Bad Krozingen, Germany	Schallstadt-Mengen (4419): rain, min/ max temp, v.p. Eshbach (706): wind speed	7.8 4.3	88102 (global radiation)
[redacted] 1992, CA 7.1.2.2.1/011	Menslage, Germany	Löningen (3044): rain, min/ max temp, v.p.	10.8	109104 (global radiation and wind speed)

Table 7.1.2.2.1-6: Availability of weather data

n.a. = not available
v.p. = vapour pressure
[1] German Meteorological Office

In accordance with EFSA guidance (2014), the weather stations from which precipitation data were derived were less than 20 km from the actual field site.

In the FOCUSPEARL 4.4.4 model, the weather data for the normalisation included a warm-up period of one year prior to the date of application, thereby accounting for seasonal effects. No irrigation was performed at the trial sites.

Soil profile settings
For the simulations with FOCUSPEARL 4.4.4, soil profiles were created based on the detailed soil properties given in the following tables.

Table 7.1.2.2.1-7: Soil characterisation for site Büchen, Germany ([redacted] 1992, CA 7.1.2.2.1/013)

Soil layer	**0 - 30 cm**	**30 - 60 cm**	**60 - 100 cm**
Soil texture (USDA)	Loamy sand	Loamy sand	Loamy sand
Sand (%)	80	80	81
Silt (%)	14	12	15
Clay (%)	6	8	4
Organic matter (%)	2.8	2.1	0.8
pH[1]	6.4	6.5	6.7
Bulk density (g/cm³)[2]	1.35	1.40	1.55
***Soil hydraulic parameters*[3]**			
Θ_{res} (m³/m³)[4]	0.025	0.025	0.025
Θ_{sat} (m³/m³)	0.4339	0.4185	0.3805
K_{sat} (m/d)	0.9845	0.4987	0.4261
α (cm^{-1})	0.0535	0.0705	0.0616
λ (-)	-1.2627	-1.6038	-0.0617
n (-)	1.3463	1.3279	1.4228
Θ_{ref} (pF 2) (m³/m³)[5]	0.2480	0.2287	0.1863

[1] Medium not reported
[2] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[3] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[4] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-8: Soil characterisation for site Klein-Zecher, Germany ([redacted] 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 60 cm	60 - 100 cm
Soil texture (USDA)	Sandy loam	Sandy loam	Sandy loam
Sand (%)	66	68	62
Silt (%)	21	15	19
Clay (%)	13	17	19
Organic matter (%)	1.9	1.2	0.2
pH[1]	7.0	7.0	7.3
Bulk density (g/cm³)[2]	1.42	1.50	1.67
Soil hydraulic parameters[3]			
Θ_{res} (m³/m³)[4]	0.025	0.025	0.01
Θ_{sat} (m³/m³)	0.4195	0.4000	0.3530
K_{sat} (m/d)	0.6876	0.3140	0.1431
α (cm^{-1})	0.0550	0.0752	0.0627
λ (-)	-2.2925	-2.8898	-1.9899
n (-)	1.2651	1.2207	1.1841
Θ_{ref} (pF 2) (m³/m³)[5]	0.2703	0.2617	0.2506

[1] Medium not reported
[2] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[3] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[4] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-9: Soil characterisation for site Unzhurst, Germany ([redacted] 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 60 cm	60 - 90 cm	90 - 100 cm[1]
Soil texture (USDA)	Loam	Sandy clay loam	Loam	Loam
Sand (%)	48	53	44	44
Silt (%)	39	31	37	37
Clay (%)	13	16	19	19
Organic matter (%)	1.8	0.6	0.3	0.15
pH[2]	6.7	5.4	5.3	5.3
Bulk density (g/cm³)[3]	1.43	1.58	1.64	1.69
Soil hydraulic parameters[4]				
Θ_{res} (m³/m³)[5]	0.01	0.01	0.01	0.01
Θ_{sat} (m³/m³)	0.4211	0.3839	0.3694	0.3560
K_{sat} (m/d)	0.3360	0.2327	0.1544	0.1071
α (cm^{-1})	0.0335	0.0429	0.0322	0.0315
λ (-)	-1.9296	-2.0472	-1.9183	-1.3176
n (-)	1.2560	1.2135	1.1800	1.1690
Θ_{ref} (pF 2) (m³/m³)[6]	0.2998	0.2765	0.2914	0.2856

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Medium not reported
[3] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[4] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[5] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[6] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-10: Soil characterisation for site Rohrbach, Germany ([redacted] 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 25 cm	25 - 35 cm	35 - 100 cm
Soil texture (USDA)	Silt loam	Silt loam	Silt loam
Sand (%)	12	13	15
Silt (%)	77	60	70
Clay (%)	11	27	15
Organic matter (%)	1.8	0.5	0.1
pH[1]	8.5	8.5	8.7
Bulk density (g/cm³)[2]	1.43	1.60	1.71
Soil hydraulic parameters[3]			
Θ_{res} (m³/m³)[4]	0.01	0.01	0.01
Θ_{sat} (m³/m³)	0.4171	0.3909	0.3518
K_{sat} (m/d)	0.0571	0.1057	0.0630
α (cm^{-1})	0.0108	0.0143	0.0083
λ (-)	-0.8235	-2.8613	0.6547
n (-)	1.3017	1.1370	1.2052
Θ_{ref} (pF 2) (m³/m³)[5]	0.3527	0.3509	0.3192

[1] Medium not reported
[2] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[3] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[4] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-11: Soil characterisation for site Herrngiersdorf, Germany ([redacted], 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 100 cm
Soil texture (USDA)	Clay loam	Silt loam
Sand (%)	23	21
Silt (%)	47	58
Clay (%)	30	21
Organic matter (%)	2.8	0.8
pH[1]	8.0	8.4
Bulk density (g/cm³)[2]	1.35	1.55
Soil hydraulic parameters[3]		
Θ_{res} (m³/m³)[4]	0.01	0.01
Θ_{sat} (m³/m³)	0.4551	0.4017
K_{sat} (m/d)	0.2175	0.1663
α (cm^{-1})	0.0311	0.0180
λ (-)	-3.5366	-2.4218
n (-)	1.1455	1.1758
Θ_{ref} (pF 2) (m³/m³)[5]	0.3760	0.3424

[1] Medium not reported
[2] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[3] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[4] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-12: Soil characterisation for site Wang-Inzkofen, Germany ([redacted] 1992, CA 7.1.2.2.1/013)

Soil layer	0 - 30 cm	30 - 100 cm[1]
Soil texture (USDA)	Silt loam	Silt loam
Sand (%)	25	25
Silt (%)	51	51
Clay (%)	24	24
Organic matter (%)	2.1	1.05
pH[2]	7.2	7.2
Bulk density (g/cm³)[3]	1.40	1.51
Soil hydraulic parameters[4]		
Θ_{res} (m³/m³)[5]	0.01	0.01
Θ_{sat} (m³/m³)	0.4356	0.4129
K_{sat} (m/d)	0.1929	0.1714
α (cm⁻¹)	0.0272	0.0241
λ (-)	-3.1300	-3.0398
n (-)	1.1767	1.1536
Θ_{ref} (pF 2) (m³/m³)[6]	0.3526	0.3478

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Medium not reported
[3] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[4] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[5] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[6] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-13: Soil characterisation for site Diegten, Switzerland [redacted] 1992, CA 7.1.2.2.1/008)

Soil layer	0 - 30 cm	30 - 100 cm[1]
Soil texture (USDA)	Sandy clay	Sandy clay
Sand (%)	47.55[2]	47.55
Silt (%)	13.29[2]	13.29
Clay (%)	39.16[2]	39.16
Organic carbon (%)	1.61	0.81
Organic matter (%)[3]	2.78	1.39
pH (KCl)	7.1	7.1
Bulk density (g/cm³)[4]	1.35	1.47
Soil hydraulic parameters[5]		
Θ_{res} (m³/m³)[6]	0.01	0.01
Θ_{sat} (m³/m³)	0.4510	0.4187
K_{sat} (m/d)	0.7132	0.1165
α (cm⁻¹)	0.0597	0.0595
λ (-)	-4.2789	-4.6174
n (-)	1.1347	1.1035
Θ_{ref} (pF 2) (m³/m³)[7]	0.3516	0.3457

Table 7.1.2.2.1-13: Soil characterisation for site Diegten, Switzerland ([redacted] 1992, CA 7.1.2.2.1/008)

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Rescaled such that sum of components = 100 %
[3] OM % = 1.724 × OC % (van Bemmelen factor)
[4] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[5] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[6] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[7] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-14: Soil characterisation for site Egerkingen, Switzerland ([redacted] 1992, CA 7.1.2.2.1/009)

Soil layer	**0 - 30 cm**	**30 - 100 cm[1]**
Soil texture (USDA)	Clay loam	Clay loam
Sand (%)	34.17[2]	34.17
Silt (%)	28.77[2]	28.77
Clay (%)	37.06[2]	37.06
Organic carbon (%)	1.55	0.78
Organic matter (%)[3]	2.67	1.34
pH (KCl)	7.33	7.33
Bulk density (g/cm³)[4]	1.36	1.48
***Soil hydraulic parameters*[5]**		
Θ_{res} (m³/m³)[6]	0.01	0.01
Θ_{sat} (m³/m³)	0.4549	0.4256
K_{sat} (m/d)	0.3819	0.1107
α (cm⁻¹)	0.0438	0.0404
λ (-)	-4.0540	-4.3228
n (-)	1.1267	1.0990
Θ_{ref} (pF 2) (m³/m³)[7]	0.3719	0.3657

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Rescaled such that sum of components = 100 %
[3] OM % = 1.724 × OC % (van Bemmelen factor)
[4] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[5] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[6] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[7] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-15: Soil characterisation for site Bad Krozingen, Germany ([redacted] 1992, CA 7.1.2.2.1/010)

Soil layer	0 - 30 cm	30 - 100 cm[1]
Soil texture (USDA)	Sandy loam	Sandy loam
Sand (%)	55.0	55.0
Silt (%)	27.1	27.1
Clay (%)	17.9	17.9
Organic carbon (%)	0.36	0.18
Organic matter (%)[2]	0.62	0.31
pH (KCl)	6.0	6.0
Bulk density (g/cm^3)[3]	1.58	1.64
Soil hydraulic parameters[4]		
Θ_{res} (m^3/m^3)[5]	0.01	0.01
Θ_{sat} (m^3/m^3)	0.3800	0.3660
K_{sat} (m/d)	0.3949	0.1733
α (cm^{-1})	0.0462	0.0466
λ (-)	-2.4670	-1.9794
n (-)	1.2249	1.1925
Θ_{ref} (pF 2) (m^3/m^3)[6]	0.2654	0.2684

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] OM % = 1.724 × OC % (van Bemmelen factor)
[3] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[4] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[5] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[6] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-16: Soil characterisation for site Menslage, Germany ([redacted]1992, CA 7.1.2.2.1/011)

Soil layer	0 - 30 cm	30 - 100 cm[1]
Soil texture (USDA)	Sand	Sand
Sand (%)	90.69[2]	90.69
Silt (%)	2.10[2]	2.10
Clay (%)	7.21[2]	7.21
Organic carbon (%)	0.25	0.13
Organic matter (%)[3]	0.43	0.22
pH (KCl)	4.73	4.73
Bulk density (g/cm^3)[4]	1.61	1.67
Soil hydraulic parameters[5]		
Θ_{res} (m^3/m^3)[6]	0.025	0.025
Θ_{sat} (m^3/m^3)	0.3370	0.3218
K_{sat} (m/d)	2.2779	0.5803
α (cm^{-1})	0.0804	0.0947
λ (-)	-0.6077	0.0465
n (-)	1.5662	1.5217
Θ_{ref} (pF 2) (m^3/m^3)[7]	0.1195	0.1159

Table 7.1.2.2.1-16: Soil characterisation for site Menslage, Germany ([redacted] 1992, CA 7.1.2.2.1/011)

[1] Not measured, properties inherited from preceding soil horizon, except for OM %, which was set to half the value of the preceding horizon
[2] Rescaled such that sum of components = 100 %
[3] OM % = 1.724 × OC % (van Bemmelen factor)
[4] Estimated with a continuous pedotransfer function (Bollen *et al.*, 1995)
[5] Calculated based on continuous HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[6] Calculated based on class HYPRES pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001)
[7] Calculated based on van Genuchten model (van Genuchten, 1980)

According to FOCUS (2000), the top soil horizon was parameterised with compartments with a layer thickness of 2.5 cm, whereas the subsoil included compartments with a layer thickness of 5 cm. The bulk density was estimated with a continuous pedotransfer function (Bollen *et al.*, 1995). The lower boundary condition of the simulation profiles was set to 'Free Drainage' by default representing common European conditions. The initial groundwater level was set to 300 cm below the ground level. For soil evaporation, the crop factor ('FacEvpSol') and reduction coefficient ('CofRedEvp') were set to the values of 1 (default for bare soils) and 0.79, respectively.

The hydraulic characteristics of the soils were parameterised in FOCUSPEARL according to the 'van Genuchten' parameters (van Genuchten, 1980). The van Genuchten parameters were estimated based on continuous or classified 'HYPRES' pedotransfer functions (Wösten *et al.*, 1999, Nemes *et al.*, 2001).

4. 10 mm criterion for $DegT50_{matrix}$ evaluation

According to EFSA (2014), for evaluation of $DegT50_{matrix}$, surface processes like photolysis and volatilisation should be excluded. Therefore, it is recommended for the kinetic evaluation to use data points following at least 10 mm of cumulative precipitation (for SFO kinetics). For this purpose, the first sampling time after 10 mm of cumulative precipitation was defined as day 0, and all later time points were adjusted accordingly.

Table 7.1.2.2.1-17: Actual and time-step normalised (temperature and moisture) sampling days for trial sites from study [redacted] 1992, CA 7.1.2.2.1/013

Büchen			**Klein-Zecher**		
DAT (d)	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**	**DAT (d)**	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**
0	0.0	-	0	0.0	-
7	2.0	0.0	7	6.2	0.0
14	4.9	2.9	14	10.7	4.5
28	11.8	9.8	28	20.9	14.7
61	27.5	25.5	61	36.6	30.4
91	46.6	44.6	91	47.4	41.3
121	67.7	65.7	119	53.7	47.5
182	103.7	101.7	201	64.4	58.2
240	120.8	118.8	244	76.7	70.5
322	131.4	129.4	298	94.0	87.8
475	198.8	196.8	479	196.1	190.0
			567	211.1	205.0
Unzhurst			**Rohrbach**		
DAT (d)	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**	**DAT (d)**	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**
0	0.0	-	0	0.0	-
7	4.5	0.0	7	8.0	-
13	8.0	3.5	14	15.4	-
27	16.5	12.0	28	26.8	-
57	38.6	34.2	56	45.8	0.0
90	68.4	64.0	85	60.8	15.0
117	95.4	91.0	231	88.8	43.0
187	132.9	128.5	282	105.4	59.6
251	146.7	142.2	418	204.0	158.2
314	155.8	151.3	582	246.0	200.2
418	201.7	197.3			
Herrngiersdorf			**Wang-Inzkofen**		
DAT (d)	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**	**DAT (d)**	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**
0	0.0		0	0	-
6	3.7	0.0	7	4.3	0.0
13	8.1	4.3	15	9.8	5.5
28	16.2	12.5	29	20.2	15.9
58	37.9	34.2	58	44.0	39.7
90	63.8	60.1	94	61.9	57.6
125	91.3	87.6	114	70.3	66.0
168	111.4	107.6	275	93.3	89.0
330	136.7	132.9	414	173.2	168.9
464	217.4	213.7	549	216.2	211.9

Table 7.1.2.2.1-18: Actual and time-step normalised (temperature and moisture) sampling days for trial sites from studies [redacted]1992, CA 7.1.2.2.1/008-CA 7.1.2.2.1/011

Diegten			**Egerkingen**		
DAT (d)	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**	**DAT (d)**	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**
0	0	-	0	0	-
7	4.0	-	7	4.1	-
15	9.0	-	15	9.0	-
30	17.4	0.0	30	17.5	0.0
62	31.1	13.7	62	31.4	13.9
194	50.3	32.9	202	53.1	35.6
282	83.8	66.4			
Bad Krozingen			**Menslage**		
DAT (d)	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**	**DAT (d)**	**t_{norm} (d)**	**t_{norm} (d) (>10 mm rainfall)**
0	0	-	0	0	-
7	4.4	-	7	3.9	0.0
15	9.6	-	15	7.3	3.5
30	19.0	0.0	30	14.7	10.8
61	34.6	15.6	60	27.1	23.2
			192	53.6	49.7
			271	80.1	76.3
			315	112.2	108.3

In the case of bi-phasic behaviour, kinetic evaluation was performed with the complete data set, and only the slow phase of the bi-phasic decline was considered for estimating half-lives following EFSA (2014).

The number of remaining data points after 10 mm of rainfall per respective trial location are presented in the following table.

Table 7.1.2.2.1-19: 10 mm rainfall criterion at field trial locations

Study	**Trial/ location**	**Total samples[1]**	**10 mm rainfall reached at**	**No. of samples after 10 mm rainfall**
[redacted]1992	Büchen, Germany	11	3 DAT	10
	Klein-Zecher, Germany	12	4 DAT	11
	Unzhurst, Germany	11	7 DAT	10
	Rohrbach, Germany	10	31 DAT	6
	Herrngiersdorf, Germany	10	3 DAT	9
	Wang-Inzkofen, Germany	10	1 DAT	9
[redacted] 1992a	Diegten, Switzerland	7	18 DAT	4
[redacted]1992b	Egerkingen, Switzerland	6	19 DAT	3
[redacted]1992c	Bad Krozingen, Germany	5	17 DAT	2[2]
[redacted]1992d	Menslage, Germany	8	3 DAT	7

[1] Number of samples after performing FOCUS correction of residue data
[2] Insufficient data points were remaining to fit the SFO model according to EFSA (2014)

5. Kinetic assessment

Kinetic models

Four kinetic models have been recommended by the FOCUS workgroup for describing the kinetic behaviour of parent substances and their metabolites in soil (FOCUS, 2006, 2014): Single first order (SFO), First order multi-compartment (FOMC), Double first order in parallel (DFOP) and Hockey stick (HS). In this report, the fitting approaches for trigger and modelling endpoints have been adopted according to FOCUS (FOCUS, 2006, 2014) and EFSA (EFSA, 2014), as appropriate.

Optimisation

The kinetic analyses were conducted using the software package CAKE 3.3. The data were initially fitted with the complete dataset and unconstrained initial concentration (M_0) for the parent substance. Iteratively Reweighted Least Squares (IRLS) was used as the solver, as implemented in CAKE. Optimisations were carried out for the initial soil residue (M_0) and degradation model parameters k, α, β, g, or t_b depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually based on the observed degradation pattern and preliminary model runs. In pathway fits for derivation of trigger endpoints, the initial amount of metabolite was fixed to 0 % by default, which was in contrast to the pathway fitting for derivation of modelling endpoints. Here, the initial amount of metabolite was not constrained to zero, as several data points from the beginning of the experimental period prior to 10 mm rainfall were cut off. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

If a pathway fit did not yield visually and/ or statistically reliable results, the kinetic model was further optimised by fixing one or more of the model parameters to either the value derived from a reliable parent-only fit (e.g. M_0, k), or to values derived from previous pathway fits with unbound parameters (e.g. ff). A stepwise fixing procedure has been applied in these cases, which is further described in the results chapter for the respective pathway fits.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually based on concentration/ residual - time plots. Generally the residuals should be distributed randomly around the zero line. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered
- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line

A statistical measure of the quality of a fit is given by the χ^2-test. The χ^2-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements. In general, for parent compounds, it is recommended that if the χ^2 error is <15 % then the model has adequately reflected the measured data (FOCUS, 2006, 2014). However, this value should only be considered as a guide and not an absolute cut-off criterion. The guidance can be relaxed for field studies where the residue data can show appreciable scatter. The same also applies for metabolites where the curve fitting is more complex.

Significance of parameters

A single-sided t-test was used to evaluate whether the optimised parameters were significantly different from zero at a chosen significance level of 5 %. In case of metabolite data, a significance level of 10 % or higher may still be acceptable due to the inherent variability that often occurs in these types of data. This is particularly relevant for the degradation rate constants (k) of the SFO, DFOP and HS kinetic models. For the FOMC kinetic model, only the significance of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The CAKE software also reports a 95 % confidence interval on the estimated parameters. As a general principle the confidence interval should be relatively tight and not contain 0 to be considered statistically robust.

Derivation of trigger and modelling endpoints

For derivation of trigger endpoints, the non-normalised dataset was considered, and the kinetic evaluation was conducted according to FOCUS guidance (2006, 2014). For the parent compound, the best-fit model was accepted for deriving trigger endpoints. For the metabolite, pathway fits were conducted using the best-fit kinetic model for the parent and SFO for the metabolite. In cases where no reliable pathway fit could be established, kinetic endpoints for the parent were derived from the corresponding parent-only fit, and decline fits were conducted for the metabolite (if possible), starting from the maximum observed concentration. The respective day was defined as 0 days after maximum concentration, and later time points were adjusted accordingly.

For derivation of modelling endpoints, the corrected residue data were combined with the normalised day length data. The resulting parent datasets were then evaluated according to EFSA (2014). For the metabolite, if the SFO parent-only fit was accepted after excluding surface processes, the SFO-SFO pathway fit was assessed. If the pathway fit was visually acceptable and resulted in statistically reliable endpoints then the fit was accepted for deriving metabolite endpoints. This is considered appropriate even if the metabolite formation phase was not completely included in the evaluation but the metabolite decline occurred after the parent compound has mostly dissipated, as in this case the metabolite degradation rate can be estimated independently. If no reliable pathway fit for the metabolite could be established, or bi-phasic models were considered for the parent-only fit, further consideration was given to whether a decline fit could be evaluated for the metabolite.

II. RESULTS AND DISCUSSION

Determination of trigger endpoints

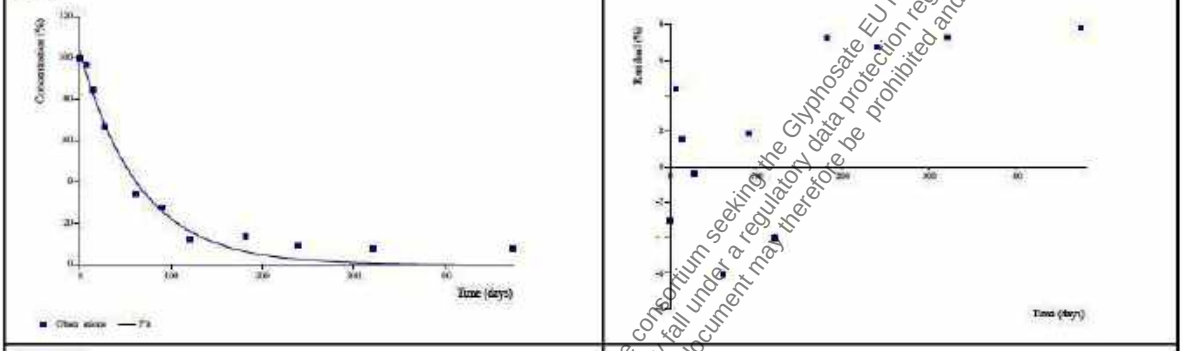
Table 7.1.2.2.1-20: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	103.0	k: 0.0153	10.1	k: <0.001	k: 0.0120	k: 0.0190	45.3	150
FOMC	Acceptable	105.8	α: 2.537 β: 127.7	8.8	-[1]	β: -78.58	β: 334	40.1	189
DFOP	Good	105.2	k_1: 0.0190 k_2: 3.38×10^{-12} g: 0.9264	6.6	k_1: <0.001 k_2: 0.5	k_1: 0.0124 k_2: -0.0061	k_1: 0.0260 k_2: 0.0060	40.7	187
HS	Not calculated								

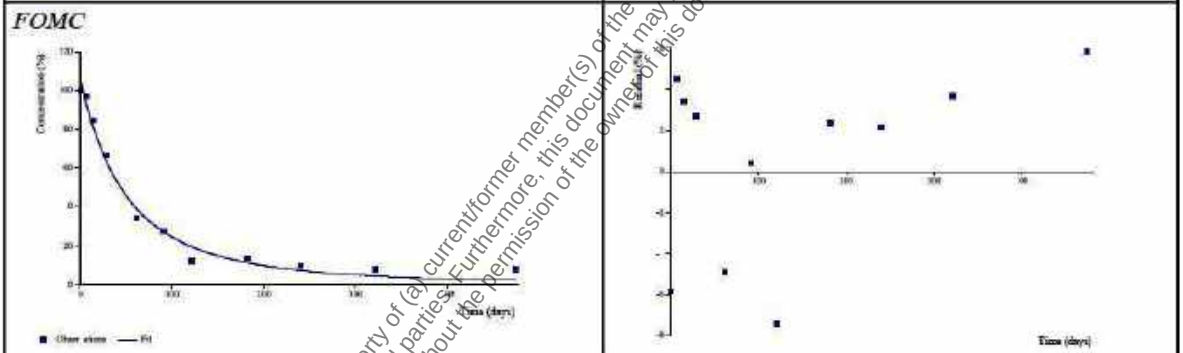
Table 7.1.2.2.1-20: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
The SFO model fit is visually (residues well described until the DT_{90}) and statistically acceptable. FOMC and DFOP models were further tested, which led to better visual fit with smaller χ^2 errors. The DFOP model provides the best visual fit and the lowest χ^2 error. The parameter k_2 of the DFOP model is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k_1 as indicated by a high value for parameter g (0.9264).									
Conclusion: DFOP to be used in pathway fit for trigger endpoints									

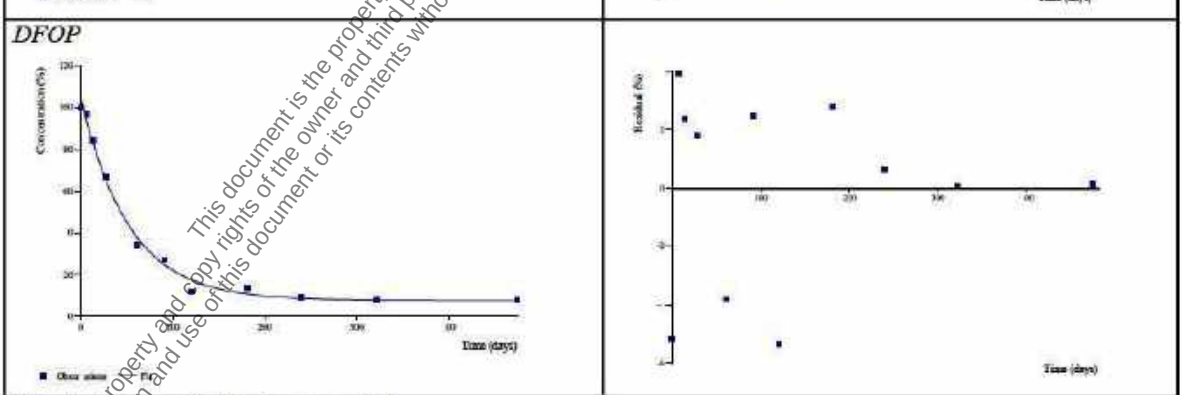
SFO



FOMC



DFOP



[1] t-test not relevant for kinetic parameter β

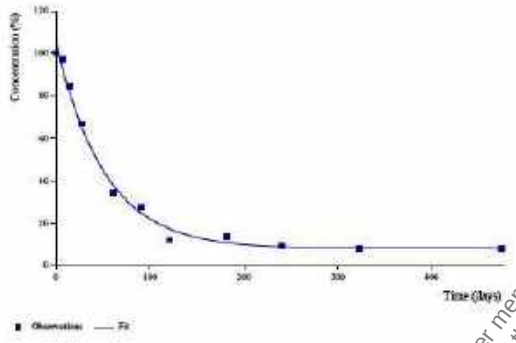
Table 7.1.2.2.1-21: Kinetic models and goodness-of-fit statistics of pathway fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

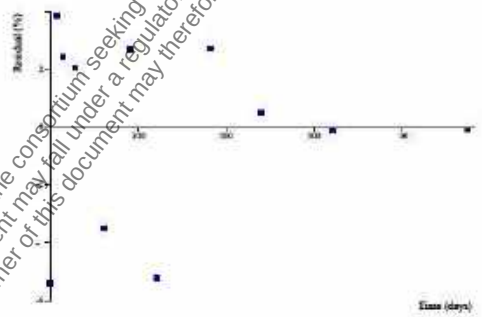
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	105.4	k_1: 0.0193 k_2: 3.93×10^{-9} g: 0.9245	6.6	k_1: <0.001. k_2: 0.5	k_1: 0.0136 k_2: -0.0051	k_1: 0.0250 k_2: 0.0050	40.3	188
AMPA: SFO	Poor	-	k: 0.0019	26.3	k: 0.0623	k: -0.0006	k: 0.0040	365	1213

The dissipation of glyphosate is well described in the pathway fit with the DFOP model. For AMPA, due to the wide scatter of residue data, the SFO model does not adequately describe the formation and decline of the metabolite. A decline fit for AMPA was not performed, as there is no clear decline phase.

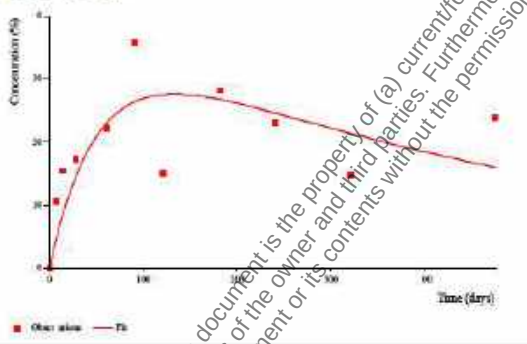
Conclusion: Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate
No reliable trigger endpoints for AMPA can be determined

Glyphosate (DFOP)





AMPA (SFO)



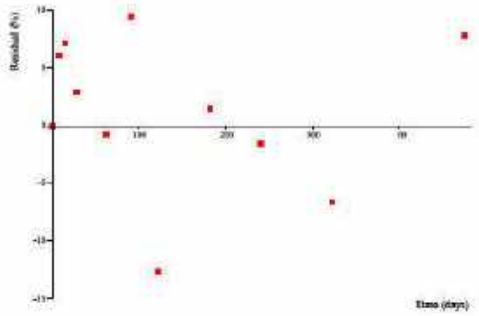


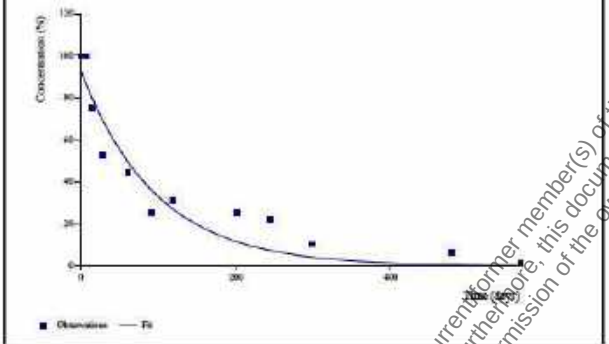
Table 7.1.2.2.1-22: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Klein-Zecher of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

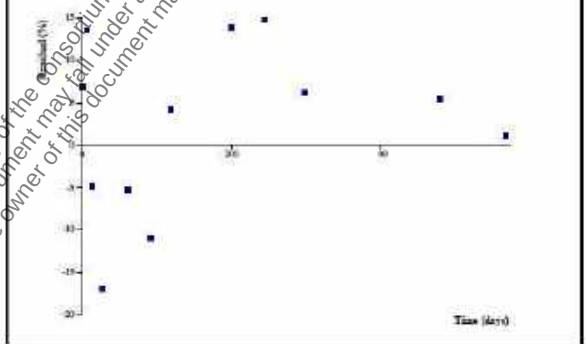
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	93.1	k: 0.0104	19.5	k: <0.001	k: 0.0061	k: 0.0150	66.8	222
FOMC	Good	104.9	α: 0.912 β: 34.66	12.6	-[1]	β: -11.64	β: 80.97	39.5	398
DFOP	Good	105.8	k_1: 0.0411 k_2: 0.0038 g: 0.583	11.5	k_1: 0.0173 k_2: 0.0169	k_1: 0.0038 k_2: 0.0004	k_1: 0.0780 k_2: 0.0090	35.5	378
HS	Not calculated								

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The DFOP model provides the best fit for the whole study duration, with small residuals scattered randomly about zero.

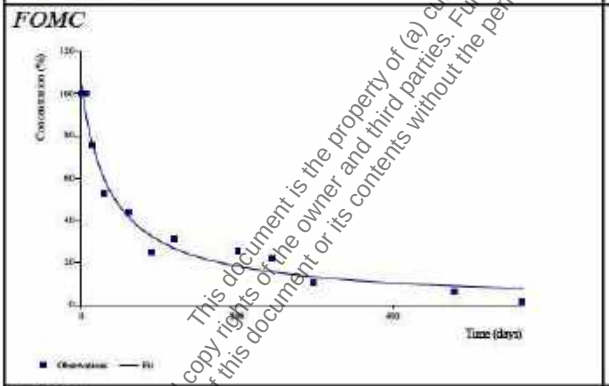
Conclusion: DFOP to be used in pathway fit for trigger endpoints

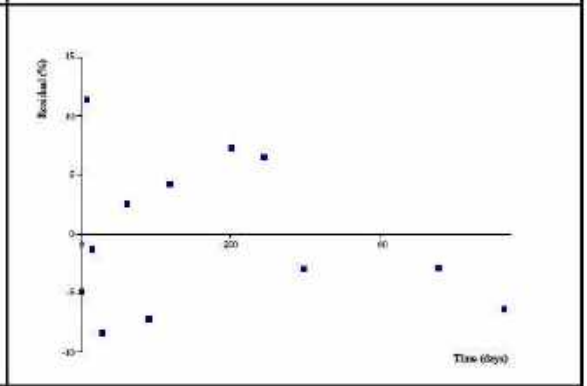
SFO



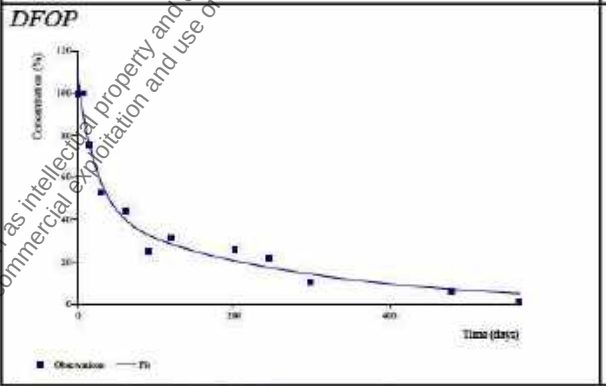


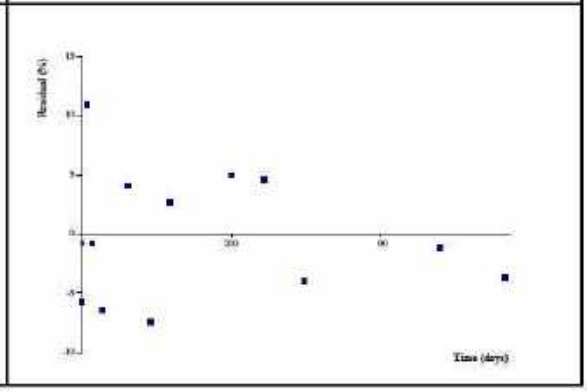
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

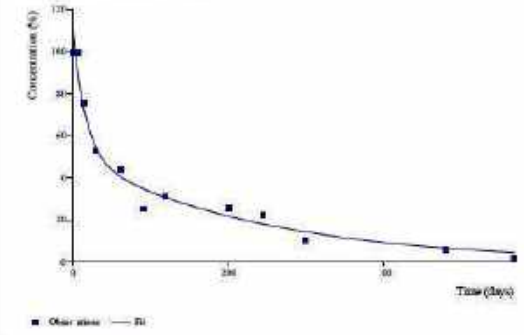
Table 7.1.2.2.1-23: Kinetic models and goodness-of-fit statistics of pathway fits for soil Klein-Zecher of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

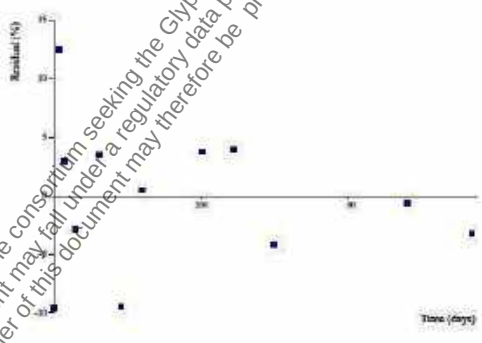
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	109.6	k_1: 0.0614 k_2: 0.0042 g: 0.5368	12.7	k_1: 0.0037 k_2: 0.0021	k_1: 0.0188 k_2: 0.0015	k_1: 0.1040 k_2: 0.0070	29.1	364
AMPA: SFO	Accep-table	-	k: 0.0013	13.9	k: 0.0089	k: 0.0003	k: 0.0020	524	>1000

The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The residuals are randomly scattered about zero and are relatively small.

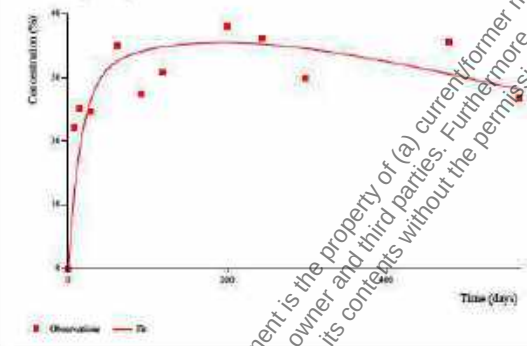
Conclusion: DFOP-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

Glyphosate (DFOP)





AMPA (SFO)



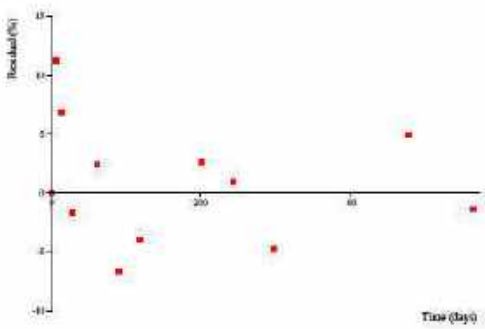


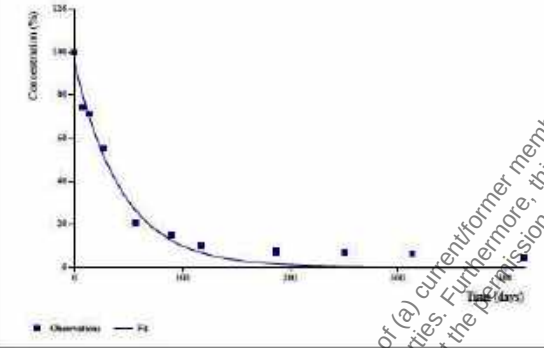
Table 7.1.2.2.1-24: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

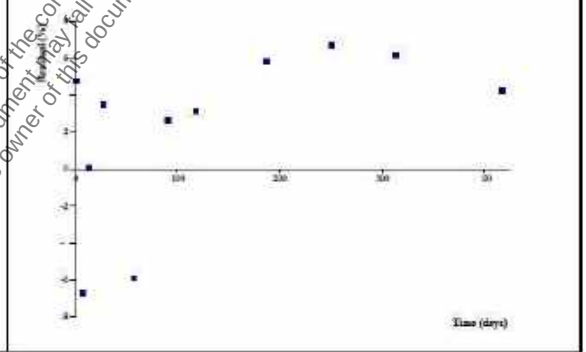
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	95.2	k: 0.0225	11.8	k: <0.001	k: 0.0170	k: 0.0280	30.8	102
FOMC	Acceptable	99.0	α: 2.025 β: 63.96	9.8	-[1]	β: -19.43	β: 147.4	26.2	135
DFOP	Good	97.3	k_1: 0.0281 k_2: 0.0009 g: 0.9214	8.4	k_1: <0.001 k_2: 0.3958	k_1: 0.0170 k_2: -0.0069	k_1: 0.0390 k_2: 0.0090	27.7	122
HS	Not calculated								

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The DFOP model provides the best fit for the whole study duration, with small residuals scattered randomly about zero. The parameter k_2 of the DFOP model is not significantly different from zero, but this can be accepted as the overall degradation is dominated by k_1 as indicated by a high value for parameter g (0.9214).

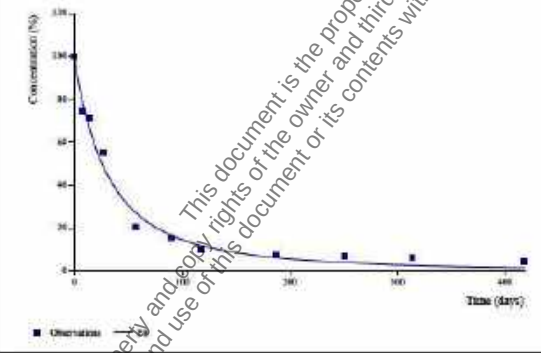
Conclusion: DFOP to be used in pathway fit for trigger endpoints.

SFO





FOMC



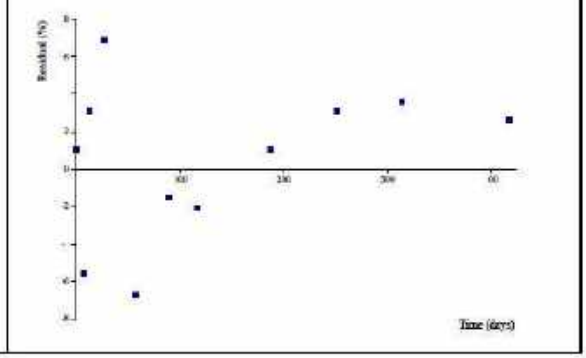
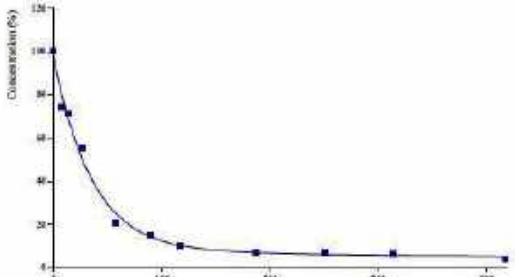
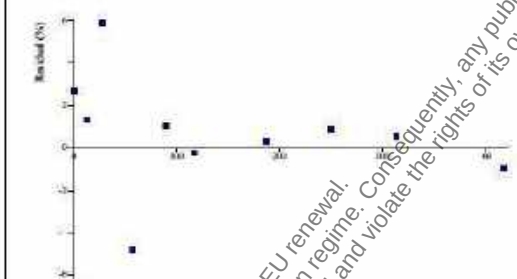


Table 7.1.2.2.1-24: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

DFOP





[1] t-test not relevant for kinetic parameter β

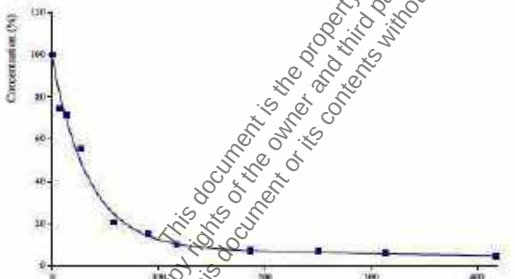
Table 7.1.2.2.1-25: Kinetic models and goodness-of-fit statistics of pathway fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	97.8	k_1: 0.0294 k_2: 0.0015 g: 0.9061	8.5	k_1: 0.001. k_2: 0.3093	k_1: 0.0197 k_2: -0.0048	k_1: 0.039 k_2: 0.0080	27.0	126
AMPA: SFO	Accep-table	-	k: 0.0011	10.9	k: 0.0252	k: -2.6×10^{-6}	k: 0.0020	634	>1000

The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. For AMPA, the SFO model provides an acceptable visual fit given the scatter in the data.

Conclusion: DFOP-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

Glyphosate (DFOP)



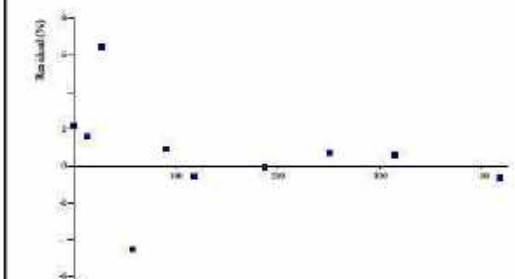
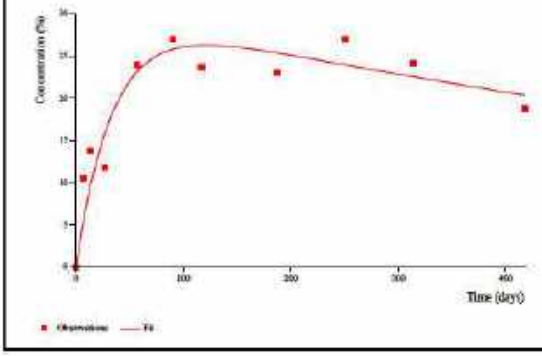


Table 7.1.2.2.1-25: Kinetic models and goodness-of-fit statistics of pathway fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
AMPA (SFO)									



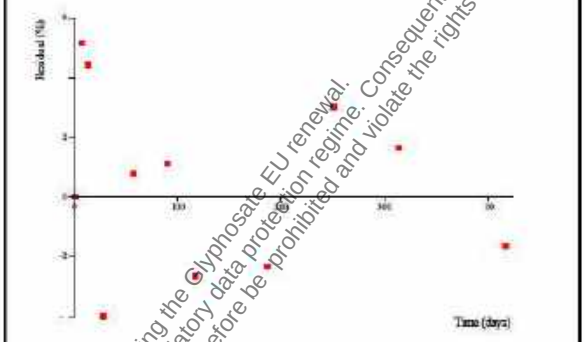
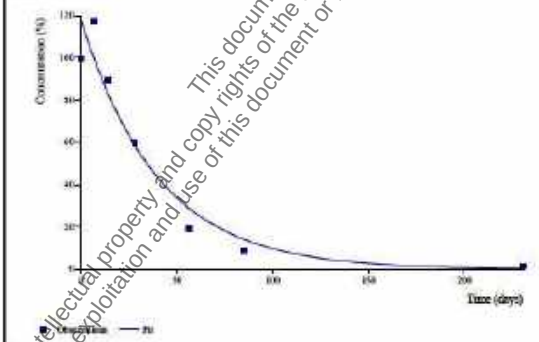


Table 7.1.2.2.1-26: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	117.8	k: 0.0250	15.4	k: 0.0030	k: 0.0109	k: 0.0390	27.8	92.2
FOMC	Acceptable	126.8	α: 5940 β: 169000	16.6	-[1]	β: 152000	β: 185000	19.7	65.3
DFOP	Not calculated								
HS	Not calculated								

The SFO model provides an acceptable visual and statistical fit. The biphasic FOMC model does not improve the fit.
Conclusion: SFO to be used in pathway fit for trigger endpoints

SFO



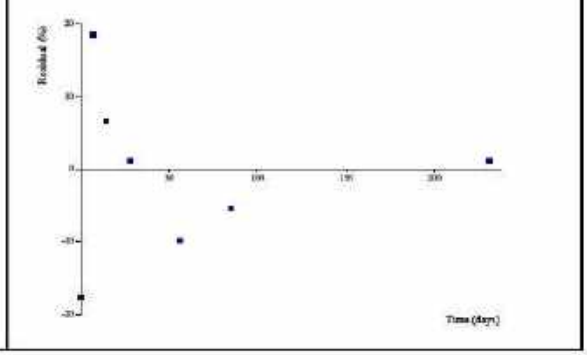
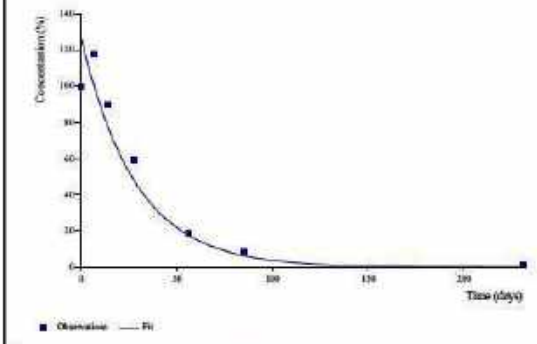
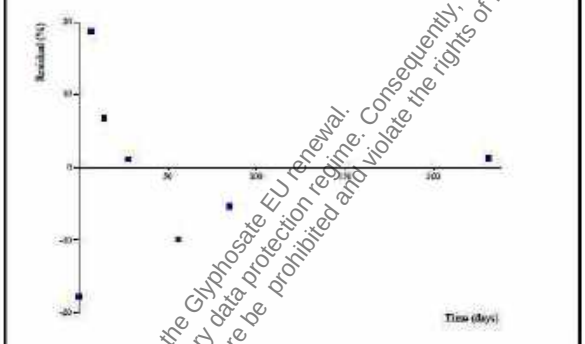


Table 7.1.2.2.1-26: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
FOMC									
									
									

[1] t-test not relevant for kinetic parameter β

Table 7.1.2.2.1-27: Kinetic models and goodness-of-fit statistics of pathway fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

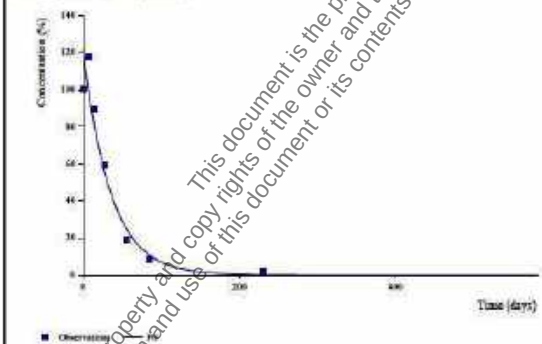
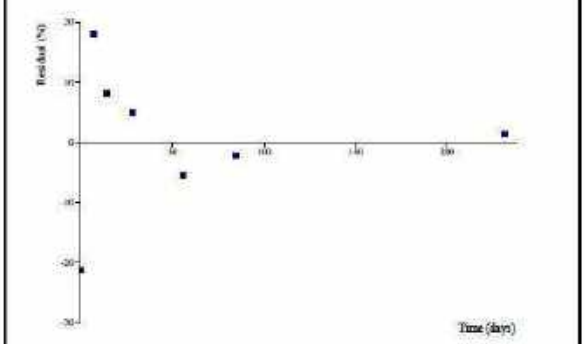
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: SFO	Accep-table	121.3	k: 0.0284	16.0	k: <0.001	k: 0.01632	k: 0.0410	24.4	81.0
AMPA: SFO	Accep-table	-	k: 0.0027	25.5	k: <0.001	k: 0.0012	k: 0.0040	255	847
The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The estimated parameters are statistically reliable. **Conclusion:** SFO-SFO to be used for deriving trigger endpoints for glyphosate and AMPA									
Glyphosate (SFO)									
									
									

Table 7.1.2.2.1-27: Kinetic models and goodness-of-fit statistics of pathway fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

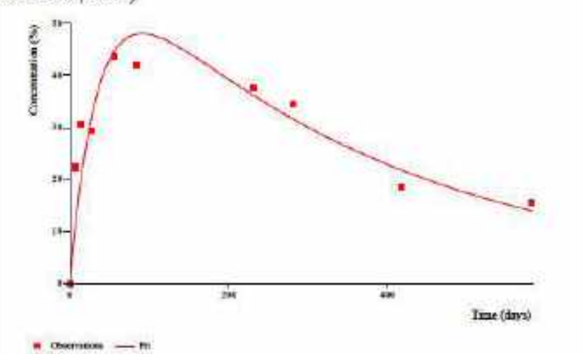
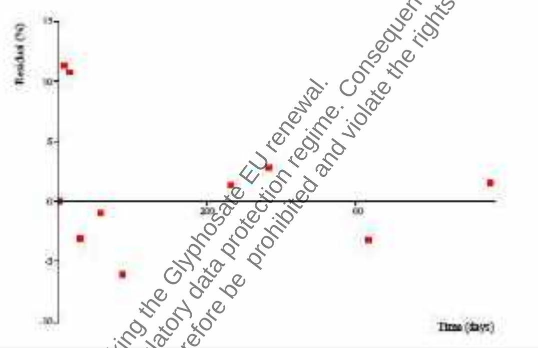
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
AMPA (SFO)									
									

Table 7.1.2.2.1-28: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

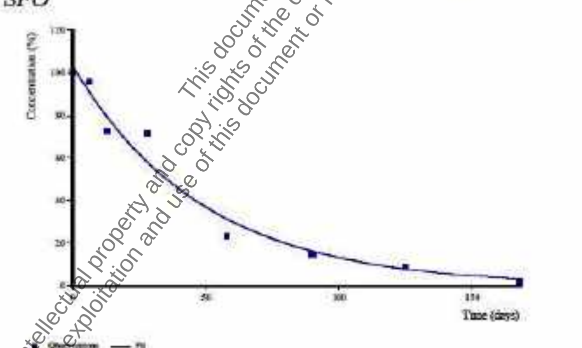
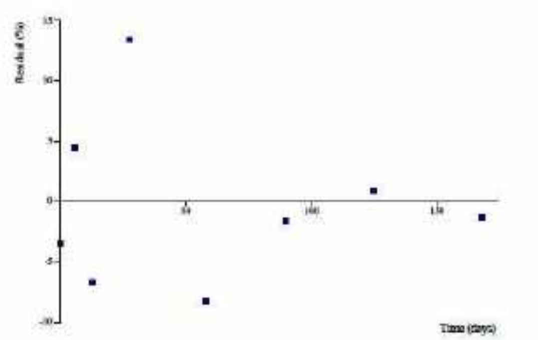
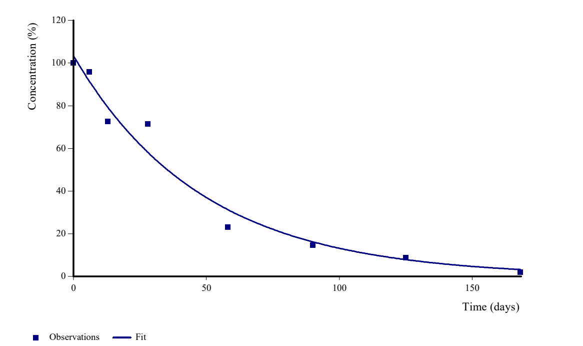
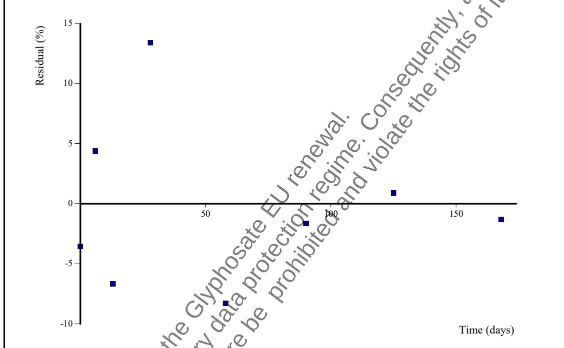
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	103.5	k: 0.0206	10.6	k: <0.001	k: 0.0139	k: 0.0270	33.7	112
FOMC	Acceptable	103.5	α: 605.7 β: 29400	11.3	-[1]	β: 2700	β: 56100	33.7	112
DFOP	Not calculated								
HS	Not calculated								
The SFO model provides an acceptable visual and statistical fit. The biphasic FOMC model does not improve the fit. **Conclusion:** SFO to be used in pathway fit for trigger endpoints									
SFO									
									

Table 7.1.2.2.1-28: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
FOMC									





[1] t-test not relevant for kinetic parameter β

Table 7.1.2.2.1-29: Kinetic models and goodness-of-fit statistics of pathway fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Pathway fit									
Glyphosate: SFO	Accep-table	104.4	k: 0.0214	10.6	k: <0.001	k: 0.0152	k: 0.0280	32.4	108
AMPA: SFO	Poor	-	k: 0.0060	29.4	k: 0.0268	k: -0.0001	k: 0.0120	115	381
Decline fit for AMPA									
AMPA: SFO	Accep-table	29.4	k: 0.0024	11.0	0.0251	k: -2.01×10^{-6}	k: 0.0050	288	958
AMPA: FOMC	Accep-table	29.4	α: 33.39 β: 13700	12.6	-[1]	β: -3.47×10^{4}	β: 6.21×10^{4}	288	980

Pathway fit: The dissipation of glyphosate is well described by the SFO model in the pathway fit. For AMPA, the SFO model does not adequately fit the data due to the scatter in the data during the decline phase. Hence, a decline fit was performed for AMPA.

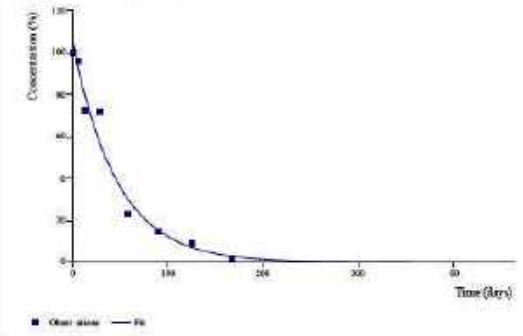
Decline fit for AMPA: The SFO model provides a visually and statistically acceptable fit. The χ^2 error above 15 % is considered acceptable as it results from the scattering of the data. The FOMC model does not improve the fit.

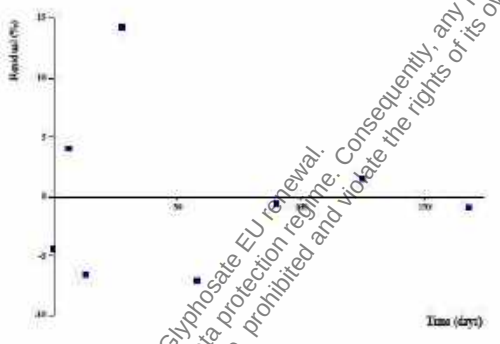
Conclusion: Parent-only SFO fit to be used for deriving trigger endpoints for glyphosate
Decline fit (SFO) to be used for deriving trigger endpoints for AMPA

Table 7.1.2.2.1-29: Kinetic models and goodness-of-fit statistics of pathway fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

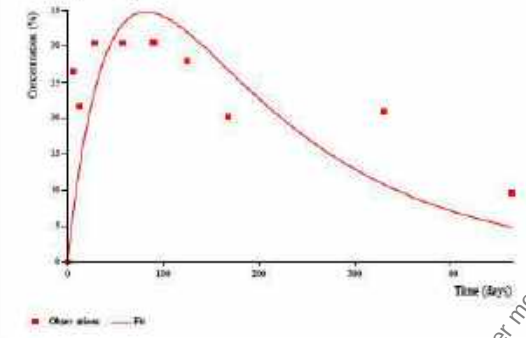
Pathway fit (SFO-SFO)

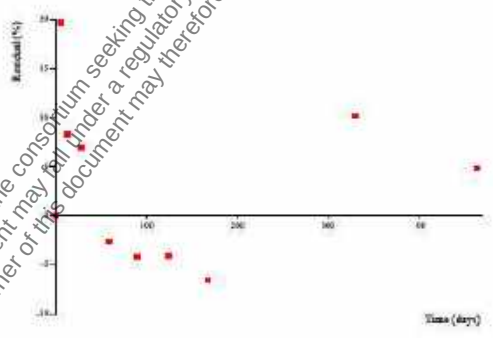
Glyphosate (SFO)





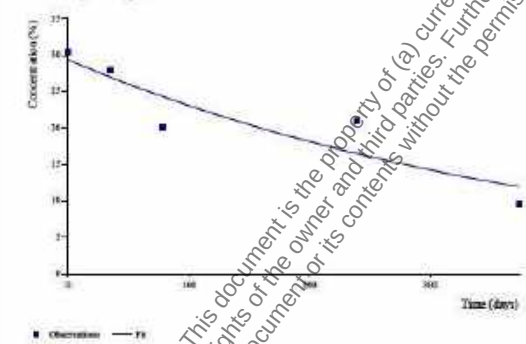
AMPA (SFO)

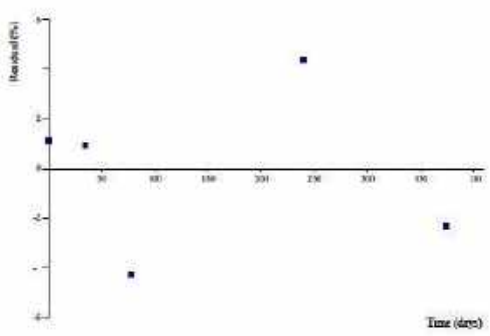




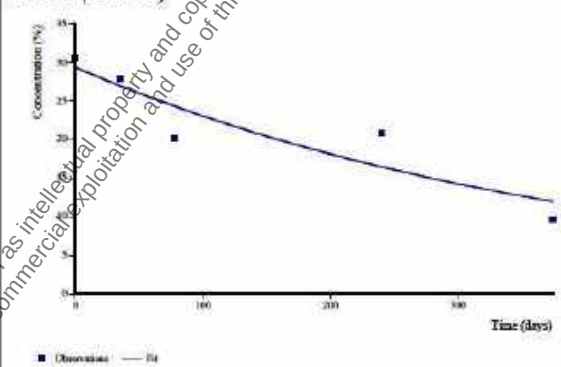
Decline fit for AMPA

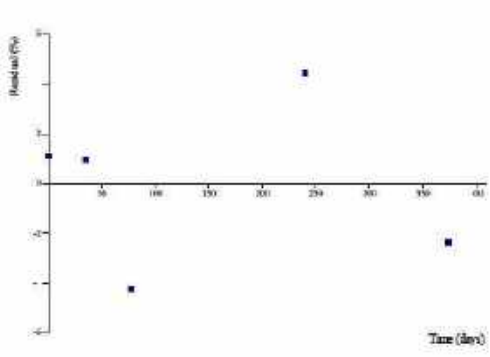
AMPA (SFO)





AMPA (FOMC)





[1] t-test not relevant for kinetic parameter β

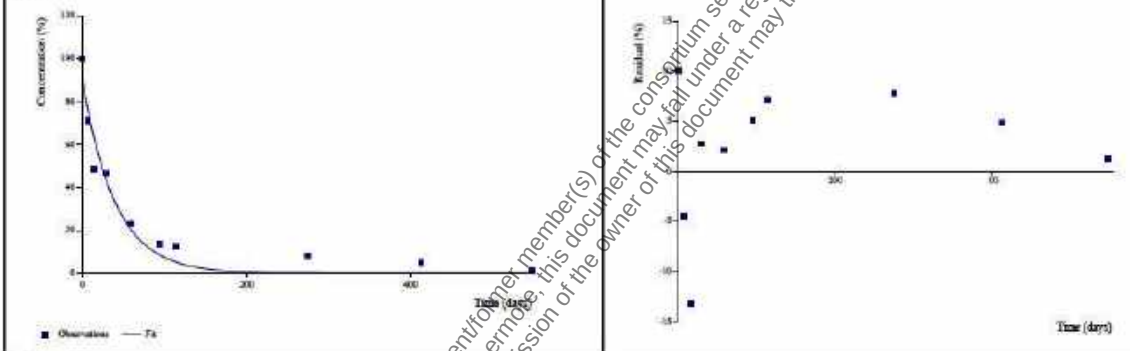
Table 7.1.2.2.1-30: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.0	k: 0.0251	16.8	k: <0.001	0.0156	k: 0.0350	27.6	91.7
FOMC	Good	99.4	α: 0.9749 β: 17.22	8.7	-[1]	β: 2.1815	β: 32.26	17.8	166
DFOP	Poor	100.3	k_1: 0.1543 k_2: 0.0148 g: 0.3839	10.3	k_1: 0.0817 k_2: 0.0039	k_1: -0.0835 k_2: -0.0056	k_1: 0.3920 k_2: 0.0286	17.6	123
HS	Not calculated								

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The FOMC model provides the best visual and statistical fit.

Conclusion: FOMC to be used in pathway fit for trigger endpoints

SFO



FOMC

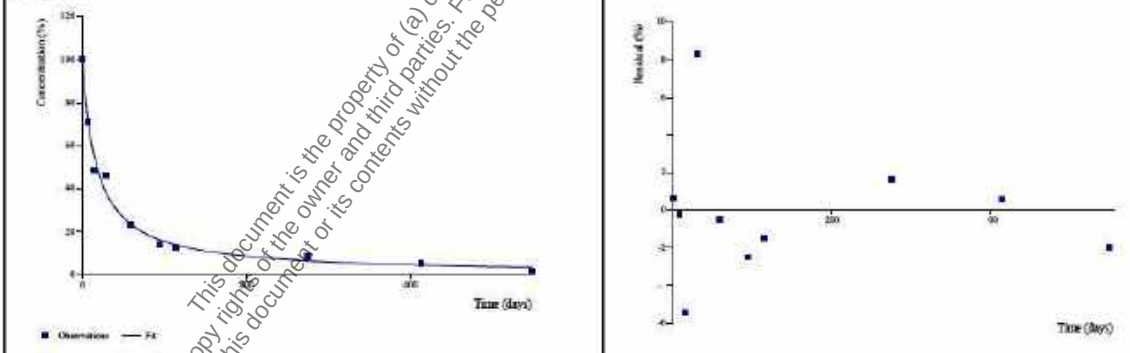
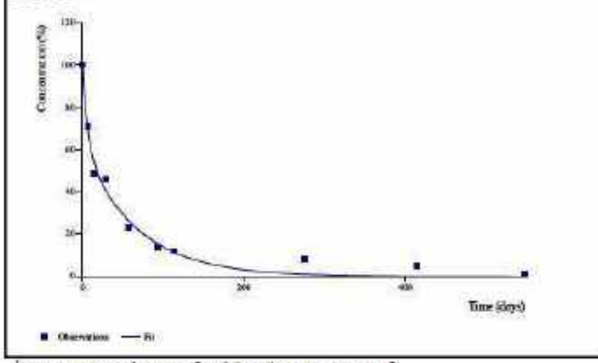
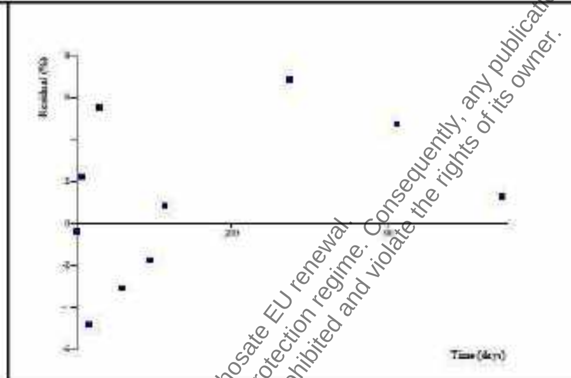


Table 7.1.2.2.1-30: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

DFOP





[1] t-test not relevant for kinetic parameter β

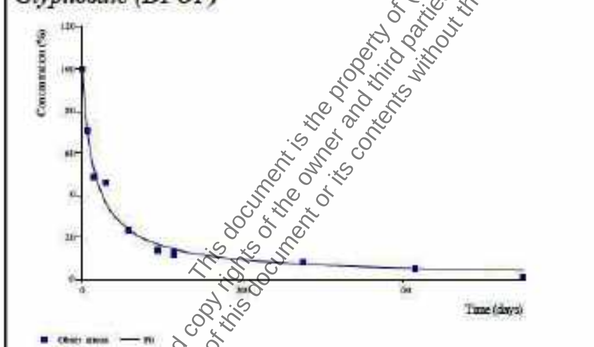
Table 7.1.2.2.1-31: Kinetic models and goodness-of-fit statistics of pathway fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: FOMC	Good	101.6	α: 0.8344 β: 12.19	9.2	-[1]	β: 3.0030	β: 21.38	15.8	180
AMPA: SFO	Accep-table	-	k: 0.0025	15.8	k: 0.011	k: 0.0011	k: 0.0040	273	907

The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. The endpoints are statistically reliable.

Conclusion: FOMC-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

Glyphosate (DFOP)



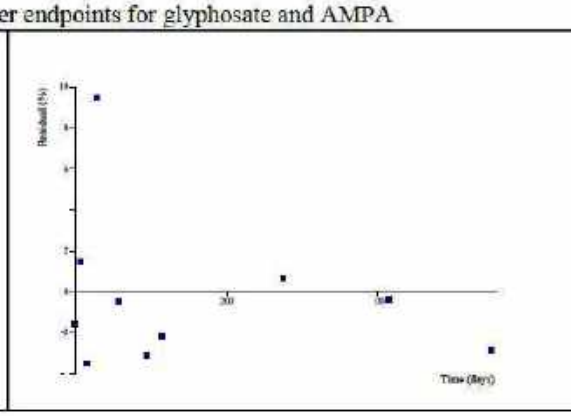
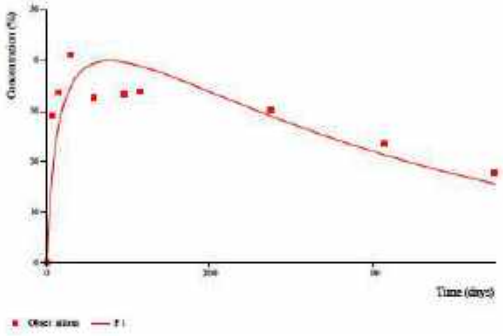
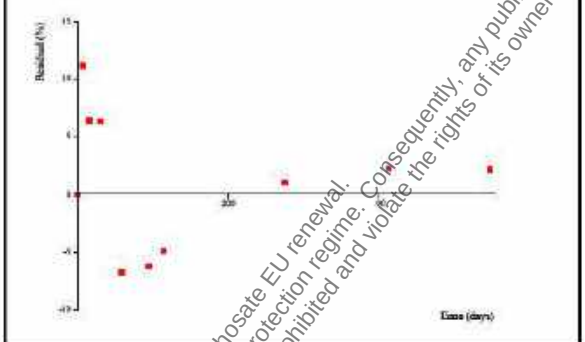


Table 7.1.2.2.1-31: Kinetic models and goodness-of-fit statistics of pathway fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – trigger endpoints

AMPA (SFO)





[1] t-test not relevant for kinetic parameter β

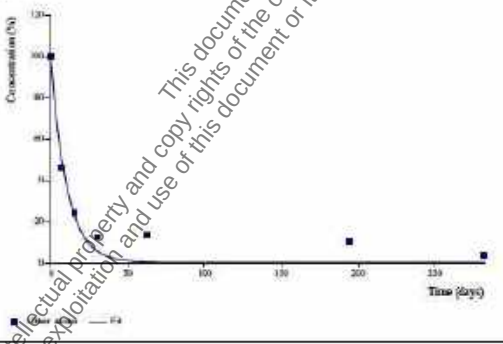
Table 7.1.2.2.1-32: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Diegten of study [redacted] (1992, CA 7.1.2.2.1/008) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	98.2	k: 0.0949	19.0	k: 0.0016	k: 0.0486	k: 0.1410	7.3	24.3
FOMC	Acceptable	100.2	α: 0.7446 β: 3.2231	10.1	[1]	β: -2.3487	β: 8.7950	5.0	67.8
DFOP	Good	100.1	k_1: 0.1425 k_2: 0.0033 g: 0.853	5.0	k_1: <0.001 k_2: 0.0602	k_1: 0.0983 k_2: -0.0016	k_1: 0.1870 k_2: 0.0080	6.1	118
HS	Not calculated								

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The DFOP model provides the best visual fit with smaller residuals, and the t-test for k_2 only marginally exceeds the 5 % level and is considered acceptable.

Conclusion: DFOP to be used in pathway fit for trigger endpoints

SFO



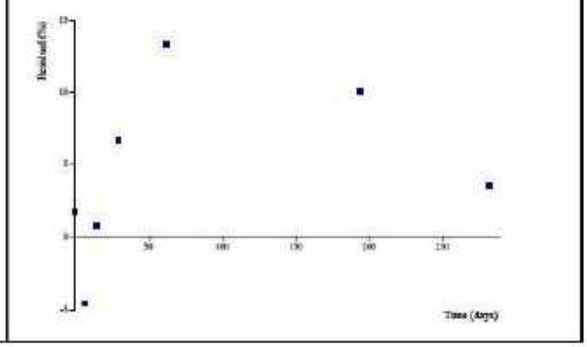
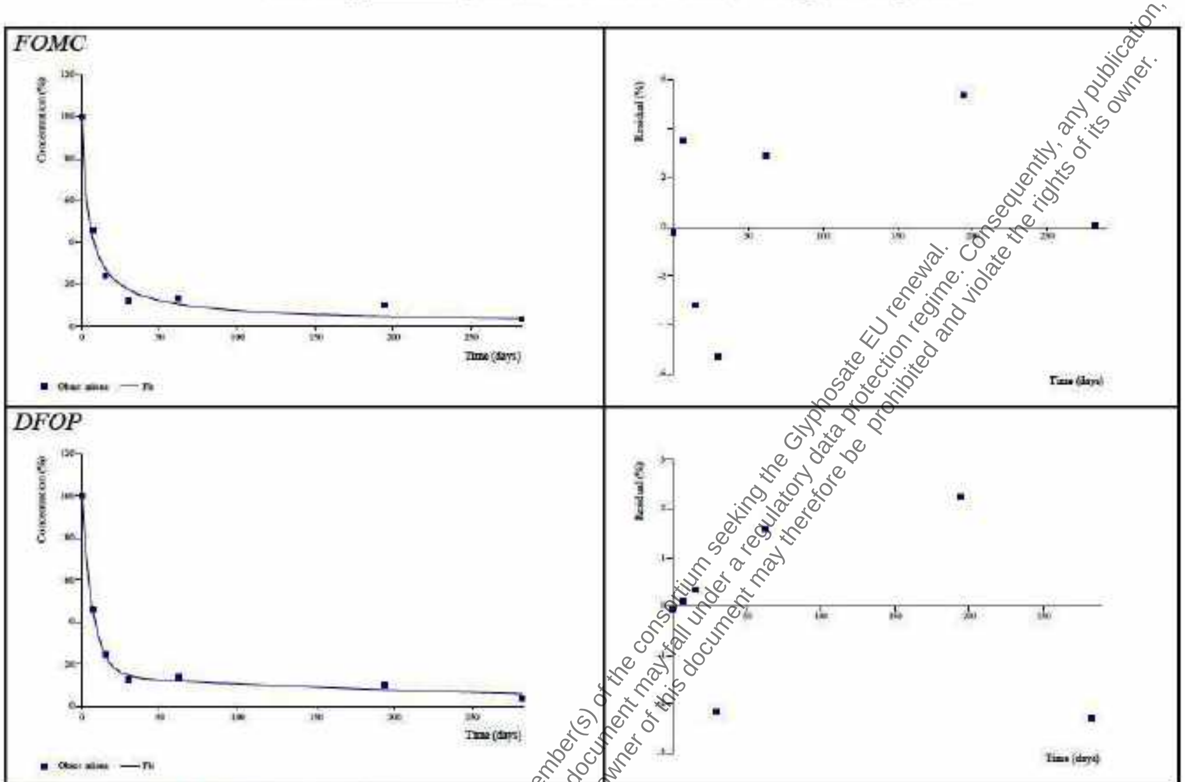


Table 7.1.2.2.1-32: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Diegten of study [redacted] (1992, CA 7.1.2.2.1/008) – trigger endpoints



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.2.1-33: Kinetic models and goodness-of-fit statistics of pathway fits for soil Diegten of study [redacted] (1992, CA 7.1.2.2.1/008) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	100.1	k_1: 0.1434 k_2: 0.0033 g: 0.8518	5.0	k_1: <0.001 k_2: 0.0252	k_1: 0.1127 k_2: -7.165×10^{-6}	k_1: 0.1740 k_2: 0.0070	6.1	119
AMPA: SFO	Poor	-	k: 0.0005	26.0	k: 0.3901	k: -0.0038	k: 0.0050	>1000	>1000
The dissipation of glyphosate is well described by the DFOP model. For AMPA, due to the wide scatter of residue data, the SFO model does not adequately describe the formation of the metabolite. A decline fit for AMPA was not performed, as there is no clear decline phase. **Conclusion:** Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate No reliable trigger endpoints for AMPA can be determined									

Table 7.1.2.2.1-33: Kinetic models and goodness-of-fit statistics of pathway fits for soil Diegten of study [redacted] (1992, CA 7.1.2.2.1/008) – trigger endpoints

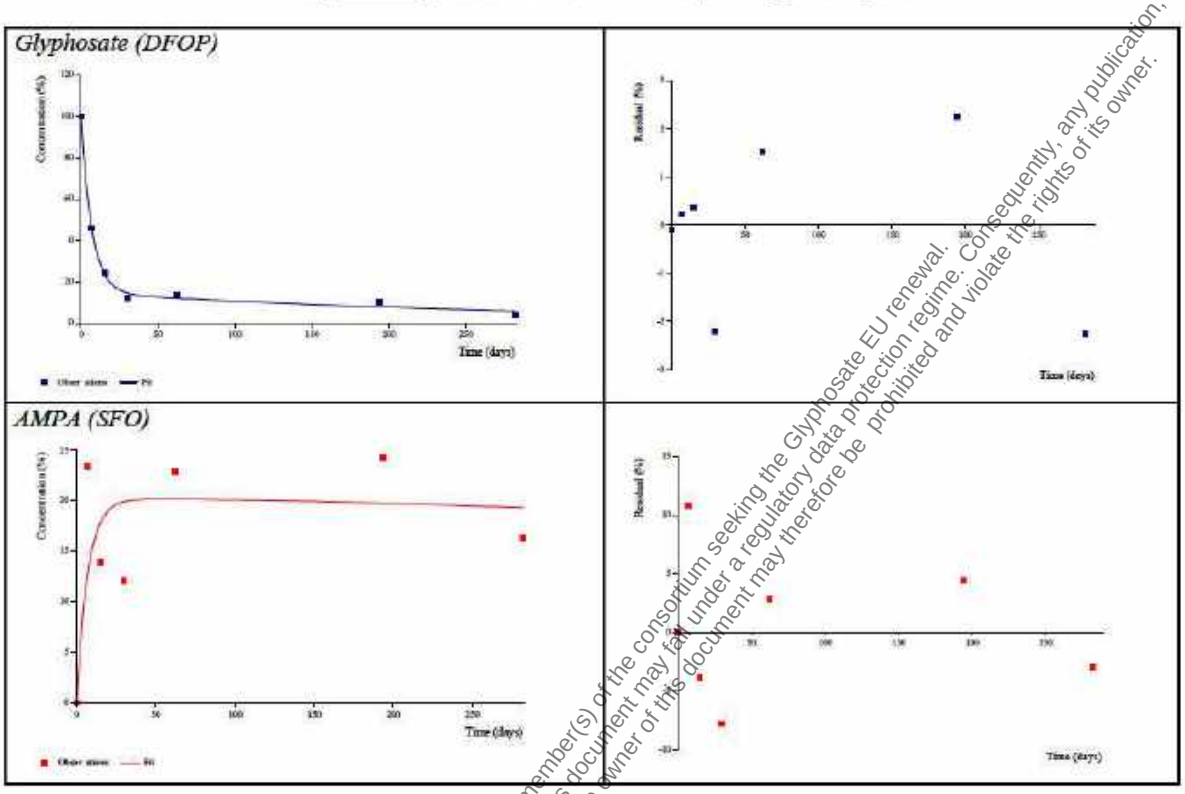


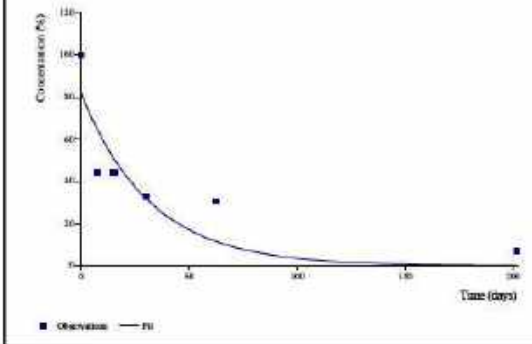
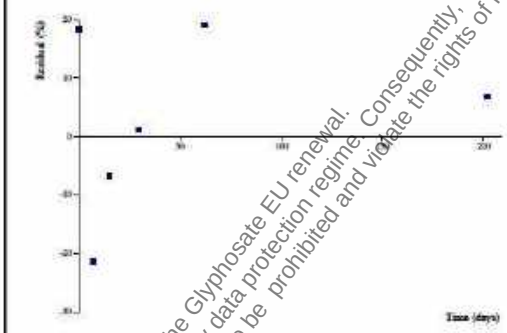
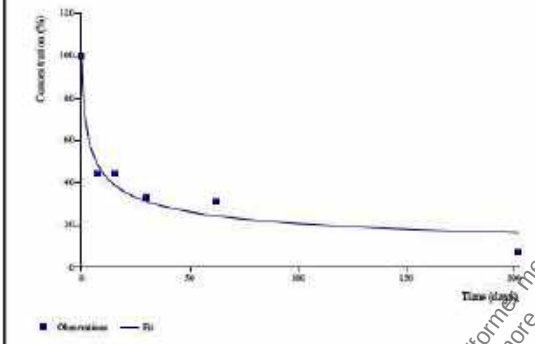
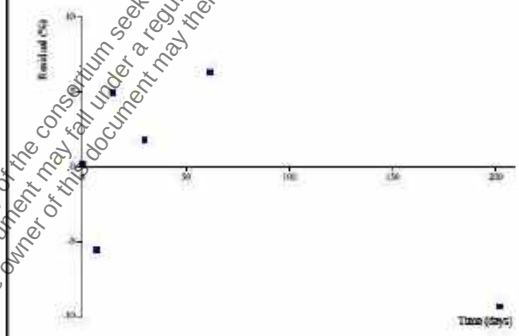
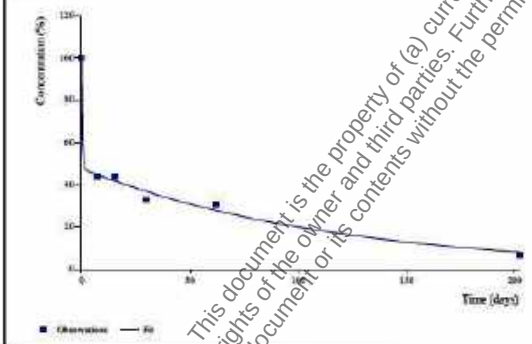
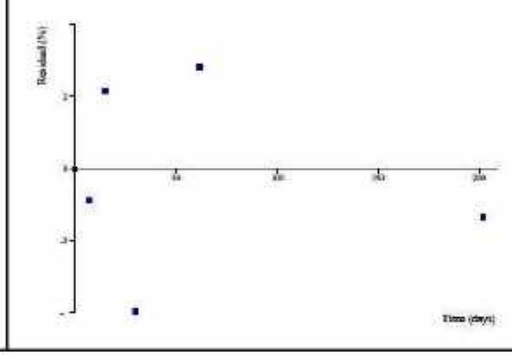
Table 7.1.2.2.1-34: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Egerkingen of study [redacted] (1992, CA 7.1.2.2.1/009) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	81.7	k: 0.0314	26.6	k: 0.0499	k: -0.0095	k: 0.0720	22.1	73.3
FOMC	Poor	99.8	α: 0.3475 β: 1.0927	11.3	-[1]	β: -3.4731	β: 5.6590	6.9	823
DFOP	Good	100.0	k_1: 2.653 k_2: 0.0087 g: 0.5228	5.3	k_1: 0.4966 k_2: 0.0211	k_1: -1183 k_2: 0.0008	k_1: 1190 k_2: 0.0170	1.1	179
HS	Not calculated								

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The FOMC model does not accurately describe the final two data points after 50 DAT well. The DFOP model provides a good visual fit. However, due to the very wide confidence interval for k_1, the endpoints are not deemed to be reliable.

Conclusion: No reliable trigger endpoints can be determined for glyphosate

Table 7.1.2.2.1-34: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Egerkingen of study [redacted] (1992, CA 7.1.2.2.1/009) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO									
FOMC									
DFOP									

[1] t-test not relevant for kinetic parameter β

As the parent-only fit for glyphosate was not acceptable, no pathway fit was tested for soil Egerkingen. For AMPA, no clear decline phase was observed; hence a decline fit was not considered.

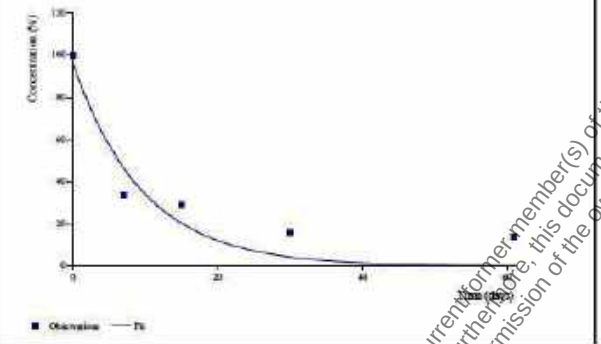
Table 7.1.2.2.1-35: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Bad Krozingen of study [redacted] (1992, CA 7.1.2.2.1/010) – trigger endpoints

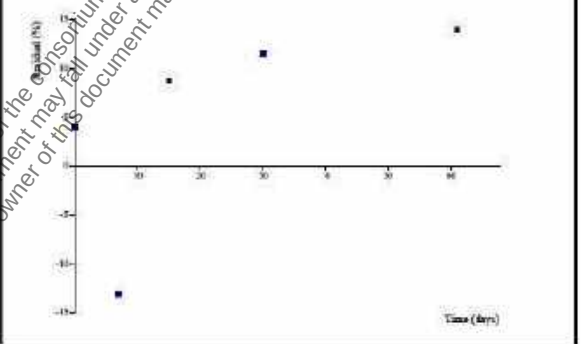
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	96.1	k: 0.1038	22.5	k: 0.0266	k: -0.0027	k: 0.2100	6.7	22.2
FOMC	Good	100.0	α: 0.45 β: 0.7373	5.3	-[1]	β: -2.106	β: 3.5800	2.7	122
DFOP	Good	100.0	k_1: 0.4281 k_2: 0.0177 g: 0.657	7.5	k_1:0.2535 k_2: 0.1860	k_1: -5.1332 k_2: -0.1312	k_1: 5.9900 k_2: 0.1650	3.1	69.6
HS	Not calculated								

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The FOMC model provides the best visual fit with the lowest χ^2 error.

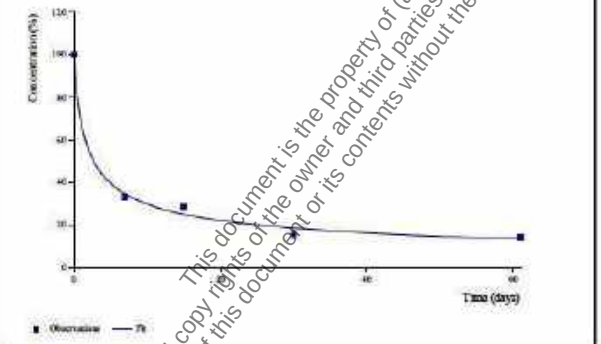
Conclusion: FOMC to be used in pathway fit for trigger endpoints.

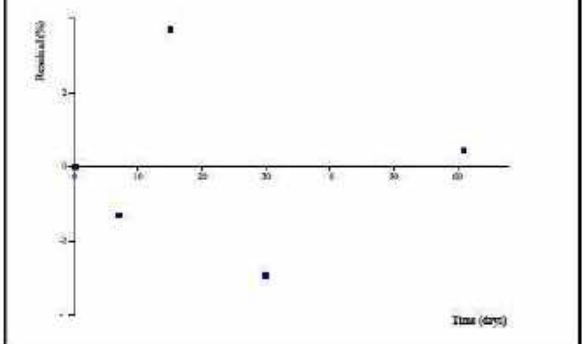
SFO



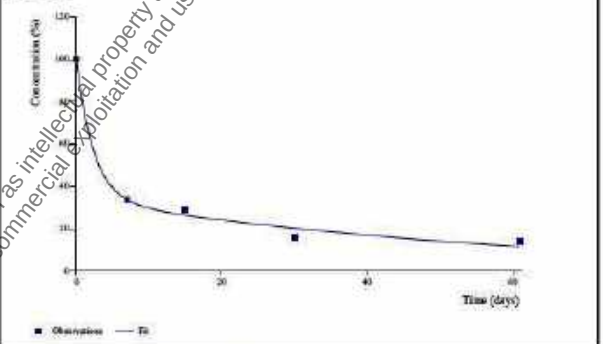


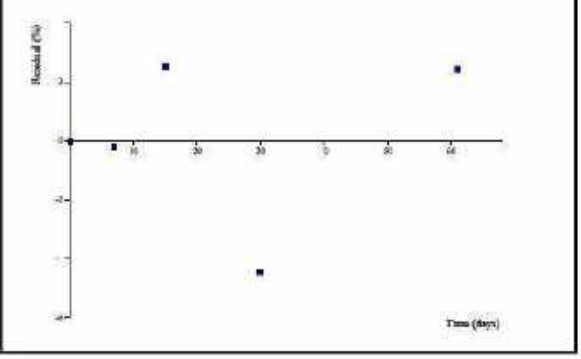
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

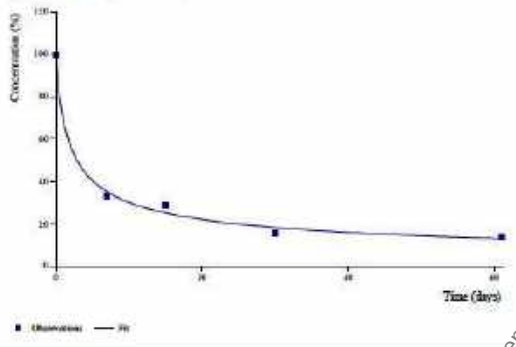
Table 7.1.2.2.1-36: Kinetic models and goodness-of-fit statistics of pathway fits for soil Bad Krozingen of study [redacted] (1992, CA 7.1.2.2.1/010) – trigger endpoints

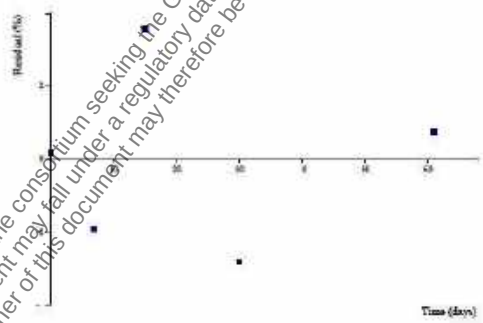
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: FOMC	Good	99.8	α: 0.4726 β: 0.873	5.3	-[1]	β: -1.1730	β: 2.9190	2.9	13
AMPA: SFO	Accep-table	-	k: 6.74×10^{-30}	11.7	k: 0.5	k: -0.0147	k: 0.015	>1000	>1000

The dissipation of glyphosate is well described by the FOMC model but the confidence interval for parameter β convincingly contains zero. For AMPA, the SFO model provides a visually acceptable fit, but the parameter k is not statistically reliable as no decline phase was observed. A decline fit for AMPA was not performed.

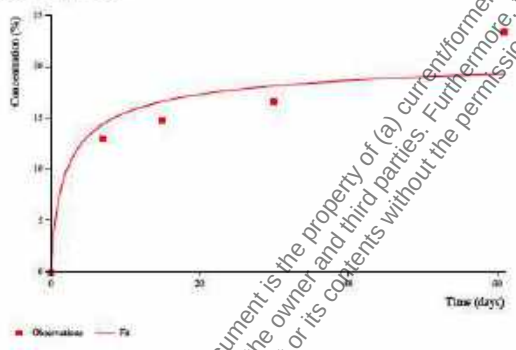
Conclusion: No reliable trigger endpoints for glyphosate or AMPA can be determined

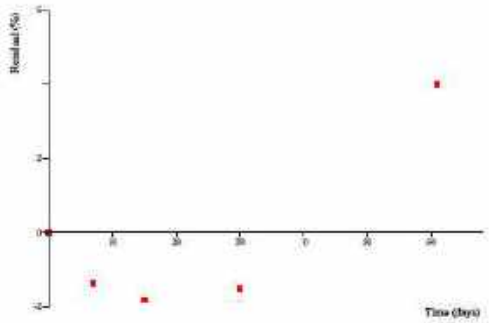
Glyphosate (FOMC)





AMPA (SFO)





[1] t-test not relevant for kinetic parameter β

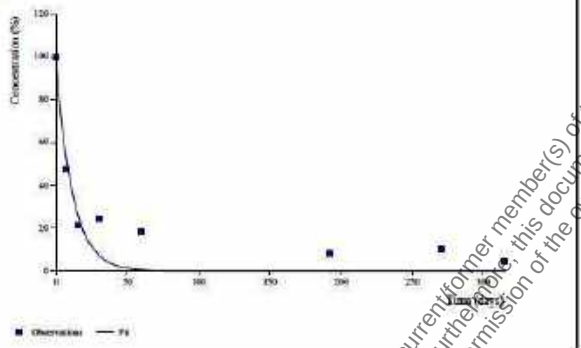
Table 7.1.2.2.1-37: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Menslage of study [redacted] (1992, CA 7.1.2.2.1/011) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	96.7	k: 0.0856	28.6	k: 0.0053	k: 0.0283	k: 0.1430	8.1	26.9
FOMC	Good	100.1	α: 0.5069 β: 1.598	12.1	-[1]	β: -1.29	β: 4.4870	4.7	149
DFOP	Good	100.4	k_1: 0.1781 k_2: 0.0041 g: 0.7704	9.4	k_1: 0.0036 k_2: 0.0315	k_1: 0.0800 k_2: -0.0004	k_1: 0.2760 k_2: 0.0090	5.8	201
HS	Not calculated								

The SFO model does not accurately represent the residue data. Dissipation of glyphosate was best described by bi-phasic models; FOMC and DFOP models were tested. The DFOP model provides the best visual fit with the lowest χ^2 error.

Conclusion: DFOP to be used in pathway fit for trigger endpoints

SFO



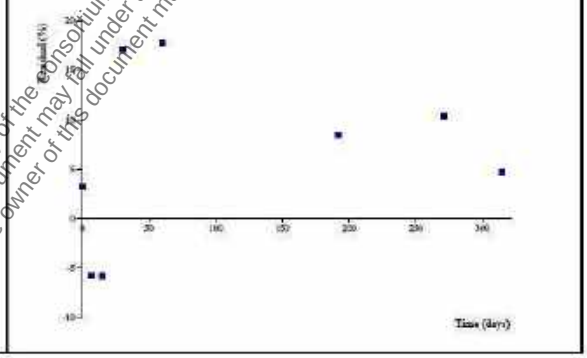
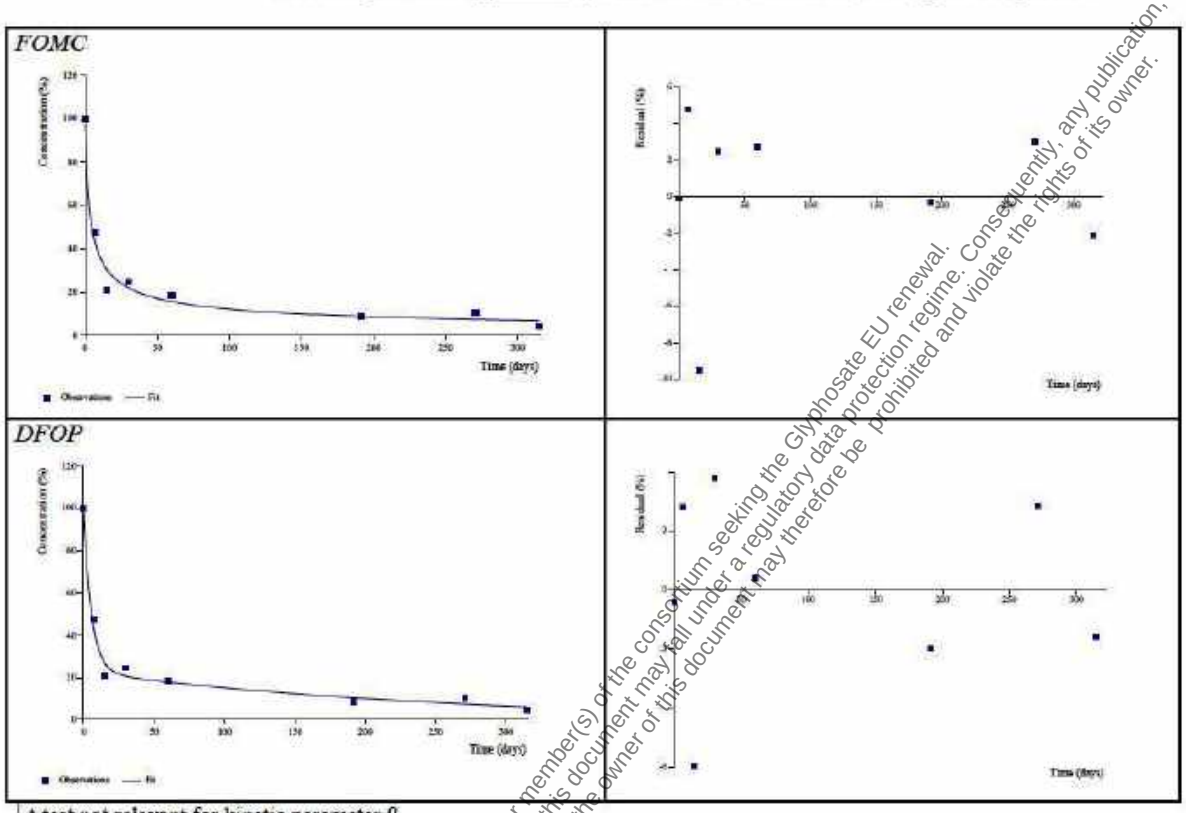


Table 7.1.2.2.1-37: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Menslage of study [redacted] (1992, CA 7.1.2.2.1/011) – trigger endpoints



[1] t-test not relevant for kinetic parameter β

Table 7.1.2.2.1-38: Kinetic models and goodness-of-fit statistics of pathway fits for soil Menslage of study [redacted] (1992, CA 7.1.2.2.1/011) – trigger endpoints

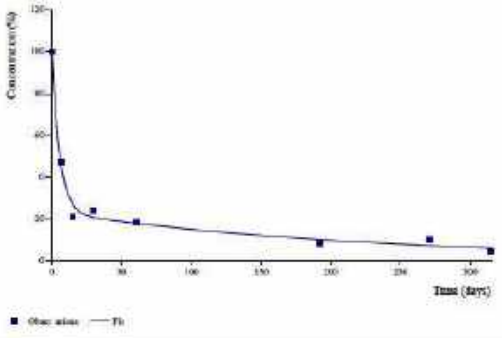
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Glyphosate: DFOP	Good	100.3	k_1: 0.1771 k_2: 0.0042 g: 0.7681	9.4	k_1: <0.001. k_2: 0.0115	k_1: 0.1023 k_2: 0.0007	k_1: 0.2520 k_2: 0.0080	5.8	199
AMPA: SFO	Poor	-	k: 1.56×10^{-20}	26.0	k: 0.5	k: -0.0031	k: 0.0030	>1000	>1000

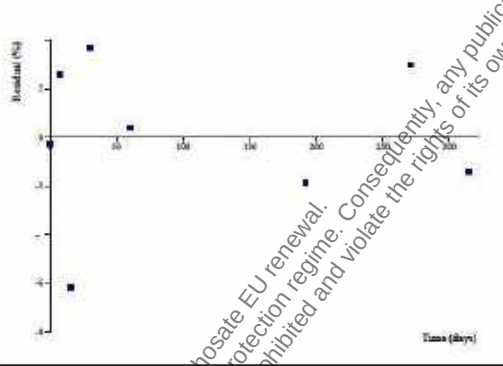
The dissipation of glyphosate is well described by the DFOP model in the pathway fit. For AMPA, the SFO model does not adequately fit the data visually or statistically. A decline fit for AMPA was not performed, as there is no clear decline phase.

Conclusion: Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate
No reliable trigger endpoints for AMPA can be determined

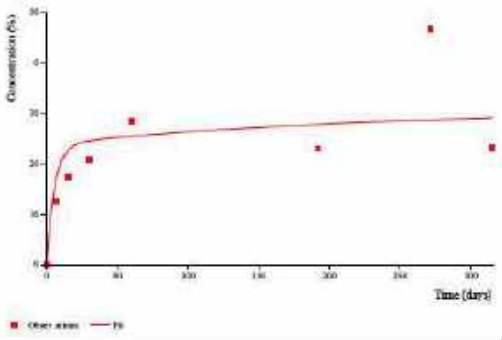
Table 7.1.2.2.1-38: Kinetic models and goodness-of-fit statistics of pathway fits for soil Menslage of study [redacted] (1992, CA 7.1.2.2.1/011) – trigger endpoints

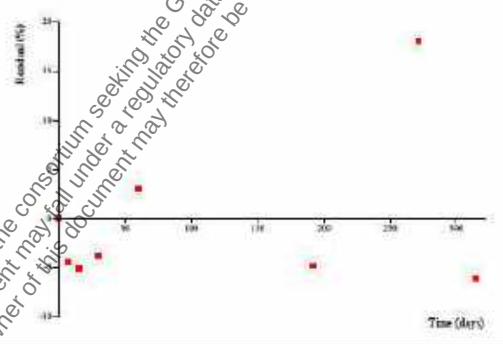
Glyphosate (DFOP)





AMPA (SFO)



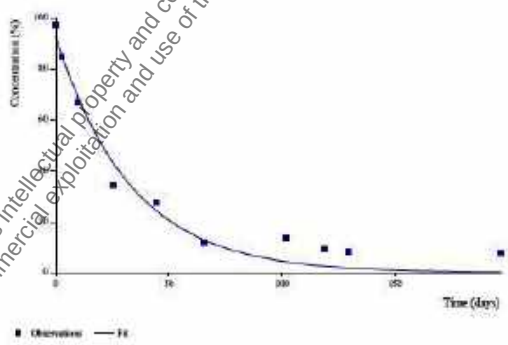


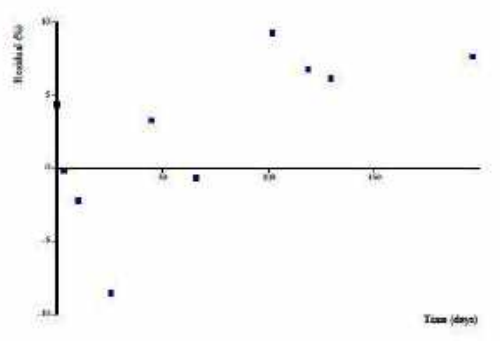
Determination of modelling endpoints

Table 7.1.2.2.1-39: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	92.6	k: 0.0301	12.9	k: <0.001	k: 0.0213	k: 0.0390	23.0	76.5
Visually, the SFO model describes the degradation of glyphosate well until the DT_{90}. Statistically the fit is acceptable (χ^2 error <15 % and the estimated degradation rate is reliable).									
Conclusion: SFO to be used in pathway fit for modelling endpoints									

SFO





[1] Representing $DegT50_{matrix}$ according to EFSA (2014)

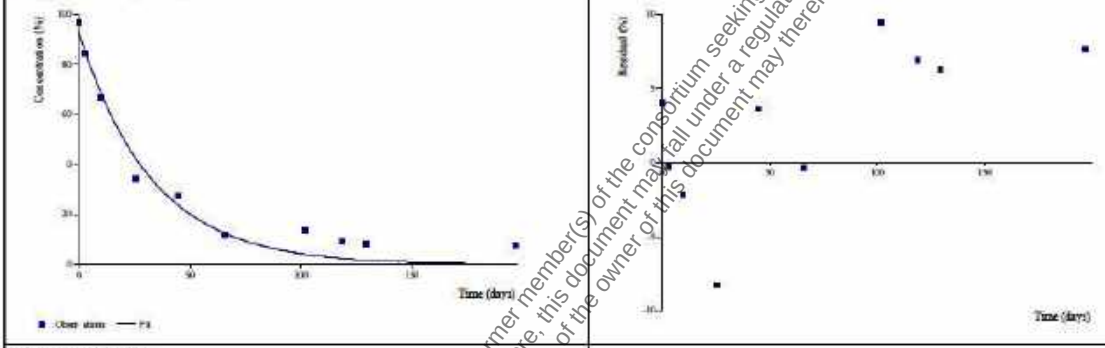
Table 7.1.2.2.1-40: Kinetic models and goodness-of-fit statistics of pathway fits for soil Büchen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic Model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	df (t std. dev.)
Glyphosate: SFO	Accep-table	92.9	k: 0.0305	12.9	k: <0.001	k: 0.0220	k 0.0390	22.7	75.6	-
AMPA: SFO	Poor	13.0	k: 0.0031	24.0	k: 0.1613	k: -0.0033	k: 0.0090	223	747	0.2085 (±0.125)

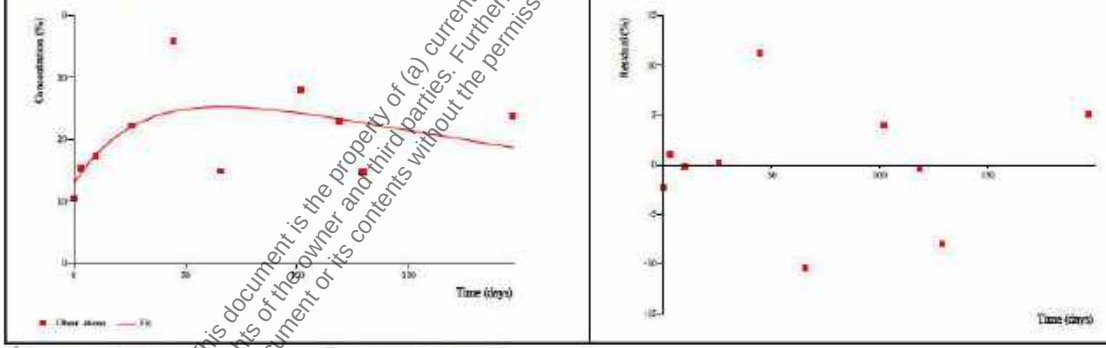
Visually, the SFO model in the pathway fit describes the degradation of glyphosate well until the DT_{90}. Statistically the fit is acceptable (χ^2 error <15 % and the estimated degradation rate is reliable).
For AMPA, the SFO model does not describe the data well visually or statistically, mainly due to the large scatter of residue data and no clear decline phase.

Conclusion: Parent-only SFO fit to be used for deriving modelling endpoints for glyphosate
No reliable modelling endpoints for AMPA can be determined

Glyphosate (SFO)

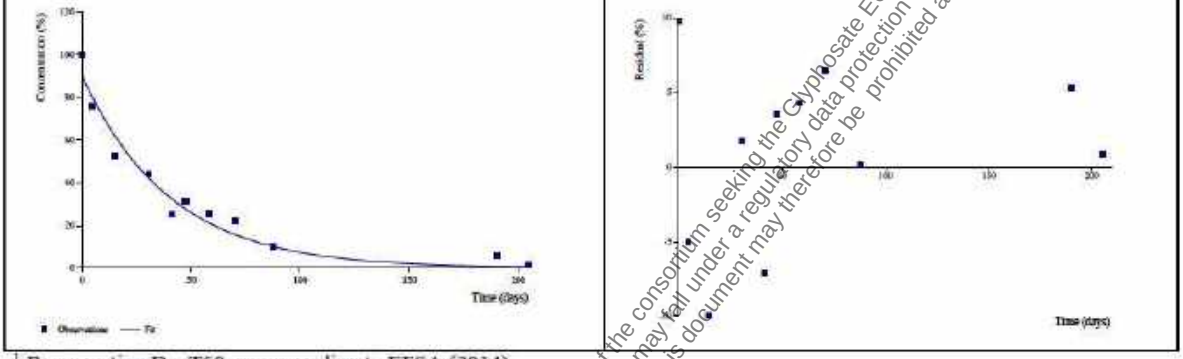


AMPA (SFO)



[1] Representing DegT50 according to EFSA (2014)

Table 7.1.2.2.1-41: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Klein-Zecher of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	90.3	k: 0.0249	13.1	k: <0.001	k: 0.0191	k: 0.0310	27.9	92.7
Visually, given the scatter in the residue data, the SFO model acceptably describes the degradation of glyphosate, with generally small residuals. Statistically the fit is also acceptable (χ^2 error <15 % and the estimated degradation rate is reliable). **Conclusion:** SFO to be used in pathway fit for modelling endpoints									
SFO 									

[1] Representing $DegT50_{matrix}$ according to EFSA (2014)

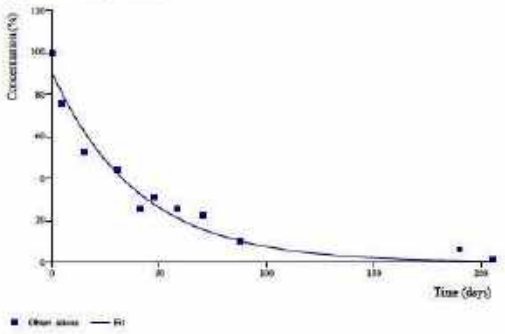
Table 7.1.2.2.1-42: Kinetic models and goodness-of-fit statistics of pathway fits for soil Klein-Zecher of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

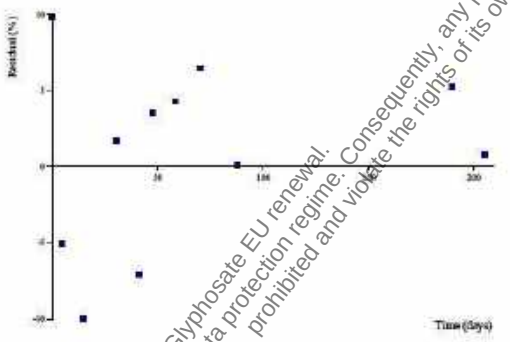
Kinetic Model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Initial fitting										
Glyphosate: SFO	Accep-table	90.3	k: 0.0248	13.1	k: <0.001	k: 0.0194	k: 0.0300	27.9	92.7	-
AMPA: SFO	Accep-table	22.2	k: 0.0015	9.6	k: 0.0708	k: -0.0005	k: 0.0030	471	>1000	0.1984 (±0.070)
Repeated fitting: AMPA ff fixed to previously estimated value										
Glyphosate: SFO	Accep-table	90.3	k: 0.0248	13.1	k: <0.001	k: 0.0197	k: 0.0300	27.9	92.7	-
AMPA: SFO	Accep-table	22.2	k: 0.0015	9.2	k: 0.0097	k: 0.0003	k: 0.0030	471	>1000	fixed to: 0.1984
Initial fitting: The dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. For AMPA, the parameter k is not significantly different from zero. However, the estimated formation fraction is reliable with a low standard deviation. Therefore, the fitting was repeated with fixing ff for AMPA to the estimated value (0.1984). **Repeated fitting:** With the formation fraction fixed, the statistical fit is improved slightly for AMPA; the parameter is significantly different from zero. **Conclusion:** SFO-SFO (repeated fitting) to be used for deriving modelling endpoints for glyphosate and AMPA											

Table 7.1.2.2.1-42: Kinetic models and goodness-of-fit statistics of pathway fits for soil Klein-Zecher of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

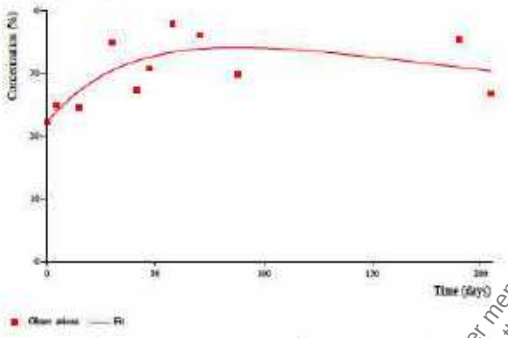
Repeated fitting: AMPA ff fixed to previously estimated value

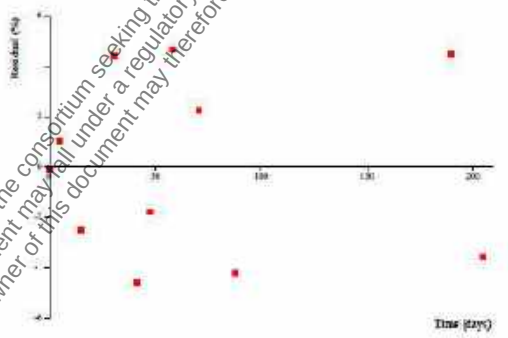
Glyphosate (SFO)





AMPA (SFO)





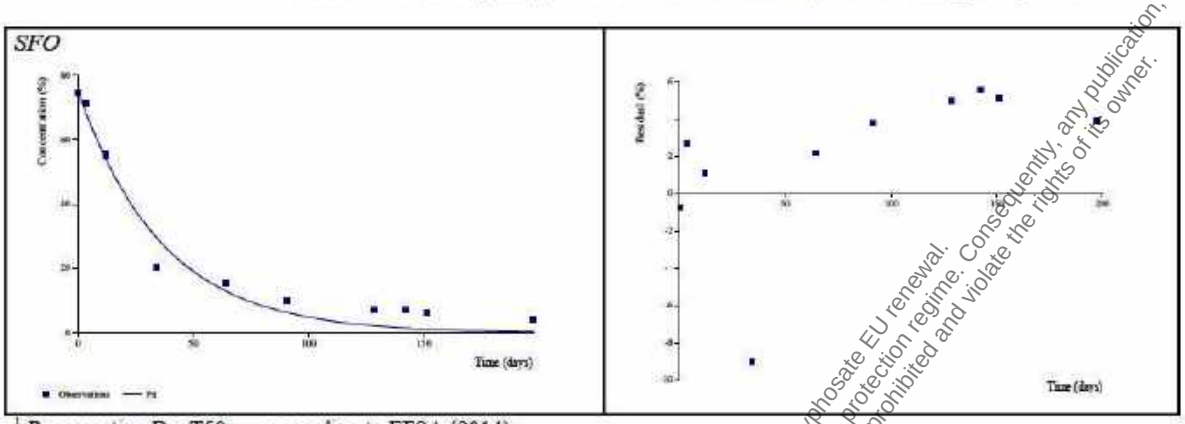
[1] Representing $DegT50_{matrix}$ according to EFSA (2014)

Table 7.1.2.2.1-43: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	75.3	k: 0.0274	13.4	k: <0.001	k: 0.0192	k: 0.0360	25.3	84.1
Visually, the SFO model acceptably describes the degradation of glyphosate. The model does overestimate degradation from 64 days, but the DT_{50} and M_0 are well represented and the residuals are small. Statistically the fit is also acceptable (χ^2 error < 15 % and the estimated degradation rate is reliable). **Conclusion:** SFO to be used in pathway fit for modelling endpoints									

Table 7.1.2.2.1-43: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

SFO



[1] Representing $DegT50_{matrix}$ according to EFSA (2014)

Table 7.1.2.2.1-44: Kinetic models and goodness-of-fit statistics of pathway fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

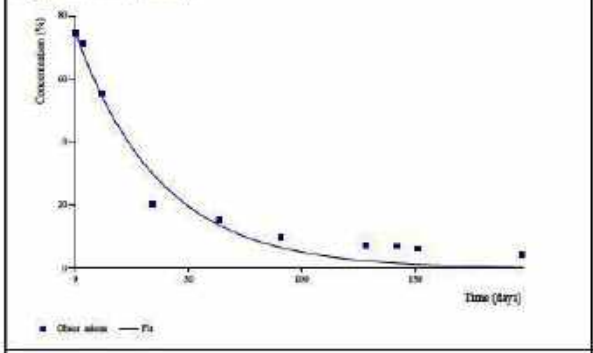
Kinetic Model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: SFO	Accep-table	75.0	k: 0.0267	13.4	k: <0.001	k: 0.0193	k 0.0340	25.9	86.2	-
AMPA: SFO	Accep-table	9.9	k: 0.0029	8.4	k: 0.0107	k: 0.0005	k: 0.0050	238	789	0.3192 (±0.068)

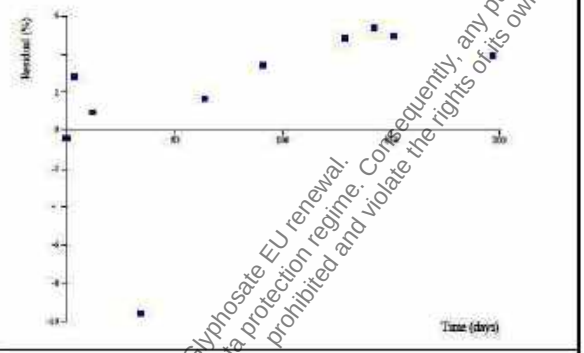
The SFO model acceptably describes the degradation of glyphosate. The model does overestimate degradation from 64 days, but the DT_{50} and M_0 are well represented and the residuals are generally small. Statistically, the χ^2 error <15 % and the estimated degradation rate is reliable. For AMPA, the SFO model well describes the data visually and statistically.

Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA

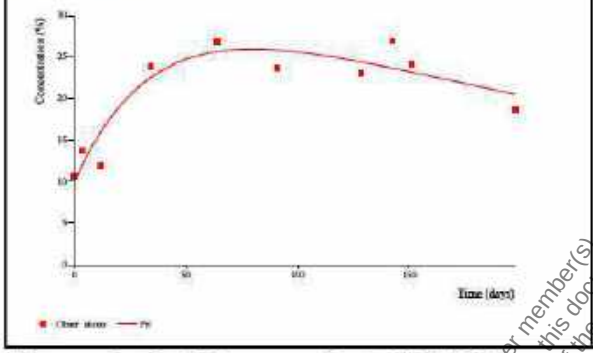
Table 7.1.2.2.1-44: Kinetic models and goodness-of-fit statistics of pathway fits for soil Unzhurst of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

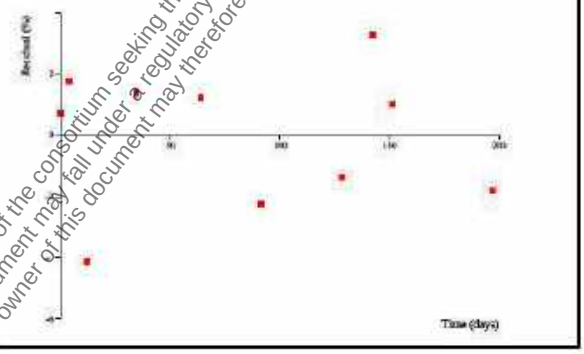
Glyphosate (SFO)





AMPA (SFO)





[1] Representing $DegT50_{matrix}$ according to EFSA (2014)

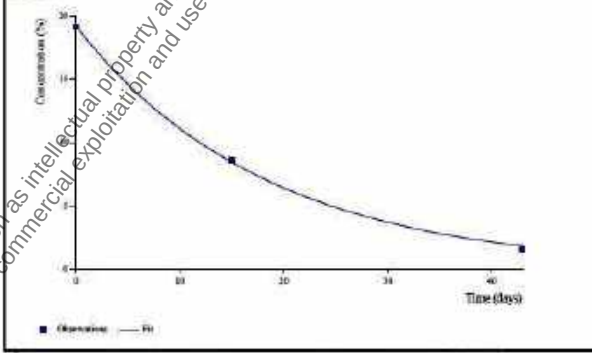
Table 7.1.2.2.1-45: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

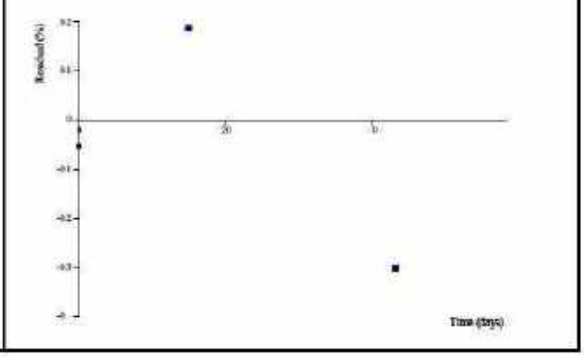
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Good	193	k: 0.0547	1.9	k: 0.0151	k: 0.0217	k: 0.088	12.7	42.1

Visually, the SFO model describes the degradation of glyphosate very well for the remaining datapoints after those prior to 10 mm rainfall have been removed. Statistically the fit is also acceptable (χ^2 error is low and the estimated degradation rate is reliable).

Conclusion: SFO to be used in pathway fit for modelling endpoints

SFO





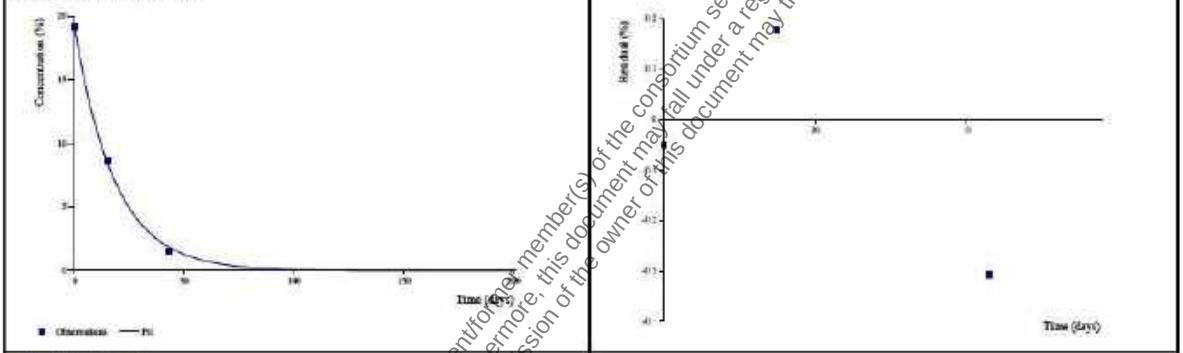
[1] Representing $DegT50_{matrix}$ according to EFSA (2014)

Table 7.1.2.2.1-46: Kinetic models and goodness-of-fit statistics of pathway fits for soil Rohrbach of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

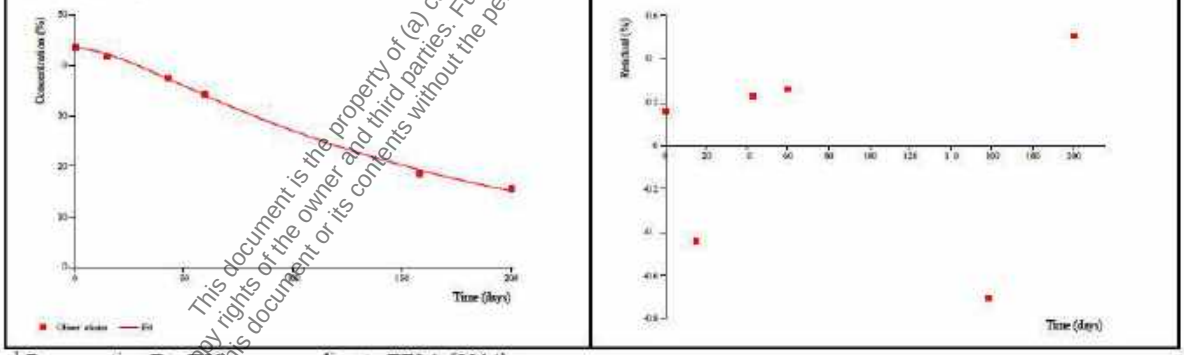
Kinetic Model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (= std. dev.)
Glyphosate: SFO	Good	19.3	k: 0.0546	1.9	k: <0.001	k: 0.0484	k 0.0610	12.7	42.2	-
AMPA: SFO	Good	43.5	k: 0.0058	1.2	k: <0.001	k: 0.0052	k: 0.0060	119	394	0.2399 (±0.060)

The degradation of glyphosate is well described by the SFO model for datapoints remaining after the 10 mm rain cutoff. The χ^2 error value is very low and the estimated degradation rate is significantly different from zero.
For AMPA, the SFO model describes the data very well visually and statistically. Although the metabolite formation phase was not completely included, the estimated parameters are reliable as the metabolite decline occurred after the parent compound has mostly dissipated and, thus, the metabolite degradation rate was estimated independently.
Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA

Glyphosate (SFO)



AMPA (SFO)



[1] Representing $DegT50_{matrix}$ according to EFSA (2014)

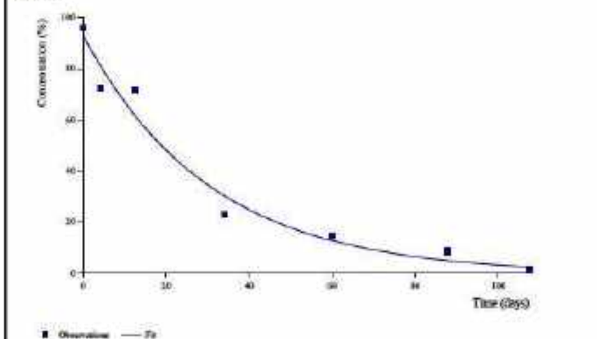
Table 7.1.2.2.1-47: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

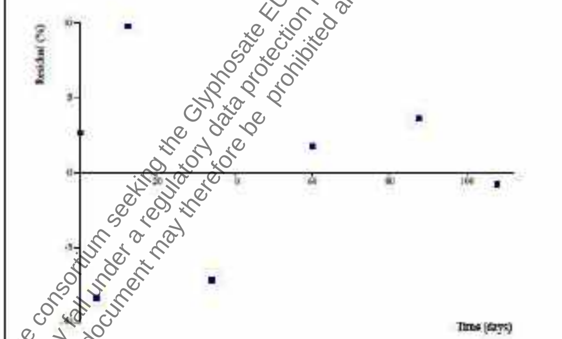
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Good	93.2	k: 0.0329	11.3	k: <0.001	k: 0.0202	k: 0.0460	21.1	70.0

The SFO model describes the degradation of glyphosate well. M_0 is well represented and residuals are generally small and randomly scattered about zero. Statistically the fit is also acceptable (χ^2 error <15 % and the estimated degradation rate is reliable).

Conclusion: SFO to be used in pathway fit for modelling endpoints

SFO





[1] Representing $DegT50_{matrix}$ according to EFSA (2014)

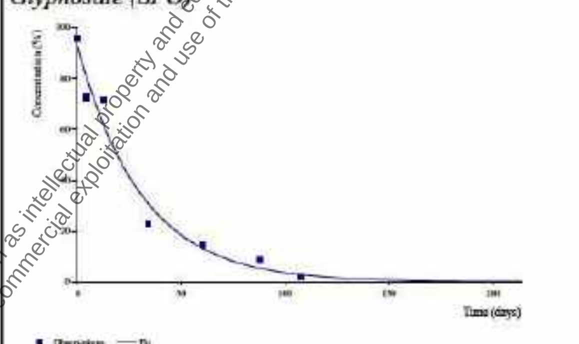
Table 7.1.2.2.1-48: Kinetic models and goodness-of-fit statistics of pathway fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

Kinetic Model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: SFO	Good	92.9	k: 0.0323	11.4	k: <0.001	k: 0.0216	k 0.0430	21.5	71.2	-
AMPA: SFO	Good	23.6	k: 0.0076	7.8	k: <0.001	k: 0.0042	k: 0.0110	90.7	301	0.2508 (±0.072)

The degradation of glyphosate is described well both visually and statistically by the SFO model. Similarly, for AMPA, the SFO model describes the data very well visually and statistically. Although the metabolite formation phase was not completely included, the estimated parameters are reliable as the metabolite decline occurred after the parent compound has mostly dissipated and, thus, the metabolite degradation rate was estimated independently.

Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA

Glyphosate (SFO)



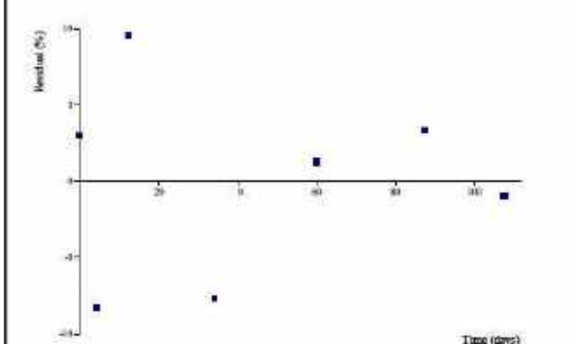
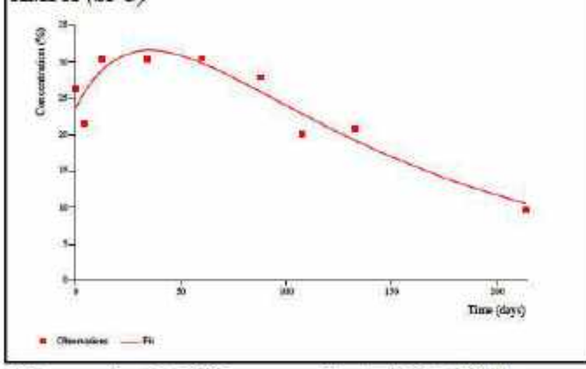
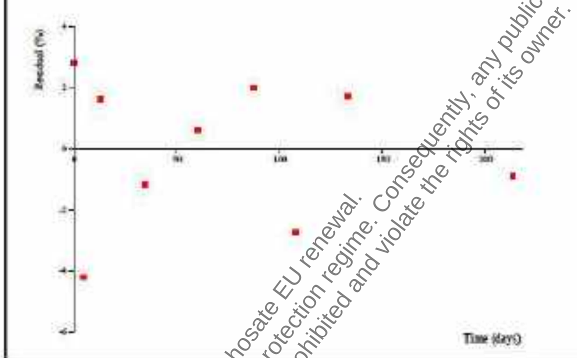


Table 7.1.2.2.1-48: Kinetic models and goodness-of-fit statistics of pathway fits for soil Herrngiersdorf of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

AMPA (SFO)





[1] Representing DegT50$_{matrix}$ according to EFSA (2014)

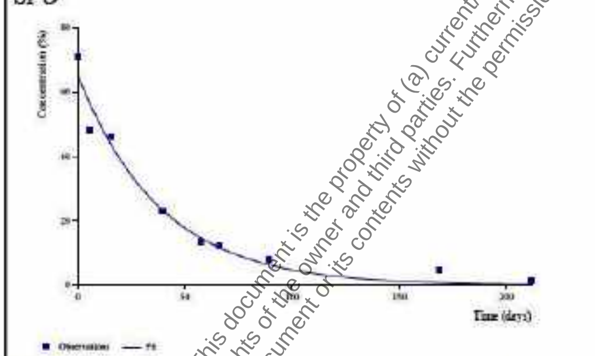
Table 7.1.2.2.1-49: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

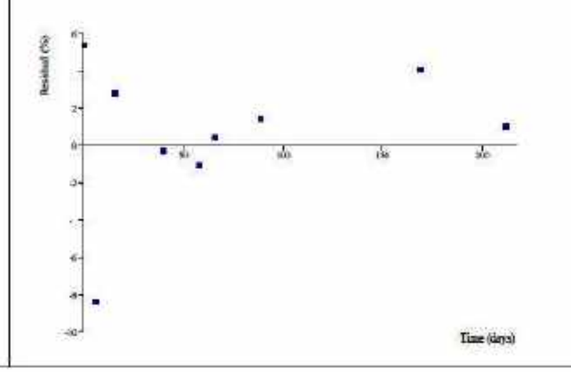
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT$_{50}$[1] (d)	DT$_{90}$ (d)
SFO	Good	65.6	k: 0.0260	11.9	k: < 0.001	k: 0.0192	k: 0.0330	26.7	88.5

The SFO model well describes the degradation of glyphosate. M_0 is accurately represented and residuals are small. Statistically the fit is also acceptable (χ^2 error < 15 % and the estimated degradation rate is reliable).

Conclusion: SFO to be used in pathway fit for modelling endpoints

SFO





[1] Representing DegT50$_{matrix}$ according to EFSA (2014)

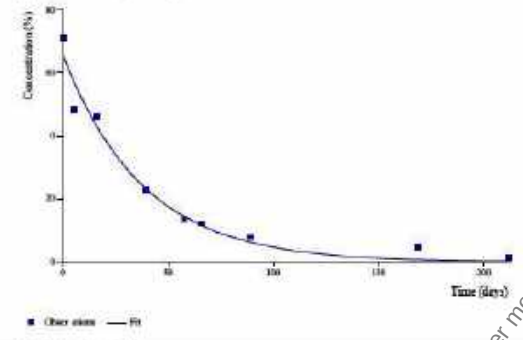
Table 7.1.2.2.1-50: Kinetic models and goodness-of-fit statistics of pathway fits for soil Wang-Inzkofen of study [redacted] (1992, CA 7.1.2.2.1/013) – modelling endpoints

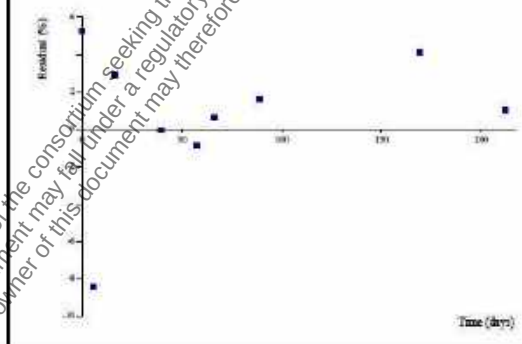
Kinetic Model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: SFO	Good	65.8	k: 0.0263	11.9	k: <0.001	k: 0.0198	k: 0.0330	26.4	87.6	
AMPA: SFO	Good	32.2	k: 0.0049	7.2	k: <0.001	k: 0.0024	k: 0.0070	142	471	0.2308 (±0.095)

The degradation of glyphosate is described well both visually and statistically by the SFO model in the pathway fit. Similarly, for AMPA, the SFO model describes the data very well visually, and statistically the estimated degradation rates are reliable. Although the metabolite formation phase was not completely included, the estimated parameters are reliable as the metabolite decline occurred after the parent compound has mostly dissipated and, thus, the metabolite degradation rate was estimated independently.

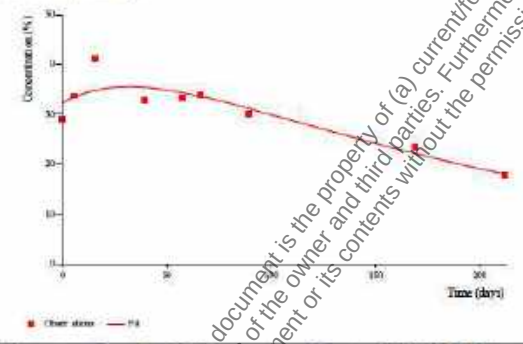
Conclusion: SFO-SFO to be used for deriving modelling endpoints for glyphosate and AMPA

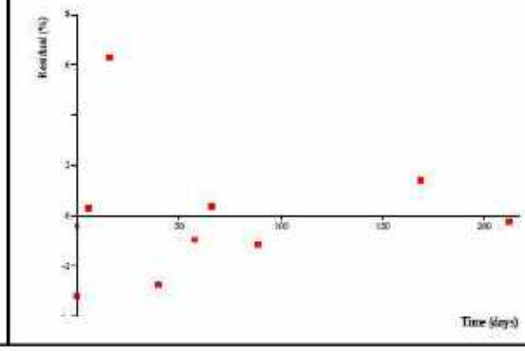
Glyphosate (SFO)





AMPA (SFO)





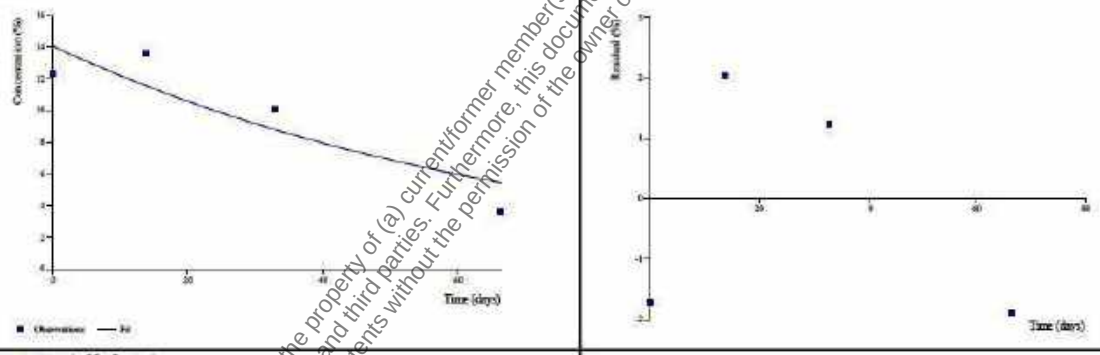
[1] Representing DegT50 according to EFSA (2014)

Table 7.1.2.2.1-51: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Diegten of study [redacted] (1992, CA 7.1.2.2.1/008) – modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	14.1	k: 0.0143	14.4	k: 0.0777	k: -0.0133	k: 0.0420	48.5	161
DFOP (full dataset)	Good	100	k_1: 0.2592 k_2: 0.0144 g: 0.8205	4.8	k_1: 0.0017 k_2: 0.0521	k_1: 0.1622 k_2: -0.0054	k_1: 0.3560 k_2: 0.0340	48.2	-
HS (full dataset, t_b fixed)	Good	99.0	k_1: 0.1728 k_2: 0.0136 t_b: fixed to 10.91	6.8	k_1: <0.001 k_2: 0.0444	k_1: 0.1417 k_2: -0.0033	k_1: 0.2040 k_2: 0.0300	51.0[2]	-

SFO model: visually, given the scatter in the data, the model describes the degradation of glyphosate adequately. But statistically the estimated degradation rate (k) is not significantly different from zero and the confidence interval includes zero. Thus, the DFOP model was alternatively fitted to the whole dataset.
DFOP model: the estimated g value is >0.75. In accordance with the EFSA (2014), the HS model was additionally fitted to the whole dataset.
HS model: in a first model run, the estimated t_b was prior to the time a 10 mm rain. Therefore, the fitting was repeated with t_b fixed to the time when rain was >10 mm (10.91 days, normalised) in accordance with EFSA (2014).
For the repeated fit, the visual fit is good with small randomly scattered residuals, and the parameter k_2 is significantly different from zero (at 5 % level).
Conclusion: Slow phase DT_{50} from HS model to be used as modelling endpoint for glyphosate

SFO



DFOP (all data)

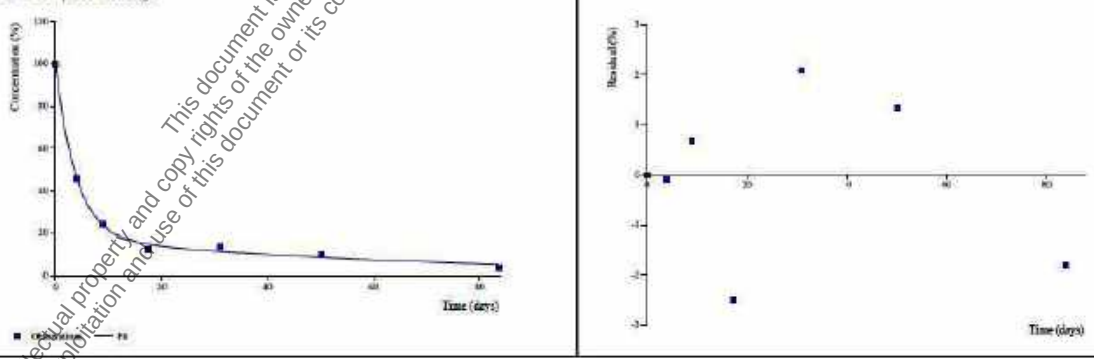
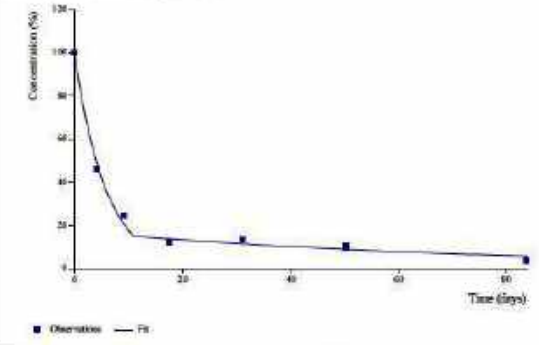
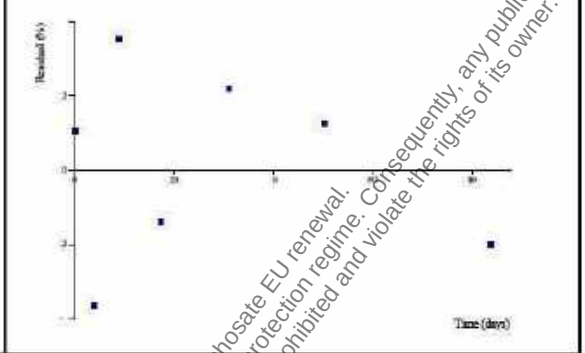


Table 7.1.2.2.1-51: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Diegten of study [redacted] (1992, CA 7.1.2.2.1/008) – modelling endpoints

HS (all data, t_b fixed)





[1] Representing $DegT50_{matrix}$ according to EFSA (2014)
[2] Calculated from the slow-phase (k_2) according to EFSA (2014)

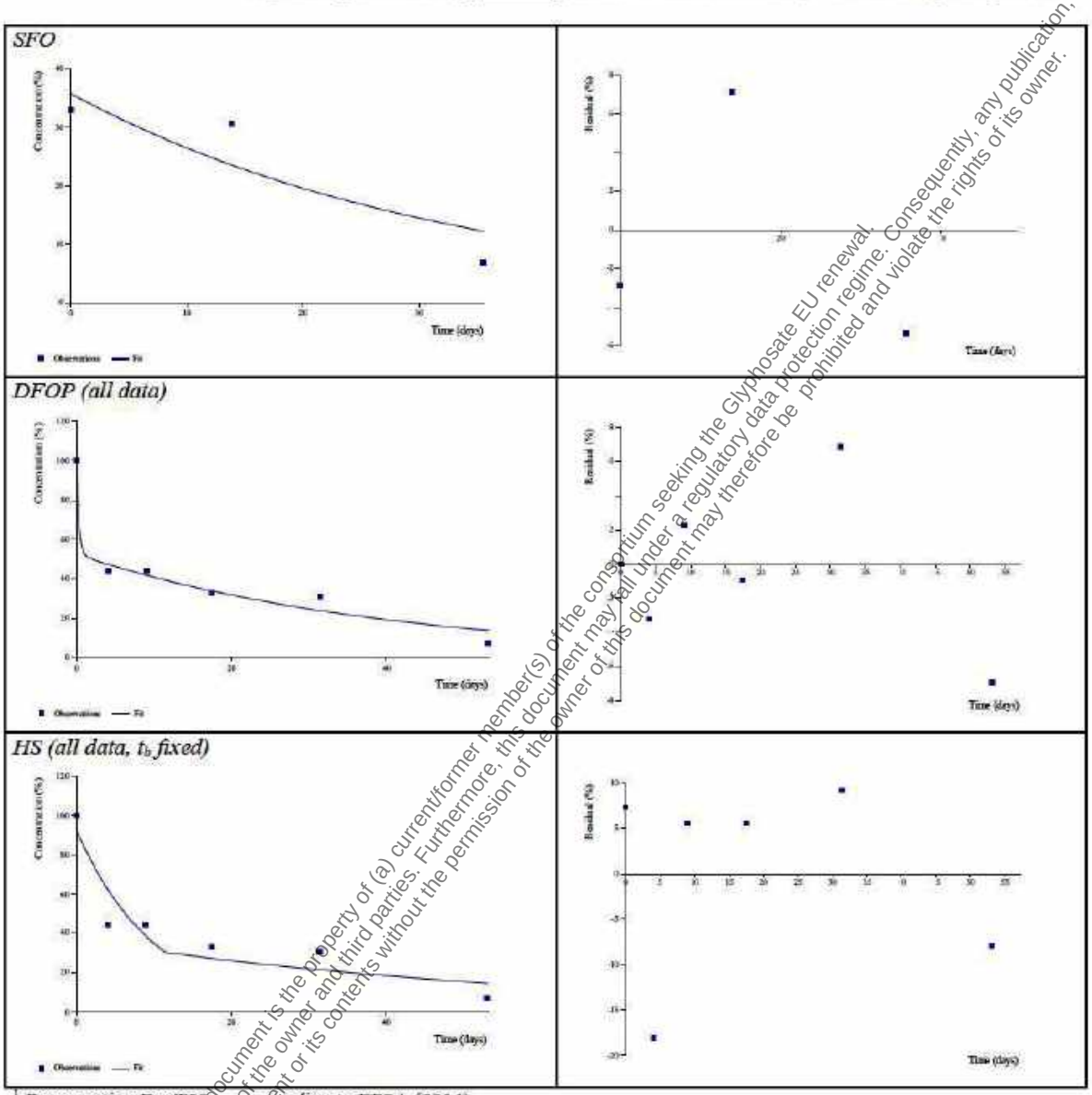
As the SFO parent-only fit for glyphosate was not acceptable, no pathway fit was tested for soil Diegten. For AMPA, since no clear decline phase was observed, a decline fit was not considered.

Table 7.1.2.2.1-52: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Egerkingen of study [redacted] (1992, CA 7.1.2.2.1/009) – modelling endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Acceptable	35.8	k: 0.0301	20.4	k: 0.1901	k: -0.2297	k: 0.2900	23.1	76.6
DFOP (full dataset)	Acceptable	100	k_1: 3.273 k_2: 0.0252 g: 0.4759	19.0	k_1: 0.0375 k_2: 0.0457	k_1: -0.8183 k_2: -0.0100	k_1: 7.365 k_2: 0.0600	27.6[2]	-
HS (full dataset, t_b fixed)	Poor	92.7	k_1: 0.0971 k_2: 0.0173 t_b: fixed to 11.48	20.1	k_1: 0.0320 k_2: 0.2460	k_1: -0.0106 k_2: -0.0532	k_1: 0.2050 k_2: 0.0880	40.1[2]	-

SFO model: visually, given the scatter in the data, the SFO model describes the degradation of glyphosate adequately. But statistically the parameter k is not significantly different from zero and the confidence interval includes zero. Thus, the DFOP model was alternatively fitted to the whole dataset.
DFOP model: the estimated g value is <0.75. The estimated degradation rates are significantly different, but the estimated DFOP breakpoint (6.8 days, normalised) was prior to the time >10 mm rain. (11.48 days, normalised). In accordance with EFSA (2014), the HS model was additionally fitted to the whole dataset.
HS model: in a first model run, the estimated t_b was prior to the time >10 mm rain. Therefore, the fitting was repeated with t_b fixed to the time when rain was >10 mm (11.48 days, normalised) in accordance with the EFSA (2014).
For the repeated fit, the visual fit is poor. Also k_2 is not significantly different from zero.
Conclusion: No acceptable modelling endpoint could be determined for glyphosate

Table 7.1.2.2.1-52: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Egerkingen of study [redacted] (1992, CA 7.1.2.2.1/009) – modelling endpoints



[1] Representing DegT50$_{Annex}$ according to EFSA (2014)
[2] Calculated from the slow phase (k_2) according to EFSA (2014)

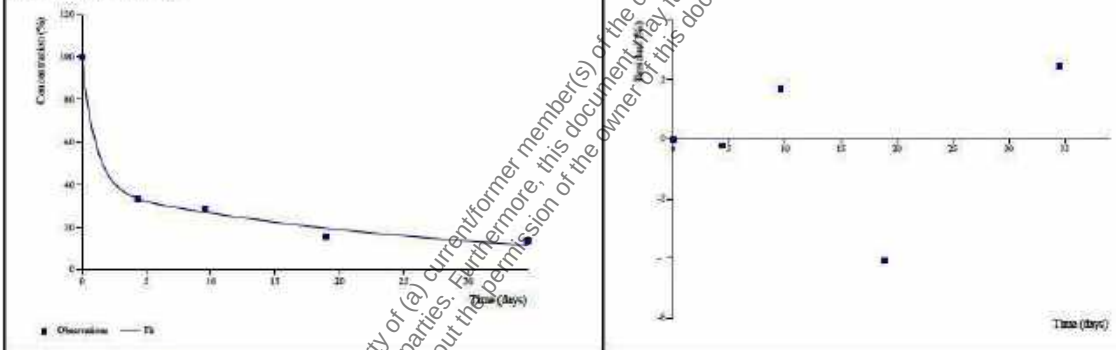
As no acceptable modelling endpoints could be determined for glyphosate, no pathway fit was tested for soil Egerkingen. For AMPA, since no clear decline phase was observed, a decline fit was not considered.

Table 7.1.2.2.1-53: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Bad Krozingen of study [redacted] (1992, CA 7.1.2.2.1/010) – modelling endpoints

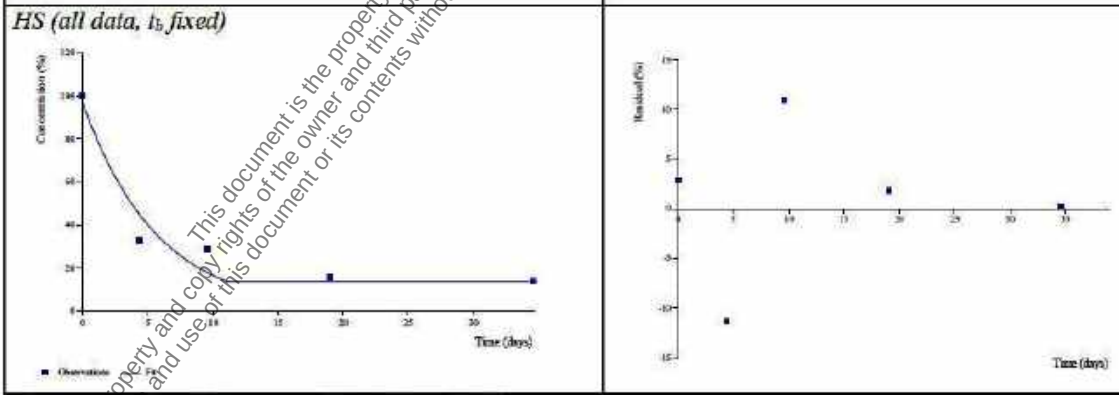
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Not calculated								
DFOP (full dataset)	Good	100	k_1: 0.9138 k_2: 0.0340 g: 0.6247	6.7	k_1: 0.3697 k_2: 0.1718	k_1: -25.856 k_2: -0.2249	k_1: 27.683 k_2: 0.2930	20.4[2]	-
HS (full dataset, t_b fixed)	Poor	97.1	k_1: 0.1762 k_2: 1.28×10^{-9} t_b: fixed to 11.08	17.2	k_1: 0.0301 k_2: 0.5	k_1: -0.0188 k_2: -0.1767	k_1: 0.3700 k_2: 0.1770	>1000[2]	-

SFO model: not applied, as after excluding residue data prior to 10 mm rain, only two datapoints remain. Hence, the DFOP model was alternatively fitted to the whole dataset.
DFOP model: the estimated g value is <0.75. However, the estimated degradation rates are not significantly different from zero. In accordance with the EFSA (2014), the HS model was additionally fitted to the whole dataset.
HS model: in a first model run, the estimated t_b was prior to the time >10 mm rain. Therefore, the fitting was repeated with t_b fixed to the time when rain was >10 mm (11.08 days, normalised), in accordance with the EFSA (2014).
For the repeated fit, the visual fit is poor, and k_2 is not significantly different different from zero.
Conclusion: No acceptable modelling endpoint could be determined for glyphosate

DFOP (all data)



HS (all data, t_b fixed)



[1] Represents DegT50$_{matrix}$ according to EFSA (2014)
[2] Calculated from the slow-phase (k_2) according to EFSA (2014)

As no acceptable modelling endpoints could be determined for glyphosate, no pathway fit was tested for soil Bad Krozingen. For AMPA, since no clear decline phase was observed, a decline fit was not considered.

Table 7.1.2.2.1-54: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Menslage of study [redacted] (1992, CA 7.1.2.2.1/011) – modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50}[1] (d)	DT_{90} (d)
SFO	Poor	35.7	k: 0.0271	27.7	k: 0.0329	k: -0.0026	k: 0.0570	25.6	85.0
DFOP (full dataset)	Good	100.5	k_1: 0.3380 k_2: 0.0135 g: 0.7581	11.4	k_1: 0.0103 k_2: 0.0650	k_1: 0.0851 k_2: -0.0062	k_1: 0.5910 k_2: 0.0330	51.4[2]	-
HS (full dataset)	Good	100.2	k_1: 0.1961 k_2: 0.0151 t_b: 7.278	6.8	k_1: <0.001 k_2: 0.0086	k_1: 0.1459 k_2: 0.0044	k_1: 0.2460 k_2: 0.0260	46.0[2]	-

SFO model: The SFO model does not describe the degradation of glyphosate adequately, especially in the initial phase of decline. Therefore the DFOP model was alternatively fitted to the whole dataset.
DFOP model: the estimated g value is >0.75. In accordance with the EFSA (2014), the HS model was additionally fitted to the whole dataset.
HS model: the estimated t_b is after the time >10 mm rain, the visual fit is good with small randomly scattered residuals and k_2 is significantly different to zero (at 5 % level).
Conclusion: Slow phase DT_{50} from HS model to be used as modelling endpoint for glyphosate

SFO

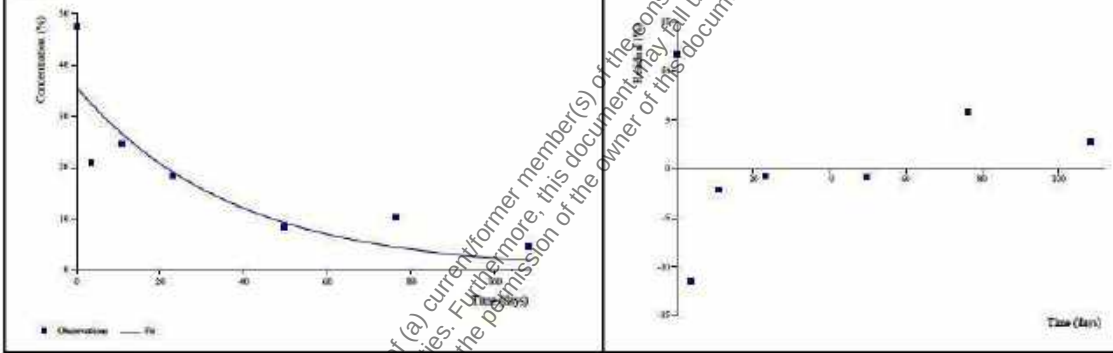
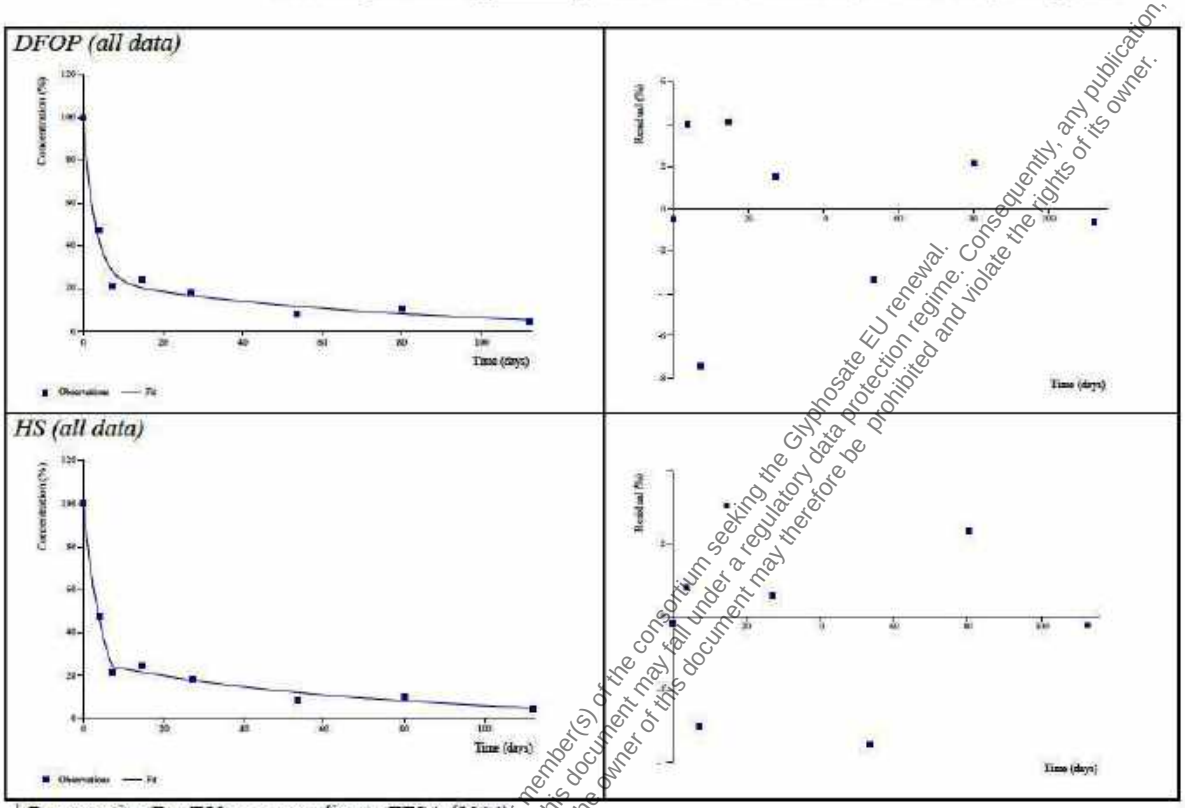


Table 7.1.2.2.1-54: Kinetic models and goodness-of-fit statistics of parent-only fits for soil Menslage of study [redacted] (1992, CA 7.1.2.2.1/011) – modelling endpoints



[1] Representing $DegT50_{matrix}$ according to EFSA (2014)
[2] Calculated from the slow-phase (k2) according to EFSA (2014)

As the SFO parent-only fit for glyphosate was not acceptable, no pathway fit was tested for soil Menslage. For AMPA, since no clear decline phase was observed, a decline fit was not considered.

Summary of trigger and modelling endpoints

Table 7.1.2.2.1-55: Summary of trigger endpoints for glyphosate

Study	Soil type (USDA)	Location	pH	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted] 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[1]	0 - 30	40.7	187	6.6	DFOP
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[1]	0 - 30	29.1	364	12.7	DFOP
	Loam (bare soil)	Unzhurst, Germany	6.7[1]	0 - 30	27.0	126	8.5	DFOP
	Silt loam (bare soil)	Rohrbach, Germany	8.5[1]	0 - 30	24.4	81.0	16.0	SFO
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[1]	0 - 30	33.7	112	10.6	SFO
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[1]	0 - 30	15.8	180	9.2	FOMC
[redacted] 1992, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[2]	0 - 30	6.1	118	5.0	DFOP
[redacted] 1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[2]	0 - 30	5.8	201	9.4	DFOP

[1] Medium not reported
[2] Measured in KCl
[3] No reliable endpoint could be determined

Table 7.1.2.2.1-56: Summary of trigger endpoints for AMPA

Study	Soil type (USDA)	Location	pH	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted] 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[1]	0 - 30	-[3]	-[3]	-	-
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[1]	0 - 30	521	>1000	13.9	DFOP-SFO
	Loam (bare soil)	Unzhurst, Germany	6.7[1]	0 - 30	634	>1000	11.9	DFOP-SFO
	Silt loam (bare soil)	Rohrbach, Germany	8.5[1]	0 - 30	255	847	15.5	SFO-SFO
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[1]	0 - 30	288	958	11.0	SFO[4]
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[1]	0 - 30	273	907	15.8	FOMC-SFO

Table 7.1.2.2.1-56: Summary of trigger endpoints for AMPA

[redacted] 1992, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[2]	0 - 30	-[3]	-[3]	-	-
[redacted] 1992, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[2]	0 - 30	-[3]	-[3]	-	
[redacted] 1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[2]	0 - 30	-[3]	-[3]		

[1] Medium not reported
[2] Measured in KCl
[3] No reliable endpoint could be determined
[4] Metabolite decline fit

Table 7.1.2.2.1-57: Summary of modelling endpoints for glyphosate

Study	Soil type (USDA)	Location	pH	Depth (cm)	$DegT_{50}$ (d) Norm.[1]	χ^2 error (%)	Kinetic model
[redacted] 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[2]	0 - 30	23.0	12.9	SFO
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[2]	0 - 30	27.9	13.1	SFO
	Loam (bare soil)	Unzhurst, Germany	6.7[2]	0 - 30	25.9	13.4	SFO
	Silt loam (bare soil)	Rohrbach, Germany	8.5[2]	0 - 30	12.7	1.9	SFO
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[2]	0 - 30	21.5	11.4	SFO
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[2]	0 - 30	26.4	11.9	SFO
[redacted] 1992, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[3]	0 - 30	51.0[4]	6.8	HS
[redacted] 1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[3]	0 - 30	-[5]	-	-
[redacted] 1992, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[3]	0 - 30	-[5]	-	-
[redacted] 1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[3]	0 - 30	46.0[4]	6.8	HS

[1] $DegT50_{matrix}$ according to EFSA (2014) and FOCUS (2006, 2014)
[2] Medium not reported
[3] Measured in KCl
[4] Calculated from the slow phase: $\ln(2)/k_2$
[5] No reliable endpoint could be determined

Table 7.1.2.2.1-58: Summary of modelling endpoints for AMPA

Study	Soil type (USDA)	Location	pH	Depth (cm)	DT_{50} (d) Norm.[1]	Formation fraction (-).	χ^2 error (%)	Kinetic model
[redacted] 1992, CA 7.1.2.2.1/013	Loamy sand (bare soil)	Büchen, Germany	6.4[2]	0 - 30	-[4]	-[4]	-	-
	Sandy loam (bare soil)	Klein-Zecher, Germany	7.0[2]	0 - 30	471	0.1984	9.2	SFO-SFO
	Loam (bare soil)	Unzhurst, Germany	6.7[2]	0 - 30	238	0.3192	8.9	SFO-SFO
	Silt loam (bare soil)	Rohrbach, Germany	8.5[2]	0 - 30	119	0.2399	1.2	SFO-SFO
	Clay loam (bare soil)	Herrngiersdorf, Germany	8.0[2]	0 - 30	90.7	0.2508	7.8	SFO-SFO
	Silt loam (bare soil)	Wang-Inzkofen, Germany	7.2[2]	0 - 30	142	0.2308	7.2	SFO-SFO
[redacted] 1992, CA 7.1.2.2.1/008	Sandy clay (bare soil)	Diegten, Switzerland	7.1[3]	0 - 30	-[4]	-[4]	-	-
[redacted] 1992, CA 7.1.2.2.1/009	Clay loam (bare soil)	Egerkingen, Switzerland	7.33[3]	0 - 30	-[4]	-[4]	-	-
[redacted] 1992, CA 7.1.2.2.1/010	Sandy loam (bare soil)	Bad Krozingen, Germany	6.0[3]	0 - 30	-[4]	-[4]	-	-
[redacted] 1992, CA 7.1.2.2.1/011	Sand (bare soil)	Menslage, Germany	4.73[3]	0 - 30	-[4]	-[4]	-	-

[1] $DegT50_{matrix}$ according to EFSA (2014)
[2] Medium not reported
[3] Measured in KCl
[4] No reliable endpoint could be determined

3. Assessment and conclusion

Assessment and conclusion by applicant:
The kinetic evaluation was performed according to the current guidances without any deviations. Thus, the study is considered valid and the provided endpoints can be used for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate: Ecoregion Crosswalk for Nineteen Terrestrial Field Dissipation Study Locations in North America
Report No	112148-005
Document No	
Guidelines followed in study	No guideline followed
Deviations from current test guideline	Not applicable; evaluation was performed with OECD ENASGIPS tool recommended in OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The fate and behaviour of the active substance glyphosate in North America was investigated at 19 trial sites in six Terrestrial Field Dissipation (TFD) studies. The study locations were evaluated using the OECD ENASGIPS tool (Europe – North American Soil Geographic Information for Pesticide Studies) aiming to determine their representativeness throughout Europe based on climate and soil similarity. The ENASGIPS tool uses the ecoregion concept to compare environmental properties such as long-term annual total rainfall, average precipitation, soil texture, soil pH, and soil organic matter to calculate a similarity index.

According to the Ecoregion crosswalk, the analysed 19 TFD trial sites are represented by twelve North American root ecoregions. A holistic similarity score of at least 80 % was observed for eight of twelve identified North American ecoregions.

In addition to the holistic similarity approach, individual scores of soil and climate were evaluated in a refined assessment. While soil conditions reached high scores for the remaining eight ecoregions, temperature as the main driving parameter for the degradation of pesticides among the climatic parameters reached low individual scores in some ecoregions. Similarity scores of temperature conditions in Europe were very low (7 to 33 %) for three of the eight North American ecoregions which indicates pronounced differences in temperature conditions between compared ecoregions in North American and Europe. For the remaining five North American ecoregions, similarity of temperature conditions in Europe was moderate to very high (66 to 100 %).

In summary, five root ecoregions representing nine North American TFD trial sites for glyphosate are considered representative for European conditions.

I. METHODS

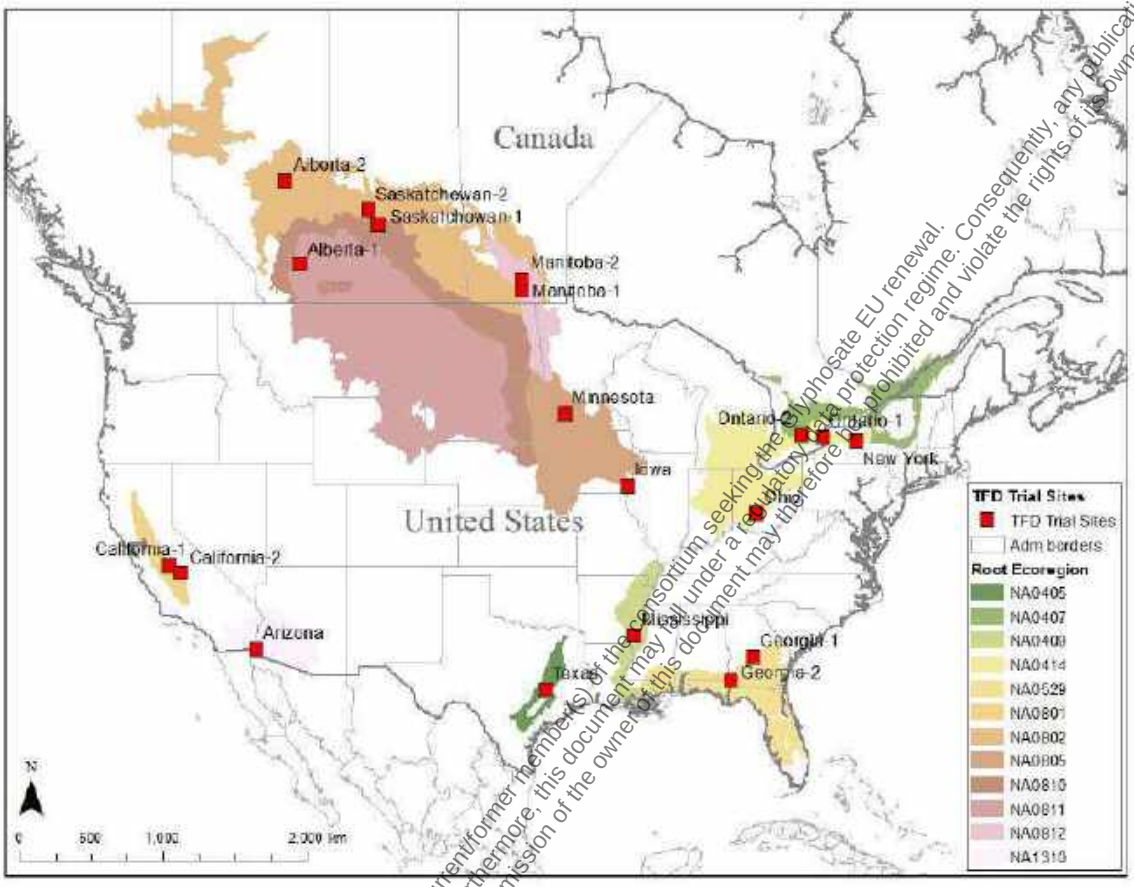
A. TRIAL SITE LOCATIONS

The similarity of 19 North American TFD trial sites was assessed. Eight of the assessed sites are located in Canada and eleven in the United States as presented in the table below. According to the OECD ENASGIPS tool (PMRA, 2015), the 19 TFD trial sites are assigned to twelve root ecoregions as presented in the figure below. These ecoregions reflect distinct combinations of regional environmental conditions and ecology, e.g. soil and climate characteristics.

Table 7.1.2.2.1-59: TFD trial sites in North America

Study	TFD Trial Site
[redacted] 1992	Ontario-1, CAN
	Saskatchewan-2, CAN
	Saskatchewan-1, CAN
	Alberta-1, CAN
	Manitoba-1, CAN
[redacted] 1993	Arizona, USA
	California-1, USA
	Iowa, USA
	Georgia-1, USA
	Minnesota, USA
	Ohio, USA
	New York, USA
	Texas, USA
[redacted] 1993	Ontario-2, CAN
	Alberta-2, CAN
	Manitoba-2, CAN
[redacted] 1989a	California-2, USA
[redacted] 1989b	Mississippi, USA
[redacted] 1989c	Georgia-2, USA

Figure 7.1.2.2.1-1: Location of assessed glyphosate TFD trial sites in North America with associated root ecoregions (PMRA, 2015)



B. ECOREGION CROSSWALK

The ecoregion crosswalk was conducted using the ENASGIPS v3.0 (Europe-North America Soil Geographic Information for Pesticide Studies) application (PMRA, 2015). The model is recommended for conducting ecoregion crosswalks by the OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies published in 2016 (OECD, 2016).

For each Canadian and American TFD trial site, the respective root ecoregion was assigned based on the geographical coordinates using the ENASGIPS tool. The 'Holistic Ecoregions Similarity' tool implemented in ENASGIPS allows the user to identify similar ecoregions in Canada, North America, and Europe. A similarity score is calculated between each North American and all European ecoregions based on soil and climate parameters such as mean annual temperature, mean annual precipitation, soil pH, soil organic carbon, and soil texture. Similarity of each of the five parameters is scored separately, and then the five scores are combined with equal weighting into an overall Similarity Score. For the present assessment, the default similarity threshold value of 80 % was used.

The Holistic Ecoregion Similarity weights all five parameters equally, i.e. low to very low scores of an individual parameter might be compensated by the high to very high score of other individual parameters. Transferring trial site conditions to the opposite continent might therefore be questionable and require a closer look to the similarity score of individual parameters.

In addition to the holistic similarity approach, individual scores of soil and climate were evaluated in a refined assessment as applying the holistic similarity approach does not account for the high impact of temperature on degradation. Thus, holistic similarity results may also include ecoregions in Europe with

very low and low temperature similarity. As a consequence, holistic matches were excluded from the final similarity results if temperature of the root ecoregion was not well represented by the comparison ecoregions in Europe.

II. RESULTS AND DISCUSSION

The 19 TFD trial sites are represented by twelve root ecoregions that cover large parts of central North America as well as parts at the south-eastern and south-western boundary of the United States (Figure 7.1.2.2.1-1). For three out of the eight root ecoregions, the area of the matching ecoregions covers >15 % of the total area of European ecoregions, for one root ecoregion the percentage area is about 4 %, and for four root ecoregions it is <2 % as presented in the table below.

Table 7.1.2.2.1-60: Root Ecoregions of 19 North American TFD trial sites and area covered by similar ecoregions in Europe (based on holistic approach, 80 % similarity)

Root Ecoregion	TFD Trial Site	Similar ecoregions Europe	
		Area[1] (km²)	Share[2] (%)
NA0414 - Southern Great Lakes forests (CA,USA)	New York Ohio Ontario-1	937,136	22.1
NA0407 - Eastern Great Lakes lowland forests (CA,USA)	Ontario-2	699,833	16.5
NA0801 - California Central Valley grasslands (USA)	California-1 California-2	647,759	15.2
NA0805 - Central tall grasslands (USA)	Iowa Minnesota	163,001	3.8
NA0811 - Northern short grasslands (CA,USA)	Alberta-1	42,624	1.0
NA0802 - Canadian Aspen forests and parklands (CA,USA)	Alberta-2 Saskatchewan-2	23,741	0.6
NA0810 - Northern mixed grasslands (CA,USA)	Saskatchewan-1	20,107	0.5
NA1310 - Sonoran desert (USA)	Arizona	2,720	0.1
NA0529 - Southeastern conifer forests (USA)	Georgia-1 Georgia-2	no similarity	-
NA0812 - Northern tall grasslands (CA,USA)	Manitoba-1 Manitoba-2	no similarity	-
NA0409 - Mississippi lowland forests (USA)	Mississippi	no similarity	-
NA0405 - East Central Texas forests (USA)	Texas	no similarity	-

[1] Area quantified with Lambert azimuthal equal-area (LAEA) coordinate map projection in ArcGIS v10.2.
[2] Share relative to area of the European Union

With the holistic approach, matching ecoregions (80 % similarity) were identified for eight of a total of twelve root ecoregions.

Individual scores of soil and climatic parameters were also assessed in their weight against each other. While soil conditions (pH, OC content and texture) reached high scores for the remaining eight ecoregions, individually and overall, temperature as the main driving parameter for the degradation of pesticides among the climatic parameters (temperature and precipitation) reached very low to low individual scores in some ecoregions. For four (NA0407, NA0414, NA0801, and NA0805) of the eight root ecoregions, individual matches of temperature reach 100 % for one or more European ecoregions. Thus, temperature characteristics of these root ecoregions are well represented by ecoregions in Europe.

NA0407-Eastern Great Lakes lowland forests

The trial site Ontario-2 ([redacted], 1993) is located within this root ecoregion. The ENASGIPS holistic similarity query identified four European ecoregions similar to the root ecoregion *Eastern Great Lakes lowland forests* based on the similarity scores summarized in the following table. The identified ecoregions cover large parts of Central Europe as well as regions in Eastern and Southeastern Europe.

Table 7.1.2.2.1-61: Similarity scores calculated by ENASGIPS for the root ecoregion *Eastern Great Lakes lowland forests* (NA0407)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0418 - Dinaric Mountains mixed forests (EU)	87	50	100	100	86	100
PA0445 - Western European broadleaf forests (EU)	81	44	63	100	100	100
PA0501 - Alps conifer and mixed forests (EU)	90	49	100	100	100	100
PA0504 - Carpathian montane forests (EU)	90	100	50	100	100	100
Average score	**87**	**61**	**78**	**100**	**97**	**100**

NA0414-Southern Great Lakes forests

The trial sites New York, Ohio ([redacted], 1993) and Ontario-1 [redacted] 1992) are located within this root ecoregion. The ENASGIPS holistic similarity query identified nine European ecoregions similar to the root ecoregion *Southern Great Lakes forests* based on the similarity scores summarized in the following table. The identified ecoregions cover large parts of Central Europe as well as regions in Northwestern, South, and Southeastern Europe.

Table 7.1.2.2.1-62: Similarity scores calculated by ENASGIPS for the root ecoregion *Southern Great Lakes forests* (NA0414)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0401 - Appenine deciduous montane forests (EU)	81	60	61	100	86	100
PA0409 - Celtic broadleaf forests (EU)	83	100	97	17	100	100
PA0418 - Dinaric Mountains mixed forests (EU)	84	100	100	53	67	100
PA0421 - English Lowlands beech forests (EU)	83	100	48	77	100	88
PA0432 - Po Basin mixed forests (EU)	80	41	90	100	70	100
PA0433 - Pyrenees conifer and mixed forests (EU)	84	95	54	88	82	100
PA0435 - Rodope montane mixed forests (EU)	91	100	56	100	100	100
PA0445 - Western European broadleaf forests (EU)	92	100	91	74	97	100
PA0504 - Carpathian montane forests (EU)	80	52	74	76	100	100
Average score	**84**	**83**	**75**	**76**	**89**	**99**

NA0801-California Central Valley grasslands

The trial sites California-1 (Oppenhuizen, 1993) and California-2 (Iwata, 1989) are located within this root ecoregion. The ENASGIPS holistic similarity query identified eight European ecoregions similar to the root ecoregion *California Central Valley grasslands* based on the similarity scores summarized in the following table. The identified ecoregions cover large parts of Southern and Southeastern Europe.

Table 7.1.2.2.1-63: Similarity scores calculated by ENASGIPS for the root ecoregion *California Central Valley grasslands* (NA0801)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0422 - Euxine-Colchic broadleaf forests (EU)	87	35	100	100	100	100
PA1201 - Aegean and Western Turkey sclerophyllous and mixed forests (EU)	85	60	100	64	100	100
PA1209 - Iberian sclerophyllous and semi-deciduous forests (EU)	84	44	100	85	89	100
PA1211 - Italian sclerophyllous and semi-deciduous forests (EU)	83	39	100	75	100	100
PA1218 - South Appenine mixed montane forests (EU)	90	50	100	100	100	100
PA1219 - Southeastern Iberian shrubs and woodlands (EU)	83	100	71	63	79	100
PA1221 - Southwest Iberian Mediterranean sclerophyllous and mixed forests (EU)	93	100	100	65	100	100
PA1222 - Tyrrhenian-Adriatic Sclerophyllous and mixed forests (EU)	97	100	100	84	100	100
Average score	**88**	**66**	**96**	**80**	**96**	**100**

NA0805-Central tall grasslands

The trial sites Iowa and Minnesota ([redacted] 1993) are located within this root ecoregion. The ENASGIPS holistic similarity query identified four European ecoregions similar to the root ecoregion *Central tall grasslands* based on the similarity scores summarized in the following table. The identified ecoregions cover parts of Southern, Southeastern and Eastern Europe.

Table 7.1.2.2.1-64: Similarity scores calculated by ENASGIPS for the root ecoregion *Central tall grasslands* (NA0805)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0401 - Appenine deciduous montane forests (EU)	91	62	100	100	93	100
PA0433 - Pyrenees conifer and mixed forests (EU)	90	100	89	100	65	97
PA0435 - Rodope montane mixed forests (EU)	98	100	100	92	100	100
PA0504 - Carpathian montane forests (EU)	86	82	100	86	62	100
Average score	**91**	**86**	**97**	**95**	**80**	**99**

Temperature similarity score of root ecoregion NA1310 with its solely similar ecoregion in Europe is 66 %, indicating a moderate similarity between the two ecoregions.

NA1310-Sonoran desert

The trial site Arizona ([redacted] 1993) is located within this root ecoregion. The ENASGIPS holistic similarity query identified one European ecoregion similar to the root ecoregion *Sonoran desert* based on the similarity scores summarized in the following table. The identified ecoregion covers small parts of the coastal area in southern Spain.

Table 7.1.2.2.1-65: Similarity scores calculated by ENASGIPS for the root ecoregion *Sonoran desert* (NA1310)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA1219 - Southeastern Iberian shrubs and woodlands (EU)	86	66	100	100	100	66

For the remaining three root ecoregions, NA0802, NA0810, and NA0811, the scores for temperature similarity range from 7 to 33 % for individual matches, indicating pronounced differences in temperature conditions between root ecoregions and their corresponding ecoregions in Europe.

NA0802-Canadian Aspen forests and parklands

The trial sites Saskatchewan-2 ([redacted] 1992) and Alberta-2 ([redacted] 1993) are located within this root ecoregion. The ENASGIPS holistic similarity query identified two European ecoregions similar to the root ecoregion *Canadian Aspen forests and parklands* based on the similarity scores summarized in the following table. The identified ecoregions cover small parts of South and Southeastern Europe.

Table 7.1.2.2.1-66: Similarity scores calculated by ENASGIPS for the root ecoregion *Canadian Aspen forests and parklands* (NA0802)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0419 - East European forest steppe (EU)	80	10	92	100	96	100
PA1204 - Corsican montane broadleaf and mixed forests (EU)	81	7	100	100	100	100
Average score	**81**	**9**	**96**	**100**	**98**	**100**

NA0810-Northern mixed grasslands

The trial site Saskatchewan-1 ([redacted] 1992) is located within this root ecoregion. The ENASGIPS holistic similarity query identified one European ecoregion similar to the root ecoregion *Northern mixed grasslands* based on the similarity scores summarized in the following table. The identified ecoregion covers small parts of Southeastern Europe.

Table 7.1.2.2.1-67: Similarity scores calculated by ENASGIPS for the root ecoregion *Northern mixed grasslands* (NA0810)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA0419 - East European forest steppe (EU) 87	87	33	100	100	100	100

NA0811-Northern short grasslands

The trial site Alberta-1 ([redacted] 1992) is located within this root ecoregion. The ENASGIPS holistic similarity query identified two European ecoregions similar to the root ecoregion *Northern short grasslands* based on the similarity scores summarized in the following table. The identified ecoregions cover parts of Southwestern and Southeastern Europe.

Table 7.1.2.2.1-68: Similarity scores calculated by ENASGIPS for the root ecoregion *Northern short grasslands* (NA0811)

Ecoregion	Similarity scores (%)					
	Holistic	Temperature	Precipitation	OC	pH	Texture class
PA1205 - Crete Mediterranean forests (EU)	81	12	92	100	100	100
PA1208 - Iberian conifer forests (EU)	86	30	100	100	100	100
Average score	**84**	**21**	**96**	**100**	**100**	**100**

III. CONCLUSIONS

In this report, an ecoregion crosswalk analysis was performed on 19 North American TFD trial sites which are represented by twelve root ecoregions. With the holistic similarity approach, matching ecoregions (80 % similarity) were identified for eight of a total of twelve root ecoregions (NA0407, NA0414, NA0801, NA0802, NA0805, NA0810, NA0811, and NA1310). This meant that six trial sites were not considered representative for European conditions based on the holistic similarity approach.

In addition to the holistic similarity approach, individual scores of temperature were evaluated in a refined assessment as temperature is known to be a main driving parameter for the degradation of pesticides. For

three of the eight root ecoregions, the scores for temperature ranged from 7 to 33 % for individual matches. This indicates that the temperature conditions of the North American root ecoregions are not well represented by the European ecoregions and thus, the three root ecoregions NA0802, NA0810 and NA0811 representing four trial sites are considered not representative for European conditions.

Based on the refined ecoregion crosswalk analysis, similar soil and climate conditions were identified for five root ecoregions: NA0407, NA0414, NA0801, NA0805 and NA1310 comprising nine trial sites of the US and Canadian TFD studies available for glyphosate. These trials are considered representative for European conditions.

Table 7.1.2.2.1-69: Overview of TFD trial sites acceptable in European conditions

Root Ecoregion	TFD Trial Site	Study	Conclusion on similarity
NA0407 - Eastern Great Lakes lowland forests (CA,USA)	Ontario-2	[redacted] 1993	Sufficient similarity; considered represenative for European conditions for further evaluation
NA0414 - Southern Great Lakes forests (CA,USA)	New York	[redacted] 1993	
	Ohio	[redacted] 1993	
	Ontario-1	[redacted] 1992	
NA0801 - California Central Valley grasslands (USA)	California-1	[redacted] 1993	
	California-2	[redacted] 1989a	
NA0805 - Central tall grasslands (USA)	Iowa	[redacted] 1993	
	Minnesota	[redacted] 1993	
NA1310 - Sonoran desert (USA)	Arizona	[redacted] 1993	
NA0802 - Canadian Aspen forests and parklands (CA,USA)	Alberta-2	[redacted] 1993	Insufficient similarity due to individual score of temperature
	Saskatchewan-2	[redacted] 1992	
NA0810 - Northern mixed grasslands (CA,USA)	Saskatchewan-1	[redacted] 1992	
NA0811 - Northern short grasslands (CA,USA)	Alberta-1	[redacted] 1992	
NA0405 - East Central Texas forests (USA)	Texas	[redacted] 1993	No similarity due to score in holistic approach
NA0409 - Mississippi lowland forests (USA)	Mississippi	[redacted] 1989b	
NA0529 - Southeastern conifer forests (USA)	Georgia-1	[redacted] 1993	
	Georgia-2	[redacted] 1989c	
NA0812 - Northern tall grasslands (CA,USA)	Manitoba-1	[redacted] 1992	
	Manitoba-2	[redacted] 1993	

3. Assessment and conclusion

Assessment and conclusion by applicant:

The evaluation was performed with OECD ENASGIPS tool recommended in OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016 and is therefore considered valid.

It is shown that 9 out of 18 field trials conducted in the US and Canada are representative for European conditions. Thus, residue data from these trials were used to derive endpoints for EU approval. The respective kinetic evaluation is summarised below ([redacted] 2020, CA 7.1.2.2.1/003).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/003
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolite AMPA from terrestrial field dissipation studies in the USA and Canada
Report No	112148-006
Document No	
Guidelines followed in study	EFSA (2014): EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662 [37 pp.]. FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp. FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUS kinetics guidance: none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not applicable for this study type
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The aim of this evaluation was to conduct a kinetic evaluation for glyphosate and its major soil metabolite AMPA using residue data from terrestrial field soil dissipation studies, in order to derive persistence endpoints that can be used for comparison against regulatory trigger values, and modelling endpoints that can be used for calculating predicted environmental concentrations (PECs) in various environmental compartments.

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics, and of the European Food Safety Authority (EFSA). Trigger endpoints were evaluated according to best-fit kinetics. For modelling endpoints, a time-step normalisation method was applied to the data (standard reference conditions of 20 °C and 100 % field capacity) and samples taken prior to 10 mm cumulative rainfall were excluded. Following normalisation, all datasets were checked for whether the field decline curve could be described well with a single first-order (SFO) model using procedures proposed by FOCUS.

The evaluation was based on soil residue data from nine field soil dissipation trials reported in four legacy field studies conducted in the United States and Canada that were found to be representative for European conditions in an ecoregion crosswalk assessment. For evaluation of trigger endpoints, all of the nine trials were considered while the evaluation for modelling endpoints was only conducted for four trials where

sufficient data for the time-step normalisation procedure was available. The evaluation was performed using the model fitting software CAKE 3.3.

I. MATERIALS AND METHODS

The FOrum for the Coordination of Pesticide Fate-Models and their USe (FOCUS) developed recommendations for the kinetic evaluation of soil degradation studies conducted in the laboratory or in the field (FOCUS, 2006, 2014). These recommendations intend to harmonise the derivation of degradation or dissipation parameters from soil studies. For modelling endpoints, further guidance has been published to help derive $DegT50_{matrix}$ values in soil (EFSA, 2014).

Glyphosate is a broadcast herbicide used widely in agricultural and non-agricultural practice. An assessment of the potential environmental impact in soil, groundwater and surface water requires an understanding of the key degradation / dissipation pathways and rates in soil.

The purpose of this evaluation was to conduct a kinetic evaluation for glyphosate and its major soil metabolite aminomethylphosphonic acid (AMPA) using data from field soil dissipation studies, in order to: i) derive DT_{50} and DT_{90} values for use in PEC_{soil} calculations and for comparison with trigger values from guidelines, and ii) derive $DegT50_{matrix}$ values for use in environmental exposure models for groundwater and surface water.

Four legacy field dissipation studies were conducted in the United States and Canada ([redacted] 1992, CA 7.1.2.2.1/014; [redacted] 1993, CA 7.1.2.2.1/006; [redacted] 1993, CA 7.1.2.2.1/005; [redacted] 1989, CA 7.1.2.2.1/016). In an ecoregion crosswalk assessment of the four studies, the locations of nine field trials were found to be representative for European conditions ([redacted] 2020, CA 7.1.2.2.1/002). Thus, the results of the nine trials were re-evaluated according to the most recent guidance (FOCUS, 2006, 2014; EFSA, 2014). For evaluation of trigger endpoints, all of the nine trials were considered while the evaluation for modelling endpoints was only conducted for four trials where sufficient data for the time-step normalisation procedure was available. The kinetic evaluation was performed using the model fitting software CAKE 3.3.

2. Description of the terrestrial field dissipation studies

The four field soil dissipation studies ([redacted] 1992, CA 7.1.2.2.1/014; [redacted] 1993, CA 7.1.2.2.1/006; [redacted] 1993, CA 7.1.2.2.1/005; [redacted] 1989, CA 7.1.2.2.1/016) included for kinetic evaluation were conducted at nine sites in USA and Canada. The locations of the nine field sites were found to be representative for European conditions as reported in an ecoregion crosswalk assessment ([redacted] 2020, CA 7.1.2.2.1/002). Different amounts of glyphosate, formulated as glyphosate-trimesium or the isopropylamine salt, were applied to bare soil. Soil samples from studies conducted with either formulation of glyphosate were analysed for glyphosate and its metabolite.

A summary of the trial locations and application data is given in the following table.

Table 7.1.2.2.1-70: Summary of trial locations and application data in field soil dissipation studies

Study	Location	Formulation	Crop	Date of Application	Duration of study (d)	Target rate (kg a.s./ha)	Actual rate (kg a.s./ha)
[redacted] 1992, CA 7.1.2.2.1/014	Ontario, Canada	Glyphosate-trimesium	Bare soil	30/09/1998	577	5.76	6.41
[redacted] 1993, CA 7.1.2.2.1/006	Arizona, USA	Isopropylamine salt	Bare soil	16/04/1991	553	7.95[1]	8.08[1]
	California, USA	Isopropylamine salt	Bare soil	18/04/1991	550	7.95[1]	8.83[1]
	Iowa, USA	Isopropylamine salt	Bare soil	06/06/1991	458	7.95[1]	7.94[1]
	Minnesota, USA	Isopropylamine salt	Bare soil	08/07/1991	475	7.95[1]	8.05[1]
	New York, USA	Isopropylamine salt	Bare soil	01/05/1991	546	7.95[1]	7.84[1]
	Ohio, USA	Isopropylamine salt	Bare soil	22/05/1991	545	7.95[1]	8.14[1]
[redacted] 1993, CA 7.1.2.2.1/005	Ontario, Canada	Isopropylamine salt	Bare soil	29/05/1991	537	4.27[2]	4.18[2,3]
[redacted] 1989, CA 7.1.2.2.1/016	California, USA	Glyphosate-trimesium	Bare soil	07/07/1987	366	4.48	n.a.

n.a. = not available
[1] lb a.e./acre
[2] kg a.e./ha
[3] Mean value; actual application rates for three replicate test plots are 4.21, 4.07 and 4.27 kg a.e./ha

The soil sampling procedure differed between the evaluated studies and a short description is given in the following.

In [redacted] (1992, CA 7.1.2.2.1/014), one trial site in Canada was included in kinetic evaluation. The treated plot was subdivided into four subplots. The zero, one and three day samples were collected up to a nominal soil depth of 10 cm. Seven to eight soil cores per subplot were taken and bulked for analysis which resulted in a total of 30 cores per sampling time. For the subsequent time intervals, soil was sampled to a depth of 30 cm. These soil cores were sectioned into three horizons (0-10 cm, 10-20 cm, 20-30 cm) and soil from each horizon was then bulked in order to obtain a representative sample.

In [redacted] (1993, CA 7.1.2.2.1/006), six trial sites in the USA were included in kinetic evaluation. For the treated plot at each site, six soil cores were randomly collected to a depth of 121.9 cm (48 inches) from each of the three subplots, sectioned into 15.2 cm (6 inches) depth increments (e.g., 0-15.2 cm, 15.2-30.5 cm, etc.), and composited to afford three representative samples per depth increment per sampling event.

In [redacted] (1993, CA 7.1.2.2.1/005), one trial site in Canada was included in kinetic evaluation. 10 soil cores to a depth of 45 cm were randomly collected from each of the three subplots, sectioned into 15 cm depth increments (e.g., 0-15 cm, 15-30 cm, and 30-45 cm), and composited to afford three representative samples per depth increment per sampling event.

In [redacted] (1989, CA 7.1.2.2.1/016), one trial site in the USA was included in kinetic evaluation. The sampling procedure for the first month after treatment was as follows: the top 7.6 cm (0-3 inches) of soil were excavated into a sample bag. Five replicates per sampling date were taken with the excavation method. Following the excavation, five cores were also taken up to a soil depth of 121.9 cm (48 inches), sectioned into six increments. Starting with the 1 month sample, the sampling probe was used to collect the samples without excavation of the 0-7.6 cm sample.

2. Data pre-processing

The data from the legacy field trials required pre-processing in order to generate appropriate input datasets for the kinetic evaluation. The standard procedures recommended by FOCUS (2006, 2014) were applied.

The time-zero concentration for the metabolite was set to zero and the initial metabolite amount was added to the parent substance accounting for the molar weight difference between the compounds.

For the two studies by [redacted] (1992, CA 7.1.2.2.1/014) and [redacted] (1989, CA 7.1.2.2.1/016), the LOQ and LOD were indistinguishable; only the 'limit of determination' is reported. Hence, the LOQ and LOD were both assigned the same value and the FOCUS guidance was then applied as follows: Values below LOD were replaced by half the LOD. If the concentrations of the applied substance in soil declined to values below LOD, the curve was cut off after the first value below LOD. For the two studies by Oppenhuizen (1993, CA 7.1.2.2.1/006) and [redacted] (1993, CA 7.1.2.2.1/005), LOD as well as LOQ were reported. Thus, values between LOQ and LOD were set to the measured value. Values below LOD were replaced by half the LOD. If the concentrations of the applied substance in soil declined to values below LOD, the curve was cut off after the first value below LOD, unless detections above LOQ were made later in the experiment (FOCUS, 2006, 2014). These corrections were performed along the time course, as well as with depth along the soil horizon, with the exception for 0 DAT, where it was assumed that residues only resided in the upper most soil layer.

The measured residues (mg/kg) in the different soil layers were converted into residues expressed in kg/ha (considering the layer depth and bulk density) and then summed up. They were then expressed as percentage values of the residue at 0 DAT (so the time zero value is 100 %). Thus, if the maximum concentration occurs after 0 DAT, the respective maximum percentage value is greater than 100 %. As the sampled soil layer depths of studies [redacted] (1993, CA 7.1.2.2.1/006) and Iwata (1989, CA 7.1.2.2.1/016) were given in inches, conversion to cm with the factor 2.54 was performed.

For the study of [redacted] (1992, CA 7.1.2.2.1/014), the horizon-specific bulk density was calculated at each sampling time using the reported soil core surface area, depth and dry weight. For the studies of [redacted] (1993, CA 7.1.2.2.1/006) and [redacted] (1993, CA 7.1.2.2.1/005), horizon-specific bulk density was given in the reports. For the study of [redacted] (1989, CA 7.1.2.2.1/016), a default value of 1.5 g/cm^3 was assumed for the bulk density.

The input values of AMPA were expressed as percentage values of the parent (glyphosate) residue at 0 DAT (correcting for molar weight differences).

According to FOCUS (2006, 2014), true replicates (and not mean concentration values) at each sampling point should be used for the kinetic evaluation, if available. For the studies [redacted] (1992, CA 7.1.2.2.1/014), [redacted] (1993, CA 7.1.2.2.1/006) and [redacted] (1993, CA 7.1.2.2.1/005), replicate treated subplots were sampled and analysed. However, either the respective replicate samples were mixed across the subplots resulting in one combined sample ([redacted] 1993, CA 7.1.2.2.1/006), or the replicate results could not be clearly assigned to the individual subplots as this information was not given in the raw data tables ([redacted] 1992, CA 7.1.2.2.1/014; [redacted] 1993, CA 7.1.2.2.1/005). Therefore, the kinetic evaluation was based on mean values.

In the study [redacted] (1989, CA 7.1.2.2.1/016), four to five samples were taken from a single treated plot. For the soil layers below 7.6 cm (3 inch), samples were mixed to one combined sample. For the uppermost soil layer (0-7.6 cm), the individual samples were analysed separately; in addition, one of the samples was further divided in two subsamples and analysed in duplicate. For kinetic evaluation, the results of the individual samples were averaged to one mean concentration for the uppermost soil layer; the results of duplicate subsample analysis were averaged separately, and the mean value was used for calculating the overall mean concentration. Thus, the evaluation was performed on single residue data per soil layer.

Processed residue data, adjusted as described above, are presented in the following tables and were used in the kinetic evaluation.

Table 7.1.2.2.1-71: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1992, CA 7.1.2.2.1/014)

Time (DAT)	Sum of horizons (0 - 20 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Ontario				
0	2.85[2]	0.00[3]	100.00	0.00
1	2.37	0.18	82.88	9.46
3	0.98	0.40	34.39	21.45
7	0.89	0.36	31.35	19.35
14	0.75	0.52	26.38	27.55
31	0.42	0.40	14.78	21.24
60	0.26	0.40	8.98	21.24
207	0.21	0.35	7.32	18.72
297	0.03	0.10	1.04	5.47
391	-[4]	0.10	-[4]	5.47
577	-[4]	0.03	-[4]	1.58

LOD = 0.05 mg/kg
Bold values = maximum residue value; potentially used for decline fit
[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0
[3] Residue value of metabolite set to 0 at day 0
[4] Data omitted according to FOCUS (2006, 2014)

Table 7.1.2.2.1-72: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993, CA 7.1.2.2.1/006)

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Arizona				
0	3.61[2]	0.00[3]	100.00	0.00
1	3.60	0.38	99.64	11.73
7	4.03	0.43	111.66	18.32
14	1.04	0.35	28.88	14.66
21	3.42	1.15	94.83	**48.33**
28	1.36	0.63	37.55	26.38
64	0.61	0.83	16.85	35.18
92	0.26	0.52	7.22	21.99
122	0.24	0.66	6.74	27.85
184	0.16	0.49	4.32	20.52
364	0.08	0.33	2.17	13.93
462	0.02	0.10	0.48	4.40
553	-	0.12	-[4]	5.13
California				
0	4.29[2]	0.00[3]	100.00	0.00
1	4.45	0.36	103.76	12.65
7	2.85	0.73	66.41	26.07
14	2.45	0.85	57.06	30.02
21	1.29	0.73	30.10	26.07
29	1.49	0.82	34.76	29.23
61	1.16	0.80	26.99	28.44
91	0.40	0.60	9.35	21.33
123	0.13	0.58	3.12	20.58

Table 7.1.2.2.1-72: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993, CA 7.1.2.2.1/006)

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
183	0.20	0.51	4.67	18.18
365	0.11	0.67	2.60	23.70
456	0.11	0.91	2.60	**32.42**
550	0.07	0.82	1.56	29.26
Iowa				
0	3.20[2]	0.00[3]	100.00	0.00
1	3.76	0.14	117.42	0.81
7	3.27	0.22	102.21	10.55
14	2.95	0.21	92.09	9.80
21	2.50	0.27	78.19	12.79
29	2.69	0.30	84.22	14.28
62	3.25	0.60	101.69	28.48
92	1.25	0.37	39.13	17.58
123	2.08	0.60	64.88	28.61
190	1.43	0.50	44.78	24.00
366	1.34	0.88	41.36	41.93
458	0.76	0.94	23.63	**44.92**
Minnesota				
0	2.11[2]	0.00[3]	100.00	0.00
1	2.37	0.36	112.34	26.16
7	2.85	0.46	134.98	33.28
15	3.11	0.40	147.42	28.53
21	2.42	0.48	114.61	34.47
35	1.00	0.63	47.54	45.14
71	0.38	0.69	17.94	49.89
95	0.46	0.74	21.84	**53.44**
129	0.40	0.71	18.79	51.07
179	0.23	0.58	10.93	41.58
372	0.08	0.28	3.92	20.23
475	0.12	0.41	5.47	29.72
New York				
0	5.10[2]	0.00[3]	100.00	0.00
1	4.43	0.19	86.82	5.66
7	3.20	0.29	62.64	8.77
14	8.01	0.88	156.96	26.33
21	7.87	0.76	154.24	22.77
30	4.77	0.88	93.52	26.33
61	2.88	0.54	56.49	16.06
90	3.61	1.01	70.74	**30.02**
120	2.10	0.68	41.17	20.16
180	2.41	0.85	47.29	25.34
362	0.95	0.38	18.70	11.36
453	0.97	0.67	19.00	20.04
546	1.09	0.74	21.44	22.21
Ohio				
0	4.24[2]	0.00[3]	100.00	0.00
1	3.08	0.62	72.78	22.24
7	1.12	0.97	26.49	34.89
14	0.90	0.82	21.34	29.44
21	1.22	1.04	28.85	**37.30**
30	1.04	0.95	24.50	34.29
61	0.22	0.65	5.13	23.45

Table 7.1.2.2.1-72: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation studies of [redacted] (1993, CA 7.1.2.2.1/006)

Time (DAT)	Sum of horizons (0 - 60 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
90	0.15	0.47	3.55	16.82
121	0.12	0.43	2.75	15.62
177	0.07	0.38	1.57	13.81
365	0.05	0.23	1.17	8.39
455	0.05	0.18	1.17	6.98
545	0.02	0.15	0.40	5.38

LOD = 0.02 mg/kg for glyphosate, 0.04 mg/kg for AMPA
LOQ = 0.05 mg/kg for glyphosate and AMPA
Bold values = maximum residue value; potentially used for decline fit
[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0
[3] Residue value of metabolite set to 0 at day 0
[4] Data omitted according to FOCUS (2006, 2014)

Table 7.1.2.2.1-73: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1993, CA 7.1.2.2.1/005)

Time (DAT)	Sum of horizons (0 - 45 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)[1]
Ontario				
0	1.69[2]	0.00[3]	100.00	0.00
1	1.08	0.24	63.73	21.64
7	1.00	0.26	59.01	23.25
14	0.82	0.28	48.39	25.23
28	0.31	0.22	18.42	19.48
57	0.12	0.20	7.21	18.04
86	0.10	0.32	6.15	**28.64**
129	0.06	0.21	3.56	18.94
177	0.07	0.29	3.91	26.31
364	0.07	0.24	4.15	21.82
537	0.01	0.03	0.59	2.69

LOD = 0.01 mg/kg for glyphosate, 0.03 mg/kg for AMPA
LOQ = 0.05 mg/kg for glyphosate and AMPA
Bold values = maximum residue value; potentially used for decline fit
[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT corrected for molar mass difference between parent and metabolite
[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol / 111.04 g/mol and added to residue value of parent at day 0
[3] Residue value of metabolite set to 0 at day 0

Table 7.1.2.2.1-74: Processed residue data (kg/ha and % of 0 DAT) of glyphosate and AMPA from the field soil dissipation study of [redacted] (1989, CA 7.1.2.2.1/016)

Time (DAT)	Sum of horizons (0 - 32 cm)			
	Glyphosate (kg/ha)	AMPA (kg/ha)	Glyphosate (% of 0 DAT)	AMPA (% of 0 DAT)
California				
0	3.16[2]	0.00[3]	100.00	0.00
1	2.86	0.11	90.70	5.24
3	1.98	0.19	62.82	9.10
7	1.45	0.31	45.80	15.16
14	0.84	0.27	26.61	12.96
31	0.35	0.43	11.04	20.67
59	0.11	0.29	3.44	14.06
205	0.09	0.14	2.72	6.89
366	0.09	0.09	2.72	4.13

LOD = 0.02 mg/kg for glyphosate, 0.04 mg/kg for AMPA
Bold values = maximum residue value; potentially used for decline fit
[1] Expressed as glyphosate equivalent = percentage of glyphosate amount at 0 DAT, corrected for molar mass difference between parent and metabolite
[2] Residue value of metabolite converted to parent equivalent with 169.10 g/mol, 113.04 g/mol and added to residue value of parent at day 0
[3] Residue value of metabolite set to 0 at day 0

3. Normalisation of field degradation half-life values to reference conditions

Time-step normalisation was conducted for four trials where sufficient data was available. The availability of the weather data for the respective trial sites are summarized in the following table.

Table 7.1.2.2.1-75: Glyphosate field trial locations and availability of weather data

Study	Trial/ location	Weather station and data availablity	Distance from test site (km)	Data sufficient for normalization?
[redacted] 1992, CA 7.1.2.2.1/014	St. Davids, Ontario, Canada	St. Catherines, Ontario: Daily weather data not available	approx. 5 km	No (no daily weather data available)
[redacted] 1993, CA 7.1.2.2.1/006	Yuma County, Arizona, USA	Arizona Meteorological Network, Yuma Valley, AZ: rain, irrigation, min/max temp., rel. humidity, soil temp., windspeed, solar rad., ET_0	-[1]	Yes (based on simulated soil temperature and moisture)
	Madera County, California, USA	On-site weather station at Pan-Ag Research Station in Madera, CA: rain, irrigation, min/max temp., rel. humidity, soil temp., windspeed	-[1]	Yes (based on measured soil temperature)
	Des Moines County, Iowa, USA	Danville, Iowa: rain, irrigation, min/max temp., soil temp.	-[1]	Yes (based on measured soil temperature)
	Redwood County, Minnesota, USA	Southwest Experiment Station, University of Minnesota, Lamberton, MN: rain, min/max temp.	-[1]	No (no radiation and soil temperature data available)
	Ontario County, New York, USA	Vegetable Research Farm, New York State Agricultural Experiment Station: rain, min/max temp., rel. humidity, solar rad., windspeed (data gap between 1.11.1991-17.05.1992)	-[1]	Yes (based on simulated soil temperature and moisture)

Table 7.1.2.2.1-75: Glyphosate field trial locations and availability of weather data

	Fayette County, Ohio, USA	NOAA Washington Courthouse Station, Division 05, Fayette County: rain, min/max temp., rel. humidity, soil temp., windspeed	-[1]	No (no radiation data, soil temperature data insufficient)
[redacted] 1993, CA 7.1.2.2.1/005	Ayr, Ontario, Canada	Shades Mill Dam, Grand River Conservation Authority weather station: rain, min/max temp.	-[1]	No (no daily weather data available)
[redacted] 1989, CA 7.1.2.2.1/016	Orange Cove, California, USA	WSO Fresno, California: rain, irrigation, min/max temp., windspeed	approx. 40 km	No (available data has poor quality)

n.a. = not available
ET_0 = evapotranspiration
[1] Weather data collected from test site research station instruments

For trials Arizona and New York ([redacted] 1993, CA 7.1.2.2.1/006), detailed weather and soil data were available. Thus, for these two trials, comprehensive normalisation procedure with regard to soil temperature and soil moisture was conducted. For trials California and Iowa ([redacted] 1993, CA 7.1.2.2.1/006), the weather data set was incomplete, but soil temperature was reported. As a conservative approach, for these two trials, normalisation was performed for soil temperature, only. The resulting modelling endpoints are worst-case estimates as normalisation for soil moisture would result in lower DT_{50} due to the fact that moisture conversion factors are defined to be below or equal to 1.

General approach
Time-step normalisation according to FOCUS (2006, 2014) and Hardy *et al.* (2003) was conducted in order to derive modelling endpoints at reference conditions (20 °C and pF 2). Daily correction factors for soil temperature (f_T) and moisture (f_Θ) were calculated for a given reference soil temperature of 20 °C and a reference soil moisture of pF 2.

According to FOCUS (2000), the exponent of the moisture response function was set to 0.7 and the temperature coefficient Q_{10} was set to 2.58, respectively.

The following limitations were applied to the normalisation procedure:
- no further increase of the degradation rate if soil moisture > reference moisture
- no degradation if soil temperature < 0 °C (resulting in a transformed day length of zero)

The obtained correction factors result in standardised transformation rates by reducing or increasing day lengths. Processed residue data, in combination with the transformed time course (*i.e.* under constant temperature and moisture conditions), were used for the evaluation of modelling endpoints according to recommendations for obtaining $DegT50_{matrix}$ values in soil from field dissipation studies for modelling purposes (FOCUS, 2006, 2014; EFSA, 2014). For the time between application and first sampling (0 DAT), no normalisation was considered and application was assumed to occur at time point zero.

Estimation of soil temperature and moisture

Weather data
For trials Arizona and New York, daily values of soil temperature and moisture data (mean of top 10 cm) were simulated with the environmental fate model FOCUSPEARL 4.4.4. Site-specific weather and soil data were used as model input. In accordance with EFSA (2014), the weather stations from which precipitation data were derived were less than 20 km away from the actual trial site.

For trial Arizona, reference evapotranspiration data were available together with minimum and maximum air temperature as well as precipitation (irrigation). Therefore, the ‘input’ option was selected for the potential evapotranspiration. As measured soil temperature at a depth of 0-10 cm was additionally available for trial Arizona, the data was used in order to verify the simulation results.

For trial New York, data were missing for the parameter ‘global radiation’ between Dec 1st, 1991 and Dec 5th, 1991. The gap was filled with the average value (i.e. 3138 kJ/m^2) of adjacent measurements (i.e. last day before gap: Nov 30th, 1991: 0 kJ/m^2; first day after gap: Dec 6th, 1991: 6276 kJ/m^2). Further, ‘windspeed’ data were missing between Nov 1st, 1991 and May 17th, 1992. Due to the large range of this gap it was decided not to use the windspeed data. Therefore, the Makkink approach (windspeed data not required) was selected in FOCUSPEARL v4.4.4 for trial New York to calculate the potential evapotranspiration. The required meteorological data for this estimation method (maximum and minimum air temperature, precipitation (irrigation), global radiation) were obtained from local meteorological stations reported in the study report as shown in the table above.

In the FOCUSPEARL 4.4.4 model, the weather data for the normalisation included a warm-up period of one year prior to the date of application, thereby accounting for seasonal effects.

Soil profile settings

For the simulations with FOCUSPEARL 4.4.4, soil profiles were created for the trials Arizona and New York based on the detailed soil properties given in the following tables.

Table 7.1.2.2.1-76: Soil characterisation for site Arizona, USA ([redacted] 1993, CA 7.1.2.2.1/006)

Soil layer (cm)[1]	**0 - 15**	**15 - 30**	**30 - 45**	**45 - 60**	**60 - 75**	**75 - 90**	**90 - 105**
Soil texture (USDA)	clay loam	clay loam	clay loam	loam	sandy loam	sandy loam	loamy sand
Sand (%)	37.3	27.3	25.3	41.3	53.3	69.3	83.3
Silt (%)	29.2	39.2	38.0	32.0	38.0	24.0	12.0
Clay (%)	33.5	33.5	36.7	26.7	8.7	6.7	4.7
Organic matter (%)	0.9	1.0	0.7	0.4	1.0	0.1	1.3
pH[2]	8.0	8.2	8.2	8.4	8.3	8.3	8.4
Bulk density (g/cm^3)[3]	1.14	1.14	1.11	1.15	1.28	1.28	1.19
Soil hydraulic parameters[4]							
θ_{res} (m^3/m^3)	0.0896	0.0909	0.0953	0.0795	0.0411	0.0398	0.0441
θ_{sat} (m^3/m^3)	0.5114	0.5116	0.5298	0.4891	0.4011	0.4265	0.4720
K_{sat} (m/d)	0.4089	0.4155	0.5024	0.3821	0.6332	1.2077	3.1132
α (cm^{-1})	0.0137	0.0109	0.0125	0.0113	0.0137	0.0313	0.0436
λ (-)	-0.5144	-0.3022	-0.4626	-0.2338	-0.2693	-0.8081	-0.7461
n (-)	1.4325	1.4692	1.4349	1.4852	1.4876	1.4670	1.6893
θ_{ref} (pF 2) (m^3/m^3)[5]	0.4067	0.4210	0.4295	0.3964	0.3045	0.2547	0.1942

[1] Converted from inch; in order to harmonize input in PEARL, 6 inch was assumed to equal 15 cm for each soil layer. Conversion differences were regarded as negligible.
[2] Buffer medium unknown
[3] Measured values derived from study report
[4] Calculated based on continuous ROSETTA pedotransfer functions (Schaap *et al.*, 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

Table 7.1.2.2.1-77: Soil characterisation for site New York, USA ([redacted] 1993, CA 7.1.2.2.1/006)

Soil layer (cm)[1]	**0 - 15**	**15 - 30**	**30 - 45**	**45 - 60**	**60 - 75**	**75 - 90**	**90 - 105**
Soil texture (USDA)	sand clay loam	clay loam	clay loam	clay	clay loam	loam	clay loam
Sand (%)	53.3	25.3	21.3	25.3	29.3	33.3	33.3
Silt (%)	24.0	42.0	46.0	32.0	38.0	40.0	39.2
Clay (%)	22.7	32.7	32.7	42.7	32.7	26.7	27.5
Organic matter (%)	2.1	0.8	0.1	0.3	0.2	0.1	0.2
pH[2]	5.8	6.4	7.3	7.3	7.5	7.6	7.8
Bulk density (g/cm^3)[3]	1.14	1.09	1.17	1.12	1.15	1.15	1.24
***Soil hydraulic parameters*[4]**							
θ_{res} (m^3/m^3)	0.0719	0.0916	0.0903	0.1003	0.0894	0.0807	0.0797
θ_{sat} (m^3/m^3)	0.4899	0.5249	0.5038	0.5401	0.5059	0.4862	0.4649
K_{sat} (m/d)	0.6273	0.5555	0.3556	0.5096	0.3900	0.4013	0.2401
α (cm^{-1})	0.0151	0.0103	0.0094	0.0161	0.0109	0.0088	0.0092
λ (-)	-0.4003	-0.2157	-0.1775	-0.8453	-0.2940	-0.0434	-0.1549
n (-)	1.4475	1.4809	1.4976	1.3758	1.4729	1.5318	1.5202
θ_{ref} (pF 2) (m^3/m^3)[5]	0.3753	0.4353	0.4239	0.4283	0.4160	0.4103	0.3898

[1] Converted from inch; in order to harmonize input in PEARL, 6 inch was assumed to equal 15 cm for each soil layer. Conversion differences were regarded as negligible.
[2] Buffer medium unknown
[3] Measured values derived from study report
[4] Calculated based on continuous ROSETTA pedotransfer functions (Schaap *et al.*, 2001)
[5] Calculated based on van Genuchten model (van Genuchten, 1980)

The soil was parameterised with 26 compartments which differed in thickness. Six numerical compartments were applied to the top soil (0 – 15 cm; converted from inch to cm) with a layer thickness of 2.5 cm each. Five numerical compartments were applied to the following 15 – 30 cm (converted from inch to cm) with a layer thickness of 3 cm each. The subsequent soil depth (30 – 105 cm; converted from inch to cm) was parameterised with 15 compartments with a layer thickness of 5 cm each. The lower boundary condition of the simulation profiles was set to 'Free Drainage' by default representing common European conditions. The initial groundwater level was set to 300 cm below the ground level. For soil evaporation, the crop factor ('FacEvpSol') and reduction coefficient ('CofRedEvp') were set to the values of 1 (default for bare soils) and 0.79, respectively.

The hydraulic characteristics of the soils were parameterised in FOCUSPEARL according to the 'van Genuchten' parameters (van Genuchten, 1980). The van Genuchten parameters were estimated based on continuous 'ROSETTA' pedotransfer functions (Schaap *et al.*, 2001).

Correction factors for soil temperature and moisture

For trials Arizona and New York, daily correction factors for soil temperature and soil moisture were calculated based on the results of the simulations in FOCUSPEARL 4.4.4 (mean of top 10 cm).

For trials California and Iowa, reported soil temperature data were used directly for calculation of daily correction factors for soil temperature.

For trial California, soil temperature data were missing between April 18th, 1991 and May 31st, 1991 due to a malfunction of the machine. The gap was filled with the reference soil temperature of 20 °C, resulting in a correction factor of 1 (i.e. no normalisation). This is regarded as a conservative approach as daily mean soil temperatures for this time period are usually below 20 °C. Comprehensive soil temperature data was available in the study report starting before the application date for trial California. Thus, average soil temperatures were calculated from available data before and after the gap. This resulted in calculated average soil temperatures of 13.8 °C for the time period April 1st to April 18th and average soil temperatures of 19.8 °C for the following month of the gap. The United States Department of Agriculture (USDA) which published soil series descriptions and classifications from across the United States found that the mean annual soil temperature in the trial area of the California trial ranges from 15.5 to 18.3 °C (60 to 65 degrees F). This finding can be regarded as a further confirmation of the appropriateness of the selected temperature for the missing time period. Another small gap in soil temperature data was detected on July 16th, 1992 which was filled with the average value (i.e. 27.5 °C) of adjacent measurements (i.e. July 15th, 1992: 26.7 °C and July 17th, 1992: 28.3 °C).

For trial Iowa, soil temperature data were missing between August 1st, 1992 and August 9th, 1992. The gap was filled with the average value (i.e. 22.5 °C) of adjacent measurements (i.e. July 31st, 1992: 20 °C and August 10th, 1992: 25 °C).

4. 10 mm criterion for DegT50$_{matrix}$ evaluations

According to EFSA (2014), for evaluation of the DegT50$_{matrix}$, surface processes like photolysis and volatilisation should be excluded. Therefore, it is recommended for the kinetic evaluation to use data points following at least 10 mm of cumulative precipitation (for SFO kinetics). For this purpose, the first sampling time after 10 mm of cumulative precipitation was defined as day 0, and all later time points were adjusted accordingly. The resulting normalised field sampling times, as well as eliminated sampling intervals (EFSA, 2014) are presented in the following table.

Table 7.1.2.2.1-78: Actual and time-step normalised (temperature and moisture) sampling days for trial sites from study [redacted] 1993, CA 7.1.2.2.1/006

Arizona			California		
DAT (d)	t_{norm} (d)	t_{norm} (d) (>10 mm rainfall)	DAT (d)	t_{norm} (d)	t_{norm} (d) (>10 mm rainfall)
0	0.0	-	0	0.0	-
1	0.7	-	1	1.0	-
7	5.6	0.0	7	7.0	0.0
14	12.5	6.9	14	14.0	7.0
21	20.0	14.4	21	21.0	14.0
28	27.9	22.3	29	29.0	22.0
64	85.4	79.8	61	61.8	54.8
92	149.5	143.9	91	101.1	94.1
122	225.3	219.7	123	146.6	139.6
184	373.5	367.9	183	209.1	202.1
364	486.8	481.2	365	267.6	260.6
462	666.4	660.8	456	391.6	384.6
553	882.2	876.6	550	514.5	507.5

Table 7.1.2.2.1-78: Actual and time-step normalised (temperature and moisture) sampling days for trial sites from study [redacted] 1993, CA 7.1.2.2.1/006

Iowa			New York		
DAT (d)	t_{norm} (d)	t_{norm} (d) (>10 mm rainfall)	DAT (d)	t_{norm} (d)	t_{norm} (d) (>10 mm rainfall)
0	0.0	-	0	0.0	-
1	0.9	-	1	0.6	-
7	7.7	0.0	7	2.7	0.0
14	16.3	8.5	14	6.9	4.3
21	24.8	17.1	21	11.6	8.9
29	43.2	35.4	30	21.3	18.6
62	89.2	81.4	61	48.1	45.5
92	126.9	119.2	90	79.1	76.4
123	155.7	148.0	120	107.7	105.0
190	183.0	175.3	180	141.7	139.0
366	243.4	235.6	362	162.3	159.6
458	339.6	331.8	453	222.0	219.3
			546	280.6	277.9

Normalisation of day lengths was applied to the four trials Arizona, California, Iowa and New York only. Normalised day lengths were determined using the correction factors for soil temperature (applicable for all four trials) and/ or moisture (applicable for trials Arizona and New York only) as described above. The number of remaining data points after 10 mm of rainfall per respective trial location are presented in the following table.

Table 7.1.2.2.1-79: 10 mm rainfall criterion at field trial locations

Study	Trial/ location	Total samples[1]	10 mm rainfall reached at	No. of samples after 10 mm rainfall
[redacted] (1993, CA 7.1.2.2.1/006)	Arizona, USA	13	7 DAT	11
	California, USA	13	5 DAT	11
	Iowa, USA	12	4 DAT	10
	New York, USA	13	2 DAT	11

[1] Number of samples after FOCUS correction of residue data

5. Kinetic assessment

Kinetic models

Three kinetic degradation models were considered to describe the degradation behaviour of the compounds in soil: single first order (SFO), first order multi-compartment (FOMC = Gustafson and Holden model) and double first order in parallel (DFOP) (FOCUS; 2006, 2014).

Optimisation

The kinetic analyses were conducted using the software package CAKE 3.3. The data were initially fitted with the complete dataset and unconstrained initial concentration (M_0) for the parent substance. Iteratively Reweighted Least Squares (IRLS) was used as the solver, as implemented in CAKE. Optimisations were carried out for the initial soil residue (M_0) and degradation model parameters k, α, β or g depending on the respective kinetic model selected. The initial estimates for the parameters were specified manually based on the observed degradation pattern and preliminary model runs. In pathway fits for derivation of trigger endpoints, the initial amount of metabolite was fixed to 0 % by default which is in contrast to the pathway fitting for derivation of modelling endpoints. Here, the initial amount of metabolite was not constrained to

zero as several data points from the beginning of the experimental period prior to 10 mm rainfall were cut off. Decline fits of the metabolite were treated similarly to parent as described above. In pathway fits for derivation of modelling endpoints, the initial amount of metabolite was not constrained. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

If a kinetic fit did not yield visually and/ or statistically reliable results, the kinetic model was further optimised by fixing one or more of the model parameters to either the measured value (e.g. M_0) or to estimated values derived from a reliable parent-only fit (e.g. k). A stepwise fixing procedure has been applied in these cases, which is further described in the results chapter for the respective pathway fits.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit
The goodness of fit of the estimated to the measured residue data was evaluated visually based on concentration/residual - time plots. Generally the residuals should be distributed randomly around the zero line. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line.
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered.
- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered.

A statistical measure of the quality of a fit is given by the χ^2-test. The χ^2-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements. In general, for parent compounds, it is recommended that if the χ^2 error is <15 % then the model has adequately reflected the measured data (FOCUS, 2006, 2014). However, this value should only be considered as a guide and not an absolute cut-off criterion. The guidance can be relaxed for field studies where the residue data can show appreciable scatter. The same also applies for metabolites where the curve fitting is more complex.

Significance of parameters
A single-sided t-test was performed to evaluate whether the optimised degradation rate constants (k) of the SFO and DFOP kinetic models were significantly different from zero at a chosen significance level of 5 %. For the FOMC kinetic model, only the confidence interval of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test and confidence interval were used as supporting information for the decision making process. The CAKE software also reports a 95 % confidence interval on the estimated parameters. It should be relatively tight and not contain 0 to be considered statistically robust.

Derivation of trigger and modelling endpoints
For derivation of trigger endpoints, the non-normalised dataset was considered and the kinetic evaluation was conducted with CAKE 3.3 according to FOCUS guidance (2006, 2014); the corresponding trigger DT_{50} and DT_{90} values are reported.

For the parent compound, the best-fit model was accepted for deriving trigger endpoints. For the metabolite, pathway fits were conducted using the best-fit kinetic model for the parent and SFO for the metabolite. In cases where no reliable pathway fit could be established, kinetic endpoints for the parent were derived from the corresponding parent-only fit, and decline fits were conducted for the metabolite (if possible), starting

from the maximum observed concentration. The respective day was defined as 0 days after maximum concentration, and later time points were adjusted accordingly.

For derivation of modelling endpoints, the corrected residue data were combined with the normalised day length data that were obtained as described above. The resulting datasets were then evaluated according to FOCUS (2006, 2014). The DT_{50} calculated from SFO model was preferably selected as modelling endpoints.

II. RESULTS AND DISCUSSION

Determination of trigger endpoints

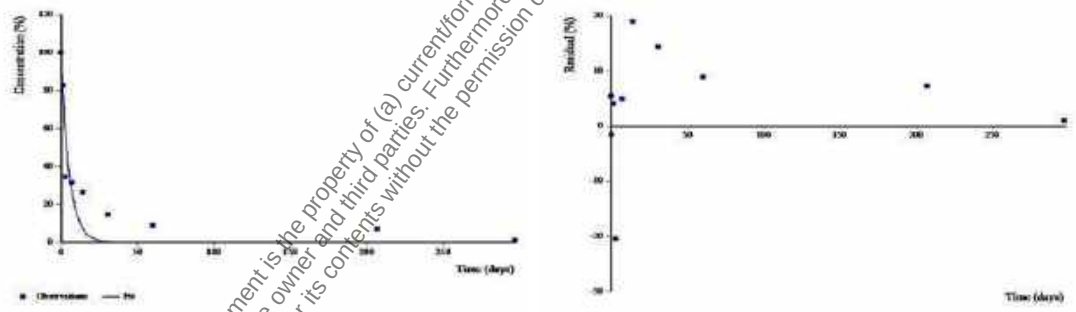
Table 7.1.2.2.1-80: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1992, CA 7.1.2.2.1/014) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	94.5	k: 0.1819	27.0	k: 0.007	k: 0.0508	k: 0.313	3.8	12.7
FOMC	Good	102.6	α: 0.6375 β: 1.3190	15.8	-[1]	β: -0.9581	β: 3.596	2.6	47.5
DFOP	Accep-table	103.6	k_1: 0.5082 k_2: 0.0171 g: 0.7169	14.4	k_1: 0.018 k_2: 0.122	k_1: 0.0526 k_2: -0.0162	k_1: 0.964 k_2: 0.050	2.3	60.7

The dissipation of glyphosate was best described by bi-phasic models. SFO model does not properly estimate the dissipation. The FOMC model provides the best fit during the whole study period. Thus, FOMC is selected as the best-fit model for parent-only fit.

Conclusion: FOMC to be used in pathway fit for trigger endpoints.

SFO



FOMC

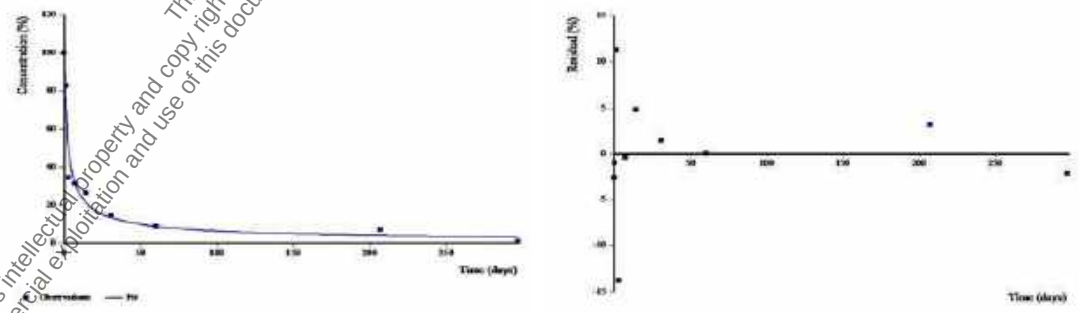
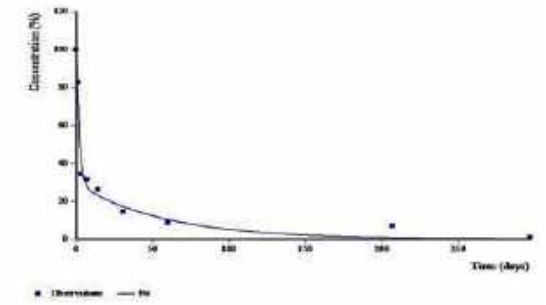
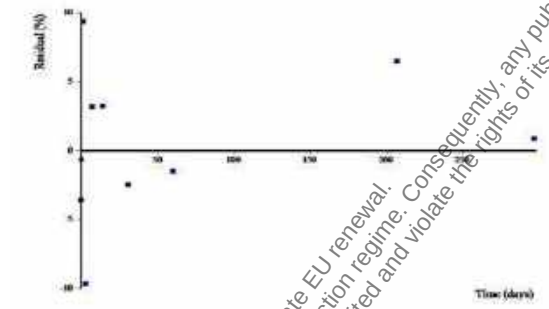


Table 7.1.2.2.1-80: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1992, CA 7.1.2.2.1/014) – trigger endpoints

DFOP





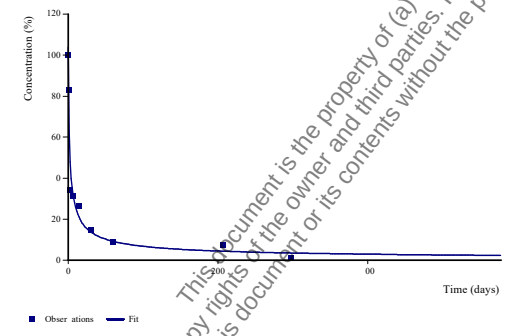
[1] t-test not relevant for kinetic parameter β

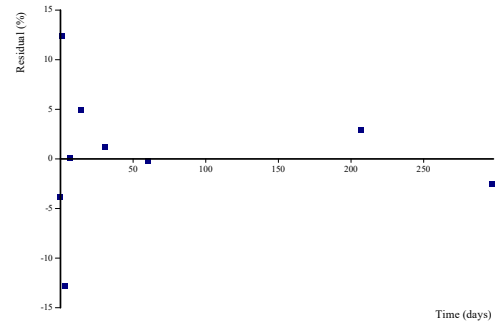
Table 7.1.2.2.1-81: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ontario of study [redacted] (1992, CA 7.1.2.2.1/014) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	103.8	α: 0.6043 β: 1.1150	15.9	-[1]	β: -0.2371	β: 2.4670	2.4	49.2	-
AMPA: SFO	Acceptable	-	k: 0.0045	16.5	k: ≤ 0.001	k: 0.0025	k: 0.0060	155	514	0.309 (±0.040)

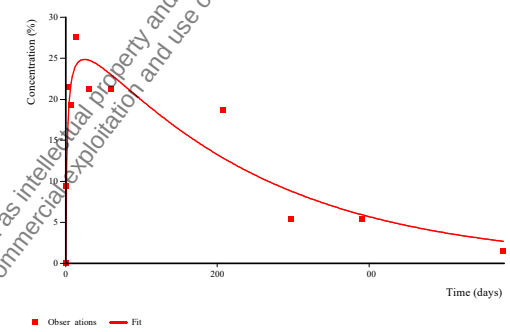
Dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit.
Conclusion: FOMC-SFO to be used for deriving trigger endpoints for glyphosate and AMPA

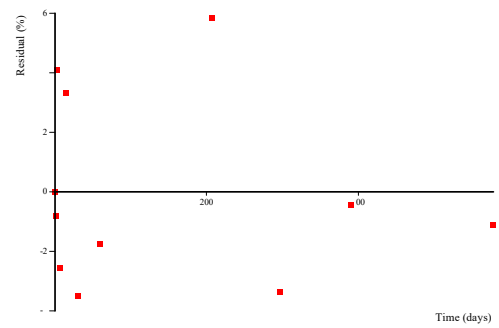
Glyphosate: FOMC





AMPA: SFO





[1] t-test not relevant for kinetic parameter β

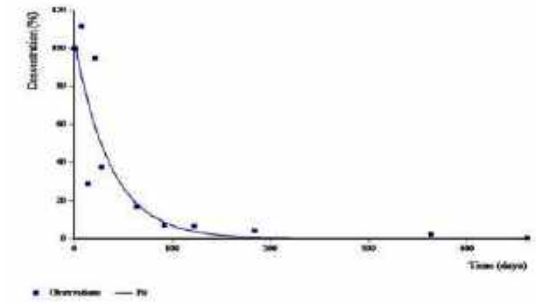
Table 7.1.2.2.1-82: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Arizona of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

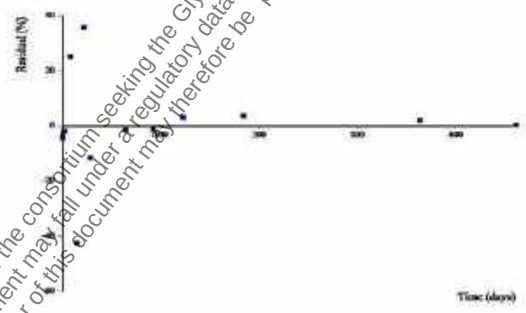
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	104.4	k: 0.0271	34.4	k: 0.007	k: 0.0068	k: 0.047	25.6	85
FOMC	Poor	104.5	α: 49.25 β: 1800	35.8	-[1]	β: -1.56×10^5	β: 1.60×10^5	25.5	86
DFOP	Poor	104.8	k_1: 0.0285 k_2: 0.0009 g: 0.9779	37.3	k_1: 0.090 k_2: 0.493	k_1: -0.0161 k_2: -0.1119	k_1: 0.073 k_2: 0.114	25.1	88

None of the applied kinetic models accurately describe the residue data of glyphosate. The visual fits are poor due to the large residuals of the first five data points and the χ^2 error is high.

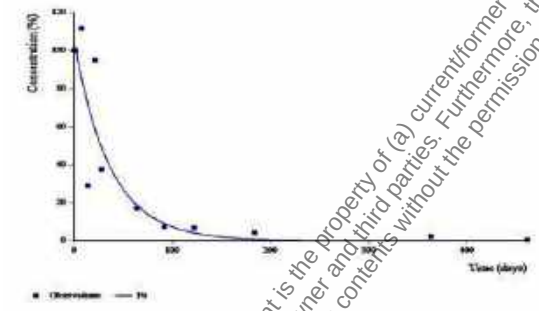
Conclusion: No trigger endpoints can be derived for glyphosate

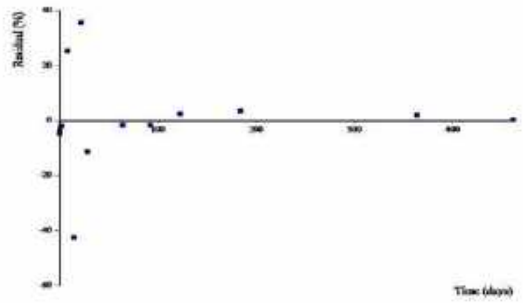
SFO



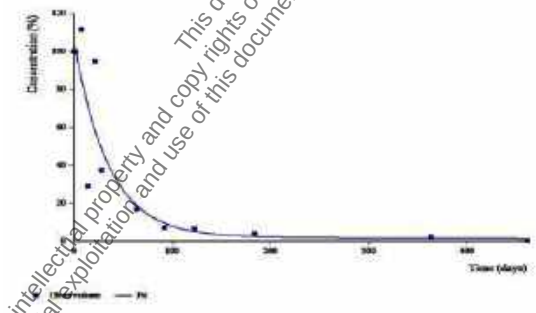


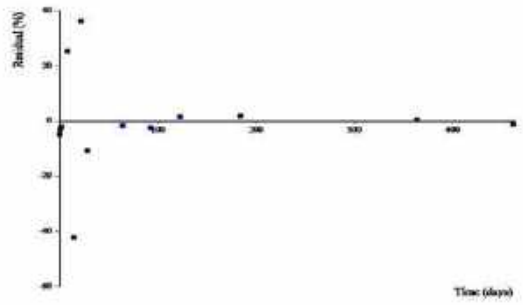
FOMC





DFOP





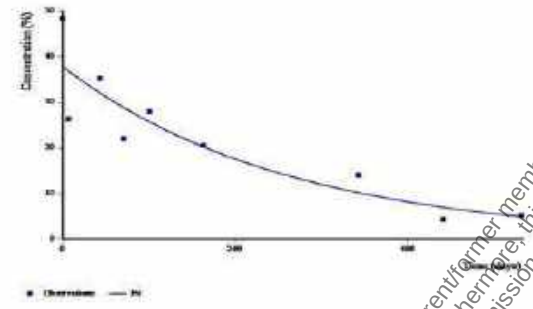
[1] t-test not relevant for kinetic parameter β

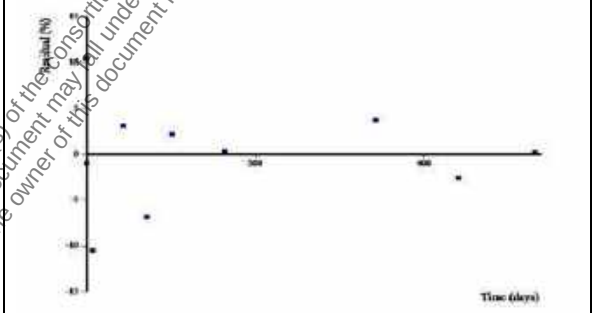
As for glyphosate, none of the tested models provided an acceptable fit, it was not possible to perform a pathway fit with the combined residue data of glyphosate and AMPA for trial Arizona. Thus, a metabolite decline fit was performed.

Table 7.1.2.2.1-83: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Arizona of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

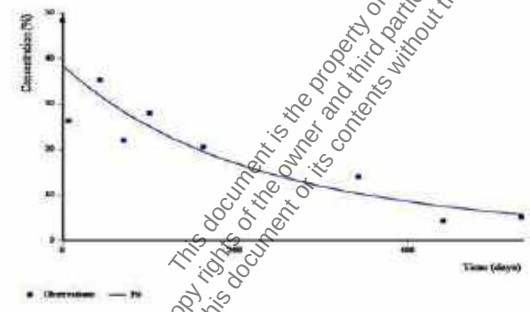
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	37.8	k: 0.0038	20.5	k: 0.005	k: 0.0012	k: 0.006	181	601
FOMC	Poor	38.5	α: 4.30 β: 956.2	21.5	-[1]	β: -1.04×10^4	β: 1.23×10^4	167	677
DFOP	Good	48.3	k_1: 1.8490 k_2: 0.0030 g: 0.3285	15.3	k_1: 0.016 k_2: 0.003	k_1: 0.2418 k_2: 0.0013	k_1: 3.457 k_2: 0.005	97.6	630
The SFO and FOMC models do not adequately describe the decline of AMPA as M_0 is clearly underestimated (measured M_0 = 48.3 %). The DFOP model provides a good visual fit with statistically reliable parameters. **Conclusion:** DFOP to be used for deriving trigger endpoints for AMPA									

SFO





FOMC



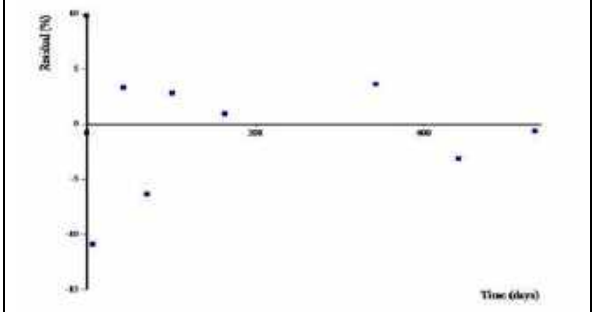
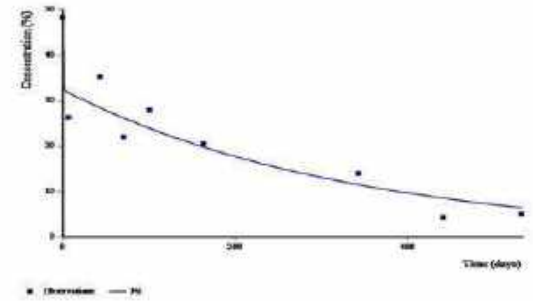
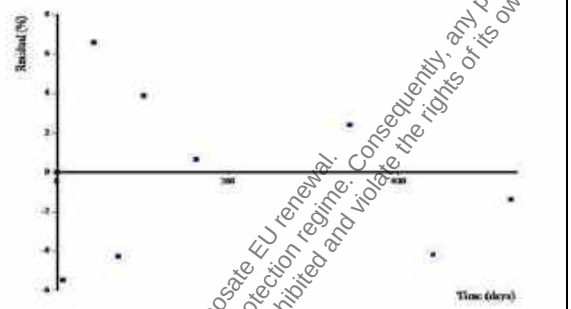


Table 7.1.2.2.1-83: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Arizona of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

DFOP





[1] t-test not relevant for kinetic parameter β

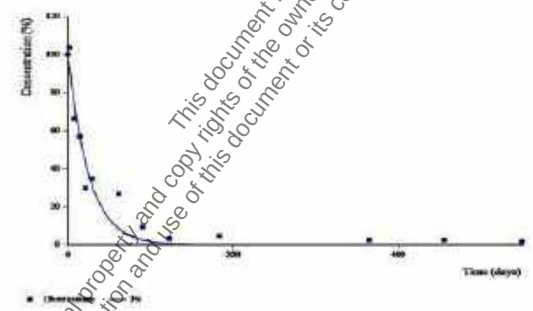
Table 7.1.2.2.1-84: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	99.3	k: 0.0400	18.1	k: <0.001	k: 0.0272	k: 0.053	17.3	57.5
FOMC	Good	104.4	α: 1.196 β: 17.25	12.7	-[1]	β: -2.377	β: 36.88	13.5	101
DFOP	Accep-table	104.7	k_1: 0.1124 k_2: 0.0148 g: 0.5490	12.7	k_1: 0.045 k_2: 0.023	k_1: -0.0214 k_2: 0.0004	k_1: 0.246 k_2: 0.029	13.0	102

The SFO model provides an acceptable visual and statistically reliable fit. The bi-phasic models further improve the visual fit. The FOMC model provides the best visual fit during the whole study period. Thus, FOMC is selected as the best-fit model for parent-only fit.

Conclusion: FOMC to be used in pathway fit for trigger endpoints.

SFO



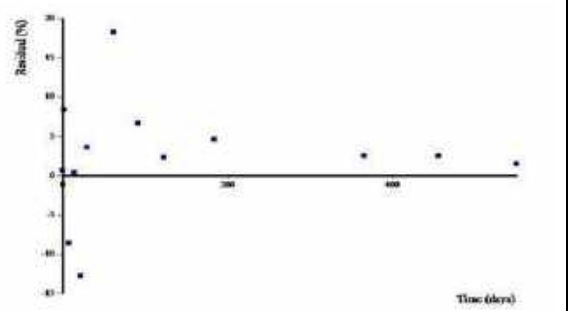
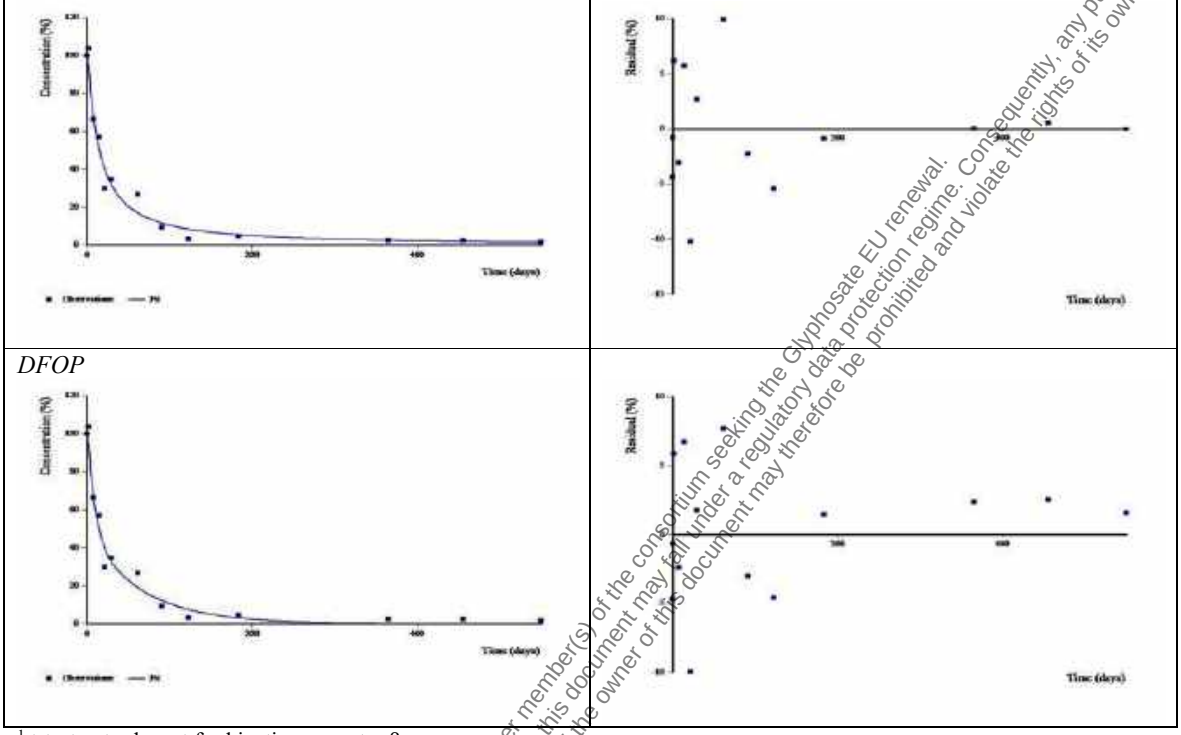


Table 7.1.2.2.1-84: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

FOMC



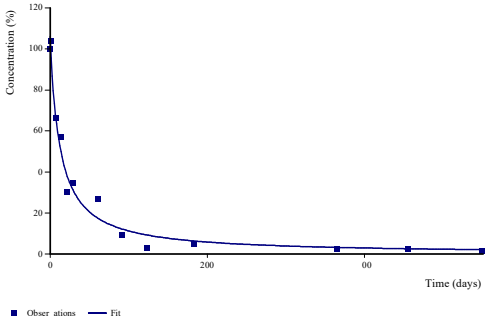
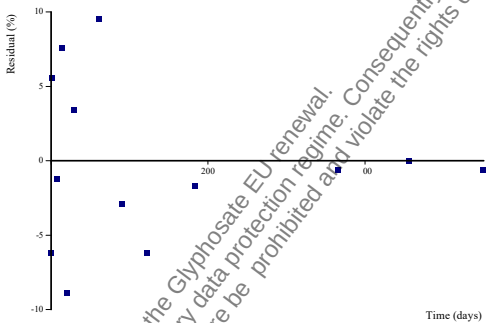
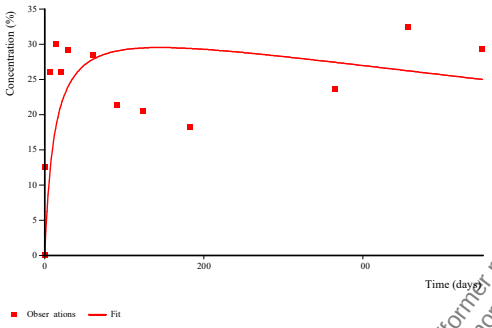
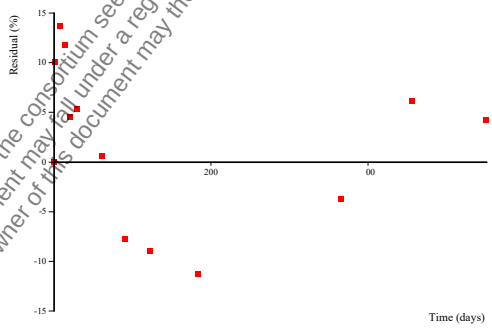
DFOP

[1] t-test not relevant for kinetic parameter β

Table 7.1.2.2.1-85: Kinetic models and goodness-of-fit statistics of pathway fits for trial California of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	106.2	α: 1.029 β: 12.74	13.0	-[1]	β: -0.0823	β: 25.57	12.3	107	-
AMPA: SFO	Poor	-	k: 0.0006	26.8	k: 0.193	k: -0.0008	k: 0.002	>1000	>1000	0.323 (±0.055)
The dissipation of glyphosate is well described by the pathway fit. The formation and decline of AMPA is not acceptably described as the residue data of the metabolite are greatly scattered. **Conclusion:** Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate No trigger endpoints can be derived for AMPA										

Table 7.1.2.2.1-85: Kinetic models and goodness-of-fit statistics of pathway fits for trial California of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: FOMC										
										
										
AMPA: SFO										
										
										

[1] t-test not relevant for kinetic parameter β

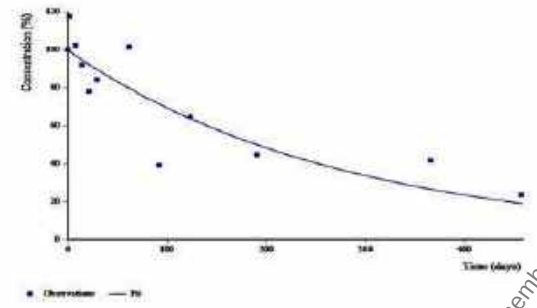
Table 7.1.2.2.1-86: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Iowa of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

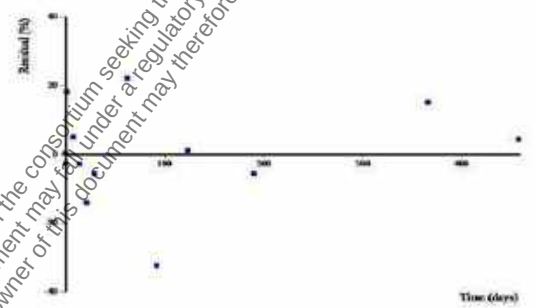
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	99.6	k: 0.0036	15.5	k: 0.001	k: 0.0016	k: 0.006	192	638
FOMC	Accep-table	106.4	α: 0.6571 β: 78.33	14.6	-[1]	β: -191.1	β: 347.8	147	>1000
DFOP	Accep-table	106.1	k_1: 0.0182 k_2: 0.0018 g: 0.3689	15.3	k_1: 0.313 k_2: 0.241	k_1:-0.0644 k_2:-0.0039	k_1: 0.101 k_2: 0.008	152	999

The SFO model provides an acceptable visual and statistically reliable fit. The bi-phasic models further improve the visual fit. The FOMC and DFOP models provide equally good visual fits but the FOMC model has the lowest χ^2. Thus, FOMC is selected as the best-fit model for parent-only fit.

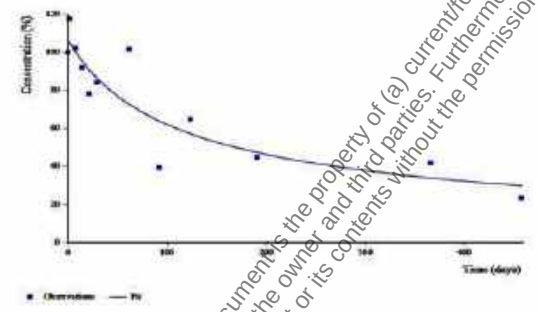
Conclusion: FOMC to be used in pathway fit for trigger endpoints.

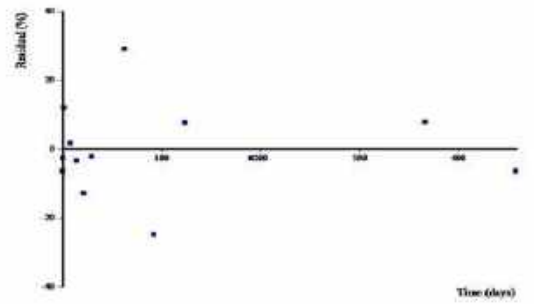
SFO



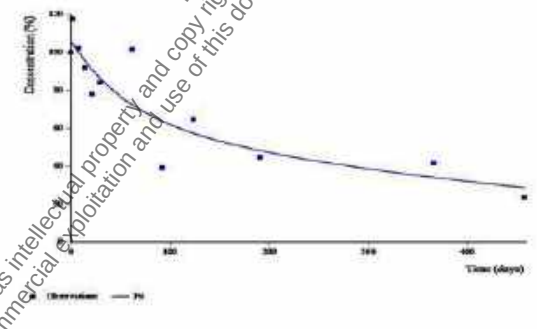


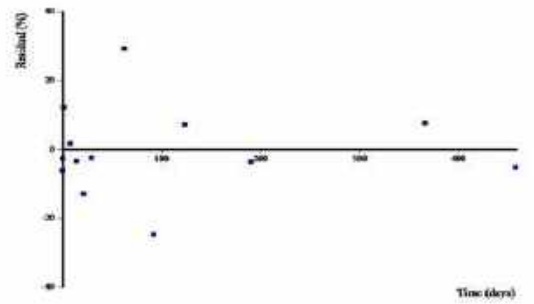
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

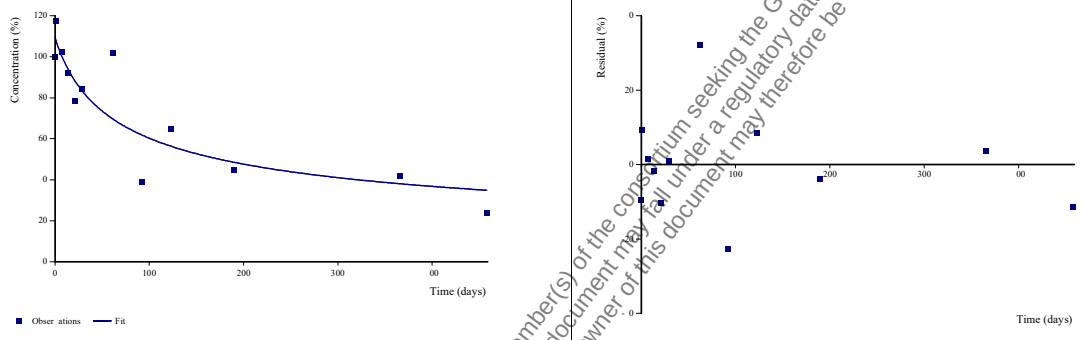
Table 7.1.2.2.1-87: Kinetic models and goodness-of-fit statistics of pathway fits for trial Iowa of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Acceptable	109.5	α: 0.4143 β: 31.01	14.9	-[1]	β: -30.51	β: 92.53	134	>1000	-
AMPA: SFO	Acceptable	-	k: 4.28×10^{-66}	19.3	k: 0.5	k: -0.0017	k: 0.002	>1000	>1000	0.542 (±0.163)

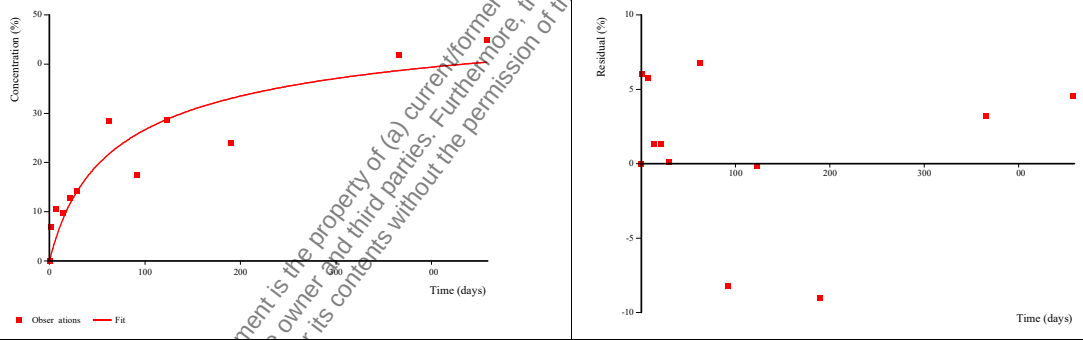
For glyphosate, the FOMC fit is acceptable. The formation of AMPA is well described by the pathway fit but the degradation rate is not significantly different from zero as the metabolite concentration is still increasing towards the end of the study. A decline fit for AMPA was not performed.

Conclusion: Parent-only FOMC fit to be used for deriving trigger endpoints for glyphosate
No trigger endpoints can be derived for AMPA

Glyphosate: FOMC



AMPA: SFO

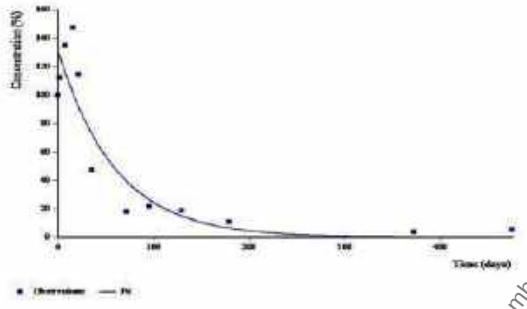


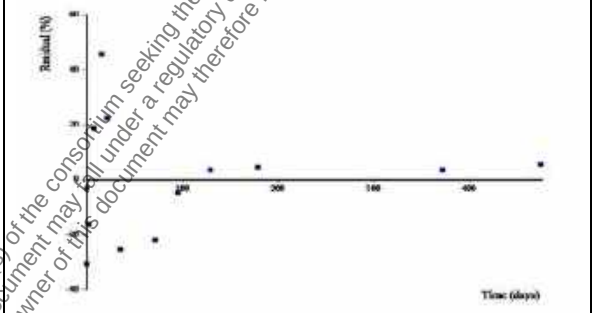
[1] t-test not relevant for kinetic parameter β

Table 7.1.2.2.1-88: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Minnesota of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

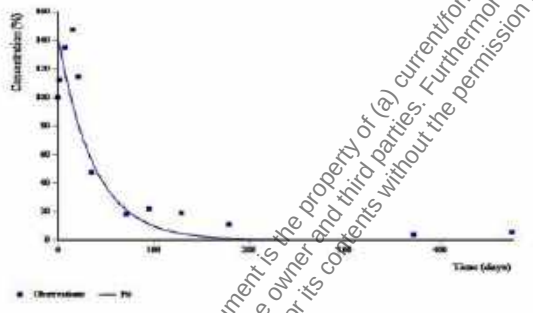
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	130.8	k: 0.0167	27.9	k: 0.003	k: 0.0060	k: 0.027	41.5	138
FOMC	Poor	141.7	α: 359.9 β: 1.35×10^4	29.1	-[1]	-[2]	-[2]	26.0	86.5
DFOP	Poor	130.8	k_1: 0.0167 k_2: 0.0167 g: 0.1314	30.4	k_1: 0.156 k_2: <0.001	k_1:-0.0190 k_2: 0.0097	k_1: 0.052 k_2: 0.024	41.5	138
None of the applied kinetic models accurately describe the residue data of glyphosate. The visual fits are poor due to an initial increase in glyphosate concentration, and the resulting residuals are large. **Conclusion:** No trigger endpoints can be derived for glyphosate.									

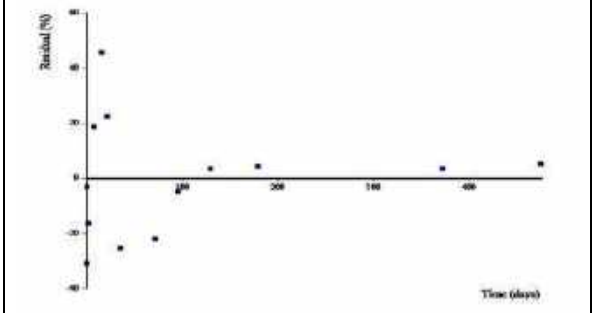
SFO



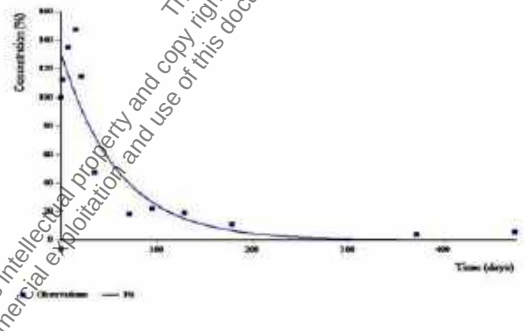


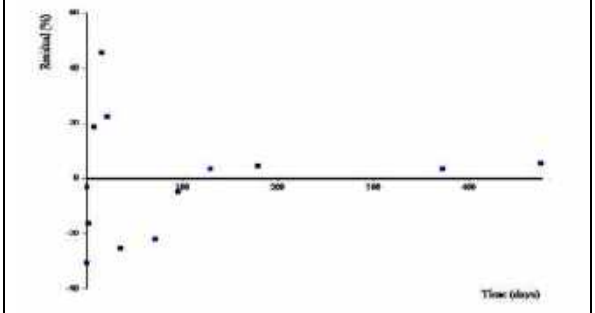
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β
[2] Errors and t-test values could not be calculated because the covariance matrix could not be created

As for glyphosate, none of the tested models provided an acceptable fit, it was not possible to perform a pathway fit with the combined residue data of glyphosate and AMPA for trial Minnesota. Thus, a metabolite decline fit was performed.

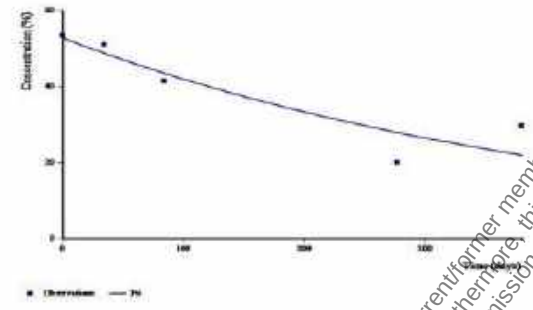
Table 7.1.2.2.1-89: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Minnesota of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

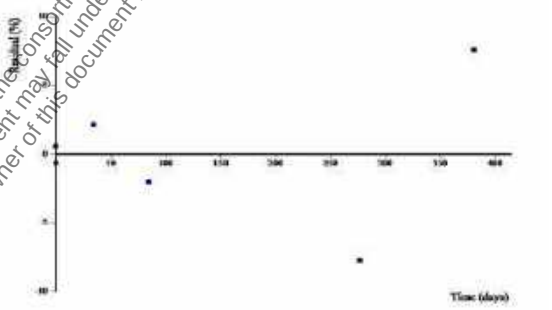
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Good	52.9	k: 0.0023	10.3	k: 0.020	k: 0.0002	k :0.004	302	>1000
FOMC	Good	55.1	α: 0.6787 β: 156.3	10.4	-[1]	β: -1768	β: 2080	278	>1000
DFOP	Good	55.6	k_1: 0.0074 k_2: 0.0000 g: 0.5915	12.0	k_1: 0.434 k_2: 0.5	k_1:-0.4369 k_2:-0.2033	k_1: 0.452 k_2: 0.203	252	>1000

The SFO model adequately describes the degradation behaviour of the measured residue data of AMPA and provides statistically reliable endpoints. The bi-phasic models do not improve the visual or statistical fit of the data.

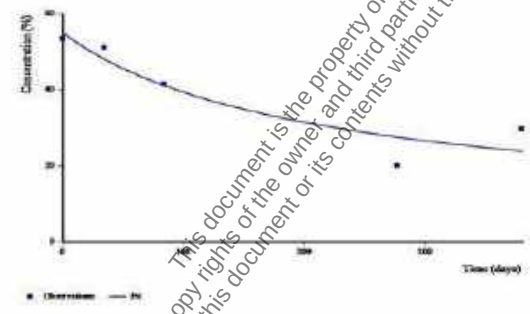
Conclusion: SFO to be used for deriving trigger endpoints for AMPA

SFO





FOMC



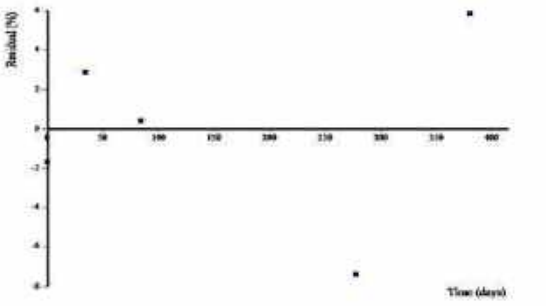
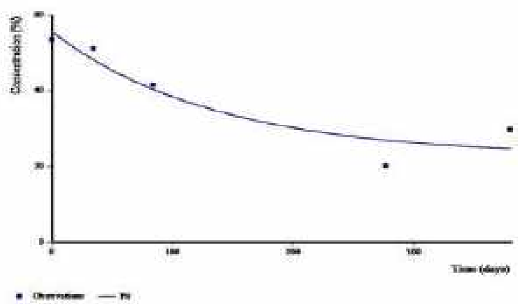
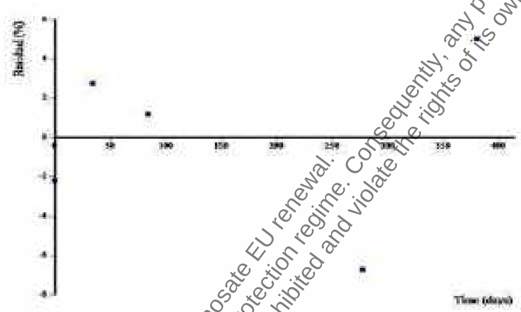


Table 7.1.2.2.1-89: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Minnesota of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

DFOP





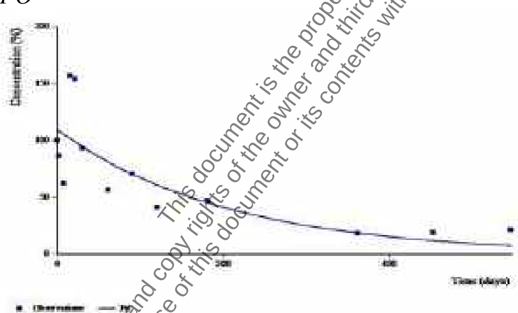
[1] t-test not relevant for kinetic parameter β

Table 7.1.2.2.1-90: Kinetic models and goodness-of-fit statistics of parent-only fits for trial New York of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5% level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	109.5	k: 0.0049	31.2	k: 0.018	k: 0.0004	k: 0.0090	142	471
FOMC	Poor	111.1	α: 3.0020 β: 511.2	32.1	-[1]	β: -4768	β: 5790	133	590
DFOP	Poor	111.8	k_1: 0.0068 k_2: 0.0000 g: 0.8729	33.2	k_1: 0.369 k_2: 0.500	k_1:-0.0378 k_2:-0.0754	k_1: 0.0510 k_2: 0.0750	125	>1000

None of the applied kinetic models adequately describe the residue data of glyphosate. The visual fits are poor due to the large scattering of the residue data, and the resulting residuals are large.
Conclusion: No trigger endpoints can be derived for glyphosate

SFO



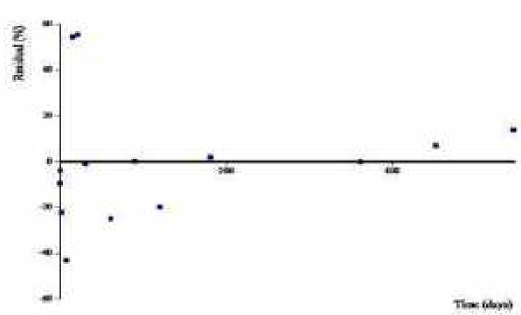
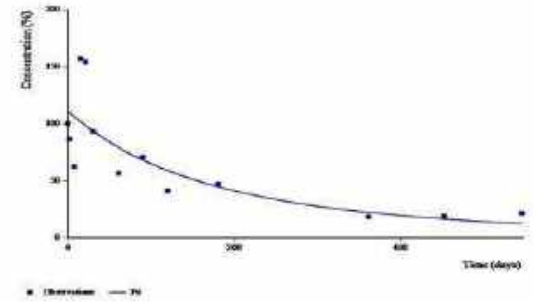
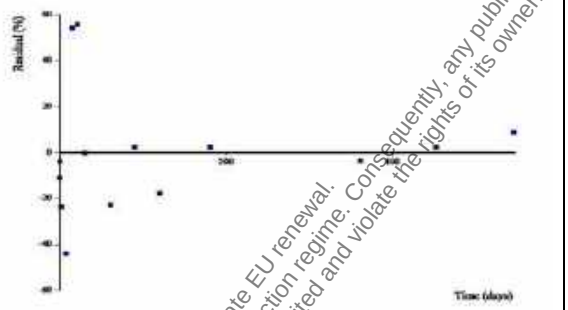


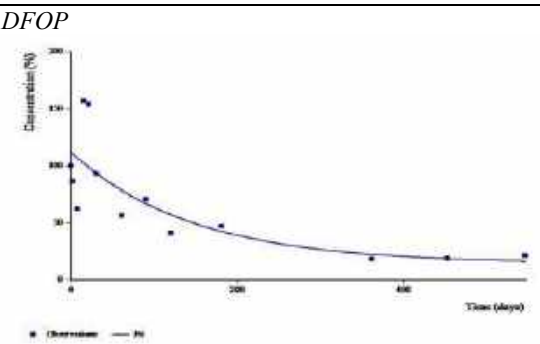
Table 7.1.2.2.1-90: Kinetic models and goodness-of-fit statistics of parent-only fits for trial New York of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

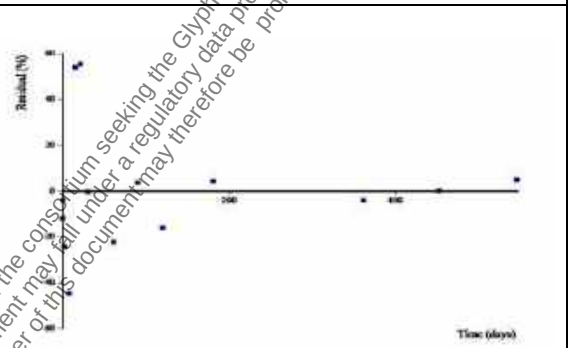
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

As for glyphosate, none of the tested models provided an acceptable fit, it was not possible to perform a pathway fit with the combined residue data of glyphosate and AMPA trial New York. As no clear decline phase was visible for AMPA, a metabolite decline fit was not performed and no trigger endpoints were derived for AMPA.

Table 7.1.2.2.1-91: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ohio of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

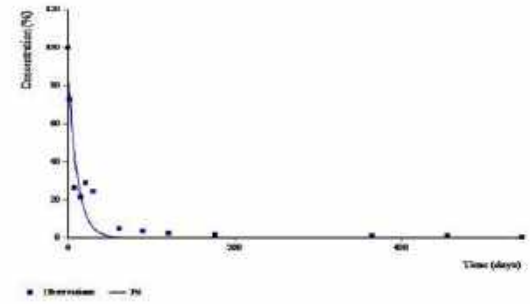
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	86.9	k: 0.0918	35.9	k: <0.001	k: 0.0450	k: 0.139	7.6	25.1
FOMC	Accep-table	100.0	α: 0.6327 β: 1.4950	19.1	-[1]	β: -0.3040	β: 3.293	3.0	55.4
DFOP	Good	100.6	k_1: 0.5430 k_2: 0.0194 g: 0.6704	13.3	k_1: 0.001 k_2: 0.002	k_1: 0.2434 k_2: 0.0076	k_1: 0.842 k_2: 0.031	2.4	61.5

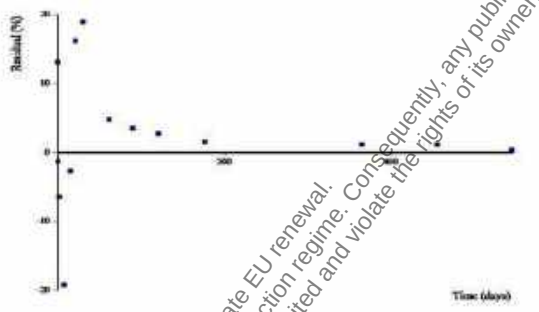
Dissipation of glyphosate was best described by bi-phasic models. SFO model does not properly estimate the dissipation. The DFOP model provides the best visual fit and the lowest χ^2 error. Thus, DFOP is selected as the best-fit model for parent-only fit.

Conclusion: DFOP to be used in pathway fits for trigger endpoints.

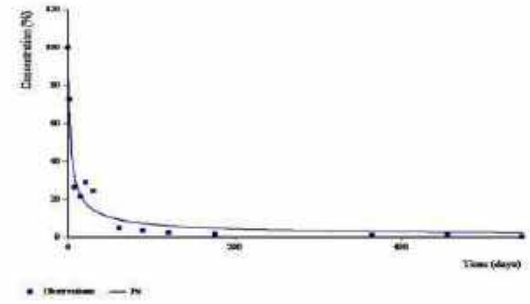
Table 7.1.2.2.1-91: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ohio of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

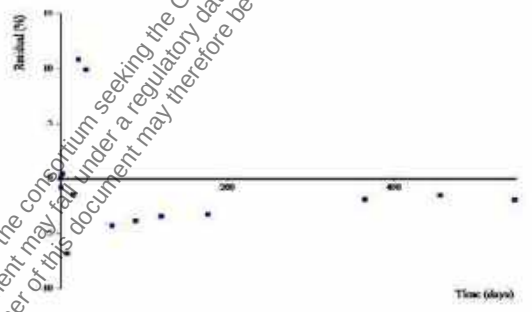
SFO



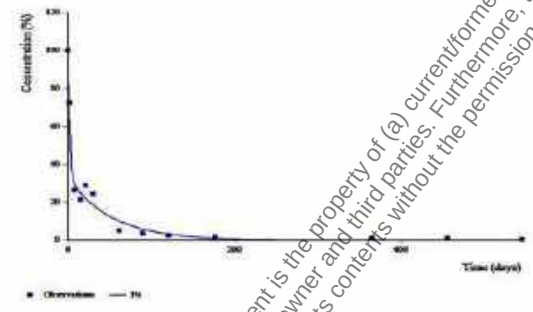


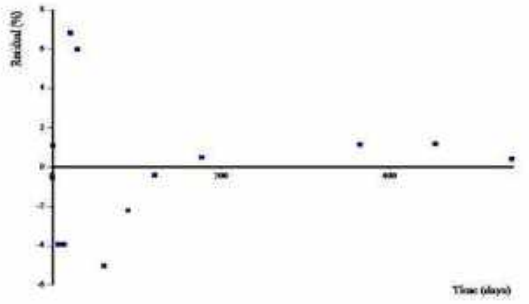
FOMC





DFOP





[1] t-test not relevant for kinetic parameter β

Table 7.1.2.2.1-92: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ohio of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate:: DFOP	Good	101.7	k_1: 0.5996 k_2: 0.0187 g: 0.6764	13.5	k_1: <0.001 k_2: <0.001	k_1: 0.3437 k_2: 0.0088	k_1: 0.856 k_2: 0.029	2.1	62.8	-
AMPA: SFO	Acceptable	-	k: 0.0107	17.5	k: <0.001	k: 0.0060	k: 0.015	65.0	216	0.510 (±0.055)
Dissipation of glyphosate is well described. The formation and decline of AMPA are acceptably described by the fit even though later data points are underestimated. **Conclusion:** DFOP-SFO to be used for deriving trigger endpoints for glyphosate and AMPA										

Table 7.1.2.2.1-92: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ohio of study [redacted] (1993, CA 7.1.2.2.1/006) – trigger endpoints

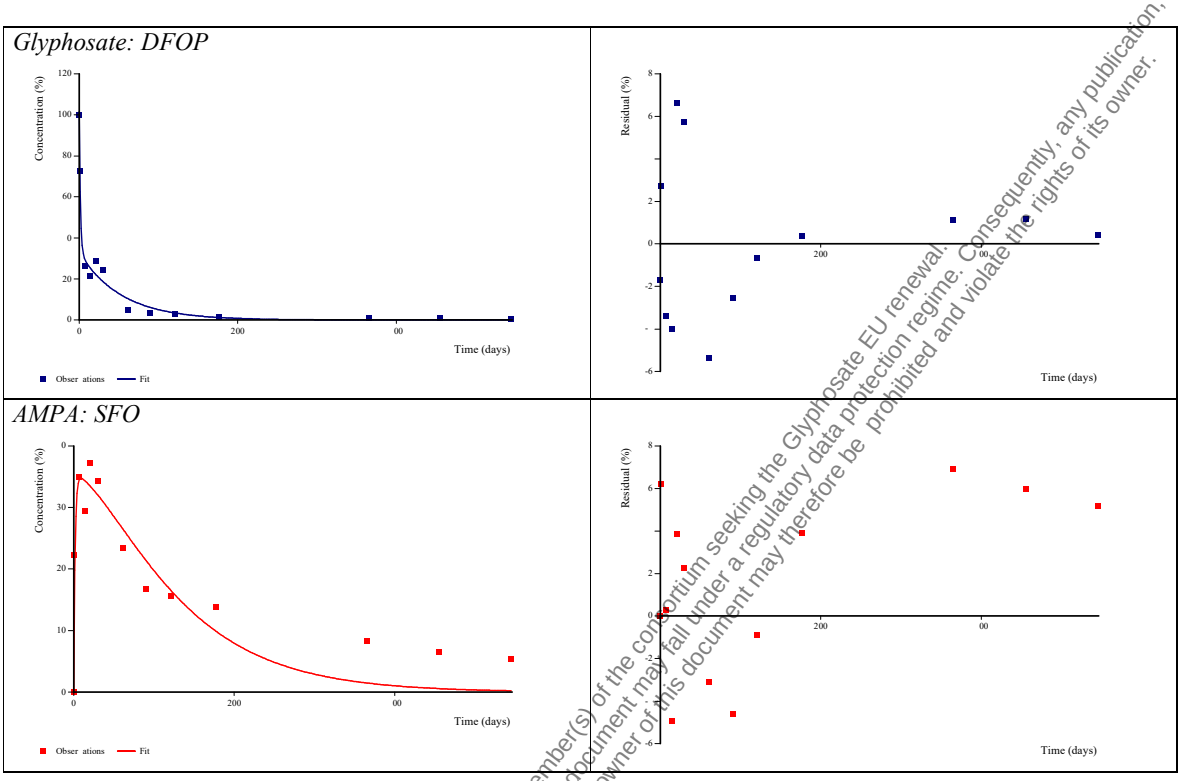
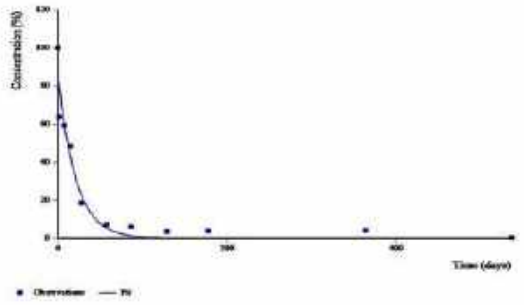


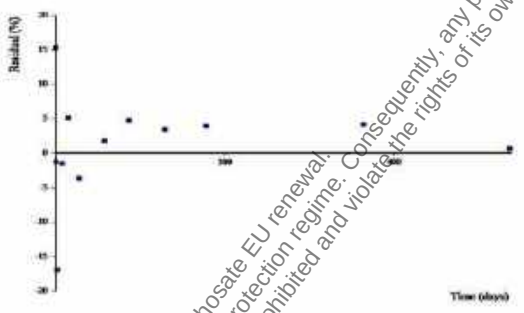
Table 7.1.2.2.1-93: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Initial fitting									
SFO	Poor	84.6	k: 0.0479	21.4	k: <0.001	k: 0.0263	k: 0.070	14.5	48.0
FOMC	Poor	86.4	α: 2.3540 β: 37.69	21.7	-[1]	β: -87.35	β: 162.7	12.9	62.5
DFOP	Poor	85.4	k_1: 0.0551 k_2: 0.0017 g: 0.9420	22.3	k_1: 0.008 k_2: 0.427	k_1: 0.0135 k_2: -0.0196	k_1: 0.097 k_2: 0.023	13.7	54.4
None of the applied kinetic models accurately describe the residue data of glyphosate in an initial fitting as M_0 was clearly underestimated. Thus, the fitting was repeated with M_0 fixed to the measured initial residue value (100 %).									

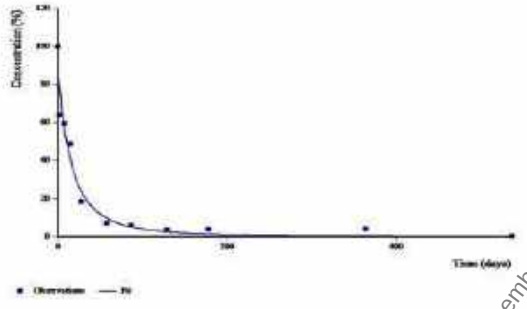
Table 7.1.2.2.1-93: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

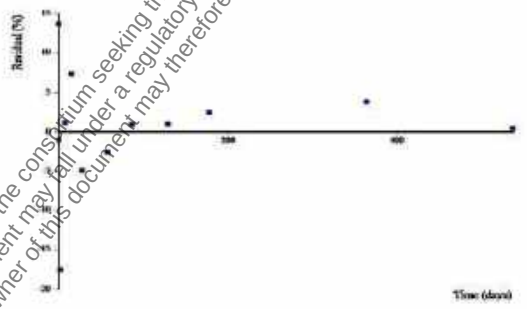
Initial fitting: SFO



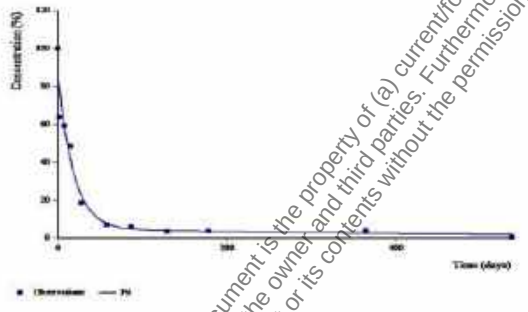


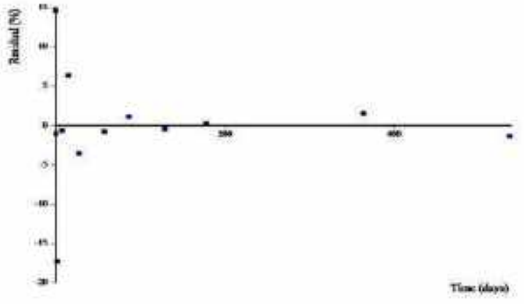
Initial fitting: FOMC





Initial fitting: DFOP





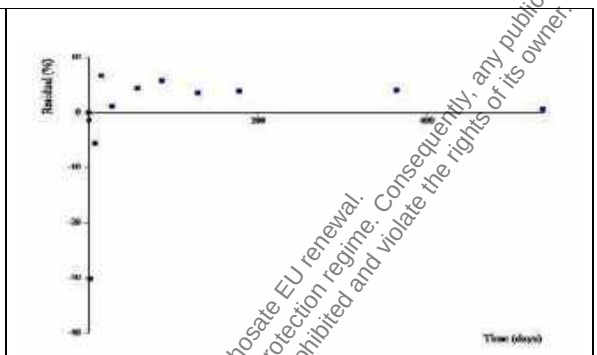
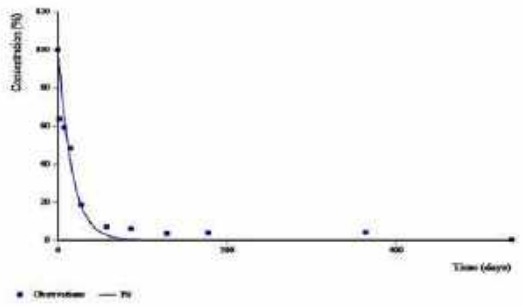
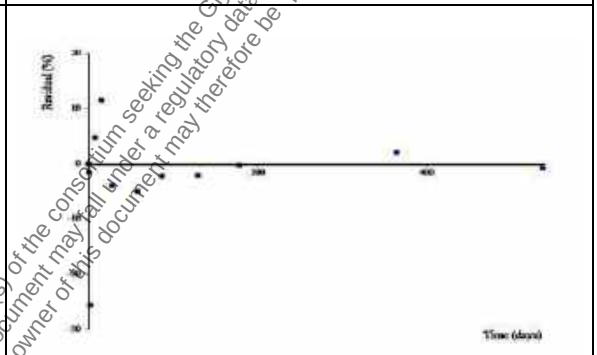
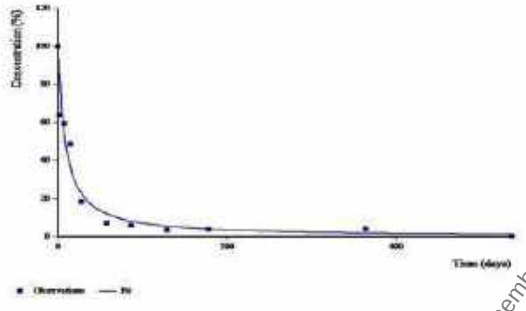
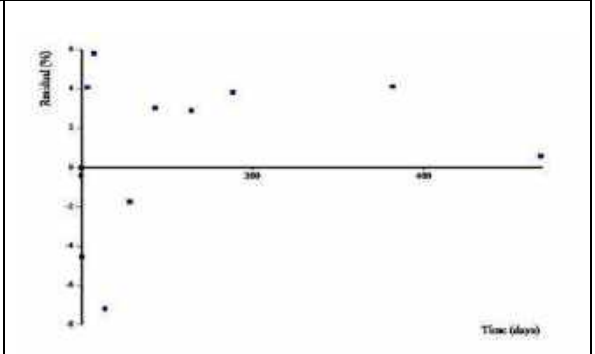
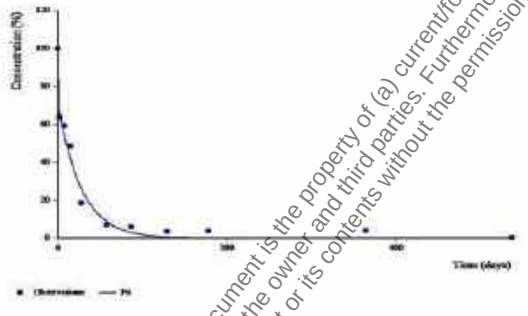
Repeated fitting: parent M_0 fixed to measured initial concentration

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	fixed to 100.0	k: 0.0625	37.3	k: <0.001	k: 0.0350	k: 0.09	11.1	36.9
FOMC	Accep-table	fixed to 100.0	α: 1.044 β: 8.7520	34.7	-[1]	β: -10.03	β: 27.53	8.3	70.6
DFOP	Accep-table	fixed to 100.0	k_1: 17.2 k_2: 0.0363 g: 0.2939	16.4	k_1: 0.486 k_2: <0.001	k_1: -1109 k_2: 0.0233	k_1: 1140 k_2: 0.049	9.5	53.9

The visual fit of improved for all models. The DFOP model provides the best visual fit and the lowest χ^2 error. Thus, DFOP is selected as the best-fit model for parent-only fit.

Conclusion: DFOP with fixed M_0 (100 %) to be used in pathway fit for trigger endpoints.

Table 7.1.2.2.1-93: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

Repeated fitting: SFO	
	
Repeated fitting: FOMC	
	
Repeated fitting: DFOP	
	

[1] t-test not relevant for kinetic parameter β

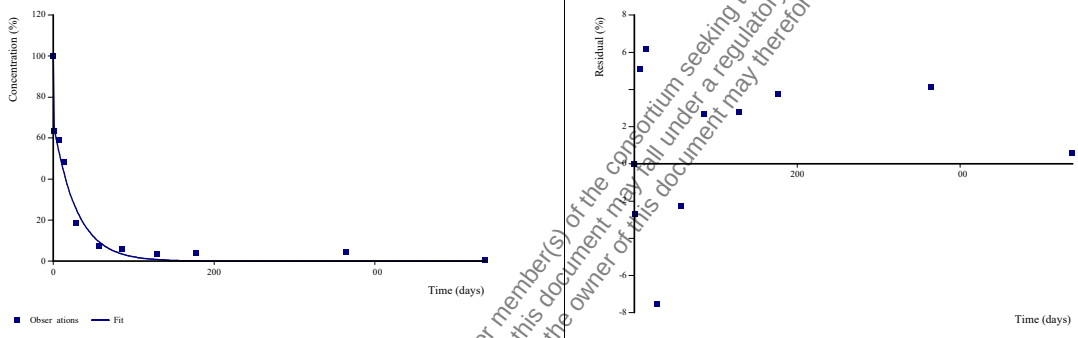
Table 7.1.2.2.1-94: Kinetic models and goodness-of-fit statistics of pathway fits for trial Ontario of study [redacted] (1993, CA 7.1.2.2.1/005) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: DFOP	Good	fixed to 100.0	fixed to k_1: 17.2 k_2: 0.0363 g: 0.2939	14.4	-[1]	-[1]	-[1]	9.5	53.9	-
AMPA: SFO	Poor	-	k: 0.0027	27.3	k: 0.020	k: 0.0001	k: 0.005	256	850	0.342 (±0.052)

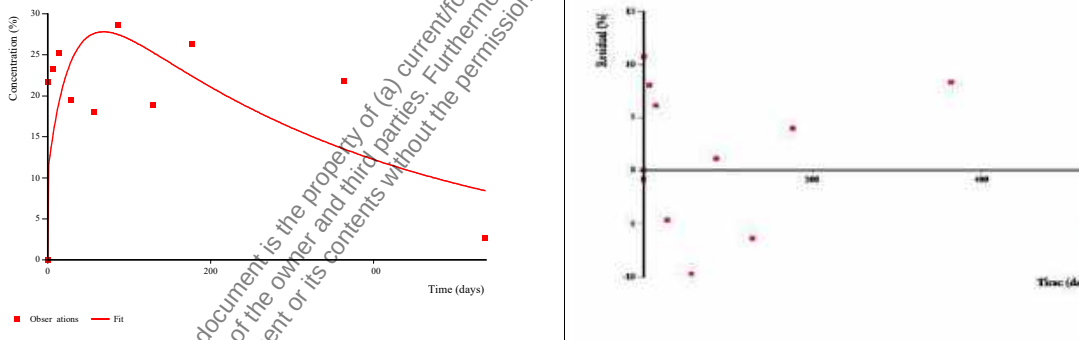
In an initial fitting, an internal error in CAKE 3.3 led to a mismatch of the plots of metabolite fit and the corresponding residuals (data not shown). Thus, the fitting was repeated with the initial parameters for the parent fixed to results from parent-only fit. The resulting visual fit for AMPA is poor due to the large scattering of the residue data.

Conclusion: Parent-only DFOP fit to be used for deriving trigger endpoints for glyphosate
No trigger endpoints can be derived for AMPA

Glyphosate: DFOP



AMPA: SFO



[1] Not determined due to fixed parameters

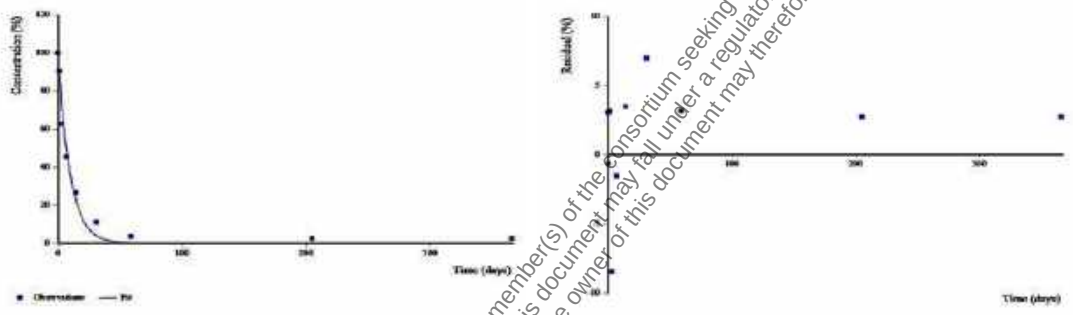
Table 7.1.2.2.1-95: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1989, CA 7.1.2.2.1/016) – trigger endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	97.0	k: 0.1024	9.3	k: <0.001	k: 0.0754	k: 0.129	6.8	22.5
FOMC	Good	101.3	α: 1.4810 β: 9.2630	5.0	-[1]	β: 1.3390	β: 17.19	5.8	34.6
DFOP	Good	101.6	k_1: 0.2811 k_2: 0.0494 g: 0.4847	5.4	k_1: 0.028 k_2: 0.013	k_1:-0.0108 k_2: 0.0092	k_1: 0.573 k_2: 0.090	5.4	33.2

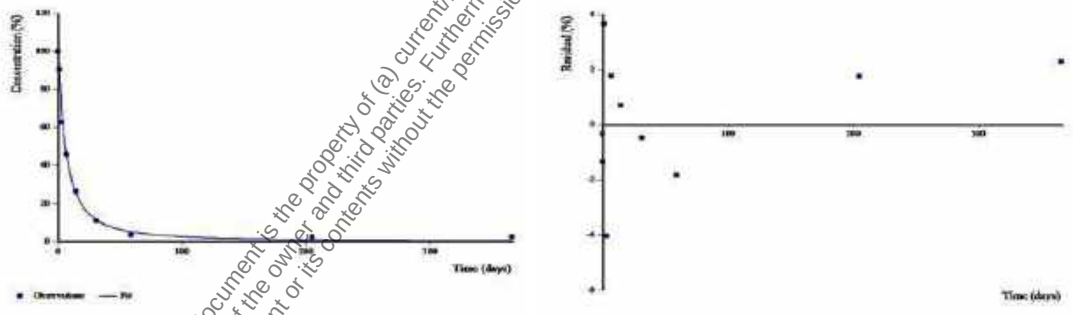
The SFO model provides an acceptable visual and statistically reliable fit. The bi-phasic models further improve the visual fit. The FOMC model provides the best visual fit during the whole study period and the lowest χ^2 error. Thus, FOMC is selected as the best-fit model for parent-only fit.

Conclusion: FOMC to be used in pathway fit for trigger endpoints.

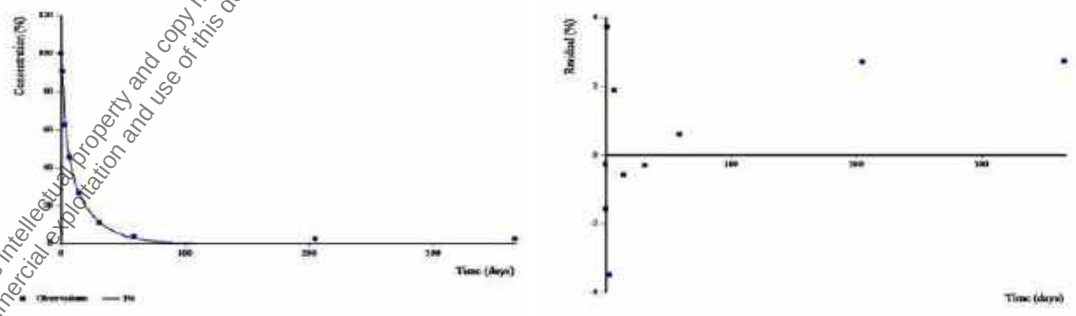
SFO



FOMC



DFOP

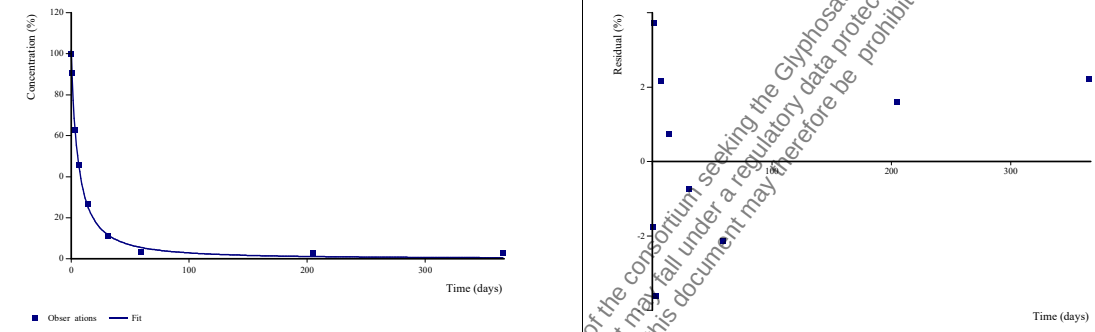


[1] t-test not relevant for kinetic parameter β

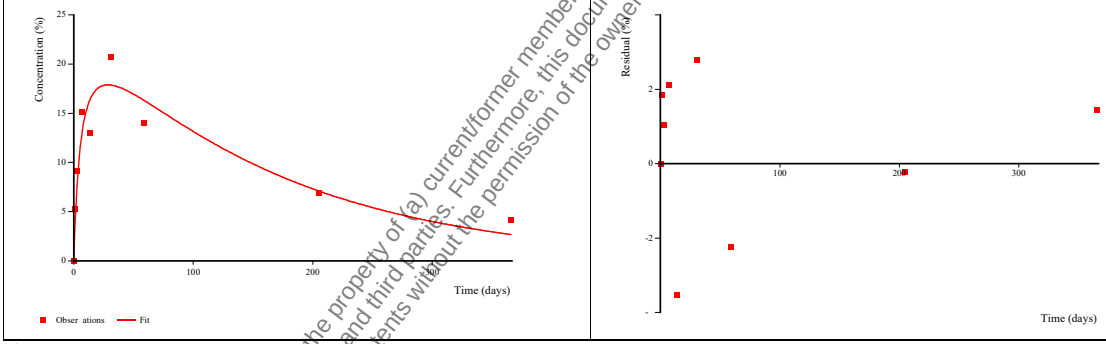
Table 7.1.2.2.1-96: Kinetic models and goodness-of-fit statistics of pathway fits for trial California of study [redacted] (1989, CA 7.1.2.2.1/016) – trigger endpoints

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: FOMC	Good	101.8	α: 1.3940 β: 8.3790	5.1	-[1]	β: 2.4360	β: 14.32	5.4	35.3	-
AMPA: SFO	Acceptable	-	k: 0.0062	15.4	k: 0.001	k: 0.0027	k: 0.0100	111	370	0.231 (±0.022)
Dissipation of glyphosate and the formation and decline of AMPA are well described by the pathway fit. **Conclusion:** FOMC-SFO to be used for deriving trigger endpoints for glyphosate and AMPA										

Glyphosate: FOMC



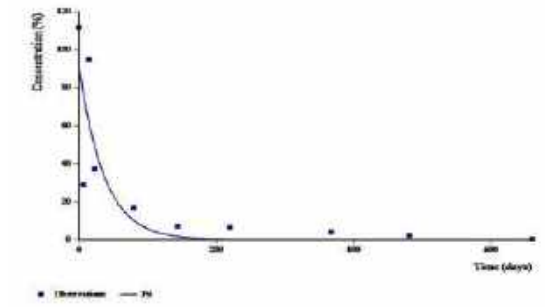
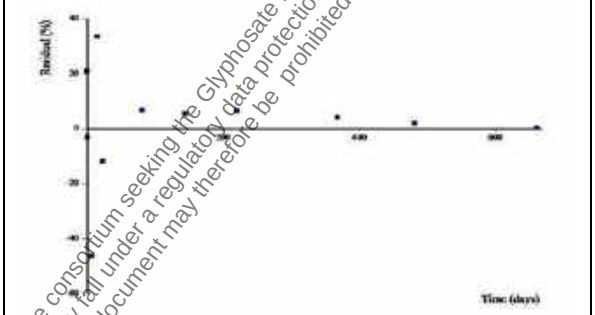
AMPA: SFO



[1] t-test not relevant for kinetic parameter β

Determination of modelling endpoints

Table 7.1.2.2.1-97: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Arizona of study [redacted] (1993, CA 7.1.2.2.1/006) – modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	90.7	k: 0.0274	51.6	k: 0.070	k:-0.0112	k:0.066	25.3	84.2
Due to the large scattering of the residue data and the resulting high χ^2 error, the SFO fit is not acceptable. **Conclusion**: No modelling endpoints can be derived for glyphosate									
SFO  									

As for glyphosate, the SFO model did not provide an acceptable fit, it was not possible to perform a pathway fit with the combined residue data of glyphosate and AMPA for trial Arizona. Thus, a metabolite decline fit was performed.

Table 7.1.2.2.1-98: Kinetic models and goodness-of-fit statistics of decline fits for AMPA for trial Arizona of study [redacted] (1993, CA 7.1.2.2.1/006) – modelling endpoints

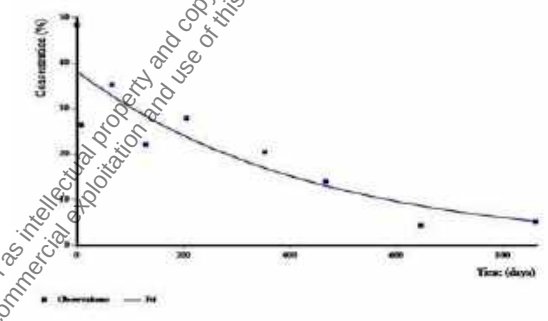
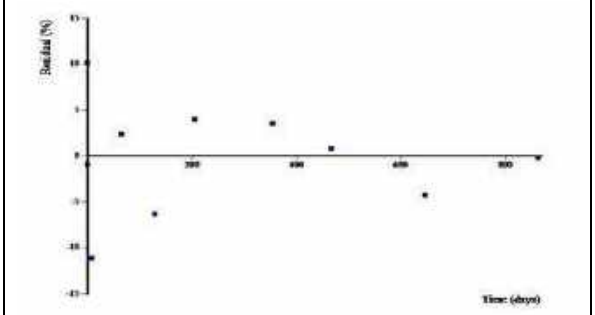
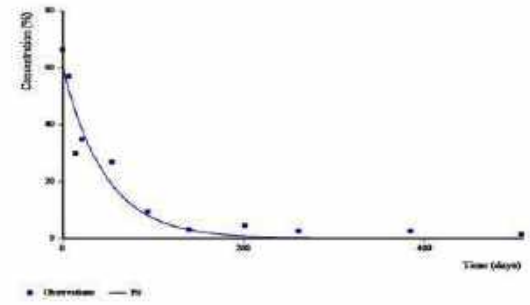
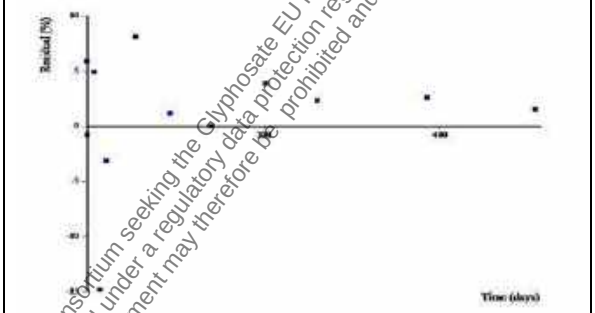
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	38.1	k: 0.0023	21.1	k: 0.004	k: 0.0008	k: 0.004	303	>1000
The SFO model provides a visually acceptable and statistically reliable fit to describe the decline of AMPA. **Conclusion:** SFO to be used for deriving modelling endpoints for AMPA									
SFO  									

Table 7.1.2.2.1-99: Kinetic models and goodness-of-fit statistics of parent-only fits for trial California of study [redacted] (1993, CA 7.1.2.2.1/006) – modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	60.5	k: 0.0213	22.0	k: <0.001	k: 0.0108	k: 0.032	32.6	108
The SFO model provides a visually acceptable and statistically reliable fit to describe the degradation of glyphosate. **Conclusion:** SFO fit to be used for deriving modelling endpoints for glyphosate.									

SFO



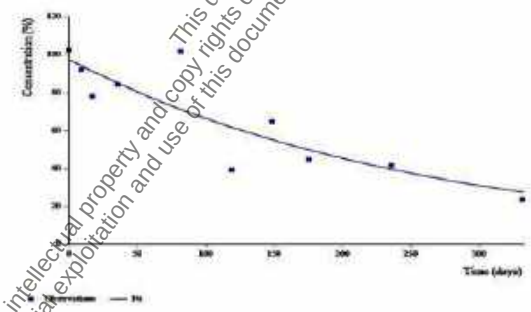


As no formation or decline phase of AMPA was observed, it was neither possible to perform a pathway fit with the combined residue data of glyphosate and AMPA nor to perform a metabolite decline fit for trial California. Thus, no modelling endpoints were derived for AMPA.

Table 7.1.2.2.1-100: Kinetic models and goodness-of-fit statistics of parent-only fits for trial Iowa of study [redacted] (1993, CA 7.1.2.2.1/006) – modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Accep-table	97.4	k: 0.0038	15.9	k: <0.05	k: 0.0017	k: 0.006	182	605
The SFO model provides a visually acceptable and statistically reliable fit to describe the degradation of glyphosate. **Conclusion:** SFO to be used in pathway fit for modelling endpoints									

SFO



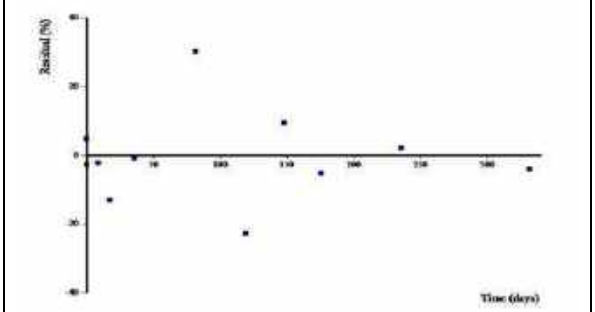


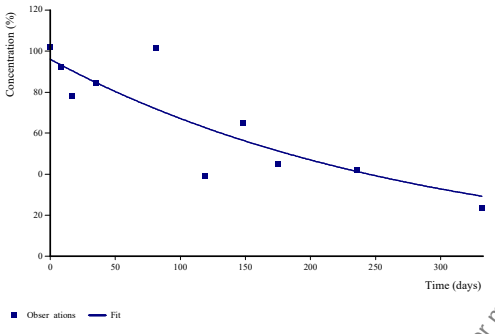
Table 7.1.2.2.1-101: Kinetic models and goodness-of-fit statistics of pathway fits for trial Iowa of study [redacted] (1993, CA 7.1.2.2.1/006) – modelling endpoints

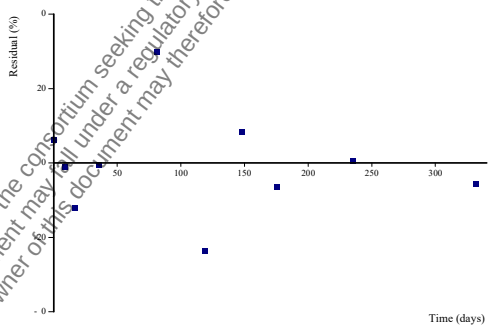
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glypho-sate: SFO	Good	96.1	k: 0.0036	15.9	k: <0.001	k: 0.0016	k: 0.006	194	643	
AMPA: SFO	Good	9.1	k: 0	16.8	k: 0.5	k: -0.0037	k: 0.004	>1000	>1000	0.502 (±0.277)

The degradation of glyphosate is well described by the pathway fit. The formation and decline of AMPA is visually well described by the fit. However, no reliable degradation endpoints can be derived as the metabolite concentration is still increasing towards the end of the study and thus, the estimated k-rate is not significantly different from zero. A decline fit for AMPA was not performed.

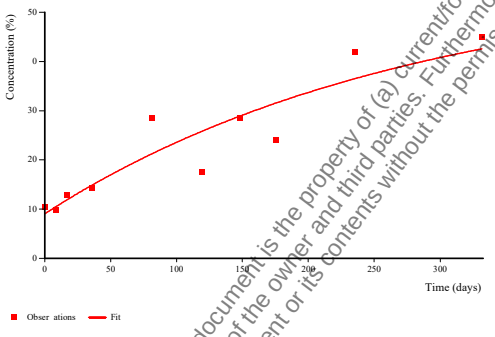
Conclusion: The pathway fit for trial Iowa is not considered acceptable for deriving modelling endpoints for parent and metabolite. Thus, modelling endpoints for glyphosate are derived from parent-only fit.

Glyphosate (SFO)





AMPA (SFO)



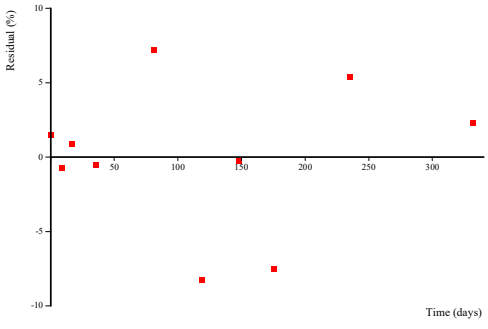
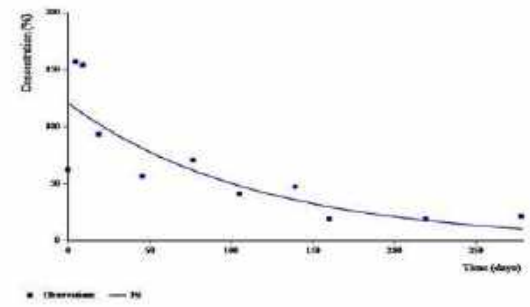
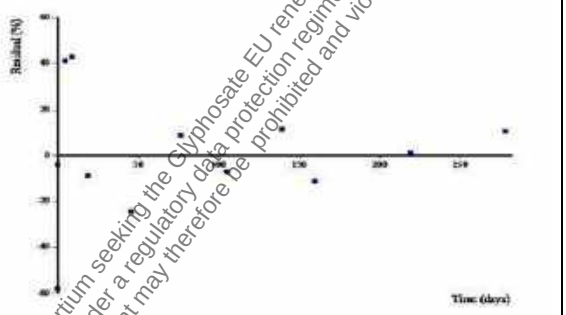


Table 7.1.2.2.1-102: Kinetic models and goodness-of-fit statistics of parent-only fits for trial New York of study [redacted] (1993, CA 7.1.2.2.1/006) – modelling endpoints

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	120.6	k: 0.0087	32.3	k: <0.05	k: 0.0019	k: 0.016	79.5	264
Due to the large scattering of the residue data, the clearly overestimated M_0 value and the resulting high χ^2 error, the SFO fit is not acceptable. **Conclusion**: No modelling endpoints can be derived for glyphosate.									
SFO  									

Summary of trigger and modelling endpoints

Table 7.1.2.2.1-103: Summary of trigger endpoints for glyphosate

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted] (1992, CA 7.1.2.2.1/014)	Silty clay	Ontario, Canada	7.9	0 – 20	2.4	49.2	15.9	FOMC
[redacted] (1993, CA 7.1.2.2.1/006)	Clay loam	Arizona, USA	8.0	0 – 60	-[2]	-[2]	-	-
	Loamy sand	California, USA	6.3	0 – 60	13.5	101	12.7	FOMC
	Silty clay loam	Iowa, USA	6.0	0 – 60	147	>1000	14.6	FOMC
	Loam	Minnesota, USA	6.5	0 – 60	-[2]	-[2]	-	-
	Sandy clay loam	New York, USA	5.8	0 – 60	-[2]	-[2]	-	-
	Loam	Ohio, USA	7.8	0 – 60	2.1	62.8	13.5	DFOP
[redacted] (1993, CA 7.1.2.2.1/005)	Loamy sand	Ontario, Canada	6.8	0 – 45	9.5	53.9	16.4	DFOP
[redacted] (1989, CA 7.1.2.2.1/016)	Sandy loam	California, USA	7.1	0 – 32	5.4	35.3	5.1	FOMC

[1] Medium unknown
[2] No reliable endpoint could be determined

Table 7.1.2.2.1-104: Summary of trigger endpoints for AMPA

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	DT_{50} (d)	DT_{90} (d)	χ^2 error (%)	Kinetic model
[redacted] (1992, CA 7.1.2.2.1/014)	Silty clay	Ontario, Canada	7.9	0 – 20	155	514	16.5	FOMC-SFO
[redacted] (1993, CA 7.1.2.2.1/006)	Clay loam	Arizona, USA	8.0	0 – 60	97.6	630	15.3	DFOP[3]
	Loamy sand	California, USA	6.3	0 – 60	-[2]	-[2]	-	-
	Silty clay loam	Iowa, USA	6.0	0 – 60	-[2]	-[2]	-	-
	Loam	Minnesota, USA	6.5	0 – 60	302	>1000	10.3	SFO[3]
	Sandy clay loam	New York, USA	5.8	0 – 60	-[2]	-[2]	-	-
	Loam	Ohio, USA	7.8	0 – 60	65.0	216	17.5	DFOP-SFO
[redacted] (1993, CA 7.1.2.2.1/005)	Loamy sand	Ontario, Canada	6.8	0 – 45	-[2]	-[2]	-	-
[redacted] (1989, CA 7.1.2.2.1/016)	Sandy loam	California, USA	7.1	0 – 32	111	370	15.4	FOMC-SFO

[1] Medium unknown
[2] No reliable endpoint could be determined
[3] Decline fit

Table 7.1.2.2.1-105: Summary of modelling endpoints for glyphosate

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	$DegT_{50}$ (d) Norm.[2]	χ^2 error (%)	Kinetic model
[redacted] (1992, CA 7.1.2.2.1/014)	Silty clay	Ontario, Canada	7.9	0 – 20	-[3]	-	-
[redacted] (1993, CA 7.1.2.2.1/006)	Clay loam	Arizona, USA	8.0	0 – 60	-[3]	-	-
	Loamy sand	California, USA	6.3	0 – 60	32.6	22.0	SFO
	Silty clay loam	Iowa, USA	6.0	0 – 60	182	15.9	SFO
	Loam	Minnesota, USA	6.5	0 – 60	-[3]	-	-
	Sandy clay loam	New York, USA	5.8	0 – 60	-[3]	-	-
	Loam	Ohio, USA	7.8	0 – 60	-[3]	-	-
[redacted] (1993, CA 7.1.2.2.1/005)	Loamy sand	Ontario, Canada	6.8	0 – 45	-[3]	-	-
[redacted] (1989, CA 7.1.2.2.1/016)	Sandy loam	California, USA	7.1	0 – 32	-[3]	-	-

[1] Medium unknown
[2] $DegT50_{matrix}$ according to EFSA (2014)
[3] No reliable endpoint could be determined

Table 7.1.2.2.1-106: Summary of modelling endpoints for AMPA

Study	Soil type (USDA)	Location	pH[1]	Depth (cm)	$DegT_{50}$ (d) Norm.[2]	ff (-)	χ^2 error (%)	Kinetic model
[redacted] (1992, CA 7.1.2.2.1/014)	Silty clay	Ontario, Canada	7.9	0 – 20	-[3]	-[3]	-	-
[redacted] (1993, CA 7.1.2.2.1/006)	Clay loam	Arizona, USA	8.0	0 – 60	303	-	21.1	SFO[4]
	Loamy sand	California, USA	6.3	0 – 60	-[3]	-[3]	-	-
	Silty clay loam	Iowa, USA	6.0	0 – 60	-[3]	-[3]	-	-
	Loam	Minnesota, USA	6.5	0 – 60	-[3]	-[3]	-	-
	Sandy clay loam	New York, USA	5.8	0 – 60	-[3]	-[3]	-	-
	Loam	Ohio, USA	7.8	0 – 60	-[3]	-[3]	-	-
[redacted] (1993, CA 7.1.2.2.1/005)	Loamy sand	Ontario, Canada	6.8	0 – 45	-[3]	-[3]	-	-
[redacted] (1989, CA 7.1.2.2.1/016)	Sandy loam	California, USA	7.1	0 – 32	-[3]	-[3]	-	-

[1] Medium unknown
[2] $DegT50_{matrix}$ according to EFSA (2014)
[3] No reliable endpoint could be determined
[4] Decline fit

3. Assessment and conclusion

Assessment and conclusion by applicant:
The kinetic evaluation was performed according to the current guidances without any deviations. Thus, the study is considered valid and the provided endpoints can be used for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/004
Report author	[redacted]
Report year	1994
Report title	Touchdown: Field dissipation study for terrestrial uses, Champaign, Illinois, 1988-1989 residue data to support registrations of products containing Glyphosate-trimesium as active ingredient
Report No	RR 93-027B
Document No	
Guidelines followed in study	US EPA 164-1

GLP/Officially recognised testing facilities	Yes
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: terrestrial field dissipation Test item: Touchdown, a.s.: glyphosate-trimesium Test sites: one locations in USA, planted with wheat Soil characterization (upper soil layer): – Silty clay loam – OM: 3.6 % – pH: 6.0 (medium not stated) Application rate: 9 kg a.s./ha, single application Application method: tractor-mounted CO_2 pressurized broadcast spray applicator; to wheat and pigweed cover Application timing: 27 May 1988, 24 days after planting, ground cover: 50 – 60 % Sampling times: 12 events, -1, 0, 1, 3, 7, 14, 28, 61, 90, 171, 382, 517 DAT Sampling method: core samplers Sampling depths: 0 – 8.9 cm, 8.9 – 39.4 cm, 39.4 - 100.3 cm Tillage: no cultural practices after application of the herbicide Sample storage: frozen directly after sampling and kept frozen until sample preparation Workup and analysis: – Mixing by hand – extraction with ammonium hydroxide and potassium phosphate – Derivatization with trifluoroacetic anhydride and heptafluorobutanol – analysis by GC/MSD, LOQ = 0.05 mg/kg Recovery in fortified samples: Glyphosate: mean: 85 %, coeff. of var. (CV): 13 % AMPA: mean: 85 %, CV: 15 %
Short description of results:	Residues: Glyphosate: (0 – 8.9 cm depth) – 2.5 mg/kg (0 DAT) Max.: 2.9 mg/kg (1 DAT) – <0.05 mg/kg (517 DAT) AMPA: (0 – 8.9 cm depth) – Max.: 0.44 mg/kg (90 DAT) – 0.17 mg/kg (517 DAT) No residues of glyphosate and AMPA >0.05 mg/kg were found in any other soil layer Half-life times: calculated based on a least-squares fit of the linear transformation of a exponential function (for the first soil layer): – Glyphosate: 79 days (r = 0.969) – AMPA: 419 days (r = 0.843) Glyphosate and AMPA show no evidence to leach below the 8.9 cm soil layer.

Reasons why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: – The test was conducted on cropped plots – High application rate (9 kg/ha)
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.2.2.1/005
Report author	[redacted]
Report year	1993
Report title	The terrestrial field dissipation of Glyphosate in Canadian soil
Report No	MSL-12605
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - rather low sampling depth (45 cm) - the missing plot management history for 2 sites weather data reported as monthly avaerges, only
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive summary

A terrestrial field dissipation study was conducted to determine the rate of dissipation and vertical mobility of glyphosate and its major soil metabolite, aminomethyl-phosphonic acid (AMPA), under actual field conditions. Glyphosate, formulated as the isopropylamine salt, is the active ingredient in Roundup. Roundup herbicide was applied at the maximum annual use rate of 4.27 kg a.s./ha related to glyphosate salt at three locations in Canada representing diverse soil types and climatic conditions. Soil samples were collected prior to and on the day of application as well as on 9-11 samplings in a range of 457 to 537 days. Residue data were obtained for glyphosate and AMPA on soil samples collected to a depth of 45 cm. This study also demonstrated that glyphosate and AMPA possess limited potential for vertical mobility in soil as the glyphosate residues in the 15-30 and 30-45 cm soil horizons were less than 0.060 and 0.039 mg/kg, respectively. Maximum average glyphosate residue levels in the 0-15 cm soil horizon were 1.081, 0.801 and 0.671 mg/kg at 0 DAT for the Alberta, Manitoba and Ontario test sites, respectively, and then dissipated to 0.084, 0.019 and below LOD, respectively, at the last sampling date. AMPA was found in the day 0 samples, demonstrating how rapidly glyphosate is degraded in soil. Maximum average AMPA residue levels in the 0-15 cm soil horizon were 0.170, 0.165, and 0.144 mg/kg and occurred at 365, 58, and 86 days after test substance application for the Alberta, Manitoba and Ontario test sites, respectively, and then dissipated to 0.128, 0.036, and below LOD, respectively, at the last sampling date.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation:	Roundup
Lot No. Alberta:	LUL-9101-2706-F
Lot No. Manitoba, Ontario:	PIT-8912-1385-A
Nominal concentration:	41 % as glyphosate salt 31 % as glyphosate

B. STUDY DESIGN

1. Test sites

Three test sites were selected, one in each of three representative provinces: Alberta, Manitoba, and Ontario. These three test sites encompass diverse climatological conditions, soil types, and geography which are representative of the wide range of conditions under which glyphosate would be used under normal agronomic practices. Four test plots were established at each test site: one untreated (control) test plot and three replicate, treated test plots. The untreated (control) test plot was separated from the nearest treated test plot by a minimum of a 38 meter buffer zone. The replicate, treated test plots were separated by a minimum of a 10 meter buffer zone. The replicate treated test plots ranged in size from 45 to 60 m². Soil cores were taken from the trial sites prior to application to determine the soil properties. An overview of the soil characterisation is given below.

Table 7.1.2.2.1-107: Characteristics of test soils

Parameter	**Lamont, Alberta test site**		
Soil depth (cm)	0-15	15-30	30-45
Textural Class (USDA)	loam	loam	sandy clay loam
Sand (50 µm – 2 mm) (%)	50.0	50.0	52.0
Silt (2 µm – 50 µmm) (%)	28.0	28.0	20.0
Clay (< 2 µm) (%)	22.0	22.0	28.0
pH[2]	6.5	6.5	6.6
Organic carbon (%)[1]	1.9	1.6	1.7
Organic matter (%)	3.3	2.8	2.9
Cation exchange capacity (meq/100 g)	15.9	18.8	19.5
Water Holding Capacity at 1/3 bar (%)	30.0	29.5	31.2
Bulk Density (disturbed) (g/cm^3)	1.07	1.05	1.05
Parameter	**Oakville, Manitoba test site**		
Soil depth (cm)	0-15	15-30	30-45
Textural Class (USDA)	loam	sandy clay loam	sandy loam
Sand (50 µm – 2 mm) (%)	44.0	50.0	68.0
Silt (2 µm – 50 µmm) (%)	32.0	26.0	14.0
Clay (< 2 µm) (%)	24.0	24.0	18.0
pH[3]	7.3	7.8	7.9
Organic carbon (%)[1]	3.5	1.7	1.0
Organic matter (%)	6.0	3.0	1.7
Cation exchange capacity (meq/100 g)	29.6	30.0	27.8
Water Holding Capacity at 1/3 bar (%)	35.9	32.1	22.5
Bulk Density (disturbed) (g/cm^3)	1.00	1.04	1.13

Table 7.1.2.2.1-107: Characteristics of test soils

Parameter	Ayr, Ontario test site		
Soil depth (cm)	0-15	15-30	30-45
Textural Class (USDA)	loamy sand	sand	sand
Sand (50 µm – 2 mm) (%)	80.0	88.0	92.0
Silt (2 µm – 50 µmm) (%)	16.0	8.0	6.0
Clay (< 2 µm) (%)	4.0	4.0	2.0
pH[2]	6.8	7.3	7.7
Organic carbon (%)[1]	1.2	0.7	0.4
Organic matter (%)	2.0	1.2	0.7
Cation exchange capacity (meq/100 g)	10.4	10.2	13.3
Water Holding Capacity at 1/3 bar (%)	16.3	13.4	10.9
Bulk Density (disturbed) (g/cm^3)	1.33	1.37	1.42

[1] Calculated from organic matter according to OC = OM × 0.58
[2] Medium not given

The Ontario test site had a known two-year history of crop and pesticide use and had not been treated with Roundup herbicide or related chemistry during the two years preceding this study. Two-year crop and pesticide use histories were not reported for the Alberta and Manitoba test sites. However, the absence of detectable levels of glyphosate and AMPA in soil samples collected prior to test substance application at the Alberta and Manitoba test sites demonstrated that there were no glyphosate residues which could potentially compromise the integrity of this study. Test plots were maintained in a weed free condition by the use of paraquat herbicide at all three locations.

Weather data were collected for each location from nearby, permanent, institutional weather recording stations. The climatological data indicate that climatic conditions at all test sites during the study were within the normal ranges and revealed no major deviations from expected weather patterns, with the exception of the Alberta test which received only about 60 % of the average 30 year historical precipitation.

2. Application
Single applications of Roundup herbicide were made to each bare ground, replicate test plot at each test site according to label directions using normal agronomic practices. At the Alberta test site, all three replicate test plots were treated at an application rate of 4.27 kg a.s./ha using a total spray solution volume of 110 L/ha. At the Manitoba test site, all three replicate test plots were treated at an application rate of 4.27 kg a.s./ha using a total spray solution volume of 122 L/ha. At the Ontario test site, the actual application rates and spray solution volumes for the .three replicate test plots were, respectively, 4.21, 4.07 and 4.27 kg a.s./ha and 147.8, 142.9 and 150.0 L/ha. Test substance application spray equipment was calibrated prior to test substance application at all three locations.

3. Sampling
Soil samples were randomly collected from both the treated and control test plots at each test site and sampling event. Early time point soil samples to define the dissipation of glyphosate were collected at 1,7,14, and 21 days after test substance application at all test sites, with the exception of the Ontario test site for which the 21 days after application samples were not collected. Longer term time point samples were collected at approximately 1,2,3,12, and 16 months after test substance application at the Alberta test site, 1,2, 3, 4, 5, 12 and 17 months after application at the Manitoba test site, and 1, 2, 3, 4, 6, 12 and 18 months after application at the Ontario test site. For the 0 days after application sampling at all three test sites and the sampling prior to application at the Alberta test site, samples were collected to a depth of 15 cm. For all other sampling events 10 soil cores to a depth of 45 cm were randomly collected from each of three replicate test plots and the untreated control test plot. Soil cores were collected using "zero contamination" commercial soil coring equipment with removable acetate liners.

4. Specimen handling and preparation

All samples were frozen within 2 hours following collection and were maintained in frozen storage until they were shipped frozen to the Sponsor's testing facility via overnight air delivery. Following receipt at the Sponsor's testing facility, the 10 samples from each test plot at each test site were sectioned into 15 cm depth increments (e.g. 0-15 cm, 15-30 cm and 30-45 cm), thawed, and composited to afford 4 representative samples per depth increment per sampling event; i.e. one composited sample for each of the three replicate, treated test plots and one composited sample for the untreated (control) test plot. Following compositing, samples were refrozen within 4 hours and maintained in frozen storage until analysis. Untreated (control) soil cores were sectioned first. Sectioning was conducted from the bottom of the soil cores to the top to prevent contamination of samples.

5. Analytical methods

Glyphosate and AMPA were extracted from soil using a 0.5 N KOH solution. The extract solution was eluted through a Chelex 100 resin in the Fe(III) form, which retains glyphosate and AMPA due to chelation to Fe(III). The retained glyphosate and AMPA iron salts are removed from the Chelex resin by elution with 6 N HCl. The isolated glyphosate and AMPA iron salts are then applied to a strong anion exchange resin and eluted with 6 N HCl to remove the iron and obtain the free acids of glyphosate and AMPA. After concentration to dryness, to remove the HCl, the samples are re-dissolved in water and analysed by high pressure liquid chromatography (HPLC). The chromatograph uses column switching and a o-phthalaldehyde post-column reactor with a fluorescence detector to separate and quantitate glyphosate and AMPA. In the post-column reactor, glyphosate is oxidised to a primary amine which then reacts with o-phthalaldehyde to form a fluorescence derivative. AMPA reacts directly with o-phthalaldehyde to form a second fluorescence derivative.

This method has been validated down to 0.05 mg/kg for both glyphosate and AMPA in 30 g soil samples and generally affords recoveries of glyphosate from fortified check samples which are greater than 70 %. AMPA recoveries are normally higher than glyphosate recoveries. The recoveries from check samples fortified over the range of 0.05 mg/kg to 2.00 mg/kg with both glyphosate and AMPA, averaged across all test sites, were 83.75 % and 81.88 %, respectively, for glyphosate and AMPA. The average recoveries of glyphosate ranged from a high of 96.61 % from soil from the Alberta test site to a low of 72.58 % from soil from the Manitoba test site. Average recoveries of AMPA ranged from a high of 87.71 % from soil from the Alberta test site to a low of 78.95 % from soil from the Ontario test site.

The limit of detection (LOD) was set at 0.01 mg/kg for glyphosate and 0.03 mg/kg for AMPA.

The stability of glyphosate and AMPA in soil was confirmed by a storage stability study (see [redacted], 1993, CA 7.1.2.2.1/007).

II. RESULTS AND DISCUSSION

A. DATA

Results of glyphosate and metabolite AMPA residues analysis in soil extracts of the 3 test sites are summarised in Table 7.1.2.2.1-108 to Table 7.1.2.2.1-113.

Table 7.1.2.2.1-108: Results of glyphosate residues (mg/kg) analysis in Alberta soil following treatment with Roundup at 4.27 kg/ha

Depth (cm)	DAT											
	Replicate	-1[5]	0	1	7	14	21	30	62	91	365	457
0-15	A	<LOD	1.081	0.680	0.616	0.393	0.384	0.220	0.212	0.252	0.178	0.128
	B	<LOD	1.036	0.624	0.801	0.331	0.375	0.679	0.170	0.416	0.147	0.119
	C	<LOD	1.125	0.482	0.499	0.265	0.273	0.519	0.176	0.013	0.113	0.005[4]
	Mean	<LOD	1.081	0.595	0.639	0.330	0.344	0.473	0.186	0.227	0.143	0.084
15-30	A	-	-	0.021	0.041	0.040	0.051	0.066	0.033	0.027	0.007[1]	0.005[1]
	B	-	-	0.069	0.026	0.020	0.031	0.045	0.026	0.034	0.010	<LOD
	C	-	-	0.033	0.020	0.021	0.034	0.040	0.030	0.211	0.009[1]	0.005[1]
	Mean	-	-	0.041	0.029[1]	0.027	0.039	0.050	0.030	0.031[2]	0.009[4]	0.003[4]
30-45	A	-	-	0.041	0.039	0.023	0.028	0.042	0.058	0.011	<LOD	0.009[1]
	B	-	-	0.033	0.058	0.019	0.025	0.042	0.005[1]	0.014	0.007[1]	0.005[1]
	C	-	-	0.035	0.084	0.022	0.027	0.034	0.010	0.006[1]	0.008[1]	0.043[3]
	Mean	-	-	0.036	0.060[1]	0.021	0.028	0.039	0.024	0.010	0.005[4]	0.007[4]

DAT: days after treatment
LOD = 0.01 mg/kg
[1] The 15-30 and 30-45 cm depth interval samples are believed to have been inadvertently reversed during sample compositing.
[2] Glyphosate residue levels of 0.027, 0.034, and 0.211 mg/kg were found in the three replicate samples for this depth interval and sampling event. The sample with the 0.211 mg/kg level was considered to be an outlier, and was not included in the calculation of the average residue level.
[3] Glyphosate residue levels of 0.009, 0.005 and 0.043 mg/kg were found in the three replicate samples for this depth interval and sampling event. The sample with the 0.043 mg/kg level was considered to be an outlier and was not included in the calculation of the average residue level.
[4] <LOD
[5] Four values were measured, all being <LOD

Table 7.1.2.2.1-109: Results of glyphosate residues (mg/kg) analysis in Manitoba soil following treatment with Roundup at 4.27 kg/ha

Depth (cm)	DAT													
	Rep.	-5	0	1	7	14	21	28	58	92	120	150	366	512
0-15	A	<LOD[2]	0.740	0.741	0.427	0.369	0.298	0.426	0.142	0.028	0.014	0.002[1]	0.009[1]	0.035
	B	<LOD	0.880	0.543	0.704	0.459	0.156	0.221	0.086	0.008[1]	0.013	0.001[1]	0.014	0.008[1]
	C	<LOD	0.783	0.497	0.495	0.481	0.338	0.271	0.062	0.009[1]	0.004[1]	0.022	<LOD	0.014
	Mean	<LOD	0.801	0.594	0.542	0.436	0.264	0.306	0.097	0.015	0.010	0.008[1]	0.008[1]	0.019
15-30	A	<LOD[3]	-	0.007[1]	0.010	0.012	0.020	0.015	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	0.050	0.006[1]	0.018	0.020	0.018	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	0.026	0.005[1]	0.016	0.025	0.018	<LOD	<LOD	<LOD	<LOD	<LOD	0.002[1]
	Mean	<LOD	-	0.028	0.007[1]	0.015	0.022	0.017	<LOD	<LOD	<LOD	<LOD	<LOD	0.001[1]
30-45	A	<LOD	-	0.020	0.007[1]	0.008[1]	<LOD	0.013	<LOD	<LOD	<LOD	<LOD	<LOD	0.003[1]
	B	<LOD	-	0.026	0.005[1]	0.012	0.019	<LOD	<LOD	<LOD	0.011	<LOD	<LOD	0.004[1]
	C	<LOD	-	0.008[1]	0.004[1]	0.012	0.014	0.016	<LOD	<LOD	<LOD	<LOD	<LOD	0.002[1]
	Mean	<LOD	-	0.018	0.005[1]	0.011	0.011	0.010	<LOD	<LOD	0.004[1]	<LOD	<LOD	0.003[3]

Table 7.1.2.2.1-109: Results of glyphosate residues (mg/kg) analysis in Manitoba soil following treatment with Roundup at 4.27 kg/ha

[1] <LOD
[2] five values were measured, all being < LOD
[3] four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.01 mg/kg
Rep. = Replicate

Table 7.1.2.2.1-110: Results of glyphosate residues (mg/kg) analysis in Ontario soil following treatment with Roundup at 4.18 kg/ha

Depth (cm)	DAT												
	Replicate	-1	0	1	7	14	28	57	86	129	177	364	537
0-15	A	<LOD [2]	0.678	0.493	0.496	0.425	0.173	0.051	0.039	0.025	0.029	0.027	0.001[1]
	B	<LOD	0.562	0.427	0.428	0.348	0.119	0.052	0.050	0.016	0.020	0.010	0.003[1]
	C	<LOD	0.773	0.686	0.562	0.442	0.160	0.064	0.052	0.034	0.035	0.054	0.010[1]
	Mean	<LOD	0.671	0.535	0.495	0.405	0.151	0.056	0.047	0.025	0.028	0.030	0.005[1]
15-30	A	<LOD [2]	-	<LOD	0.004[1]	<LOD	0.003[1]	<LOD	<LOD	<LOD	<LOD	<LOD	-0.002[1]
	B	<LOD	-	<LOD	0.004[1]	<LOD	0.001[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	<LOD	0.009[1]	<LOD	0.004[1]	<LOD	<LOD	<LOD	<LOD	<LOD	-0.002[1]
	Mean	<LOD	-	<LOD	0.006[1]	<LOD	0.003[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-45	A	<LOD	-	<LOD	<LOD	<LOD	-0.001[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	<LOD	0.003[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	-	<LOD	0.001[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[1] <LOD
[2] Four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.01 mg/kg

Table 7.1.2.2.1-111: Results of AMPA residues (mg/kg) analysis in Alberta soil following treatment with Roundup at 4.27 kg/ha

Depth (cm)	DAT											
	Replicate	-1 [2]	0	1	7	14	21	30	62	91	365	457
0-15	A	<LOD	0.035	0.024[1]	0.037	0.032	0.045	0.034	0.063	0.061	0.270	0.145
	B	<LOD	0.031	0.028[1]	0.056	0.043	0.057	0.085	0.049	0.121	0.104	0.240
	C	<LOD	0.037	0.020[1]	0.045	0.037	0.042	0.083	0.051	<LOD	0.136	<LOD
	Mean	<LOD	0.034	0.024[1]	0.046	0.037	0.048	0.067	0.054	0.061	0.170	0.128
15-30	A	-	-	<LOD	<LOD	<LOD	<LOD	0.016[1]	0.014[1]	<LOD	0.028[1]	<LOD
	B	-	-	0.017[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.019[1]	<LOD
	C	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.080[1]	<LOD	<LOD
	Mean	-	-	0.006[1]	<LOD	<LOD	<LOD	0.005[1]	0.005[1]	0.027[1]	0.016[1]	<LOD

Table 7.1.2.2.1-111: Results of AMPA residues (mg/kg) analysis in Alberta soil following treatment with Roundup at 4.27 kg/ha

30-45	A	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	0.056	<LOD	<LOD	<LOD
	B	-	-	<LOD	0.002[1]	<LOD	<LOD	0.013[1]	<LOD	<LOD	<LOD	<LOD
	C	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.064
	Mean	-	-	<LOD	0.001[1]	<LOD	<LOD	0.004[1]	0.019[1]	<LOD	<LOD	0.021[1]

[1] <LOD
[2] Four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.03 mg/kg

Table 7.1.2.2.1-112: Results of AMPA (mg/kg) residues analysis in Manitoba soil following treatment with Roundup at 4.27 kg/ha

Depth (cm)	DAT													
	Repli-cate	-5	0	1	7	14	21	28	58	92	120	150	366	512
0-15	A	<LOD [2]	0.049	0.061	0.070	0.049	0.043	0.067	0.197	0.079	0.084	0.073	0.065	0.049
	B	<LOD	0.057	0.052	0.105	0.054	0.028[1]	0.046	0.143	0.061	0.087	0.067	0.072	0.031
	C	<LOD	0.050	0.043	0.075	0.064	0.075	0.054	0.155	0.060	0.074	0.074	0.033	0.027[1]
	Mean	<LOD	0.052	0.052	0.083	0.056	0.049	0.056	0.165	0.067	0.082	0.071	0.057	0.036
15-30	A	<LOD [3]	-	<LOD	<LOD	<LOD	0.012[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	0.012[1]	0.013[1]	<LOD	<LOD	<LOD	<LOD	<LOD	0.011[1]
	C	<LOD	-	0.011[1]	<LOD	<LOD	<LOD	0.012[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	-	0.004[1]	<LOD	<LOD	0.008[1]	0.008[1]	<LOD	<LOD	<LOD	<LOD	<LOD	0.004[1]
30-45	A	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.015[1]
	C	<LOD	-	<LOD	0.014[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	-	<LOD	0.005[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.005[1]

[1] <LOD
[2] Five values were measured, all being <LOD
[3] Four values were measured, all being <LOD
DAT: days after treatment
LOD = 0.03 mg/kg

Table 7.1.2.2.1-113: Results of AMPA residues (mg/kg) analysis in Ontario soil following treatment with Roundup at 4.18 kg/ha

Depth (cm)	DAT												
	Replicate	-1	0	1	7	14	28	57	86	129	177	364	537
0-15	A	<LOD [2]	0.118	0.105	0.116	0.137	0.119	0.082	0.133	0.084	0.163	0.099	0.008[1]
	B	0.028[1]	0.115	0.097	0.107	0.102	0.069	0.079	0.153	0.066	0.090	0.040	0.003[1]
	C	0.011[1]	0.114	0.112	0.118	0.137	0.092	0.093	0.147	0.120	0.140	0.178	0.024[1]
	Mean	0.013[1]	0.116	0.105	0.114	0.125	0.093	0.085	0.144	0.090	0.131	0.106	0.012[1]
15-30	A	<LOD [3]	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.015[1]	<LOD
	Mean	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.005[1]	<LOD
30-45	A	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[1] <LOD
[2] Four values were measured, being: < LOD, 0.028, 0.011 and 0.011
[3] Four values were measured, all being < LOD
DAT: days after treatment
LOD = 0.03 mg/kg

B. CHARACTERISATION OF RESIDUES

1. Alberta test site

The maximum average residue level of glyphosate in the 0 to 15 cm soil layer was 1.081 mg/kg at 0 days after treatment (DAT). Average glyphosate residues declined to 0.330 mg/kg by 14 DAT, increased to 0.473 mg/kg at 30 DAT, and then dissipated to 0.084 mg/kg at 457 DAT. Average glyphosate residues declined with depth and were below the LOD at 365 DAT for the 15-30 and 30-45 cm soil layers.

The average residue level of AMPA in the 0 to 15 cm soil layer was 0.034 mg/kg on the day of treatment and gradually increased to a maximum of 0.170 mg/kg by 365 DAT. AMPA residue levels were 0.128 mg/kg at the final sampling at 457 DAT. In the deeper soil layers the residue levels were below the LOD.

2. Manitoba test site

The average residue level of glyphosate in the 0 to 15 cm layer was 0.801 mg/kg at 0 DAT. Average glyphosate residues gradually dissipated to 0.019 mg/kg at 512 DAT. Average glyphosate residues greater than 0.01 mg/kg (the lower limit of detection) were only found in the 15-30 and 30-45 cm soil horizons for the 1, 14, 21 and 28 days after application sampling events. Average glyphosate residues declined with depth and were below the LOD at 58 DAT for the 15-30 and 30-45 cm soil layers.

The average AMPA residue level in the top 0-15 cm of soil measured 0.052 mg/kg at 0 DAT. Average AMPA residues reached a maximum concentration of 0.165 mg/kg at 58 DAT, and then declined to 0.036 mg/kg at 512 DAT. AMPA residues were less than 0.01 mg/kg in all soil samples taken below 15 cm.

3. Ontario test site

The maximum average residue level of glyphosate in the 0 to 15 cm soil layer was 0.671 mg/kg at 0 DAT, and declined steadily below LOD at 537 DAT. The average residue level of AMPA in the 0 to 15 cm soil layer measured 0.116 mg/kg at 0 DAT. Average AMPA residues reached a maximum of

0.144 mg/kg at 86 DAT, and then declined to 0.012 mg/kg at 537 DAT. Average glyphosate and AMPA residues were less than 0.01 mg/kg for all samples taken below 15 cm.

C. KINETICS

An Ecoregion Crosswalk exercise was performed ([redacted] 2020, CA 7.1.2.2.1/002). The trial in Ontario was found to be representative for European conditions and included in kinetic evaluation (Sachers, 2020, CA 7.1.2.2.1/003).

III. CONCLUSIONS

Maximum average glyphosate residue levels in the 0-15 cm soil horizon were 1.081, 0.801 and 0.671 mg/kg at 0 DAT for the Alberta, Manitoba and Ontario test sites, respectively, and then dissipated to 0.084, 0.019 and below LOD, respectively, at the last sampling date. AMPA was found in the day 0 samples, demonstrating how rapidly glyphosate is degraded in soil. Maximum average AMPA residue levels in the 0-15 cm soil horizon were 0.170, 0.165, and 0.144 mg/kg and occurred at 365, 58, and 86 days after test substance application for the Alberta, Manitoba and Ontario test sites, respectively, and then dissipated to 0.128, 0.036, and below LOD, respectively, at the last sampling date.

The results of this study also demonstrate that glyphosate and AMPA possess very limited potential for vertical mobility in soil, consistent with previous laboratory and field studies. The results obtained from the Alberta test site may be consistent with low levels of vertical mobility of glyphosate in the soil profile. However, for this location, it has been determined that the maximum glyphosate residues in the 15-30 and 30-45 cm soil horizons resulting from vertical mobility of glyphosate are less than 0.060 and 0.039 mg/kg, respectively. For the Manitoba test site, average glyphosate residues greater than 0.01 mg/kg (the lower limit of detection) were found in the 15-30 and 30-45 cm soil horizons for the 1, 14, 21, and 28 days after application sampling events. However, these residues can be attributed to contamination during sampling rather than vertical mobility of glyphosate in the soil. At the Ontario location, no glyphosate residues greater than or equal to 0.01 mg/kg were found in the 15-30 or 30-45 cm soil horizons at any sampling time. For all three test sites, AMPA residues in the 15-30 and 30-45 cm soil horizons were always less than 0.03 mg/kg (limit of detection).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study provides detailed information on the dissipation behavior of glyphosate under Canadian field conditions at different testing conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the rather low sampling depth (45 cm), the missing plot management history and the reporting of monthly averaged weather data. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/006
Report author	[redacted]
Report year	1993
Report title	The terrestrial field dissipation of glyphosate: Final report
Report No	MSL-12651
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: None
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive summary

A terrestrial field dissipation study was conducted to determine the rate of dissipation and vertical mobility of glyphosate and its major soil metabolite, aminomethyl-phosphonic acid (AMPA), under actual field conditions. Glyphosate, formulated as the isopropylamine salt, is the active ingredient in Roundup herbicide. Roundup was applied at the maximum annual use rate of 9.0 kg a.s./ha at eight locations across the United States representing a diversity of soil types and climatic conditions. Residue data were obtained for glyphosate and AMPA on soil samples collected to a depth of 121.9 cm. The vertical mobility of glyphosate and AMPA did not exceed 30.5 cm.

Maximum average glyphosate residue levels in the 0-15.2 cm soil horizon were 2.23, 0.62, 3.06, 2.34, 1.82, 4.58, 2.01 and 1.93 mg/kg occurred at 7, 0, 0, 1, 15, 14, 0 and 0 days after test substance application for the Arizona, California, Georgia, Iowa, Minnesota, New York, Ohio and Texas test sites, respectively, and then dissipated close to or below LOD, respectively, at the last sampling date. AMPA was found in the day 0 samples, demonstrating how rapidly glyphosate is degraded in soil. Maximum average AMPA residue levels in the 0-15 cm soil horizon were 0.56, 0.36, 0.60, 0.36, 0.43, 0.48, 0.60 and 0.27 mg/kg and occurred at 21, 14, 61, 62, 95, 90, 21 and 11 days after test substance application for the Arizona, California, Georgia, Iowa, Minnesota, New York, Ohio and Texas test sites, respectively, and then dissipated close to or below LOD, respectively, at the last sampling date with exception of the New York test side with a AMPA concentration of 0.36 mg/kg at 546 DAT.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation:	Roundup
Lot No. Alberta:	LUL-9101-2706-F
Nominal concentration:	41.0 % as glyphosate salt 30.4 % as glyphosate equivalent

B. STUDY DESIGN

1. Test sites

Eight test sites were selected, one in each of three representative provinces: Arizona, California, Georgia, Iowa, Minnesota, New York and Texas. These eight test sites encompass diverse climatological conditions, soil types, and geography which are representative of the wide range of conditions under which glyphosate would be used under normal agronomic practices. Two test plots were established at each test site: one untreated (control) test plot and one treated test plots. The treated test plot was divided in 3 subplots. The untreated (control) test plot was separated from the nearest treated test plot by a minimum of a 61 meter buffer zone. The replicate treated test plots ranged in size from 45 to 60 m². Soil cores were taken from the trial sites prior to application to determine the soil properties. An overview of the soil characterisation is given in Table 7.1.2.2.1-114 to Table 7.1.2.2.1-121.

Table 7.1.2.2.1-114: Characteristics of test soil for Arizona test site

Parameter	**Result**			
Soil depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
Textural Class (USDA)	clay loam	clay loam	clay loam	loam
Sand (50 µm – 2 mm) (%)	37.3	27.3	25.3	41.3
Silt (2 µm – 50 µmm) (%)	29.2	39.2	38.0	32.0
Clay (< 2 µm) (%)	33.5	33.5	36.7	26.7
pH[1]	8.0	8.2	8.2	8.4
Organic carbon (%)[2]	0.5	0.6	0.4	0.2
Organic matter (%)	0.9	1.0	0.7	0.4
Cation exchange capacity (meq/100 g)	32.3	31.5	29.7	26.8
Water Holding Capacity at 1/3 bar (%)	27.2	26.2	28.6	27.9
Bulk Density (disturbed) (g/cm³)	1.14	1.14	1.11	1.15
Parameter				
Soil depth (cm)	61.0-76.2	76.2-91.4	91.4-106.7	106.7-121.9
Textural Class (USDA)	sandy loam	sandy loam	loamy sand	-
Sand (50 µm – 2 mm) (%)	53.3	69.3	83.3	-
Silt (2 µm – 50 µmm) (%)	38.0	24.0	12.0	-
Clay (< 2 µm) (%)	8.7	6.7	4.7	-
pH[1]	8.3	8.3	8.4	-
Organic carbon (%)[2]	0.6	0.06	0.2	
Organic matter (%)	1.0	0.1	1.3	-
Cation exchange capacity (meq/100 g)	20.9	18.9	18.9	-
Water Holding Capacity at 1/3 bar (%)	25.4	22.7	27.1	-
Bulk Density (disturbed) (g/cm³)	1.28	1.28	1.19	-

[1] Medium not given
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-115: Characteristics of test soil for California test site

Parameter	Result			
Soil depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
Textural Class (USDA)	loamy sand	loamy sand	loamy sand	sand
Sand (50 µm – 2 mm) (%)	79.3	83.3	83.3	88.5
Silt (2 µm – 50 µmm) (%)	15.2	11.2	11.2	8.0
Clay (< 2 µm) (%)	5.5	5.5	5.5	3.5
pH[1]	6.3	6.3	6.5	6.5
Organic carbon (%)[2]	0.1	0.1	0.0	0.2
Organic matter (%)	0.2	0.2	0.0	0.3
Cation exchange capacity (meq/100 g)	5.1	4.4	4.4	3.9
Water Holding Capacity at 1/3 bar (%)	10.3	9.1	7.9	12.4
Bulk Density (disturbed) (g/cm^3)	1.46	1.47	1.48	1.42
Parameter				
Soil depth (cm)	61.0-76.2	76.2-91.4	91.4-106.7	106.7-121.9
Textural Class (USDA)	sand	loamy sand	loamy sand	-
Sand (50 µm – 2 mm) (%)	91.3	84.5	78.5	-
Silt (2 µm – 50 µmm) (%)	5.2	12.0	16.0	-
Clay (< 2 µm) (%)	3.5	3.5	5.5	-
pH[1]	6.9	7.0	7.1	-
Organic carbon (%)[2]	0.06	0.1	2.3	
Organic matter (%)	0.1	0.2	4.0	-
Cation exchange capacity (meq/100 g)	4.0	3.4	20.4	-
Water Holding Capacity at 1/3 bar (%)	10.5	12.7	16.5	-
Bulk Density (disturbed) (g/cm^3)	1.43	1.37	1.35	-

[1] Medium not given
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-116: Characteristics of test soil for Georgia test site

Parameter	Result			
Soil depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
Textural Class (USDA)	sand	loamy sand	sandy loam	sandy clay loam
Sand (50 µm – 2 mm) (%)	89.3	83.3	76.5	69.3
Silt (2 µm – 50 µmm) (%)	8.0	8.0	8.0	7.2
Clay (< 2 µm) (%)	2.7	8.7	15.5	23.5
pH[1]	6.8	5.8	4.8	4.9
Organic carbon (%)[2]	0.6	0.2	0.06	0.2
Organic matter (%)	1.1	0.4	0.1	0.3
Cation exchange capacity (meq/100 g)	3.2	2.8	4.4	6.0
Water Holding Capacity at 1/3 bar (%)	5.9	7.2	-	21.2
Bulk Density (disturbed) (g/cm^3)	1.56	1.47	1.40	1.27
Parameter				
Soil depth (cm)	61.0-76.2	76.2-91.4	91.4-106.7	106.7-121.9
Textural Class (USDA)	sandy clay loam	silt loam	sandy clay loam	sandy clay loam
Sand (50 µm – 2 mm) (%)	69.3	67.3	69.3	67.3

Table 7.1.2.2.1-116: Characteristics of test soil for Georgia test site

Silt (2 µm – 50 µmm) (%)	7.2	5.2	5.2	7.2
Clay (< 2 µm) (%)	23.5	27.5	25.5	25.5
pH[1]	4.9	4.8	4.3	4.3
Organic carbon (%)[2]	0.1	0.06	0.0	0.06
Organic matter (%)	0.2	0.1	0.0	0.1
Cation exchange capacity (meq/100 g)	6.4	6.6	5.5	5.3
Water Holding Capacity at 1/3 bar (%)	22.5	23.9	23.7	22.6
Bulk Density (disturbed) (g/cm^3)	1.27	1.26	1.28	1.26

[1] Medium not given
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-117: Characteristics of test soil for Iowa test site

Parameter	**Result**			
Soil depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
Textural Class (USDA)	silty clay loam	silty clay loam	silty clay	silty clay loam
Sand (50 µm – 2 mm) (%)	13.3	13.3	9.3	9.3
Silt (2 µm – 50 µmm) (%)	58.0	53.2	50.0	51.2
Clay (< 2 µm) (%)	28.7	33.5	40.7	39.5
pH[1]	6.0	6.0	5.7	6.2
Organic carbon (%)[2]	1.4	1.0	0.7	043
Organic matter (%)	2.4	1.7	1.2	0.6
Cation exchange capacity (meq/100 g)	20.5	22.6	23.7	20.9
Water Holding Capacity at 1/3 bar (%)	35.2	37.8	41.3	47.6
Bulk Density (disturbed) (g/cm^3)	1.03	1.09	1.06	0.89
Parameter				
Soil depth (cm)	61.0-76.2	76.2-91.4	91.4-106.7	106.7-121.9
Textural Class (USDA)	silty clay	silty clay loam	silty clay loam	silty clay loam
Sand (50 µm – 2 mm) (%)	11.3	9.3	13.3	9.3
Silt (2 µm – 50 µmm) (%)	45.2	53.2	49.2	53.2
Clay (< 2 µm) (%)	43.5	37.5	37.5	37.5
pH[1]	6.2	6.5	6.8	7.1
Organic carbon (%)[2]	0.2	0.4	0.1	0.1
Organic matter (%)	0.4	0.7	0.2	0.2
Cation exchange capacity (meq/100 g)	26.9	23.0	23.3	22.4
Water Holding Capacity at 1/3 bar (%)	42.2	44.1	44.4	45.6
Bulk Density (disturbed) (g/cm^3)	1.12	1.01	0.99	0.89

[1] Medium not given
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-118: Characteristics of test soil for Minnesota test site

Parameter	Result			
Soil depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
Textural Class (USDA)	loam	loam	loam	loam
Sand (50 µm – 2 mm) (%)	50.5	50.5	48.5	48.5
Silt (2 µm – 50 µmm) (%)	32.0	32.0	30.0	28.0
Clay (< 2 µm) (%)	17.5	17.5	21.5	23.5
pH[1]	6.5	6.8	7.1	7.6
Organic carbon (%)[2]	3.1	2.3	1.1	0.8
Organic matter (%)	5.3	3.9	1.9	1.3
Cation exchange capacity (meq/100 g)	7.0	22.3	20.1	20.7
Water Holding Capacity at 1/3 bar (%)	37.8	37.0	35.2	33.0
Bulk Density (disturbed) (g/cm^3)	1.08	1.11	1.15	1.15
Parameter				
Soil depth (cm)	61.0-76.2	76.2-91.4	91.4-106.7	106.7-121.9
Textural Class (USDA)	loam	silt loam	sandy loam	sandy loam
Sand (50 µm – 2 mm) (%)	48.5	26.0	57.3	67.3
Silt (2 µm – 50 µmm) (%)	28.0	53.3	23.2	14.0
Clay (< 2 µm) (%)	23.5	20.7	19.5	18.7
pH[1]	7.9	8.1	8.2	8.3
Organic carbon (%)[2]	0.5	0.1	0.1	0.1
Organic matter (%)	0.8	0.2	0.2	0.2
Cation exchange capacity (meq/100 g)	28.1	26.1	26.8	22.2
Water Holding Capacity at 1/3 bar (%)	30.5	29.5	26.2	26.1
Bulk Density (disturbed) (g/cm^3)	1.11	1.20	1.16	1.07

[1] Medium not given
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-119: Characteristics of test soil for New York test site

Parameter	Result			
Soil depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
Textural Class (USDA)	sandy clay loam	clay loam	clay loam	clay
Sand (50 µm – 2 mm) (%)	53.3	25.3	21.3	25.3
Silt (2 µm – 50 µmm) (%)	24.0	42.0	46.0	32.0
Clay (< 2 µm) (%)	22.7	32.7	32.7	42.7
pH[1]	5.8	6.4	7.3	7.3
Organic carbon (%)[2]	1.2	0.5	0.06	0.2
Organic matter (%)	2.1	0.8	0.1	0.3
Cation exchange capacity (meq/100 g)	10.6	13.6	25.9	29.3
Water Holding Capacity at 1/3 bar (%)	19.2	44.0	28.9	32.3
Bulk Density (disturbed) (g/cm^3)	1.14	1.09	1.17	1.12
Parameter				
Soil depth (cm)	61.0-76.2	76.2-91.4	91.4-106.7	106.7-121.9
Textural Class (USDA)	clay loam	loam	clay loam	loam
Sand (50 µm – 2mm) (%)	29.3	33.3	33.3	41.3
Silt (2 µm – 50 µmm) (%)	38.0	40.0	39.2	36.0

Table 7.1.2.2.1-119: Characteristics of test soil for New York test site

Clay (< 2 µm) (%)	32.7	26.7	27.5	22.7
pH[1]	7.5	7.6	7.8	8.1
Organic carbon (%)[2]	0.1	0.06	0.1	0.0
Organic matter (%)	0.2	0.1	0.2	0.0
Cation exchange capacity (meq/100 g)	28.8	25.5	24.6	24.3
Water Holding Capacity at 1/3 bar (%)	34.6	21.5	23.9	21.0
Bulk Density (disturbed) (g/cm^3)	1.15	1.15	1.24	1.21

[1] Medium not given
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-120: Characteristics of test soil for Ohio test site

Parameter	**Result**			
Soil depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
Textural Class (USDA)	loam	clay loam	clay	clay
Sand (50 µm – 2 mm) (%)	27.3	33.3	23.3	21.3
Silt (2 µm – 50 µmm) (%)	49.2	39.2	33.2	33.2
Clay (< 2 µm) (%)	23.5	27.5	43.5	45.5
pH[1]	7.8	7.6	7.5	7.7
Organic carbon (%)[2]	0.8	0.7	0.6	0.5
Organic matter (%)	1.3	1.2	1.1	0.9
Cation exchange capacity (meq/100 g)	17.6	12.2	18.6	21.0
Water Holding Capacity at 1/3 bar (%)	28.8	29.2	34.5	35.7
Bulk Density (disturbed) (g/cm^3)	1.10	1.06	1.11	1.11
Parameter				
Soil depth (cm)	61.0-76.2	76.2-91.4	91.4-106.7	106.7-121.9
Textural Class (USDA)	clay loam	clay loam	loam	loam
Sand (50 µm – 2 mm) (%)	31.3	30.0	34.0	36.0
Silt (2 µm – 50 µmm) (%)	31.2	37.3	39.3	37.3
Clay (< 2 µm) (%)	37.5	32.7	26.7	26.7
pH[1]	8.0	8.2	8.4	8.4
Organic carbon (%)[2]	0.5	0.3	0.4	0.2
Organic matter (%)	0.8	0.5	0.7	0.3
Cation exchange capacity (meq/100 g)	20.9	26.4	30.1	23.3
Water Holding Capacity at 1/3 bar (%)	32.8	28.6	24.0	22.8
Bulk Density (disturbed) (g/cm^3)	1.13	1.21	1.26	1.26

[1] Medium not given
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-121: Characteristics of test soil for Texas test site

Parameter	**Result**			
Soil depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
Textural Class (USDA)	silt loam	loam	loam	silt loam
Sand (50 µm – 2 mm) (%)	22.0	34.0	38.0	30.0
Silt (2 µm – 50 µmm) (%)	57.3	45.3	39.3	53.3
Clay (< 2 µm) (%)	20.7	20.7	22.7	16.7
pH[1]	8.2	8.3	8.3	8.3
Organic carbon (%)[2]	0.5	0.4	0.4	0.4
Organic matter (%)	0.9	0.7	0.7	0.6
Cation exchange capacity (meq/100 g)	26.7	26.2	27.6	25.0
Water Holding Capacity at 1/3 bar (%)	31.5	31.1	28.4	28.9
Bulk Density (disturbed) (g/cm^3)	1.25	1.26	1.31	1.27
Parameter				
Soil depth (cm)	61.0-76.2	76.2-91.4	91.4-106.7	106.7-121.9
Textural Class (USDA)	sandy loam	loam	loam	sandy loam
Sand (50 µm – 2 mm) (%)	56.0	48.0	51.3	59.3
Silt (2 µm – 50 µmm) (%)	33.3	37.3	39.2	33.2
Clay (< 2 µm) (%)	10.7	14.7	9.5	7.5
pH[1]	8.3	8.2	7.8	8.1
Organic carbon (%)[2]	0.2	0.3	0.2	0.3
Organic matter (%)	0.3	0.5	0.4	0.5
Cation exchange capacity (meq/100 g)	23.0	24.3	24.0	22.3
Water Holding Capacity at 1/3 bar (%)	25.8	26.9	25.2	23.4
Bulk Density (disturbed) (g/cm^3)	1.26	1.26	1.28	1.23

[1] Medium not given
[2] Calculated from organic matter according to OC = OM × 0.58

All test sites had a known two-year history of crop and pesticide use, and none of the test sites had been treated with Roundup herbicide or related chemistry during the two years preceding this study. Test plots were maintained in a weed free condition by hand weeding and/or the use of maintenance herbicides which were approved in advance. Irrigation was applied when necessary and where it was consistent with the local crop-growing practices or required to compensate for deficiencies of rainfall. Weather data was collected at each location from test site research station instruments and/or from nearby, permanent, institutional weather recording stations (NOAA and others). The climatological data indicate that environmental conditions at all test sites during the study were within the normal conditions and revealed no major deviations from expected weather patterns. The prescribed sampling schedule was not significantly altered by climatological factors at any of the eight test sites, with the exception of the Iowa, Minnesota, and Texas test sites. The final 18 months after treatment samples were not taken at the Iowa and Minnesota test sites due to frozen ground conditions. As a result of extensive flooding of the Texas test site on December 23-25, 1991, the Texas test site was terminated on March 6, 1992. Therefore, no sampling from the Texas test site occurred following the 6 months after application sampling event.

2. Application

Single applications of Roundup herbicide were made to each bare ground, replicate test plot at each test site according to label directions using normal agronomic practices. The average application rates used for this study was 9.07 kg a.s./ha and ranged from a high rate of 9.90 kg a.s./ha at the California test Site to a low rate of 8.79 kg a.s./ha at the New York test Site. The total test substance spray solution volume ranged from 137.9 L/ha to 246.1 L/ha. Test substance application spray equipment was calibrated prior to application.

3. Sampling

Soil samples were randomly collected from both the treated and control test plots at each test site and sampling event. Early time point soil samples to define the dissipation of glyphosate were collected at 1, 7, 14, and 21 days after test substance application at all test sites with the exception of the Minnesota and Texas test sites. In the case of the Minnesota test site, the 14 days after application sampling event occurred 15 days after application. For the Texas test site, the 7, 14 and 21 days after application sampling events occurred on 12, 15, and 28 days after application. Longer term time point samples were collected at approximately 1, 2, 3, 4, 6, 12, 15, and 18 months after test substance application, with the exception of the Iowa and Minnesota test sites. For each sampling event, 18 soil core samples were collected from the treated test plot (6 from each of three subplots) to a depth of 121.9 cm. For the control test plot, 4 soil cores to a depth of 121.9 cm were collected at each sampling event. The untreated plot was always sampled first followed by the treated plot. With the exception of the 0-15 cm pre-excavation samples, soil samples at all sites were collected using "zero contamination" commercial soil coring equipment with removable acetate liners.

4. Specimen handling and preparation

The cores were cut into 15 cm sections in a clean area away from the field. Check (untreated) cores were sectioned first. Sectioning was performed from the bottom of the cores to the top of the cores to prevent contamination. Replicate soil cores for each sampling event for a given 15 cm depth increment were packaged together for storage and subsequent shipment to Monsanto. All samples were frozen within 4 hours of collection and were kept frozen during storage at the test site prior to shipment to Monsanto with one exception. The storage temperature for the 11 days after application (DAA) samples for the Texas location rose above freezing for approximately 24 hours due to equipment failure before the samples were transferred to another freezer and refrozen. All samples were shipped frozen to Monsanto, and all shipments were accompanied by an inventory list of the samples in the shipment that served as sample transfer document or chain-of-custody record.

5. Analytical methods

Glyphosate and AMPA were extracted from soil using a 0.5 N KOH solution. The extract solution was eluted through a Chelex 100 resin in the Fe(III) form, which retains glyphosate and AMPA due to chelation to Fe(III). The retained glyphosate and AMPA iron salts are removed from the Chelex resin by elution with 6 N HCl. The isolated glyphosate and AMPA iron salts are then applied to a strong anion exchange resin and eluted with 6 N HCl to remove the iron and obtain the free acids of glyphosate and AMPA. After concentration to dryness to remove the HCl, the samples are re-dissolved in water and analysed by high pressure liquid chromatography (HPLC). The chromatograph uses column switching and an o-phthalaldehyde post-column reactor with a fluorescence detector to separate and quantitate glyphosate and AMPA. In the post-column reactor, glyphosate is oxidised to a primary amine which then reacts with o-phthalaldehyde to form a fluorescence derivative. AMPA reacts directly with o-phthalaldehyde to form a second fluorescence derivative. This method has been validated down to 0.05 mg/kg for both glyphosate and AMPA in 30 g soil samples. Due to the varying degrees of glyphosate adsorption to different soil types, glyphosate recoveries from fortified check samples vary with soil type, and obtaining consistent recoveries of glyphosate is occasionally difficult. Nonetheless, the analytical method used generally affords recoveries of glyphosate from fortified check samples which are greater than 70 %. AMPA recoveries are normally higher than glyphosate recoveries. The recoveries from check samples fortified over the range of 0.05 mg/kg to 5.00 mg/kg with both glyphosate and AMPA, averaged across all test sites, were 77.8 % and 85.4 %, respectively, for glyphosate and AMPA. The average recoveries of glyphosate ranged from a high of 88.8 % from soil from the Georgia test site to a low of 65.0 % from soil from the Iowa test site. Average recoveries of AMPA ranged from a high of 87. 5 % from soil from the Minnesota test site to a low of 81.0 % from soil from the Iowa test site.

The limit of detection (LOD) was set at 0.02 mg/kg for glyphosate and 0.04 mg/kg for AMPA.

The stability of glyphosate and AMPA in soil was confirmed by a storage stability study (see [redacted] 1993, CA 7.1.2.2.1/007).

II. RESULTS AND DISCUSSION

A. DATA

Results of glyphosate and metabolite AMPA residues analysis in soil extracts of the 8 test sites are summarised in Table 7.1.2.2.1-122 to Table 7.1.2.2.1-137.

Table 7.1.2.2.1-122: Results of glyphosate residues (mg/kg) analysis in Arizona soil following treatment with Roundup at 9.05 kg/ha

Depth (cm)	DAT														
	Rep.	-1	0	1	7	14	21	28	64	92	122	184	364	462	553
0-15.2	A	<LOD	1.34	2.45	2.23	0.63	1.05	1.28	0.41	0.16	0.16	0.04	0.03	-0.01[1]	0.01[1]
	B	<LOD	1.27	2.57	2.67	0.53	4.50	0.57	0.37	0.13	0.12	0.02	0.04	-0.01[1]	<LOD
	C	<LOD	3.11	1.16	1.79	0.60	0.19	0.45	0.24	0.13	0.10	0.10	0.04	<LOD	<LOD
	Mean	<LOD	1.91	2.06	2.23	0.59	1.91	0.77	0.34	0.14	0.13	0.05	0.04	-0.01[1]	<LOD
15.2-30.5	A	<LOD	0.01[1]	0.01[1]	0.01[1]	<LOD	0.01[1]	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	0.01[1]	0.01[1]	0.18	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	-	0.02	<LOD	0.06	<LOD	0.03	<LOD	<LOD	<LOD	<LOD	0.08	0.01[1]	0.03	<LOD
	Mean	<LOD	0.01[1]	0.01[1]	0.08	<LOD	0.01[1]	<LOD	0.01[1]	<LOD	<LOD	0.03	<LOD	0.01[1]	<LOD
30.5-45.7	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
	B	<LOD	<LOD	<LOD	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	-	<LOD	<LOD	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
	Mean	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
45.7-61.0	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	0.01[1]	<LOD	-	-	-
	C	0.01[1]	<LOD	<LOD	<LOD	<LOD	0.15	<LOD	-	0.01[1]	<LOD	<LOD	-	-	-
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	0.05	<LOD	-	<LOD	<LOD	<LOD	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	-0.02[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	-0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD [2]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] A fourth value was measured, being < LOD (0.00)
Rep. = Replicate
DAT= days after treatment
LOD = 0.02 mg/kg

Table 7.1.2.2.1-123: Results of metabolite AMPA (mg/kg) residues analysis in Arizona soil following treatment with Roundup at 9.05 kg/ha

Depth (cm)	DAT													
	Rep.	**-1**	**0**	**1**	**7**	**14**	**21**	**28**	**64**	**92**	**122**	**184**	**364**	**462**	**553**
0-15.2	A	<LOD	0.07	0.16	0.21	0.21	0.46	0.47	0.48	0.26	0.37	0.27	0.14	0.08	0.07
	B	<LOD	0.08	0.11	0.23	0.16	1.16	0.37	0.52	0.27	0.34	0.15	0.14	0.05	0.05
	C	-	0.17	0.15	0.24	0.17	0.07	0.19	0.38	0.30	0.36	0.35	0.22	0.01[1]	0.04
	Mean	<LOD	0.11	0.14	0.23	0.18	0.56	0.34	0.46	0.28	0.36	0.26	0.17	0.04	0.05
15.2-30.5	A	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	0.04	<LOD	<LOD	<LOD	<LOD	<LOD	0.03[1]
	B	<LOD	<LOD	<LOD	0.02[1]	<LOD	<LOD	<LOD	0.03[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.09	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	0.02[1]	<LOD	<LOD	0.03[1]	<LOD	<LOD	0.01[1]
30.5-45.7	A	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
	Mean	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
45.7-61.0	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	0.03[1]	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	C	<LOD	<LOD	<LOD	<LOD	<LOD	0.18	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	Mean	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.06	<LOD	-	<LOD	<LOD	<LOD	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
Rep. = Replicate
DAT: days after treatment
LOD = 0.04 mg/kg

Table 7.1.2.2.1-124: Results of glyphosate residues (mg/kg) analysis in California soil following treatment with Roundup at 9.90 kg/ha

Depth (cm)	DAT														
	Rep.	-8	0	1	7	14	21	29	61	91	123	183	365	456	550
0-15.2	A	<LOD	1.36	2.11	1.47	0.56	0.44	0.58	0.48	0.19	0.05	0.07	0.04	0.03	<LOD
	B	<LOD	0.72	1.79	1.40	0.94	0.65	0.62	0.52	0.10	0.04	0.07	0.04	0.04	0.04
	C	-	1.27	1.75	0.85	1.78	0.45	0.67	0.46	0.17	0.06	0.09	0.04	0.04	0.03
	Mean	<LOD	1.12	1.94	1.24	1.09	0.51	0.62	0.49	0.15	0.05	0.08	0.04	0.04	0.02
15.2-30.5	A	<LOD	0.18	0.04	0.04	<LOD	0.03	0.03	<LOD	0.02	0.02	<LOD	<LOD	0.02	<LOD
	B	<LOD	0.38	0.04	0.04	<LOD	0.05	0.03	<LOD	0.01[1]	0.01[1]	<LOD	0.01[1]	<LOD	<LOD
	C	-	0.33	0.06	<LOD	<LOD	0.04	<LOD	0.06	0.02	<LOD	<LOD	0.01[1]	<LOD	<LOD
	Mean	<LOD	0.30	0.05	0.03	<LOD	0.04	0.02	0.02	0.02	0.01[1]	<LOD	0.01[1]	0.01[1]	<LOD
30.5-45.7	A	<LOD	0.24	0.01[1]	0.01[1]	0.01[1]	0.03	0.02	<LOD	<LOD	0.02	<LOD	<LOD	<LOD	<LOD
	B	0.01[1]	0.23	0.01[1]	0.01[1]	0.01[1]	0.01[1]	0.03	<LOD	0.01[1]	0.02	<LOD	<LOD	<LOD	<LOD
	C	0.01[1]	0.17	0.02	0.01[1]	0.01[1]	0.01[1]	<LOD	<LOD	0.01[1]	0.04	<LOD	<LOD	<LOD	<LOD
	Mean	0.01[1]	0.21	0.01[1]	0.01[1]	0.01[1]	0.02	0.02	<LOD	0.01[1]	0.03	<LOD	<LOD	<LOD	<LOD
45.7-61.0	A	<LOD	0.12	0.01[1]	<LOD	0.01[1]	0.01[1]	0.01[1]	-	0.01[1]	<LOD	<LOD	-	-	-
	B	0.01[1]	0.06	<LOD	<LOD	<LOD	<LOD	0.01[1]	-	<LOD	<LOD	<LOD	-	-	-
	C	-	0.11	<LOD	<LOD	0.01[1]	0.02	0.01[1]	-	0.01[1]	<LOD	<LOD	-	-	-
	Mean	0.01[1]	0.10	<LOD	<LOD	0.01[1]	0.01[1]	<LOD	-	0.01[1]	<LOD	<LOD	-	-	-
61.0-76.2	A	<LOD [2]	0.05	-	-	-	-	-	-	-	-	-	-	-	-
	B	0.01[1]	0.03	-	-	-	-	-	-	-	-	-	-	-	-
	C	0.01[1]	0.03	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.01[1]	0.04	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	0.01[1]	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.01[1]	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD [2]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] A fourth value was measured, being <LOD (0.00)
Rep. = Replicate
DAT = days after treatment
LOD = 0.02 mg/kg

Table 7.1.2.2.1-125: Results of metabolite AMPA (mg/kg) residues analysis in California soil following treatment with Roundup at 9.90 kg/ha

Depth (cm)	DAT														
	Rep.	-8	0	1	7	14	21	29	61	91	123	183	365	456	550
0-15.2	A	<LOD	0.16	0.15	0.34	0.20	0.26	0.33	0.34	0.25	0.25	0.20	0.26	0.31	0.25
	B	<LOD	0.11	0.14	0.35	0.24	0.35	0.38	0.34	0.25	0.16	0.23	0.27	0.34	0.33
	C	-	0.13	0.13	0.23	0.63	0.33	0.33	0.33	0.26	0.19	0.19	0.32	0.40	0.34
	Mean	<LOD	0.13	0.14	0.31	0.36	0.31	0.35	0.34	0.25	0.20	0.21	0.28	0.35	0.31
15.2-30.5	A	<LOD	<LOD	<LOD	<LOD	<LOD	0.03[1]	0.04	0.04	0.04	0.04	<LOD	0.03[1]	0.06	0.07
	B	<LOD	<LOD	<LOD	<LOD	0.02[1]	0.02[1]	0.01[1]	0.01[1]	0.03[1]	0.05	<LOD	0.02[1]	0.04	0.02[1]
	C	-	<LOD	<LOD	<LOD	0.02[1]	0.02[1]	0.01[1]	0.05	<LOD	0.03[1]	<LOD	0.04	0.02[1]	0.03[1]
	Mean	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.02[1]	0.02[1]	0.03[1]	0.02[1]	0.04	<LOD	0.03[1]	0.04	0.04
30.5-45.7	A	<LOD	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	0.02[1]
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	0.01[1]
	C	<LOD	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]
	Mean	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	0.02[1]
45.7-61.0	A	<LOD	0.03[1]	<LOD	<LOD	<LOD	0.02[1]	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	0.04	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	C	-	0.03[1]	<LOD	<LOD	<LOD	-0.01[1]	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	Mean	<LOD	0.02[1]	<LOD	<LOD	0.01[1]	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	-	-
61.0-76.2	A	<LOD [2]	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	0.01 [1]	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	0.03 [1]	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.02 [1]	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD [2]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] A fourth value was measured, being <LOD (0.00)
Rep. = Replicate
DAT = days after treatment
LOD = 0.04 mg/kg

Table 7.1.2.2.1-126: Results of glyphosate residues (mg/kg) analysis in Georgia soil following treatment with Roundup at 8.95 kg/ha

Depth (cm)	DAT														
	Rep.	-2	0	1	7	14	21	31	61	94	123	188	368	459	550
0-15.2	A	<LOD	3.36	1.79	1.61	1.21	0.76	0.73	0.38	0.24	0.11	0.15	0.12	0.05	0.03
	B	<LOD	2.81	3.11	1.64	1.54	1.01	0.48	0.60	0.14	0.08	0.24	0.10	0.06	0.03
	C	-	3.02	2.91	1.66	1.32	1.05	0.48	0.33	0.18	0.10	0.14	0.07	0.05	0.04
	Mean	<LOD	3.06	2.60	1.64	1.36	0.94	0.56	0.44	0.19	0.10	0.18	0.10	0.05	0.03
15.2-30.5	A	0.0 1[1]	<LOD	<LOD	0.01[1]	0.02	0.02	0.02	<LOD	<LOD	0.03	<LOD	0.01[1]	0.02	<LOD
	B	0.01 [1]	<LOD	0.02	0.01[1]	0.01[1]	0.02	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	-	<LOD	0.01[1]	0.01[1]	0.01[1]	0.02	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
	Mean	0.01 [1]	<LOD	0.01[1]	0.01[1]	0.01[1]	0.02	0.02	<LOD	<LOD	0.01[1]	<LOD	<LOD	0.01[1]	<LOD
30.5-45.7	A	<LOD [2]	<LOD	<LOD	0.02	0.01[1]	0.01[1]	0.01[1]	<LOD	-0.03[1]	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	<LOD	0.02	0.03	0.01[1]	0.01[1]	<LOD	-0.01[1]	-0.03[1]	<LOD	0.01[1]	<LOD	<LOD	<LOD
	C	0.01 [1]	<LOD	0.02	0.03	0.01[1]	0.01[1]	0.01[1]	<LOD	<LOD	0.01[1]	0.01[1]	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	0.01[1]	0.03	0.01[1]	0.01[1]	0.01[1]	<LOD	-0.02[1]	<LOD	0.01[1]	<LOD	<LOD	<LOD
45.7-61.0	A	<LOD	<LOD	<LOD	<LOD	0.02	<LOD	<LOD	-	0.01[1]	0.01[1]	<LOD	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	0.02	0.02	-	-	-
	C	-	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	-	0.02	0.02	<LOD	-	-	-
	Mean	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD		0.01[1]	0.02	0.01[1]	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.01 [1]	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD [2]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	0.01 [1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	-0.04[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	-0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-
	C		-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.01 [1]	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] A fourth value was measured, being <LOD (0.00)
Rep. = Replicate
DAT = days after treatment
LOD = 0.02 mg/kg

Table 7.1.2.2.1-127: Results of metabolite AMPA (mg/kg) residues analysis in Georgia soil following treatment with Roundup at 8.95 kg/ha

Depth (cm)	DAT														
	Rep.	-2	0	1	7	14	21	31	61	94	123	188	368	459	550
0-15.2	A	0.02 [1]	0.07	0.06	0.30	0.27	0.21	0.47	0.47	0.62	0.39	0.56	0.49	0.23	0.18
	B	0.02 [1]	0.05	0.09	0.27	0.32	0.30	0.27	0.86	0.38	0.26	0.50	0.44	0.28	0.28
	C	-	0.07	0.08	0.26	0.30	0.34	0.29	0.48	0.39	0.32	0.52	0.38	0.26	0.27
	Mean	0.02 [1]	0.06	0.08	0.28	0.30	0.28	0.34	0.60	0.46	0.32	0.53	0.44	0.26	0.24
15.2-30.5	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	0.01[1]	<LOD	0.02[1]	<LOD	0.06
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	0.02[1]
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.04	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	0.03[1]	<LOD	0.03[1]
30.5-45.7	A	<LOD [2]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]
	B	<LOD	<LOD	0.08	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	0.03[1]
	C	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD
	Mean	<LOD	<LOD	0.03[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.01[1]	0.02[1]
45.7-61.0	A	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	-	-
	C	-	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	-	0.01[1]	<LOD	<LOD	-	-	-
	Mean	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD [2]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] A fourth value was measured, being <LOD (0.00)
Rep. = Replicate
DAT = days after treatment
LOD = 0.04 mg/kg

Table 7.1.2.2.1-128: Results of glyphosate residues (mg/kg) analysis in Iowa soil following treatment with Roundup at 8.90 kg/ha

Depth (cm)	DAT													
	Rep.	-1	0	1	7	14	21	29	62	92	123	190	366	458
0-15.2	A	0.01[1]	2.29	1.59	1.69	1.47	1.76	1.25	1.21	0.33	2.71	0.40	0.52	0.55
	B	0.01[1]	1.36	2.70	2.75	1.42	1.09	0.78	3.42	1.07	0.54	0.83	1.09	0.30
	C	0.01[1]	2.02	2.74	1.54	2.23	1.61	2.63	1.45	0.58	0.48	1.19	0.87	0.51
	Mean	0.01[1]	1.89	2.34	1.99	1.71	1.49	1.55	2.03	0.66	1.24	0.81	0.83	0.45
15.2-30.5	A	<LOD	0.05	0.08	0.06	0.14	0.08	0.12	0.02	0.03	0.07	0.04	0.01[1]	0.03
	B	<LOD	0.04	0.01[1]	0.08	0.10	0.06	0.10	0.03	0.11	0.03	0.03	<LOD	<LOD
	C	-	0.02	0.04	0.05	0.11	0.08	0.12	0.03	0.23	-	0.04	0.01[1]	0.03
	Mean	<LOD	0.04	0.04	0.06	0.12	0.07	0.11	0.03	0.12	0.05	0.04	0.01[1]	0.02
30.5-45.7	A	<LOD	0.05	<LOD	0.02	0.04	0.03	0.07	0.01[1]	<LOD	-0.01[1]	0.06	<LOD	0.01[1]
	B	0.01[1]	0.01[1]	0.03	0.02	0.02	0.02	0.03	<LOD	<LOD	0.04	0.04	<LOD	<LOD
	C	-	<LOD	<LOD	0.02	0.02	0.02	0.03	<LOD	<LOD	-	0.04	<LOD	<LOD
	Mean	0.01[1]	0.02	0.01[1]	0.02	0.03	0.02	0.04	<LOD	<LOD	0.02	0.05	<LOD	<LOD
45.7-61.0	A	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	-	0.02	0.01[1]	<LOD	-	-
	B	<LOD	<LOD	0.04	<LOD	0.01[1]	<LOD	0.01[1]	-	0.02	<LOD	0.01[1]	-	-
	C	-	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	-	0.02	0.01[1]	0.01[1]	-	-
	Mean	<LOD	<LOD	0.01[1]	<LOD	0.01[1]	<LOD	<LOD	-	0.02	0.01[1]	0.01[1]	-	-
61.0-76.2	A	<LOD [2]	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	-0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] A fourth value was measured, being <LOD (-0.01)
Rep. = Replicate
DAT = days after treatment
LOD = 0.02 mg/kg

Table 7.1.2.2.1-129: Results of metabolite AMPA (mg/kg) residues analysis in Iowa soil following treatment with Roundup at 8.90 kg/ha

Depth (cm)	DAT													
	Rep.	-1	0	1	7	14	21	29	62	92	123	190	366	458
0-15.2	A	<LOD	0.05	0.05	0.12	0.10	0.16	0.11	0.27	0.08	0.62	0.16	0.38	0.61
	B	<LOD	0.04	0.07	0.16	0.10	0.09	0.06	0.53	0.22	0.16	0.40	0.67	0.41
	C	-	0.05	0.08	0.09	0.14	0.19	0.34	0.27	0.09	0.17	0.33	0.56	0.73
	Mean	<LOD	0.05	0.07	0.12	0.11	0.15	0.17	0.36	0.13	0.32	0.30	0.54	0.58
15.2-30.5	A	<LOD	<LOD	<LOD	<LOD	0.03[1]	0.02[1]	0.02[1]	0.01[1]	0.04	0.03[1]	<LOD	0.02[1]	0.05
	B	<LOD	<LOD	<LOD	<LOD	0.03[1]	<LOD	0.02[1]	0.02[1]	0.07	0.05	0.04	0.01[1]	<LOD
	C	-	<LOD	<LOD	<LOD	<LOD	0.02[1]	0.03[1]	<LOD	0.12	-	0.05	0.02[1]	<LOD
	Mean	<LOD	<LOD	<LOD	<LOD	0.02[1]	0.01[1]	0.02[1]	0.01[1]	0.08	0.04	0.03[1]	0.02[1]	0.02[1]
30.5-45.7	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	-	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	0.01[1]	<LOD	<LOD	<LOD	0.01[1]
45.7-61.0	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD		<LOD	<LOD	<LOD	-	-
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	0.01[1]	-	-
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	0.01[1]	-	-
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	0.01[1]	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	0.04	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
Rep. = Replicate
DAT = days after treatment
LOD = 0.04 mg/kg

Table 7.1.2.2.1-130: Results of glyphosate residues (mg/kg) analysis in Minnesota soil following treatment with Roundup at 9.02 kg/ha

Depth (cm)	DAT													
	Rep.	-3	0	1	7	15	21	35	71	95	129	179	372	475
0-15.2	A	<LOD [2]	1.19	1.59	1.82	2.41	1.45	0.40	0.35	0.46	0.26	0.22	0.05	0.05
	B	<LOD	0.92	0.90	1.04	1.50	0.79	0.49	0.15	0.19	0.15	0.08	0.03	0.09
	C	0.03	1.01	1.64	2.09	1.54	2.09	0.91	0.17	0.17	0.19	0.10	0.05	0.03
	Mean	0.01 [1]	1.04	1.38	1.65	1.82	1.44	0.60	0.22	0.27	0.21	0.13	0.04	0.06
15.2-30.5	A	<LOD	0.02	0.06	0.05	0.03	0.03	0.01 [1]	0.02	0.01 [1]	0.01 [1]	0.01 [1]	<LOD	<LOD
	B	<LOD	0.02	0.06	0.10	0.10	0.02	<LOD	-0.02 [1]	0.01 [1]	0.02	<LOD	<LOD	<LOD
	C	-	<LOD	0.03	0.07	0.04	0.02	<LOD	-0.02 [1]	0.01 [1]	0.02	0.02	<LOD	<LOD
	Mean	<LOD	0.01 [1]	0.05	0.07	0.06	0.02	<LOD	-0.01 [1]	0.01 [1]	0.02	0.01 [1]	<LOD	<LOD
30.5-45.7	A	<LOD	<LOD	0.01 [1]	<LOD	0.02	<LOD	<LOD	<LOD	<LOD	0.01 [1]	<LOD	<LOD	<LOD
	B	<LOD	<LOD	<LOD	<LOD	0.02	<LOD	<LOD	<LOD	<LOD	0.01 [1]	0.01 [1]	<LOD	<LOD
	C	<LOD	<LOD	<LOD	0.01 [1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	<LOD	0.01 [1]	<LOD	<LOD	<LOD	<LOD	0.01 [1]	<LOD	<LOD	<LOD
45.7-61.0	A	<LOD	<LOD	0.01 [1]	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	-
	B	0.01 [1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	-
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	-
	Mean	0.01 [1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	0.01 [1]	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] A fourth value was measured, being <LOD (0.00)
Rep. = Replicate
DAT= days after treatment
LOD = 0.02 mg/kg

Table 7.1.2.2.1-131: Results of metabolite AMPA (mg/kg) residues analysis in Minnesota soil following treatment with Roundup at 9.02 kg/ha

Depth (cm)	DAT													
	Rep.	-3	0	1	7	15	21	35	71	95	129	179	372	475
0-15.2	A	<LOD	0.15	0.26	0.23	0.24	0.31	0.27	0.66	0.62	0.42	0.45	0.19	0.21
	B	-	0.17	0.13	0.25	0.21	0.17	0.30	0.27	0.37	0.39	0.25	0.11	0.35
	C	<LOD	0.15	0.20	0.29	0.22	0.34	0.51	0.26	0.29	0.28	0.28	0.16	0.14
	Mean	<LOD	0.16	0.20	0.26	0.22	0.27	0.36	0.40	0.43	0.41	0.33	0.15	0.23
15.2-30.5	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.03[1]	0.02[1]	0.02[1]	<LOD	<LOD
	B	<LOD	0.03[1]	<LOD	<LOD	0.02[1]	<LOD	<LOD	<LOD	0.02[1]	<LOD	0.02[1]	<LOD	<LOD
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	0.02[1]	<LOD	0.01[1]
	Mean	<LOD	0.01 [1]	<LOD	<LOD	0.01 [1]	<LOD	<LOD	<LOD	0.02 [1]	0.01 [1]	0.02 [1]	<LOD	<LOD
30.5-45.7	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.03[1]	<LOD	<LOD	<LOD	<LOD
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]
	C	<LOD	<LOD	<LOD	<LOD	<LOD	0.03[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	0.01 [1]	<LOD	<LOD	0.01 [1]	<LOD	<LOD	<LOD	0.03[1]
45.7-61.0	A	<LOD	0.04	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	-
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	-
	Mean	<LOD	0.01 [1]	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
Rep. = Replicate
DAT= days after treatment
LOD = 0.04 mg/kg

Table 7.1.2.2.1-132: Results of glyphosate residues (mg/kg) analysis in New York soil following treatment with Roundup at 8.79 kg/ha

Depth (cm)	DAT														
	Rep.	-1	0	1	7	14	21	30	61	90	120	180	362	453	546
0-15.2	A	<LOD [2]	4.65	2.48	1.45	4.84	3.71	2.04	1.70	2.18	1.26	1.47	0.40	0.79	0.38
	B	<LOD	1.64	2.81	1.62	3.84	5.57	4.47	1.34	2.28	0.71	0.88	0.88	0.72	0.84
	C	0.02	1.95	2.34	2.41	5.05	4.27	1.44	1.82	1.48	1.48	1.62	0.26	<LOD	0.64
	Mean	0.01 [1]	2.75	2.54	1.83	4.58	4.52	2.65	1.62	1.98	1.15	1.32	0.51	0.50	0.62
15.2-30.5	A	<LOD [3]	<LOD	<LOD	0.03	0.01[1]	0.03	0.20	0.03	<LOD	0.02	0.02	0.02	0.03	<LOD
	B	<LOD	<LOD	0.01[1]	0.01[1]	0.03	<LOD	0.02	0.02	0.11	0.04	0.05	0.01[1]	0.06	0.01[1]
	C	0.02	0.05	0.01[1]	<LOD	0.03	<LOD	0.06	0.03	0.09	0.02	0.01[1]	0.03	0.06	0.03
	Mean	0.01 [1]	0.02	0.01[1]	0.01[1]	0.02	0.01[1]	0.09	0.03	0.07	0.03	0.03	0.02	0.05	0.01[1]
30.5-45.7	A	-0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.03	0.03	<LOD	<LOD	<LOD
	B	<LOD	<LOD	<LOD	0.01[1]	<LOD	0.01[1]	<LOD	0.01[1]	0.02	<LOD	0.02	-0.01[1]	0.02	<LOD
	C	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.03	0.02	0.02	<LOD	0.01[1]	0.02
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.02	0.02	0.02	<LOD	0.01[1]	0.01[1]
45.7-61.0	A	<LOD	-0.01[1]	-0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.01[1]	0.02	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.01[1]	<LOD	0.01[1]	0.02	0.02	-	-	-
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	0.02	-	-	-
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	0.01[1]	0.01[1]	0.02	-	-	-
61.0-76.2	A	<LOD [3]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD [4]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] Five values were measured, all being <LOD : 0.00 / 0.00 / 0.00 / 0.02 / 0.02
[3] A fourth value was measured, being <LOD (0.00)
[4] Five values were measured, all being <LOD (0.00)
Rep. = Replicate
DAT= days after treatment
LOD = 0.02 mg/kg

Table 7.1.2.2.1-133: Results of metabolite AMPA (mg/kg) residues analysis in New York soil following treatment with Roundup at 8.79 kg/ha

Depth (cm)	DAT														
	Rep.	-1	0	1	7	14	21	30	61	90	120	180	362	453	546
0-15.2	A	0.02[1, 2]	0.17	0.10	0.12	0.39	0.39	0.41	0.23	0.54	0.30	0.46	0.19	0.28	0.21
	B	0.03[1]	0.09	0.10	0.14	0.36	0.44	0.62	0.27	0.58	0.29	0.25	0.27	0.56	0.49
	C	0.04	0.08	0.08	0.20	0.55	0.44	0.26	0.32	0.32	0.41	0.57	0.14	<LOD	0.37
	Mean	0.03[1]	0.11	0.09	0.15	0.43	0.42	0.43	0.27	0.48	0.33	0.43	0.20	0.28	0.36
15.2-30.5	A	0.03[1]	0.06	0.02[1]	0.05	0.04	0.06	0.08	0.04	<LOD	0.03[1]	0.03[1]	0.03[1]	0.08	<LOD
	B	<LOD [3]	<LOD	0.01[1]	<LOD	0.06	<LOD	0.04	0.02[1]	0.06	0.06	0.05	0.02[1]	0.09	0.06
	C	0.05	<LOD	<LOD	<LOD	0.08	0.02[1]	0.05	0.03[1]	0.06	0.04	0.03[1]	0.03[1]	0.10	0.10
	Mean	0.02[1]	0.02[1]	0.01[1]	0.02[1]	0.06	0.03[1]	0.06	0.03[1]	0.04	0.04	0.04	0.03[1]	0.09	0.05
30.5-45.7	A	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.01[1]	0.02[1]	<LOD	0.04	0.04	0.05	<LOD	<LOD	<LOD
	B	<LOD	<LOD	<LOD	0.04	0.01[1]	0.02[1]	<LOD	0.02[1]	0.03[1]	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	0.04	0.04	0.04	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	0.01[1]	0.01[1]	0.01[1]	0.01[1]	0.01[1]	0.04	0.03[1]	0.03[1]	<LOD	<LOD	<LOD
45.7-61.0	A	<LOD	<LOD	<LOD	-0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-
	B	0.01[1]	<LOD	<LOD	-0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-
	C	-	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-
	Mean	0.01[1]	<LOD	<LOD	-0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD [4]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C		-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] Five values were measured, all being <LOD : 0.00 / 0.02 / 0.03 / 0.04 / 0.04
[3] A fourth value was measured, being <LOD (0.00)
[4] Five values were measured, all being <LOD (0.00)
Rep. = Replicate
DAT= days after treatment
LOD = 0.04 mg/kg

Table 7.1.2.2.1-134: Results of glyphosate residues (mg/kg) analysis in Ohio soil following treatment with Roundup at 9.12 kg/ha

Depth (cm)	DAT														
	Rep.	-1	0	1	7	14	21	30	61	90	121	177	365	455	545
0-15.2	A	<LOD [2]	2.30	2.32	0.70	0.51	0.73	0.44	0.12	0.07	0.05	0.02	0.03	0.02	<LOD
	B	0.01[1]	1.45	1.34	0.49	0.37	0.75	0.78	0.15	0.09	0.03	0.02	0.03	0.02	0.02
	C	0.02	2.29	1.83	0.78	0.65	0.62	0.56	0.09	0.08	0.09	0.06	0.01[1]	0.02	0.01[1]
	Mean	0.01[1]	2.01	1.83	0.66	0.51	0.70	0.59	0.12	0.08	0.06	0.03	0.02	0.02	0.01[1]
15.2-30.5	A	-0.01[1]	<LOD	-0.01[1]	<LOD	0.01[1]	0.03	0.02	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD
	B	<LOD	<LOD	0.01[1]	<LOD	0.03	0.02	0.03	<LOD	0.02	<LOD	<LOD	<LOD	<LOD	<LOD
	C	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.02	<LOD	0.02	0.02	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	<LOD	0.02	0.02	0.02	0.01[1]	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD
30.5-45.7	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	C	-	<LOD	<LOD	<LOD	<LOD	0.02	<LOD	0.01[1]	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD
45.7-61.0	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	-0.01[1]	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
[2] A fourth value was measured, being <LOD (-0.01)
Rep. = Replicate
DAT = days after treatment
LOD = 0.02 mg/kg

Table 7.1.2.2.1-135: Results of metabolite AMPA (mg/kg) residues analysis in Ohio soil following treatment with Roundup at 9.12 kg/ha

Depth (cm)	DAT														
	Rep.	-1	0	1	7	14	21	30	61	90	121	177	365	455	545
0-15.2	A	<LOD	0.29	0.43	0.56	0.33	0.69	0.45	0.34	0.22	0.18	0.18	0.12	0.10	0.05
	B	<LOD	0.28	0.28	0.47	0.34	0.58	0.74	0.41	0.32	0.17	0.14	0.15	0.08	0.07
	C	0.02[1]	0.45	0.33	0.65	0.61	0.53	0.46	0.37	0.25	0.38	0.30	0.10	0.09	0.08
	Mean	0.01[1]	0.34	0.35	0.56	0.43	0.60	0.55	0.37	0.26	0.24	0.21	0.12	0.09	0.07
15.2-30.5	A	<LOD	<LOD	<LOD	<LOD	0.04	0.04	<LOD	<LOD	<LOD	0.03[1]	<LOD	<LOD	0.03[1]	<LOD
	B	<LOD	<LOD	<LOD	<LOD	0.04	0.05	0.05	0.03[1]	<LOD	<LOD	<LOD	0.02[1]	<LOD	<LOD
	C	<LOD	<LOD	<LOD	<LOD	0.03[1]	<LOD	<LOD	0.04	0.03[1]	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	<LOD	0.04	0.03[1]	0.02[1]	0.02[1]	0.01[1]	0.01[1]	<LOD	0.01[1]	0.01[1]	<LOD
30.5-45.7	A	<LOD	<LOD	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD	<LOD
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	<LOD
45.7-61.0	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] <LOD
Rep. = Replicate
DAT = days after treatment
LOD = 0.04 mg/kg

Table 7.1.2.2.1-136: Results of glyphosate residues (mg/kg) analysis in Texas soil following treatment with Roundup at 8.80 kg/ha

Depth (cm)	DAT											
	Rep.	-1	0	1	11	14	27	30	61	91	122	183
0-15.2	A	<LOD	1.46	1.68	0.95	<LOD	0.04	0.16	0.02	0.02	0.02	-0.01[1]
	B	<LOD	1.58	1.49	1.21	<LOD	0.15	0.19	0.01[1]	0.03	-0.01[1]	<LOD
	C	0.04	2.75	1.64	1.17	0.04	0.21	0.11	0.01[1]	0.02	-0.01[1]	<LOD
	Mean	<LOD	1.93	1.60	1.11	0.01[1]	0.13	0.15	0.01[1]	0.02	<LOD	<LOD
15.2-30.5	A	<LOD	0.06	0.05	0.01[1]	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	B	<LOD	0.05	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	<LOD	0.01[1]
	C	0.02	0.07	0.01[1]	0.01[1]	<LOD	<LOD	<LOD	-0.01[1]	<LOD	<LOD	<LOD
	Mean	0.01[1]	0.06	0.03	0.01[1]	0.01[1]D	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30.5-45.7	A	<LOD	<LOD	<LOD	<LOD	0.01[1]D	<LOD	<LOD	<LOD	<LOD	<LOD	0.04
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-0.01[1]	<LOD	<LOD	<LOD
	C	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]
45.7-61.0	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]D	-	-	-	-
	C	--	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-
	B	0.01[1]	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.01[1]	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.01[1]	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	<LOD	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-

[1] <LOD
Rep. = Replicate
DAT= days after treatment
LOD = 0.02 mg/kg

Table 7.1.2.2.1-137: Results of metabolite AMPA (mg/kg) residues analysis in Texas soil following treatment with Roundup at 8.80 kg/ha

Depth (cm)	DAT											
	Rep.	-1	0	1	11	14	27	30	61	91	122	183
0-15.2	A	0.02[1]	0.10	0.13	0.20	<LOD	<LOD	0.27	0.12	0.08	0.14	0.02[1]
	B	<LOD	0.10	0.11	0.31	<LOD	0.24	0.34	0.15	0.16	0.06	0.02[1]
	C	<LOD	0.12	0.13	0.29	<LOD	0.22	0.20	0.07	0.07	0.08	0.02[1]
	Mean	0.01[1]	0.11	0.12	0.27	<LOD	0.15	0.27	0.11	0.10	0.09	0.02[1]
15.2-30.5	A	<LOD	0.03[1]	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.02[1]	<LOD
	B	<LOD	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	-0.01	0.02[1]	<LOD	<LOD
	C	<LOD	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	0.03[1]	0.01[1]	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	0.01[1]	<LOD
30.5-45.7	A	<LOD	0.02[1]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.03[1]	<LOD	0.04
	B	<LOD	<LOD	<LOD	<LOD	0.03[1]	<LOD	<LOD	<LOD	0.03[1]	<LOD	<LOD
	C	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Mean	<LOD	0.01[1]	<LOD	<LOD	0.01[1]	<LOD	<LOD	<LOD	0.02[1]	<LOD	0.01[1]
45.7-61.0	A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-
	B	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-
	C	-	<LOD	<LOD	<LOD	<LOD	<LOD	0.04	-	-	-	-
	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01[1]	-	-	-	-
61.0-76.2	A	<LOD	-	-	-	-	-	-	-	-	-	-
	B	<LOD	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-
76.2-91.4	A	<LOD	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-
91.4-106.7	A	0.03[1]	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
	Mean	0.03[1]	-	-	-	-	-	-	-	-	-	-
106.7-121.9	A	<LOD	-	-	-	-	-	-	-	-	-	-
	B		-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-
	Mean	<LOD	-	-	-	-	-	-	-	-	-	-

[1] <LOD
Rep. = Replicate
DAT = days after treatment
LOD = 0.04 mg/kg

B. CHARACTERISATION OF RESIDUES

1. Arizona test site

Residues of glyphosate averaged less than 0.05 mg/kg in all soil samples taken below 15.2 cm, with two exceptions. The 15.2-30.5 cm sample at 7 DAT contained an average glyphosate residue level of 0.08 mg/kg. In addition, one of the three replicate samples from the 45.7-61.0 cm sample at 21 DAT contained 0.15 mg/kg glyphosate. However, the other two replicate samples from the 45.7-61.0 cm depth at 21 DAT failed to contain detectable amounts of glyphosate. Since only one of the three replicate samples had measurable glyphosate residues and all the other samples that bracket this sample by depth and time contained no glyphosate residues, contamination of this sample is suspected. These residues were also attributed to contamination during sampling rather than vertical mobility due to the absence of supporting residues in the 15.2-30.5 cm and 30.5-45.7 cm soil horizons at 21 days after treatment and the absence of residues below 30.5 cm in subsequent soil samples.

AMPA residues were below LOD in all soil samples taken below 15.2 cm except for 0.18 mg/kg, which was detected in the same 45.7-61.0 cm soil sample at 21 DAT described above which contained 0.15 mg/kg glyphosate. Since the other two replicate samples from this depth interval and sampling event did not contain detectable amounts of AMPA, contamination of this sample was suspected.

2. California test site

The average residue level of glyphosate in the 0-15.2 cm layer was 1.12 mg/kg at 0 DAT. Average glyphosate residues increased to a maximum of 1.94 mg/kg at 1 DAT, and then gradually dissipated to less than 0.02 mg/kg at 550 DAT. The average AMPA residue level in the top 15.2 cm of soil measured 0.13 mg/kg at 0 DAT. Average AMPA residues reached a maximum concentration of 0.36 mg/kg at 14 DAT, and then declined to 0.31 mg/kg at 550 DAT. California was one of two locations at which pre-excavation of the top 15.2 cm of the soil was not performed prior to soil core sampling. Consequently, the day zero samples showed evidence of contamination at lower depths. Residues of glyphosate were found in all the 0 DAT samples analysed to a depth of 61.0 cm. For day 0, the average glyphosate residues were 0.30 mg/kg in the 15.2-30.5 cm layer, 0.21 mg/kg in the 30.5-45.7 cm layer, 0.10 mg/kg in the 45.7-61.0 cm layer, and 0.04 mg/kg in the 61.0-76.2 cm layer. These residues were attributed to contamination during sampling rather than vertical mobility due to the depth of the residues at such an early time point following test substance application and the absence of supporting concentrations of glyphosate below 30.5 cm in subsequent soil samples. In all other samples below 15.2 cm, glyphosate residues were equal or less than 0.05 mg/kg. AMPA residues were less than 0.05 mg/kg in all soil samples taken below 15.2 cm.

3. Georgia test site

The maximum average residue level of glyphosate in the 0 to 15.2 cm soil layer was 3.06 mg/kg at 0 DAT, and declined steadily to 0.03 mg/kg at 550 DAT. The average residue level of AMPA in the top 15.2 cm measured 0.06 mg/kg at 0 DAT. Average AMPA residues reached a maximum of 0.60 mg/kg at 61 DAT, and then declined to 0.24 mg/kg at 550 DAT. Average glyphosate and AMPA residues were less than 0.05 mg/kg for all samples taken below 15.2 cm, demonstrating that glyphosate and AMPA did not move vertically in the soil profile at this test site.

4. Iowa test site

The average residue level of glyphosate in the 0 to 15.2 cm layer was 1.89 mg/kg at 0 DAT, and reached a maximum concentration of 2.34 mg/kg at 1 DAT. Average glyphosate residues then declined slowly to 0.45 mg/kg at 458 DAT.

The average AMPA residue level in the top to 15.2 cm was 0.05 mg/kg at 0 DAT. Average AMPA residues rose to 0.36 mg/kg at 62 DAT, declined slightly to 0.31 mg/kg at 366 DAT, and increased again to 0.38 mg/kg at 458 DAT sampling.

Average glyphosate residues ranging from 0.05 to 0.12 mg/kg were found in the 15.2-30.5 cm layer of all sampling events between 7 and 123 DAT, except for the 62 DAT sampling. Glyphosate residues averaged less than 0.05 mg/kg for all samples taken below 30.5 cm with one exception; an average glyphosate residue level of 0.05 mg/kg was found in the 30.5-45.7 cm soil horizon at 190 days after treatment. However, this

residue was attributed to contamination during sampling rather than vertical mobility due to the absence of supporting residues in the 15.2-30.5 cm soil horizon at 190 days after treatment and the absence of residues below 30.5 cm in subsequent soil samples. AMPA residues were less than 0.05 mg/kg in all soil samples taken below 30.5 cm except for an average level of 0.08 mg/kg found in the 15.2-30.5 cm layer at 92 DAT.

Iowa was the second of two locations, at which pre-excavation of the top 30.5 cm of the soil was not performed prior to soil core sampling. Since Iowa did have more instances and generally higher residues of glyphosate and AMPA in the 15.2-30.5 cm soil layer than other locations, it was postulated that contamination during sampling contributed, at least in part, to the residues found in the 15.2-30.5 cm layer.

The 18 months after application sampling was not taken at the Iowa because the ground was frozen too hard to permit sampling.

5. Minnesota test site

The average residue level of glyphosate in the 0 to 15.2 cm layer measured 1.04 mg/kg at 0 DAT. The maximum average glyphosate residue was 1.82 mg/kg at 15 DAT, after which it rapidly decreased to 0.27 mg/kg by 95 DAT and then continued to decline at a slower rate to 0.06 mg/kg at 475 DAT.

The average AMPA residue level in the 0 to 15.2 cm soil layer was 0.16 mg/kg at 0 DAT. Average AMPA residues peaked at 95 DAT, reaching 0.43 mg/kg, and then declined to 0.23 mg/kg at 475 DAT. Average glyphosate residues of 0.05, 0.07 and 0.06 mg/kg were found in the 15.2 to 30.5 cm layer at the 1, 7 and 15 DAT sampling events, respectively. Glyphosate residues averaged less than 0.05 mg/kg for all other samples taken below 15.2 cm. AMPA residues were less than 0.05 mg/kg in all soil samples taken below 15.2 cm.

The 18 months after application sampling was not taken at the Minnesota test site because the ground was frozen too hard to permit sampling.

6. New York test site

The average residue level for glyphosate in the 0 to 15.2 cm horizon was 2.75 mg/kg at 0 DAT. The maximum average glyphosate residue was 4.58 mg/kg at 14 DAT, after which average residues decreased steadily and were measured at 0.50 mg/kg on day 453. Average glyphosate residues were 0.62 mg/kg at 546 DAT, the final sampling. Average residue levels of AMPA were measured at 0.11 mg/kg on 0 DAT and increased to 0.48 mg/kg at 90 DAT. After 90 DAT, AMPA residues declined to 0.20 mg/kg at 362 DAT and then rose again to 0.36 mg/kg at 546 DAT.

Average glyphosate residue levels of 0.09 and 0.07 mg/kg were found in the 15.2-30.5 cm layers at 30 and 90 DAT, respectively. With the exception of these samples, no average glyphosate residues above 0.05 mg/kg were found below the 0 to 15.2 cm layer. AMPA was detected in the 15.2-30.5 cm layer on 14 and 30 days after treatment when the average residue levels reached 0.06 mg/kg at both times. AMPA was also detected in the 15.2-30.5 cm layer on 453 and 546 days after application when the average residue levels reached 0.09 and 0.05 mg/kg, respectively. No other AMPA residues averaging 0.05 mg/kg or greater were measured below 15.2 cm.

7. Ohio test site

The maximum average residue level of glyphosate in the 0 to 15.2 cm layer was 2.01 mg/kg at 0 DAT. Glyphosate residue levels decreased steadily and rapidly to less than LOD at 545 DAT. Average AMPA residues were measured at 0.34 mg/kg at the 0 DAT sampling. The highest average AMPA residue level of 0.60 mg/kg was found at 21 DAT. After this time, the AMPA levels decreased steadily to 0.07 mg/kg at 545 DAT. Residues of glyphosate and AMPA averaged less than 0.05 mg/kg in all soil samples taken below 15.2 cm at all sampling times. These results demonstrate that glyphosate and AMPA did not move vertically in the soil profile at this test site.

8. Texas test site

The maximum average residue level of glyphosate in the 0 to 15.2 cm soil layer was 1.93 mg/kg at 0 DAT. Average glyphosate residue levels decreased rapidly to less than 0.05 mg/kg at 14 DAT, the increased to 0.15 mg/kg at 30 DAT, and then decreased to less than 0.05 mg/kg at all other sampling times. The average AMPA residue level at the 0 DAT sampling was measured at 0.11 mg/kg. The highest average AMPA residue level of 0.27 mg/kg was found at both 11 and 30 DAT. After this time, AMPA residue levels decreased to less than 0.05 mg/kg at 183 DAT. Residues of glyphosate averaged less than 0.05 mg/kg in all soil samples taken below 15.2 cm except for the 15.2 to 30.5 cm depth sample at 0 DAT which contained an average glyphosate residue level of 0.06 mg/kg. No AMPA residues were found to exceed 0.05 mg/kg below 15.2 cm for any sampling times.

The test plots and surrounding areas at the Texas location were flooded with approximately three feet of water for three days (December 23, 24, and 25 1991) due to a record rainfall during December 1991. As a result, all sampling from this location was stopped after the flood, and no 12, 15 or 18 months after treatment samples were collected. Due to the very rapid degradation of glyphosate at this location, the existing sampling events through six months after treatment were sufficient to define the dissipation of glyphosate.

C. KINETICS

An Ecoregion Crosswalk exercise was performed (see [redacted] 2020, CA 7.1.2.2.1/002). The trials in New York, Ohio, California, Iowa, Minnesota and Arizona were found to be representative for European conditions and included in kinetic evaluation ([redacted] 2020, CA 7.1.2.2.1/003).

III. CONCLUSIONS

Maximum average glyphosate residue levels in the 0-15.2 cm soil horizon were 2.23, 0.62, 3.06, 2.34, 1.82, 4.58, 2.01 and 1.93 mg/kg occurred at 7, 0, 0, 1, 15, 14, 0 and 0 days after test substance application for the Arizona, California, Georgia, Iowa, Minnesota, New York, Ohio and Texas test sites, respectively, and then dissipated close to or below LOD, respectively, at the last sampling date. AMPA was found in the day 0 samples, demonstrating how rapidly glyphosate is degraded in soil. Maximum average AMPA residue levels in the 0-15 cm soil horizon were 0.56, 0.36, 0.60, 0.36, 0.43, 0.48, 0.60 and 0.27 mg/kg and occurred at 21, 14, 61, 62, 95, 90, 21 and 11 days after test substance application for the Arizona, California, Georgia, Iowa, Minnesota, New York, Ohio and Texas test sites, respectively, and then dissipated close to or below LOD, respectively, at the last sampling date with exception of the New York test site with a AMPA concentration of 0.36 mg/kg at 546 DAT.

The results of this study demonstrate that glyphosate and AMPA had little propensity to leach through the soil. Glyphosate degradation was typically rapid. The AMPA metabolite residue levels initially increased as glyphosate degraded, and then declined as it also degraded, demonstrating that it was a non-persistent metabolite. AMPA was found in the day 0 samples, demonstrating how rapidly glyphosate degraded in soil. As glyphosate degraded, the levels of AMPA rose and reached a maximum concentration between days 11 and 95 at seven of eight test sites. AMPA has also been demonstrated to dissipate with time. Average AMPA residue levels decreased from a maximum of 0.27 mg/kg, found at both the 11 and 30 days after treatment sampling events, to 0.02 mg/kg at 6 months after application at the Texas test site. At the Georgia and Ohio test sites, the average AMPA residue levels in the 0-15.2 cm soil horizon decreased from a maximum of 0.60 mg/kg at 6 and 21 days after treatment, respectively, to 0.24 mg/kg and 0.07 mg/kg, respectively, at 18 months after treatment.

The results of this study demonstrate that glyphosate and AMPA possess very limited potential for vertical mobility in soil. Average glyphosate and AMPA residues greater than 0.05 mg/kg (the lower limit of method validation) were never detected below 30.5 cm in the soil profile with three exceptions. For the sampling event on the day of test substance application at the California test site, average glyphosate residues of 0.21 mg/kg and 0.10 mg/kg, respectively, where found in the 30.5-45.7 cm and 45.7-61.0 cm soil horizons. However, these residues were attributed to contamination during sampling rather than vertical mobility of glyphosate due to the depth of the residues at such an early time point following test substance application and the absence of supporting concentrations of glyphosate below 30.5 cm in subsequent soil

samples. In addition, average glyphosate and AMPA residues of 0.05 and 0.06 mg/kg, respectively, were found in the 45.7-61.0 cm soil horizon at 21 days after treatment at the Arizona test site and an average glyphosate residue level of 0.05 mg/kg was found in the 30.5-45.7 cm soil horizon at 190 days after treatment at the Iowa test site. These residues were also attributed to contamination during sampling rather than vertical mobility due to the absence of supporting residues in the 15.2-30.5 cm and 30.5-45.7 cm soil horizons at 21 days after treatment at the Arizona test site, the absence of residues in the 15.2-30.5 cm soil horizon at 190 days after treatment at the Iowa test sites, and the absence of residues below 30.5 cm in subsequent soil samples from both test sites. Lack of pre-excavation at the Iowa test site may be responsible for the residues found at lower depths at that location.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides detailed information on the dissipation behavior of glyphosate under field conditions at different testing conditions according to the relevant guideline. It is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/007
Report author	[redacted]
Report year	1993
Report title	Storage stability of Glyphosate and AMPA in Soil and Stream sediment
Report No	MSL-12682
Document No	
Guidelines followed in study	None
Deviations from current test guideline	Not relevant
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
A storage stability study was conducted to determine the stability of glyphosate and its major soil metabolite, aminomethylphos-phonic acid (AMPA), in soil under frozen conditions. Every six months, glyphosate and AMPA were applied to soil and sediment samples that were then stored frozen. After 975 to 977 days of storage, the samples were removed and analysed for recovery of glyphosate and AMPA. Statistical analysis of the results demonstrated a small but statistically significant decrease in glyphosate recovery with increasing storage time in the two soils and one sediment studied. AMPA was stable in the sediment, but one of the soils also showed a small but statistically significant decrease in recovery with increasing storage time. Based on the data, the percentage remaining after 500 days in storage ranges from 88 to 94 % for glyphosate and ≥ 95 % for AMPA.

I. MATERIAL AND METHODS

A. MATERIALS

During the course of the study, three different lots of glyphosate and four different lots of AMPA were used as presented below.

1. Test Material:

Identification:	Glyphosate
Lot No.:	PIT-90001-1524-A
Chemical purity:	≥ 98.8 %
Lot No.:	PIT-8906-666-A
Chemical purity:	99.7 %
Lot No.:	RUD-9203-3961-A
Chemical purity:	99.8 %
Identification:	AMPA
Lot No.:	SIG-8912-1253-A
Chemical purity:	≥ 97.4 %
Lot No.:	SIG-8912-1253-A-5
Chemical purity:	99 %
Lot No.:	PIT-8912-1385-A
Chemical purity:	99.1 %
Lot No.:	PIT-8912-1385-A-2
Chemical purity:	99.1 %

2. Soil:

For this study, soil from Georgia and Iowa and one stream sediment from Oregon were used. These matrices are representative of the soils and sediment to which glyphosate would be applied under normal agronomic practices. The samples were taken from test sites that had a known two-year history of crop and pesticide use, and none of the test sites had been treated with Roundup herbicide or related chemistry during the two years preceding this study.

Soil characterization data for each soil type are presented in the table below.

Table 7.1.2.2.1-138: Characteristics of test soils

Parameter	Results		
Soil	Georgia soil (Climax)	Iowa soil (Danville)	Oregon sediment (Corvallis)
Textural Class (USDA)	Sandy loam	Silt loam	Sandy clay loam
Sand (50 µm – 2 mm) (%)	76	20	56
Silt (2 µm – 50µmm) (%)	14	54	23
Clay (< 2 µm) (%)	10	26	21
pH [1]	4.7	6.0	5.8
Organic matter (%)	1.1	4.4	7.2
Organic carbon (%) [2]	0.64	2.55	4.18
Cation exchange capacity (meq/100 g)	2.3	17.8	18.2
Water Holding Capacity at 1/3 bar (%)	7.02	33.65	40.37
Bulk Density (disturbed) (g/cm^3)	1.29	1.17	0.99

[1] Medium not stated
[2] Calculated from organic matter according to OC=OM × 0.58
DAT = days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions and sampling

Untreated control soil from Climax, Georgia and Danville, Iowa and sediment from Corvallis, Oregon were pre-weighed as 30.0 g aliquots into 250 mL polypropylene centrifuge bottles. The uniquely labelled sample bottles were capped securely to prevent loss of moisture and placed into closed cardboard boxes. The boxes were transferred to a restricted access freezer and stored at a temperature < -17 °C in the dark. The closed boxes excluded light from the samples and provided a small degree of insulation from temperature changes in the freezer due to door openings.

At approximately every six months, three unfortified samples from each location were removed from frozen storage, thawed, fortified, and then returned to frozen storage. In the case of the Georgia soil and Oregon sediment, fortifications were made 0, 65, 247, 429, 611, 793 and 975 days prior to analysis. In the case of the Iowa soil, fortifications were made 0, 67, 249, 431, 613, 795 and 977 days prior to analysis. Fortifications were made by pipetting the test solution directly onto the soil matrix at a level of 1.0 mg/kg each of glyphosate and AMPA. Immediately after fortification, the samples were re-capped securely and taped to further prevent the lids from loosening.

Samples were removed from frozen storage and analysed in sets consisting of unfortified samples, method recovery samples (day 0 samples fortified at the time of analysis) and fortified storage stability samples for each fortification interval. Of the three samples fortified at each time point, only two were analysed; the third was kept frozen. The method recovery samples served as day 0 analyses for comparison to the stored fortified samples.

After the initial chromatographic analysis, the sample extracts for all samples were returned to refrigerated storage (3 – 6 °C) in the dark. After a period ranging from 35 to 42 days, four random sample extracts from each of the three locations were reanalysed to determine if there were any gross changes in recovery due to sample extract storage. With average changes of -1.8% for glyphosate and 3.5 % for AMPA between initial analyses and analyses after storage, stability in sample extracts was demonstrated.

2. Analytical procedures

Glyphosate and AMPA were extracted from soil using a 0.5 N KOH solution. The extract solution was eluted through a Chelex 100 resin in the Fe(III) form, which retains glyphosate and AMPA due to chelation to Fe(III). The retained glyphosate and AMPA iron salts are removed from the Chelex resin by elution with 6 N HCl. The isolated glyphosate and AMPA iron salts are then applied to a strong anion exchange resin and eluted with 6 N HCl to remove the iron and obtain the free acids of glyphosate and AMPA. After concentration to dryness, to remove the HCl, the samples are re-dissolved in water and analysed by high pressure liquid chromatography (HPLC). The chromatograph uses column switching and an o-phthalaldehyde post-column reactor with a fluorescence detector to separate and quantitate glyphosate and AMPA. In the post-column reactor, glyphosate is oxidised to a primary amine which then reacts with o-phthalaldehyde to form a fluorescence derivative. AMPA reacts directly with o-phthalaldehyde to form a second fluorescence derivative.

This method has been validated down to 0.05 mg/kg for both glyphosate and AMPA in 30 g soil samples.

Due to the varying degrees of glyphosate adsorption to different soil types, glyphosate recovery from fortified check samples varies with soil type, and obtaining consistent recoveries of glyphosate is occasionally difficult. Nonetheless, the analytical method used generally affords recoveries of glyphosate from fortified check samples that are greater than 70 %. The percentage recoveries from samples fortified on the day of analysis (day 0) with both glyphosate and AMPA averaged across all three soil matrices were 79.44 % and 77.45 %, respectively.

II. RESULTS AND DISCUSSION

A. DATA

Summary tables of residues for untreated control and fortified frozen field samples are presented below. Analyses of duplicate samples (uncorrected for recovery) are reported for all time points.

Table 7.1.2.2.1-139: Summary of residues (mg/kg) of glyphosate and AMPA in Georgia soil after frozen storage

Fortification rate (mg/kg)	Days in storage	Glyphosate (mg/kg)				AMPA (mg/kg)			
		Rep. 1	Rep. 2	Mean	In %	Rep. 1	Rep. 2	Mean	In %
0	0	0	0	0		0	0.0067	0.003	
1	0	0.8001	0.8365	0.818		0.7778	0.8152	0.797	
1	65	0.8246	0.8314	0.828	101.19	0.8046	0.8115	0.808	101.45
1	247	0.7866	0.8351	0.811	97.93	0.7588	0.7905	0.775	95.87
1	429	0.7928	0.8411	0.817	100.75	0.7689	0.8098	0.789	101.90
1	611	0.7376	0.7431	0.740	90.62	0.7100	0.7278	0.719	91.07
1	793	0.6768	0.7284	0.703	94.90	0.6639	0.6983	0.681	94.74
1	975	0.7770	0.7928	0.785	111.71	0.7623	0.7730	0.768	112.71

Table 7.1.2.2.1-140: Summary of residues (mg/kg) of glyphosate and AMPA in Iowa soil after frozen storage

Fortification rate (mg/kg)	Days in storage	Glyphosate (mg/kg)				AMPA (mg/kg)			
		Rep. 1	Rep. 2	Mean	In %	Rep. 1	Rep. 2	Mean	In %
0	0	0	0	0		0	0	0	
1	0	0.7205	0.7940	0.757		0.6868	0.7692	0.728	
1	67	0.7333	0.8109	0.772	101.96	0.7210	0.7912	0.756	103.86
1	249	0.7712	0.8346	0.803	103.99	0.7554	0.8401	0.798	105.51
1	431	0.7211	0.7408	0.731	91.04	0.7486	0.7734	0.761	95.39
1	613	0.6969	0.6974	0.697	95.38	0.7688	0.7761	0.772	101.50
1	795	0.6100	0.6759	0.643	92.23	0.6983	0.7698	0.734	95.03
1	977	0.6717	0.6804	0.676	105.15	0.7881	0.8066	0.797	108.62

Table 7.1.2.2.1-141: Summary of residues (mg/kg) of Glyphosate and AMPA in Oregon sediment after frozen storage

Fortification rate (mg/kg)	Days in storage	Glyphosate (mg/kg)				AMPA (mg/kg)			
		Rep. 1	Rep. 2	Mean	In %	Rep. 1	Rep. 2	Mean	In %
0	0	0	0	0		0	0	0	
1	0	0.7943	0.8208	0.808		0.7944	0.8033	0.799	
1	65	0.8723	0.8975	0.885	109.58	0.8361	0.8504	0.843	105.56
1	247	0.7909	0.8321	0.812	91.71	0.7997	0.8110	0.805	95.51
1	429	0.5946	0.6863	0.640	78.92	0.6216	0.7014	0.662	82.14
1	611	0.7122	0.7623	0.737	115.11	0.7663	0.8567	0.812	122.68
1	793	0.5816	0.6712	0.626	84.96	0.6714	0.7891	0.730	89.99
1	975	0.6931	0.7249	0.709	113.19	0.7944	0.8779	0.836	114.50

B. CHARACTERISATION OF RESIDUES

The results from all three soil matrices show that average recoveries of glyphosate and AMPA residues fortified at 1.0 mg/kg generally range from 0.65 to 0.85 mg/kg.

The average recoveries of the Georgia soil fortified on the date of extraction (day 0 samples) were 0.82 and 0.80 mg/kg for glyphosate and AMPA, respectively. The average recoveries of the Iowa soil fortified on day 0 were 0.76 and 0.73 mg/kg for glyphosate and AMPA, respectively. The average recoveries of the Oregon sediment fortified on the date of extraction were 0.81 and 0.80 mg/kg for glyphosate and AMPA, respectively.

After 975 days amounts of 0.79 and 0.77 mg/kg glyphosate and AMPA, respectively, were found in the Georgia soil. In the Iowa soil 0.68 and 0.80 mg/kg glyphosate and AMPA, respectively, were found after 977 days and in the Oregon sediment amounts of 0.71 mg/kg glyphosate and 0.84 mg/kg AMPA were found after 975 days.

Statistical analysis of the data included fitting the data to a simple first-order decay model and testing the hypothesis that the slope is equal to zero.

The results showed a small but statistically significant decrease in the amount of glyphosate recovered with increasing time in storage for all three soil matrices. AMPA also showed a statistically significant decrease in the Georgia soil. No statistical trend was found in the Iowa soil and Oregon sediment indicating stability of the AMPA residues in those two matrices.

Based on the respective first-order decay models the percentage remaining after 500 days ranges from 88 to 94 % for glyphosate and 95 % or above for AMPA.

The observed decrease of glyphosate recovery with increasing storage time is postulated to be due to increased adsorption of glyphosate to the soil and binding to metallic cations. If microbial, chemical, or photolytic degradation were the cause of the decreased glyphosate recoveries, the AMPA residues would be expected to increase with increasing time, which is not the case. Moreover, since the samples were stored in the dark at <-17 °C, photodecomposition and microbial degradation can be eliminated.

III. CONCLUSION

The results of this study demonstrate that glyphosate had a small but statistically significant decrease in recovery with increasing storage time for the soils and sediment used. AMPA was determined to be stable in one of the two soils and the sediment, but it also had a small but statistically significant decrease in recovery with increasing storage time in the Georgia sandy loam soil tested. Glyphosate loss in soil at less

than -17 °C in the dark is typically very slow with the percentage remaining after 500 days ranging from 88 to 94 %. The AMPA metabolite loss is slower, yet, with 95 % or greater of the AMPA still extractable at 500 days.

3. Assessment and conclusion

Assessment and conclusion by applicant:
It was shown that glyphosate and AMPA residues in soil are stable for up to three years when stored at -17 °C. The study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/008
Report author	[redacted]
Report year	1992
Report title	Field soil dissipation rate determination of Glyphosate 360 (Diegten, Switzerland)
Report No	273565
Document No	
Guidelines followed in study	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland (BBA): Richtlinien für die amtliche Prufung von Pflanzenschutzmitteln. Teil IV, 4-1,Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus; Stand: Dezember 1986.
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available, while treated samples were mixed from 20 sampling points - Verification of application rate was not conducted - No information on transport and processing
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary
In the course of this study the rate of field soil dissipation of glyphosate following application of Glyphosate 360 was determined under weather conditions which are typical for the Swiss Jura. The soil used was characterised and found to be typical for this area. The residue levels of Glyphosate 360 (parent compound) and of AMPA (metabolite) were determined at different soil depths and in predetermined intervals until the DT-90 value of glyphosate was achieved or could be calculated. This study should provide a rational basis for an assessment of the degradability of the test article in soil.

To determine the degradation of glyphosate in soil, an untreated and a treated plot were chosen. The treated plot was sprayed by means of a hand driven sprayer at an application rate of 1961.4 L application solution/ha (1.8 g a.s./L) corresponding to 3530.5 g glyphosate/ha. This treatment resulted in a residue level of 2.065 mg glyphosate per kg soil in the soil layer 0-10 cm (estimated value = 2.354 mg/kg). The control plot was left untreated. During the study, temperature, sunlight and precipitation were measured. Soil samples were taken from the treated plot before application, 60 minutes after application and at the time intervals 7, 15, 30, 62, 194 and 282 days. Soil samples from the untreated plot were taken before application and after 7, 15, 30 62, 194 and 282 days.

On each sampling date, 20 cores were taken from the treated plot and 5 cores from the untreated plot. Segments of the same sampling date, plot and depth were combined and blended to give the field sample.

The residue concentrations of the parent compound glyphosate and of the metabolite AMPA were determined by HPLC with post column derivatisation and fluorescence detection. It was shown that only small quantities of glyphosate leached from the top layer. The residue concentrations decreased with the depth of soil segments to reach levels close to the limit of quantification in the 20-30 cm soil layers. A maximum concentration of 0.362 mg AMPA/kg soil (or 0.551 mg/kg calculated as glyphosate equivalent) was observed after 7 days in the 0-10 cm soil segment. Only a small quantity was found in soil segment 20-30 cm after 194 days.

The analytical method was validated with recovery experiments performed at seven fortification levels. The mean recovery of glyphosate was 82.2 % with a relative standard deviation of 21.9 %. The mean recovery of AMPA was 84.1 % with a relative standard deviation of 16.1 %.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation:	Glyphosate 360
Lot No.:	229-Jak-24-1/F
Nominal concentration:	360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The field trial was located in Diegten, Switzerland. Two plots were installed at the field trial, one serving as treated plot and one serving as untreated control plot. One plot served as the control and was at a distance of about 100 m from the treated plot. In each of these plots, a 22 m^2 area was constructed. The 22 m^2 area of the treated plot was divided with cord into 22 subplots, each with an area of 1 m^2.

Soil cores were taken from the trial sites prior to application to determine the soil properties. An overview of the soil characterisation is given in Table 7.1.2.2.1-142.

Table 7.1.2.2.1-142: Soil characteristics of the test plots

Parameter		Result
Cation Exchange Capacity(meq/100 g)		31.0
Particle Size Analysis (USDA) (%) [1]	sand	47.6
	silt	13.3
	clay	39.2
Soil Type		sandy clay
Organic Carbon (%)		1.61
Organic Matter (%) [2]		2.78
pH-Value (KCl)		7.1
Max. water holding capacity (g H_2O/100 g soil dw)		70.1

Table 7.1.2.2.1-142: Soil characteristics of the test plots

Parameter	Result
Biomass before application (mg microb. C/100 g dry soil)	180.0
Biomass 62d after application (mg microb. C/100 g dry soil)	170.0
Biomass 282d after application (mg microb. C/100 g dry soil)	240.5

[1] Due to rounding differences the sum may not correspond to 100 percent.
[2] Calculated from organic carbon according to OM = OC / 0.58

Daily weather data during the entire study from September 1990 to June 1991 was recorded using the weather station "Rünenberg" (altitude: 610 m), about 7 km straight line from the trial site. Reported daily parameters include minimum and maximum air temperatures, total daily precipitation and daily sunlight hours, averaged over a period of one month. Soil temperature and soil moisture measurements from the plots were not reported. Prior to this field experiment, neither the treated nor the untreated field had been treated with any pesticides containing glyphosate as active substance for at least 3 years. After harvest on July 1990, the field was ploughed and afterwards meadow was sown. Immediately before application, the plots were milled by means of a tiller (almost no grass was obtained at this time).

2. Application
Applications at the plots were conducted on 5th September 1990 with a calibrated hand sprayer to bare soil. 35 mL of glyphosate formulation (360 g/L) was placed in a 5 L flask, filled up to the mark with tap water and manually shaken for 2-3 minutes and then transferred to the sprayer. The tank was filled up to 7 L with tap water and stirred inside the sprayer to obtain a homogeneous solution. The application time was determined with a pre-test to ensure a homogeneous distribution and resulted in 4.5 min application time on the 22 m² plot. 4315 mL of the application solution were used corresponding to an actual application rate of 3530.5 g a.s./ha. Stability of the application solution was assessed before and after application with mean values of 86.9 % and 88.0 %.

3. Sampling
Samples for method validation, soil characterisation, water holding capacity and biomass determination were taken shortly before the application.

Residue soil specimens were taken from treated plots before application, 60 minutes after application and at the time intervals 7, 15, 30, 62, 194 and 282 days after application (DAA). Soil samples from untreated plots were taken before application and after 7, 15, 30 62, 194 and 282 DAA. Soil cores were taken by means of a soil corer which contained a plastic tube (length=30 cm, diameter=3.5 cm). From the untreated plot, one sample consisted of 5 cores of 30 cm length and taken at different sites of the plot (not specified). From the treated plot, one sample consisted of 20 cores of 30 cm length, each taken at certain sites of the plot. The sampling points of each plot were noted. During soil sampling the plots were weeded and the plucked weed was left on the plots. No later than 6 h after the sampling, the samples were stored at -20 °C in a deep-freezer.

4. Specimen handling and preparation
At the test facility the core specimens were thawed and cut into segments of 0-10 cm. 10-20 cm and 20-30 cm length by a metal saw in order of increasing concentration. Segments of the same sampling date, plot and depth were combined and homogenised to form a bulk sample representing one specific soil layer per plot and day. Afterwards the samples were transferred to 1 kg plastic screw top bottles and stored in a freezer until the analyses were performed.

To prove the stability of glyphosate and AMPA in the test system during the storage period, untreated samples were fortified with the test compounds and stored under the same conditions (-20 °C) as the field samples. The storage stability test of samples mentioned above was performed and is reported in [redacted] 1995 (CA 7.1.2.2.1/012).

5. Analytical methods

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bidistilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the coeluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Glyphosate and AMPA were quantified separately by HPLC equipped with a post column derivatisation unit and a fluorescence detector. Glyphosate was oxidised with sodium hypochlorite to obtain glycine. Glycine and AMPA were coupled with o-phthaldialdehyde and mercaptoethanol to give fluorescent compounds. The residue was dissolved in 10.0 mL of 0.001 M EDTA solution and analysed by HPLC. If the concentration of the injected sample was above the highest calibration point, samples were diluted with 0.001 M EDTA solution.

This method of analysis was validated with recovery experiments. Stock solutions of glyphosate and AMPA were prepared by dissolving an appropriate amount in bidistilled water. Seven fortification levels from 0.02 mg/kg up to 3.0 mg/kg were prepared for each compound. The mean recovery for glyphosate was 82.2 % with a relative standard deviation of 21.9 %. The mean recovery of AMPA was 84.1 % with a relative standard deviation of 16.1 %. The solutions used for fortification were stored in the refrigerator. The stability of the solutions was checked by analysis before and after the experiments.

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Results of glyphosate and metabolite AMPA residues analysis in soil extracts are summarised in Table **7.1.2.2.1-143** to Table 7.1.2.2.1-144.

Table 7.1.2.2.1-143: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)	**Soil depth (cm)**	**Concentration (mg/kg)**	**Concentration (mg/kg)**
-1	0 - 10	< LOQ	-
	10 - 20	< LOQ	-
	20 - 30	< LOQ	-
0	0 - 10	2.065	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
7	0 - 10	1.033	0.065
	10 - 20	0.054	0.028
	20 - 30	LOQ	< LOQ
15	0 - 10	0.586	0.038
	10 - 20	< LOQ	0.029
	20 - 30	< LOQ	< LOQ
30	0 - 10	0.245	< LOQ
	10 - 20	0.028	0.057
	20 - 30	0.025	0.026
62	0 - 10	0.308	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

Table 7.1.2.2.1-143: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
194	0 - 10	0.175	0.039
	10 - 20	0.039	0.031
	20 - 30	0.028	0.037
282	0 - 10	0.066	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

Table 7.1.2.2.1-144: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.
-1	0 - 10	< LOQ	< 0.030	-	-
	10 - 20	< LOQ	< 0.030	-	-
	20 - 30	< LOQ	< 0.030	-	-
0	0 - 10	0.266	0.344	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
7	0 - 10	0.362	0.551	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
15	0 - 10	0.211	0.321	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
30	0 - 10	0.181	0.276	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	0.024	< 0.037
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
62	0 - 10	0.343	0.522	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
194	0 - 10	0.337	0.513	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	0.036	0.055	< LOQ	< 0.030
282	0 - 10	0.238	0.362	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

Conversion factor AMPA to glyphosate = 1.5226; eq = equivalent

B. CHARACTERISATION OF RESIDUES

The highest level of residue was observed with 2.065 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 and decreased rapidly to 0.066 mg/kg at DAA 282. Only small quantities of glyphosate were found in the soil segments 10-20 cm. In the 20-30 cm soil layers, concentrations close to the limit of quantification were found. Hence it follows that only small quantities of glyphosate leached from the top layer. The maximum concentration of the metabolite AMPA (0.362 mg/kg) was observed in the soil layer 0-10 cm, 7 days after application. This value corresponds to 0.551 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers, except for a concentration of 0,036 mg/kg in the 20-30 cm soil segment after 194 days.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in Robinson (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

The dissipation behaviour of glyphosate was assessed in the field following an application of 3530.5 g a.s./ha. This treatment resulted in a residue level of 2.065 mg glyphosate/kg in the soil layer 0-10 cm on the day of application, declining to 0.066 mg/kg on DAA 282. Only small quantities of glyphosate were found in the soil segments 10-20 cm. In the 20-30 cm soil layers residues were generally below LOQ or close to LOQ for single samplings. Hence it follows that only small quantities of glyphosate leached from the top layer. Thus, leaching is not expected to present a relevant decline process and to impact on degradation kinetics. The maximum concentration of the metabolite AMPA (0.362 mg/kg) was observed in the soil layer 0-10 cm, 7 days after application. This value corresponds to 0.551 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers, except for a concentration of 0.036 mg/kg in the 20-30 cm soil segment after 194 days.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study provides information on the dissipation behavior of glyphosate under European field conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the missing replicates, the missing verification of the application rate and the missing information on transport of samples. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/009
Report author	[redacted]
Report year	1992
Report title	Field soil dissipation rate determination of Glyphosate 360 (Egerkingen, Switzerland)
Report No	280416
Document No	
Guidelines followed in study	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland (BBA): Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln. Teil IV, 4-1, Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus; Stand: Dezember 1986.
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available, while treated samples were mixed from 20 sampling points - Verification of application rate was not conducted - No information on transport and processing
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

In the course of this study the rate of field soil dissipation of glyphosate following application of Glyphosate 360 was determined under weather conditions which are typical for the area between the Swiss Jura and the Swiss alps. The soil used was characterised and found to be typical for this area. The residue levels of Glyphosate 360 (parent compound) and of AMPA (metabolite) were determined at different soil depths and in predetermined intervals until the DT_{90} value of glyphosate was achieved or could be calculated. This study should provide a rational basis for an assessment of the degradability of the test article in soil.

To determine the degradation of glyphosate in soil, an untreated and a treated plot were chosen. The treated plot was sprayed by means of a hand driven sprayer at an application rate of 2152.3 L application solution/ha (1.8 g a.s./L) corresponding to 3874.1 g glyphosate/ha. This treatment resulted in a residue level of 1.317 mg glyphosate per kg soil in the soil layer 0-10 cm (estimated value = 2.583 mg/kg). The control plot was left untreated. During the study, temperature, sunlight and precipitation were measured. Soil samples were taken from the treated plot before application, 60 minutes after application and at the time intervals 7, 15, 30, 62 and 202 days. Soil samples from the untreated plot were taken before application and after 7, 15, 30 62 and 202 days.

On each sampling date, 20 cores were taken from the treated plot and 5 cores from the untreated plot. Segments of the same sampling date, plot and depth were combined and blended to give the field sample.

The residue concentrations of the parent compound glyphosate and of the metabolite AMPA were determined by HPLC with post column derivatisation and fluorescence detection. Glyphosate was found at a concentration of 1.317 mg/kg in the 0-10 cm layer on the day of application and decreased rapidly to 0.091 mg/kg on DAA 202. It was shown that glyphosate was not leached from the top layer. A maximum concentration of 0.328 mg AMPA/kg soil (or 0.500 mg/kg calculated as glyphosate equivalent) was observed after 62 days in the 0-10 cm soil segment.
The analytical method was validated with recovery experiments performed at six fortification levels. The mean recovery of glyphosate was 79.3 % with a relative standard deviation of 25.2 %. The mean recovery of AMPA was 78.9 % with a relative standard deviation of 15.2 %.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation:	Glyphosate 360
Lot No.:	229-Jak-24-1/F
Nominal concentration:	360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The field trial was located in Egerkingen, Switzerland. Two plots were installed at the field trial, one serving as treated plot and one serving as untreated control plot. One plot served as the control and was at a distance of about 100 m from the treated plot. In each of these plots, a 22 m^2 area was constructed. The 22 m^2 area of the treated plot was divided with cord into 22 subplots, each with an area of 1 m².

Soil cores were taken from the trial sites prior to application to determine the soil properties. An overview of the soil characterisation is given in Table 7.1.2.2.1-145.

Table 7.1.2.2.1-145: Soil characteristics of the test plots

Parameter		Result
Cation Exchange Capacity(meq/100 g)		31.3
Particle Size Analysis (USDA) (%) [1]	sand	34.2
	silt	28.8
	clay	37.1
Soil Type		clay loam
Organic Carbon (%)		1.55
Organic Matter (%) [2]		2.67
pH-Value (KCl)		7.33
Max. water holding capacity (g H_2O/100 g soil dw)		69.6
Biomass before application (mg microb. C/100 g dry soil)		187.0
Biomass 62d after application (mg microb. C/100 g dry soil)		200.7
Biomass 202d after application (mg microb. C/100 g dry soil)		211.0

[1] Due to rounding differences the sum may not correspond to 100 percent.
[2] Calculated from organic carbon according to OM = OC / 0.58.

Daily weather data during the entire study from September 1990 to March 1991 was recorded using the weather station "Wynau", about 7 km straight line from the trial site. Reported daily parameters include minimum and maximum air temperatures, total daily precipitation and daily sunlight hours, averaged over a period of one month. Soil temperature and soil moisture measurements from the plots were not reported. Prior to this field experiment, neither the treated nor the untreated field had been treated with any pesticides containing glyphosate as active substance for at least 3 years. After harvest on August 1990, the field was ploughed and afterwards milled by means of a tiller.

2. Application

Applications at the plots were conducted on 5th September 1990 with a calibrated hand sprayer to bare soil. 35 mL of glyphosate formulation (360 g/L) was placed in a 5 L flask, filled up to the mark with tap water and manually shaken for 2-3 minutes and then transferred to the sprayer. The tank was filled up to 7 L with tap water and stirred inside the sprayer to obtain a homogeneous solution. The application time was determined with a pre-test to ensure a homogeneous distribution and resulted in 4.6 min application on the 22 m² plot. 4735 mL of the application solution were used corresponding to an actual application rate of 3874.1 g a.s./ha. Stability of the application solution was assessed before and after application with mean values of 92.6 % and 93.5 %.

3. Sampling

Samples for method validation, soil characterisation, water holding capacity and biomass determination were taken shortly before the application.

Residue soil specimens were taken from treated plots before application, 60 minutes after application and at the time intervals 7, 15, 30, 62 and 202 days after application (DAA). Soil samples from untreated plots were taken before application and after 7, 15, 30 62 and 202 days DAA. Soil cores were taken by means of a soil corer which contained a plastic tube (length=30 cm, diameter=3.5 cm). From the untreated plot, one sample consisted of 5 cores of 30 cm length and taken at different sites of the plot (not specified). From the

treated plot, one sample consisted of 20 cores of 30 cm length, each taken at certain sites of the plot. The sampling points of each plot were noted. During soil sampling the plots were weeded and the plucked weed was left on the plots. No later than 6 h after the sampling, the samples were stored at -20 °C in a deep-freezer.

4. Specimen handling and preparation

At the test facility the core specimens were thawed and cut into segments of 0-10 cm. 10-20 cm and 20-30 cm length by a metal saw in order of increasing concentration. Segments of the same sampling date, plot and depth were combined and homogenised to form a bulk sample representing one specific soil layer per plot and day. Afterwards the samples were transferred to 1 kg plastic screw top bottles and stored in a freezer until the analyses were performed.

To prove the stability of glyphosate and AMPA in the test system during the storage period, untreated samples were fortified with the test compounds and stored under the same conditions (-20 °C) as the field samples. The storage stability test of samples mentioned above was performed and is reported in [redacted] 1995 (CA 7.1.2.2.1/012).

5. Analytical methods

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bidistilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the coeluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Glyphosate and AMPA were quantified separately by HPLC equipped with a post column derivatisation unit and a fluorescence detector. Glyphosate was oxidised with sodium hypochlorite to obtain glycine. Glycine and AMPA were coupled with o-phthaldialdehyde and mercaptoethanol to give fluorescent compounds. The residue was dissolved in 10.0 mL of 0.001 M EDTA solution and analysed by HPLC. If the concentration of the injected sample was above the highest calibration point, samples were diluted with 0.001 M EDTA solution.

This method of analysis was validated with recovery experiments. Stock solutions of glyphosate and AMPA were prepared by dissolving an appropriate amount of glyphosate or AMPA in bi-distilled water. Six fortification levels from 0.02 mg/kg up to 2.0 mg/kg were prepared. The mean recovery for glyphosate was 79.3 % with a relative standard deviation of 25.2 %. The mean recovery of AMPA was 78.9 % with a relative standard deviation of 15.2 %. The solutions used for fortification were stored in the refrigerator. The stability of the solutions was checked by analysis before and after the experiments.

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to about double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Results of glyphosate and metabolite AMPA residues analysis in soil extracts are summarised in Table **7.1.2.2.1-146** to Table 7.1.2.2.1-147.

Table 7.1.2.2.1-146: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)]	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
-1	0 - 10	0.040	-
	10 - 20	< LOQ	-
	20 - 30	< LOQ	-
0	0 - 10	1.317	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
7	0 - 10	0.637	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
15	0 - 10	0.637	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
30	0 - 10	0.472	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
62	0 - 10	0.440	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
202	0 - 10	0.091	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

Table 7.1.2.2.1-147: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.
-1	0 - 10	< LOQ	< 0.030	-	-
	10 - 20	< LOQ	< 0.030	-	-
	20 - 30	< LOQ	< 0.030	-	-
0	0 - 10	0.096	0.146	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
7	0 - 10	0.115	0.175	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
15	0 - 10	0.235	0.358	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
30	0 - 10	0.302	0.460	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
62	0 - 10	0.328	0.500	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
202	0 - 10	0.217	0.330	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

Conversion factor AMPA to glyphosate = 1.5226; eq = equivalent

B. CHARACTERISATION OF RESIDUES

The highest level of residue was observed with 1.317 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 and decreased rapidly to 0.091 mg/kg at DAA 202. In the deeper soil segments, 10-20 cm and 20-30 cm, no concentrations above the limit of quantification were found. Hence it follows that glyphosate was not leached from the top layer. The maximum concentration of the metabolite AMPA (0.328 mg/kg) was observed in the soil layer 0-10 cm, 62 days after application. This value corresponds to 0.500 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

The dissipation behaviour of glyphosate was assessed in the field following an application of 3530.5 g a.s./ha. This treatment resulted in a residue level of 1.317 mg glyphosate/kg in the soil layer 0-10 cm on the day of application, declining to 0.091 mg/kg on DAA 202. In the deeper soil segments 10-20 cm and 20-30 cm, no concentrations above the limit of quantification were found. The maximum concentration of the metabolite AMPA (0.328 mg/kg) was observed in the soil layer 0-10 cm, 62 days after application. This value corresponds to 0.500 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study provides information on the dissipation behavior of glyphosate under European field conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the missing replicates, the missing verification of the application rate and the missing information on transport of samples. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/010
Report author	[redacted]
Report year	1992
Report title	Field soil dissipation rate determination of Glyphosate 360 (Bad Krozingen, Germany
Report No	280427
Document No	
Guidelines followed in study	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland (BBA): Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln. Teil IV, 4-1,Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus; Stand: Dezember 1986.
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available, while treated samples were mixed from 20 sampling points - Verification of application rate was not conducted - No information on transport and processing - Study was terminated at decline of a.s. to ca. 16 % of initial
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

In the course of this study the rate of field soil dissipation of glyphosate following application of Glyphosate 360 was determined under weather conditions which are typical for the south-west of Germany. The soil used was characterised and found to be typical for this area. The residue levels of Glyphosate 360 (parent compound) and of AMPA (metabolite) were determined at different soil depths and in predetermined intervals until the DT-90 value of glyphosate was achieved or could be calculated. This study should provide a rational basis for an assessment of the degradability of the test article in soil.

To determine the degradation of glyphosate in soil, an untreated and a treated plot were chosen. The treated plot was sprayed by means of a hand driven sprayer at an application rate of 2036.4 L application solution/ha (1.8 g a.s./L) corresponding to 3665.5 g glyphosate/ha. This treatment resulted in a residue level of 2.456 mg glyphosate per kg soil in the soil layer 0-10 cm (estimated value = 2.444 mg/kg). The control plot was left untreated. During the study, temperature, sunlight and precipitation were measured. Soil samples were taken from the treated plot before application, 60 minutes after application and at the time intervals 7, 15, 30 and 61 days. Soil samples from the untreated plot were taken before application and after 7, 15, 30 and 61 days.

On each sampling date, 20 cores were taken from the treated plot and 5 cores from the untreated plot. Segments of the same sampling date, plot and depth were combined and blended to give the field sample.

The residue concentrations of the parent compound glyphosate and of the metabolite AMPA were determined by HPLC with post column derivatisation and fluorescence detection. Glyphosate was found at a concentration of 2.456 mg/kg in the 0-10 cm layer on the day of application and decreased rapidly to

0.390 mg/kg on DAA 61. It was shown that glyphosate was not leached from the top layer. Only small quantities (0.046 mg/kg) were found between 10 and 20 cm depth after 7 days, no residues were observed above LOQ in the 20-30 cm layer. A maximum concentration of 0.425 mg AMPA/kg soil (or 0.647 mg/kg calculated as glyphosate equivalent) was observed after 61 days in the 0-10 cm soil segment.

The analytical method was validated with recovery experiments performed at six fortification levels. The mean recovery of glyphosate was 81.3 % with a relative standard deviation of 7.8 %. The mean recovery of AMPA was 86.2 % with a relative standard deviation of 5.4 %.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation:	Glyphosate 360
Lot No.:	229-Jak-24-1/F
Nominal concentration:	360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The field trial was located in Bad Krozingen, Germany. Two plots were installed at the field trial, one serving as treated plot and one serving as untreated control plot. One plot served as the control and was at a distance of about 150 m from the treated plot. In each of these plots, a 22 m^2 area was constructed. The 22 m² area of the treated plot was divided with cord into 22 subplots, each with an area of 1 m².

Soil cores were taken from the trial sites prior to application to determine the soil properties. An overview of the soil characterisation is given in Table 7.1.2.2.1-148.

Table 7.1.2.2.1-148: Soil characteristics of the test plots

Parameter		Result
Cation Exchange Capacity(meq/100 g)		8.9
Particle Size Analysis (USDA) (%) [1]	sand	55.0
	silt	27.1
	clay	17.9
Soil Type		sandy loam
Organic Carbon (%)		0.36
Organic Matter (%) [2]		0.62
pH-Value (KCl)		6.0
Max. water holding capacity(g H_2O/100 g soil d)		32.3
Biomass before application (mg microb. C/100 g dry soil)		19.5
Biomass 61d after application (mg microb. C/100 g dry soil)		47.1

[1] Due to rounding differences the sum may not correspond to 100 percent.
[2] Calculated from organic carbon according to OM = OC / 0.58

Daily weather data during the entire study from September to November 1990 was recorded using the weather stations “Schallstadt-Mengen” and “Bremgarten”, about 7 km and 4 km straight line from the trial site, respectively. Reported daily parameters include minimum and maximum air temperatures, total daily precipitation and daily sunlight hours, averaged over a period of one month. Soil temperature and soil moisture measurements from the plots were not reported. Prior to this field experiment, neither the treated nor the untreated field had been treated with any pesticides containing glyphosate as active substance for at least 3 years. After harvest on August 1990, the field was ploughed and afterwards harrowed.

2. Application
Applications at the plots were conducted on 4th September 1990 with a calibrated hand sprayer to bare soil. 35 mL of glyphosate formulation (360 g/L) was placed in a 5 L flask, filled up to the mark with tap water and manually shaken for 2-3 minutes and then transferred to the sprayer. The tank was filled up to 7 L with tap water and stirred inside the sprayer to obtain a homogeneous solution. The application time was determined with a pre-test to ensure a homogeneous distribution and resulted in 4.4 min application time on the 22 m² plot. 4480 mL of the application solution were used corresponding to an actual application rate of 3665.5 g a.s./ha. The stability of the application solution was tested in the field dissipation studies RCC 273565 and RCC 280416. The solutions were considered to be stable under the application conditions.

3. Sampling
Samples for method validation, soil characterisation, water holding capacity and biomass determination were taken shortly before the application.

Residue soil specimens were taken from treated plots before application, 60 minutes after application and at the time intervals 7, 15, 30 and 61 days after application (DAA). Soil samples from untreated plots were taken before application and after 7, 15, 30 and 61 days DAA. Soil cores were taken by means of a soil corer which contained a plastic tube (length=30 cm, diameter=3.5 cm). From the untreated plot, one sample consisted of 5 cores of 30 cm length and taken at different sites of the plot (not specified). From the treated plot, one sample consisted of 20 cores of 30 cm length, each taken at certain sites of the plot. The sampling points of each plot were noted. During soil sampling the plots were weeded and the plucked weed was left on the plots. No later than 6 h after the sampling, the samples were stored at -20 °C in a deep-freezer.

4. Specimen handling and preparation
At the test facility the core specimens were thawed and cut into segments of 0-10 cm, 10-20 cm and 20-30 cm length by a metal saw in order of increasing concentration. Segments of the same sampling date, plot and depth were combined and homogenised to form a bulk sample representing one specific soil layer per plot and day. Afterwards the samples were transferred to 1 kg plastic screw top bottles and stored in a freezer until the analyses were performed.

To prove the stability of glyphosate and AMPA in the test system during the storage period, untreated samples were fortified with the test compounds and stored under the same conditions (-20 °C) as the field samples. The storage stability test of samples mentioned above was performed and is reported in [redacted] 1995 (CA 7.1.2.2.1/012).

5. Analytical methods
25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bidistilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the coeluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Glyphosate and AMPA were quantified separately by HPLC equipped with a post column derivatisation unit and a fluorescence detector. Glyphosate was oxidised with sodium hypochlorite to obtain glycine. Glycine and AMPA were coupled with o-phthaldialdehyde and mercaptoethanol to give fluorescent compounds. The residue was dissolved in 10.0 mL of 0.001 M EDTA solution and analysed by HPLC. If the concentration of the injected sample was above the highest calibration point, samples were diluted with 0.001 M EDTA solution.

This method of analysis was validated with recovery experiments. Stock solutions of glyphosate and AMPA were prepared by dissolving an appropriate amount of glyphosate or AMPA in bidistilled water. Six fortification levels from 0.05 mg/kg up to 2.5 mg/kg were prepared. The mean recovery of glyphosate was 81.3 % with a relative standard deviation of 7.8 %. The mean recovery of AMPA was 86.2 % with a relative standard deviation of 5.4 %. The solutions used for fortification were stored in the refrigerator. The stability of the solutions was checked by analysis before and after the experiments.

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to about double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Results of glyphosate and metabolite AMPA residues analysis in soil extracts are summarised in Table **7.1.2.2.1-149** to Table 7.1.2.2.1-150.

Table 7.1.2.2.1-149: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)	**Soil depth (cm)**	**Concentration (mg/kg)**	**Concentration (mg/kg)**
-1	0 - 10	< LOQ	-
	10 - 20	< LOQ	-
	20 - 30	< LOQ	-
0	0 - 10	2.456	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
7	0 - 10	0.893	< LOQ
	10 - 20	0.046	< LOQ
	20 - 30	< LOQ	< LOQ
15	0 - 10	0.812	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
30	0 - 10	0.436	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
61	0 - 10	0.390	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

Table 7.1.2.2.1-150: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.
-1	0 - 10	< LOQ	< 0.030	-	-
	10 - 20	< LOQ	< 0.030	-	-
	20 - 30	< LOQ	< 0.030	-	-
0	0 - 10	0.253	0.385	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
7	0 - 10	0.233	0.355	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
15	0 - 10	0.266	0.405	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
30	0 - 10	0.300	0.457	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
61	0 - 10	0.425	0.647	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

Conversion factor AMPA to glyphosate = 1.5226; eq = equivalent

B. CHARACTERISATION OF RESIDUES

The highest level of residue was observed with 2.456 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 and decreased rapidly to 0.390 mg/kg at DAA 61. Only small quantities of glyphosate (0.046 mg/kg) were found in the 10-20 cm soil segment, 7 days after application, no residues were encountered above LOQ in the 20-30 cm layer. Hence it follows that glyphosate was not leached from the top layer. The maximum concentration of the metabolite AMPA (0.425 mg/kg) was observed in the soil layer 0-10 cm, 61 days after application. This value corresponds to 0.647 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

The dissipation behaviour of glyphosate was assessed in the field following an application of 3665.5 g a.s./ha. This treatment resulted in a residue level of 2.456 mg glyphosate/kg in the soil layer 0-10 cm on the day of application, declining to 0.390 mg/kg on DAA 61 at approximately 84 % glyphosate decline compared to initial. Glyphosate was not prone to leaching; as a maximum of 0.046 mg a.s./kg was found in the 10-20 cm layer. No residues > LOQ were encountered in the 20-30 cm layer. The maximum concentration of the metabolite AMPA (0.425 mg/kg) was observed in the soil layer 0-10 cm, 61 days after application. This value corresponds to 0.647 mg equivalents glyphosate/kg soil. No AMPA concentrations above the determination limit were found in deeper soil layers.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides information on the dissipation behavior of glyphosate under European field conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the missing replicates, the missing verification of the application rate and the missing information on transport of samples. These deficiencies do not have a serious impact on the results of the study.
The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/011
Report author	[redacted]
Report year	1992
Report title	Field soil dissipation rate determination of Glyphosate 360 (Menslage, Germany)
Report No	RCC 280438
Document No	
Guidelines followed in study	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesrepublik Deutschland (BBA): Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln. Teil IV, 4-1,Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus; Stand: Dezember 1986.
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available, while treated samples were mixed from 20 sampling points - Verification of application rate was not conducted - No information on transport and processing
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary
In the course of this study the rate of field soil dissipation of glyphosate following application of Glyphosate 360 was determined under weather conditions which are typical for the north-west of Germany. The soil used was characterised and found to be typical for this area. The residue levels of Glyphosate 360 (parent compound) and of AMPA (metabolite) were determined at different soil depths and in predetermined intervals until the DT-90 value of glyphosate was achieved or could be calculated. This study should provide a rational basis for an assessment of the degradability of the test article in soil.

To determine the degradation of glyphosate in soil, an untreated and a treated plot were chosen. The treated plot was sprayed by means of a hand driven sprayer at an application rate of 2036.4 L application solution/ha (1.8 g a.s./L) corresponding to 3665.5 g glyphosate/ha. This treatment resulted in a residue level of 2.659 mg glyphosate per kg soil in the soil layer 0-10 cm (estimated value = 2.444 mg/kg). The control plot was left untreated. During the study, temperature, sunlight and precipitation were measured. Soil samples were taken from the treated plot before application, 60 minutes after application and at the time intervals 7, 15, 30, 60, 192, 271 and 315 days. Soil samples from the untreated plot were taken before application and after 7, 15, 30, 60, 192, 271 and 315 days.

On each sampling date, 20 cores were taken from the treated plot and 5 cores from the untreated plot. Segments of the same sampling date, plot and depth were combined and blended to give the field sample.

The residue concentrations of the parent compound glyphosate and of the metabolite AMPA were determined by HPLC with post column derivatisation and fluorescence detection. It was shown that glyphosate was not leached from the top layer. A maximum of 2.659 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 was observed and decreased rapidly to 0.122 mg/kg at DAA 315. The residue concentrations decrease with the depth of soil segments to reach levels to the limit of quantification in the 10-20 and 20-30 cm soil layers. A maximum concentration of 0.853 mg AMPA/kg soil (or 1.299 mg/kg calculated as glyphosate equivalent) was observed after 271 days in the 0-10 cm soil segment.

The analytical method was validated with recovery experiments performed at eight fortification levels. The mean recovery of glyphosate was 74.7 % with a relative standard deviation of 16.0 %. The mean recovery of AMPA was 78.7 % with a relative standard deviation of 19.3 %.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation:	Glyphosate 360
Lot No.:	229-Jak-24-1/F
Nominal concentration:	360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The field trial was located in Menslage, Germany. Two plots were installed at the field trial, one serving as treated plot and one serving as untreated control plot. One plot served as the control and was at a distance of about 150 m from the treated plot. In each of these plots, a 22 m^2 area was constructed. The 22 m² area of the treated plot was divided with cord into 22 subplots, each with an area of 1 m².

Soil cores were taken from the trial sites prior to application to determine the soil properties. An overview of the soil characterisation is given in Table 7.1.2.2.1-151.

Table 7.1.2.2.1-151: Soil characteristics of the test plots

Parameter		Result
Cation Exchange Capacity(meq/100 g)		4.9
Particle Size Analysis (USDA) (%) [1]	sand	90.6
	silt	2.1
	clay	7.2
Soil Type		sandy soil
Organic Carbon (%)		0.25
Organic Matter (%) [2]		0.43
pH-Value (KCl)		4.73
Max. water holding capacity (g H_2O/100 g soil dw)		33.3
Biomass before application (mg microb. C/100 g dry soil)		11.2
Biomass 60d after application (mg microb. C/100 g dry soil)		24.6
Biomass 271d after application (mg microb. C/100 g dry soil)		18.8

[1] Due to rounding differences the sum may not correspond to 100 percent.
[2] Calculated from organic carbon according to OM = OC / 0.58

Daily weather data during the entire study from September 1990 to July 1991 was recorded using the weather stations "Löningen" and "Menslage-Borg", about 10 km and 0.2 km straight line from the trial site, respectively. Reported daily parameters include minimum and maximum air temperatures, total daily precipitation and daily sunlight hours, averaged over a period of one month. Soil temperature and soil moisture measurements from the plots were not reported. Prior to this field experiment, neither the treated nor the untreated field had been treated with any pesticides containing glyphosate as active substance for at least 3 years. Prior to application, the grown corn was cut and the soil hoed.

2. Application
Applications at the plots were conducted on 7th September 1990 with a calibrated hand sprayer to bare soil. 35 mL of glyphosate formulation (360 g/L) was placed in a 5 L flask, filled up to the mark with tap water and manually shaken for 2-3 minutes and then transferred to the sprayer. The tank was filled up to 7 L with tap water and stirred inside the sprayer to obtain a homogeneous solution. The application time was determined with a pre-test to ensure a homogeneous distribution and resulted in 4.3 min application time on the 22 m² plot. 4480 mL of the application solution were used corresponding to an actual application rate of 3665.5 g a.s./ha. The stability of the application solution was tested in the field dissipation studies RCC 273565 and RCC 280416. The solutions were considered to be stable under the application conditions.

3. Sampling
Samples for method validation, soil characterisation, water holding capacity and biomass determination were taken shortly before the application.

Residue soil specimens were taken from treated plots before application, 60 minutes after application and at the time intervals 7, 15, 30, 60, 192, 271 and 315 days after application (DAA). Soil samples from untreated plots were taken before application and after 7, 15, 30, 60, 192, 271 and 315 days DAA. Soil cores were taken by means of a soil corer which contained a plastic tube (length=30 cm, diameter=3.5 cm). From the untreated plot, one sample consisted of 5 cores of 30 cm length and taken at different sites of the plot (not specified). From the treated plot, one sample consisted of 20 cores of 30 cm length, each taken at certain sites of the plot. The sampling points of each plot were noted. During soil sampling the plots were weeded and the plucked weed was left on the plots. No later than 6 h after the sampling, the samples were stored at -20 °C in a deep-freezer.

4. Specimen handling and preparation
At the test facility the core specimens were thawed and cut into segments of 0-10 cm. 10-20 cm and 20-30 cm length by a metal saw in order of increasing concentration. Segments of the same sampling date, plot and depth were combined and homogenised to form a bulk sample representing one specific soil layer per plot and day. Afterwards the samples were transferred to 1 kg plastic screw top bottles and stored in a freezer until the analyses were performed.

To prove the stability of glyphosate and AMPA in the test system during the storage period, untreated samples were fortified with the test compounds and stored under the same conditions (-20 °C) as the field samples. The storage stability test of samples mentioned above was performed and is reported in Morgenroth, 1995 (CA 7.1.2.2.1/012).

5. Analytical methods

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bidistilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the coeluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Glyphosate and AMPA were quantified separately by HPLC equipped with a post column derivatisation unit and a fluorescence detector. Glyphosate was oxidised with sodium hypochlorite to obtain glycine. Glycine and AMPA were coupled with o-phthaldialdehyde and mercaptoethanol to give fluorescent compounds. The residue was dissolved in 10.0 mL of 0.001 M EDTA solution and analysed by HPLC. If the concentration of the injected sample was above the highest calibration point, samples were diluted with 0.001 M EDTA solution.

This method of analysis was validated with recovery experiments. Stock solutions of glyphosate and AMPA were prepared by dissolving an appropriate amount of glyphosate or AMPA in bidistilled water. Eight fortification levels from 0.05 mg/kg up to 2.5 mg/kg were prepared. The mean recovery of glyphosate was 74.7 % with a relative standard deviation of 16.0 %. The mean recovery of AMPA was 78.7 % with a relative standard deviation of 19.3 %. The solutions used for fortification were stored in the refrigerator. The stability of the solutions was checked by analysis before and after the experiments.

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to about double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Results of glyphosate and metabolite AMPA residues analysis of test item and metabolite in soil extracts are summarised in Table 7.1.2.2.1-152 to Table 7.1.2.2.1-153.

Table 7.1.2.2.1-152: Results of glyphosate residues analysis

Glyphosate		Treated plot	Untreated plot
DAA (d)	Soil depth (cm)	Concentration (mg/kg)	Concentration (mg/kg)
-1	0 - 10	< LOQ	-
	10 - 20	< LOQ	-
	20 - 30	< LOQ	-
0	0 - 10	2.659	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	0.030[1]	< LOQ
7	0 - 10	1.319	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
15	0 - 10	0.580	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
30	0 - 10	0.678[2]	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
60	0 - 10	0.506	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
192	0 - 10	0.277	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
271	0 - 10	0.281	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ
315	0 - 10	0.122	< LOQ
	10 - 20	< LOQ	< LOQ
	20 - 30	< LOQ	< LOQ

[1] Peak is probably not caused by glyphosate; therefore, this concentration is not used for interpretation
[2] Sample was re-analysed since the peak is probably not caused by glyphosate; consequently, the result of the second analysis is presented (result of first analysis: 0.299 mg/kg)

Table 7.1.2.2.1-153: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.
-1	0 - 10	< LOQ	< 0.030	-	-
	10 - 20	< LOQ	< 0.030	-	-
	20 - 30	< LOQ	< 0.030	-	-
0	0 - 10	0.094	0.143	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
7	0 - 10	0.224	0.341	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
15	0 - 10	0.312	0.475	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
30	0 - 10	0.374[1]	0.569	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
60	0 - 10	0.515	0.784	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

Table 7.1.2.2.1-153: Results of metabolite AMPA residues analysis

AMPA		Treated plot		Untreated plot	
DAA (d)	Soil depth (cm)	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.	Concentration (mg/kg) as AMPA	Concentration (mg/kg) as glyphosate eq.
192	0 - 10	0.416	0.633	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
271	0 - 10	0.853	1.299	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030
315	0 - 10	0.417	0.635	< LOQ	< 0.030
	10 - 20	< LOQ	< 0.030	< LOQ	< 0.030
	20 - 30	< LOQ	< 0.030	< LOQ	< 0.030

[1] Sample was re-analysed due to unsatisfactory result; consequently, the result of the second analysis is presented (result of the first analysis: 0.241 mg/kg)
Conversion factor AMPA to glyphosate = 1.5226; eq = equivalent

B. CHARACTERISATION OF RESIDUES

The highest level of residue was observed with 2.659 mg glyphosate/kg in the soil layer 0-10 cm at DAA 0 and decreased rapidly to 0.122 mg/kg at DAA 315. No residues above the limit of quantification (0.02 mg/kg) were found in the soil layers 10-20 and 20-30 cm. Hence it follows that no glyphosate was leached from the top layer. The maximum concentration of the metabolite AMPA (0.853 mg/kg) was observed in the soil layer 0-10 cm, 271 days after application. This value corresponds to 1.299 mg glyphosate/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

The dissipation behaviour of glyphosate was assessed in the field following an application of 3665.5 g a.s./ha. This treatment resulted in a residue level of 2.659 mg glyphosate/kg in the soil layer 0-10 cm on the day of application, declining to 0.122 mg/kg on DAA 315. No residues above the limit of quantification (0.02 mg/kg) were found in the soil layers 10-20 and 20-30 cm. Hence it follows that glyphosate was not leached from the top layer. The maximum concentration of the metabolite AMPA (0.853 mg/kg) was observed in the soil layer 0-10 cm, 271 days after application. This value corresponds to 1.299 mg glyphosate equivalent/kg soil. No AMPA concentrations above the quantification limit were found in deeper soil layers.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study provides information on the dissipation behavior of glyphosate under European field conditions. It is mainly consistent with the current guideline showing no major deficiencies. Minor deficiencies are the missing replicates, the missing verification of the application rate and the missing information on transport of samples. These deficiencies do not have a serious impact on the results of the study.

The study is therefore considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/012
Report author	[redacted]
Report year	1995
Report title	Storage stability of Glyphosate and AMPA in soil
Report No	303625
Document No	
Guidelines followed in study	Biologische Bundesanstalt (BBA) Richtlinie Teil IV, Reihe 2: Rückstandsanalytik (1986), BBA-Merkblatt Nr. 58. Rückstandsuntersuchungen - Richtlinien zur Durchführung der Analysen (1983) Industrieverband Agrar (IVA) Guidelines „Rückstandsversuche“
Deviations from current test guideline	Not relevant
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary

This study was initiated to determine the storage stability of glyphosate and its metabolite AMPA in soil when stored in a deepfreeze compartment at about -20 °C. The soil control sample used was obtained from a local farmer (CH-4457 Diegten) in the Swiss Jura, and derived from RCC project 273565 (= Field soil dissipation rate determination of Glyphosate 360, Dietgen, Switzerland). The soil was of a sandy clay soil type (organic carbon content = 1.6 %), taken from a depth of 0-30 cm and the moisture content was determined to be 20.3 %.

Glyphosate and AMPA were analysed separately by high performance liquid chromatography with post column derivatization and fluorescence detection, according to the method used in RCC project 273565 and in a preliminary test.

Soil samples fortified at levels of 1.0 mg glyphosate/kg wet soil or 0.5 mg AMPA /kg wet soil were put into storage in a deepfreeze compartment (about -20 °C) in the dark until the analyses were performed. The analyses were performed one week after preparation of storage stability samples (day 7), about six months (day 188), about nine months (day 292), and about one year (day 404) later. Storage time was considered from the date of sample fortification to the date of extraction.

No residues of glyphosate or AMPA above the limit of determination of 0.02 mg/kg were found in the control samples, except for one control sample assumed to be contaminated during the analytical procedure.

The recovery percentages for glyphosate in soil storage stability samples were calculated to be 90.1 % one week after sample fortification, 56.1 % after about six months, 111.8 % after about nine months, and 76.2 % after about one year of storage time. The overall mean recovery was determined to be 83.6 % with a relative standard deviation of 28.1 % (n = 4). The recovery percentages for AMPA in soil storage stability samples were calculated to be 77.3 % one week after sample fortification, 58.9 % after about six months, 84.9 % after about nine months, and 73.4 % after about one year of storage time. The overall mean recovery was determined to be 73.6 % with a relative standard deviation of 14.8 % (n = 4).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate Analytical standard
Lot No.:	185-ff-131
Chemical purity:	99.5 %

Identification:	AMPA
Lot No.:	108F3811
Chemical purity:	Appr. 99 %

2. Soil:

The soil control samples were obtained from a local farmer (CH-4457 Diegten) in the Swiss Jura, and derived from RCC project 273565 (please refer to [redacted] 1992, CA 7.1.2.2.1/008). The soil cores were of a sandy clay soil type (organic carbon content = 1.6 %), taken from a depth of 0-30 cm, and the moisture content was determined to be 20.3 %. The control samples were stored deep frozen until the storage stability test.

B. STUDY DESIGN

1. Experimental conditions

To prepare the storage stability test samples, the deep frozen untreated soil sample (about 1 kg) was thawed to room temperature. Afterwards, analytical size portions of 25 g wet soil were taken and transferred into 50 ml plastic screw-top bottles on August 08, 1991. These samples were immediately fortified with 240 µl of the glyphosate stock solution or 130 ul of the AMPA stock solution, corresponding to concentration levels of 1.0 mg glyphosate/kg wet soil and 0.5 mg AMPA/kg wet soil, respectively. To achieve a nearly homogeneous distribution, the fortification solution was slowly injected by circular movements of the microliter syringe.

Additionally, two control samples were stored for each time interval under equal conditions as the storage stability test samples.

Immediately after fortification, the plastic bottles were put in storage in a deepfreeze compartment (at about -20 °C) in the dark until the analyses were performed. Samples were taken for analysis one week after preparation of storage stability sample (day 7), and about six months (day 188), about nine months (day 292), and about one year (day 404) later. At each time interval, the storage stability test sample and the corresponding control sample were removed from the freezer and analysed for glyphosate and its metabolite AMPA.

For method validation, at least one procedural recovery at a level of 1.0 mg glyphosate/kg wet soil or 0.5 mg AMPA/kg wet soil was freshly prepared per sample series by fortifying untreated control samples with calculated amounts of glyphosate or AMPA solutions. These fortified samples were analysed according to the same analytical procedures as the storage stability samples. The procedural recoveries provided were an indication of the method efficiency on that day.

2. Analytical procedures

25 g of wet soil was placed in a 250 mL wide neck bottle. 150 mL of 1 M ammonium hydroxide solution was added and shaken for 30 minutes at 180 movements per minute using a lab shaker. Then, the mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant liquid was transferred to a 2 L beaker. This extraction step was repeated twice. Afterwards, the combined extracts were adjusted to pH 2.0±0.4 using about 30 mL of 32 % hydrochloric acid and 20-30 mL of 1 M hydrochloric acid. After dilution to 1.6 L with bi-distilled water, the pH value was checked and, if necessary, re-adjusted to pH 2.0±0.4. The sediment was allowed to settle for about 30 minutes and the supernatant was decanted and collected. The extract was

cleaned-up on a Fe (III) loaded Chelex 100 resin. Glyphosate and AMPA were eluted with hydrochloric acid and the co-eluted Fe (III) ions were removed from the eluates using an ion-exchange resin. Afterwards, the resulting eluate was concentrated to dryness by means of a rotary-evaporator.

Procedural Recoveries

This method of analysis was validated with recovery experiments. Stock solutions were prepared by dissolving an appropriate amount of glyphosate or AMPA in 0.001 mol/L EDTA solution. These stock solutions were diluted with 0.001 mol/L EDTA solution to yield concentrations of 10 µg/ml. Fortified samples were prepared by adding calculated volumes of the latter solutions to the analytical material of untreated control samples based on the lowest concentrations successfully used in RCC project 273565 (please refer to [redacted] 1992, CA 7.1.2.2.1/008).

The limit of quantification (LOQ) was set at 0.02 mg/kg and corresponds to about double the limit of detection (LOD) of 0.01 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA

Residues for glyphosate and AMPA after frozen storage are presented in the tables below.

Table 7.1.2.2.1-154: Summary of residues (mg/kg) of glyphosate in sandy clay soil after frozen storage

DAT	Control	Procedural recoveries		Storage stability sample	
	Residue (mg/kg)	Residue (mg/kg)	Recovery (%)	Residue (mg/kg)	Recovery (%)
0	< 0.02	0.916 (1.017) [2]	90.1	0.916 (1.017) [2]	90.1
188	< 0.02	0.676 (1.000) [2]	67.6	0.571 (1.017) [2]	56.1
292	0.048 [1]	0.747 (1.000) [2]	74.7	1.137 (1.017) [2]	111.8
404	< 0.02	0.821 (1.000) [2]	82.2	0.775 (1.017) [2]	76.2
Mean			78.6		83.6

[1] Sample was assumed to be contaminated during analytical procedure. The storage stability was not correct for the control sample
[2] Fortification level in brackets

Table 7.1.2.2.1-155: Summary of residues (mg/kg) of AMPA in sandy clay soil after frozen storage

DAT	Control	Procedural recoveries		Storage stability sample	
	Residue (mg/kg)	Residue (mg/kg)	Recovery (%)	Residue (mg/kg)	Recovery (%)
0	< 0.02	n.a. [1]	n.a. [1]	0.401 (0.519) [2]	77.3
188	< 0.02	0.278 (0.500) [2]	55.7	0.306 (0.519) [2]	58.9
292	< 0.02	0.396 (0.500) [2]	79.2	0.440 (0.519) [2]	84.9
404	< 0.02	0.400 (0.500) [2]	80.0	0.381 (0.519) [2]	73.4
Mean			73.6		71.6

[1] na = not evaluated due to technical reasons
[2] Fortification level in brackets

B. CHARACTERISATION OF RESIDUES

The recovery percentages for glyphosate in soil storage stability samples were calculated to be 90.1 % one week after sample fortification, 56.1 % after about six months, 111.8 % after about nine months, and 76.2 % after about one year of storage time. The overall mean recovery was determined to be 83.6 % with a relative standard deviation of 28.1 % (n = 4). The recovery percentages for AMPA in soil storage stability samples were calculated to be 77.3 % one week after sample fortification, 58.9 % after about six months, 84.9 %

after about nine months, and 73.4 % after about one year of storage time. The overall mean recovery was determined to be 73.6 % with a relative standard deviation of 14.8 % (n = 4).

No residues of glyphosate or AMPA above the limit of determination of 0.02 mg/kg were found in the control samples, except for the glyphosate control sample analysed after about nine months (292 days). This control sample was assumed to be contaminated during the analytical procedure.

The efficiency of the analytical method on the day of analysis was determined with freshly prepared procedural recoveries performed at the fortification levels of the stored samples, namely 1.0 mg/kg for glyphosate and 0.5 mg/kg for AMPA. The mean procedural recovery for glyphosate was 78.6 % with a relative standard deviation of 12.3 % (n = 4). The mean procedural recovery for AMPA was 71.6 % with a relative standard deviation of 19.3 % (n = 4).

The recoveries of glyphosate and AMPA were not corrected for control values and the storage stability results were not corrected for procedural recoveries or control values.

III. CONCLUSIONS

In conclusion, the results indicate that glyphosate and AMPA are stable in the tested soil for at least 404 days at about -20 °C. The recoveries of stored fortified samples were nearly identical to that of the procedural fortification samples.

3. Assessment and conclusion

Assessment and conclusion by applicant:
It was shown that glyphosate and AMPA residues in soil are stable for up to one year storage time under deep frozen conditions. The relative standard deviation is fairly high due to the low recovery on day 188. Due to the improved recovery at day 292 and 404, the overall conclusion is seen as sufficiently reliable. The study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/013
Report author	[redacted]
Report year	1992
Report title	Glyphosate-trimesium: Soil Dissipation Study (Germany 1990-1992)
Report No	RJ1294B
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No replicate residue data available, while treated samples were mixed from 20 sampling points - No verification transport and processing
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A study was carried out in Germany during 1990-1992 to monitor the rate of dissipation of glyphosate-trimesium in soil following a single application at a nominal rate of 4.80 kg a.s./ha. Trials were carried out at six locations; Buchen, Kleinzecher, Unzhurst, Rohrbach, Herrngiersdorf and Wang-lnzkofen. Samples were taken on 10 to 12 sampling dates after application distributed over periods of 15 or 19 months after application.

Samples were analysed for residues of trimesium (TMS+) (trimethylsulfonium cation), glyphosate (PMG) (n-Phosphomethylglycine) and AMPA (aminomethylphosphonic acid).

Residues of glyphosate-trimesium (TMS+) in the top layer ranged from 0.98 to 1.6 mg/kg at day 0 and decreased to <LOD in four trials after 61 to 298 days. In the other two trials, Unzhurst (RS-9027/E1) and Wang-Inzkofen (RS-9027G2), background levels of natural DMS was present. These were equivalent to means of 7-10 % of the applied chemical.

Initial residues in the top layer ranged from 1.9 to 3.2 mg/kg at day 0 and decreased to < LOD in four trials after 168 to 549 days. In the remaining two trials, Buchen (RS-9027/B1) and Unzhurst (RS-9027/E1), residues of 0.15 and 0.07 mg/kg were detected by the end of the study. No residues of glyphosate (PMG) were measured in any trial, on any occasion, in the soil horizon below 10 cm.

Residues of AMPA did not exceed 0.5 mg/kg at any interval in any trial and dissipated to between 0.06 and 0.33 mg/kg during the study period. No residues greater than 0.10 mg/kg^{-} of N-phosphonomethylglycine (PMG), aminomethylphosphonic acid (AMPA) or trimethylsulphonium cation (TMS+) were determined in the second depth profile (10-20 cm) at any sampling interval, at any trial.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate as glyphosate-trimesium (ICIA 0224)
Product:	Sulfosate (YF7712A), 48 % SL formulation
Lot No.:	D4875/160
Nominal concentration:	48 % glyphosate-trimesium

B. STUDY DESIGN

1. Test sites

The study (field work and analysis) was carried out between April 1990 and June 1992. Test sites were chosen to present typical soils and climates of proposed use areas of the product. Pesticide history was reported over three years for each trial.

At each of the trial sites the area was divided into two plots, a treated plot and an untreated or control plot, separated bv a buffer zone. The size of the treated and control plots for ail trials was 2.5 m x 22 m (except trial RS-9027/G2 where the size was 2.5 m x 20 m). Each of the treated plot areas was subdivided into four sub-plots from which a total of 20 core samples (generally 5 from each subplot) were taken.

At each of the trial sites (except for Buchen (RS-9027/B1) and Wang-Inzkofen (RS-9027/G2)) at least one soil pit was dug and samples were taken from three horizons to a depth of at least 90 cm in all cases. Between 0.5 and 1 kg of soil was then bulked from each horizon and sent to Jealott's Hill Research Station, Bracknell, UK, for physico-chemical characterization.

An overview of the soil characterization is given in Table 7.1.2.2.1-156 to Table 7.1.2.2.1-161

Table 7.1.2.2.1-156: Soil characteristics of the Buchen (RS-9027/B1) test site

Parameter		Horizon		
Soil depth (cm)		**0-30**	**30-60**	**60-100**
Cation Exchange Capacity (meq/100g)		6.5	5.5	3.5
Particle Size Analysis (USDA) (%)	sand	80	80	81
	silt	14	12	15
	clay	6	8	4
Soil Type		Loamy Sand		
Organic matter (%)		2.8	2.1	0.8
Organic carbon (%) [2]		1.624	1.218	0.464
Soil pH [3]		6.4	6.5	6.7
Soil Bulk Density (g/L) [1]		1.4	-	-
Field capacity (% soil moisture at 1/3 bar)		12.72	9.33	6.95

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM × 0.58
[3] Medium not stated

Table 7.1.2.2.1-157: Soil characteristics of the Kleinzecher (RS-9027/B2) test site

Parameter		Horizon		
Soil depth (cm)		**0-30**	**30-60**	**60-100**
Cation Exchange Capacity (meq/100g)		7.7	8.7	10.4
Particle Size Analysis (USDA) (%)	sand	66	68	62
	silt	21	15	19
	clay	13	17	19
Soil Type		Sandy loam		
Organic matter (%)		1.9	1.2	0.2
Organic carbon (%) [2]		1.102	0.696	0.116
Soil pH		7.0	7.0	7.3
Soil Bulk Density (g/L) [1]		1.6	-	-
Field capacity (% soil moisture at 1/3 bar)		13.71	14.60	15.19

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-158: Soil characteristics of the Unzhurst (RS-9027/E1) test site

Parameter		Horizon		
Soil depth (cm)		**0-30**	**30-60**	**60-90**
Cation Exchange Capacity (meq/100g)		6.6	6.1	6.2
Particle Size Analysis (USDA) (%)	sand	48	53	44
	silt	39	31	37
	clay	13	16	19
Soil Type		Loam	Sandy clay loam	Loam
Organic matter (%)		1.8	0.6	0.3
Organic carbon (%) [2]		1.044	0.348	0.174
Soil pH		6.7	5.4	5.3
Soil Bulk Density (g/L) [1]		1.4	-	-
Field capacity (% soil moisture at 1/3 bar)		15.57	16.50	16.88

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-159: Soil characteristics of the Rohrbach (RS-9027/E2) test site

Parameter		Horizon		
Soil depth (cm)		**0-25**	**25-35**	**35-105**
Cation Exchange Capacity (meq/100g)		12.7	12.1	5.4
Particle Size Analysis (USDA) (%)	sand	12	13	15
	silt	77	60	70
	clay	11	27	15
Soil Type		Silt Loam		
Organic matter (%)		1.8	0.5	0.1
Organic carbon (%) [2]		1.044	0.290	0.058
Soil pH		8.5	8.5	8.7
Soil Bulk Density (g/L) [1]		1.3	-	-
Field capacity (% soil moisture at 1/3 bar)		23.10	21.28	18.95

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-160: Soil characteristics of the Herrngiersdorf (RS-9027/G1) test site

Parameter		Horizon [2]	
Soil depth (cm)		**Upper**	**Lower**
Cation Exchange Capacity (meq/100g)		14.4	9.3
Particle Size Analysis (USDA) (%)	sand	23	21
	silt	47	58
	clay	30	21
Soil Type		Clay loam	Silt loam
Organic matter (%)		2.8	0.8
Organic carbon (%) [3]		1.624	0.464
Soil pH		8.0	8.4
Soil Bulk Density (g/L) [1]		1.5	-
Field capacity (% soil moisture at 1/3 bar)		24.31	21.18

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] The soil horizons were not measured. The two horizons were sampled from a pit dug to a depth of 1 m
[3] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-161: Soil characteristics of the Wang-Inzkofen (RS-9027/G2) test site

Parameter		Horizon
Soil depth (cm)		**0-30**
Cation Exchange Capacity (meq/100g)		14.0
Particle Size Analysis (USDA) (%)	sand	25
	silt	51
	clay	24
Soil Type		Silt loam
Organic matter (%)		2.1
Organic carbon (%) [2]		1.218
Soil pH		7.2
Soil Bulk Density (g/L) [1]		1.6
Field capacity (% soil moisture at 1/3 bar)		24.53

[1] Data included from AIR 2 Application for Renewal of Approval, Annex II, Document M, Point 7, May 2012
[2] Calculated from organic matter according to OC = OM × 0.58

Long-term air temperatures (daily or monthly mean), precipitation data as well as sunshine hours (as sum daily or monthly) were reported. No information on irrigation was reported.

Details on weather data are presented in the table below.

Table 7.1.2.2.1-162: Weather station and reporting time

Test site	Reporting time	Weather station
Buchen (RS-9027/B1)	April 1990 – September 1991	Mölln-Grambek (T, N), Lübeck-Blankensee (S)
Kleinzecher (RS-9027/ B2)	July 1990 – December 1991	Mölln-Grambek (T, N), Lübeck-Blankensee (S)
Unzhurst (RS-9027/E1)	Daily: April 1990 –November 1990 Monthly: December 1990 – November 1991	Rheinau-Freistett (T, N, S)
Rohrbach (RS-9027/E2)	Daily: July 1990 – November 1990 Monthly: December 1990 – February 1992	Daily: Bad Bergzabern (T, S), Landau/Pfalz (N) Monthly: Bad Bergzabern (T, N, S)

Table 7.1.2.2.1-162: Weather station and reporting time

Herrngiersdorf (RS-9027/G1)	April 1990 – December 1991	Regensburg (T, N, S)
Wang-Inzkofen (RS-9027/G2)	July 1990 – January 1992	Freising-Weihenstephan

2. Application

Glyphosate-trimesium (as YF7712A) was applied as 48 % SL formulation (sulfosate) at each trial, at a nominal application rate of 4.80 kg a.s./ha (ICIA 0224). One batch of spray solution was mixed to cover the entire plot. The application was made in all cases using a band held CO_2 pressurised sprayer equipped with a 2.5 m boom. A single application was made at each trial site. Conditions during application are detailed in the table below.

Table 7.1.2.2.1-163: Conditions during application

Treatment No.	Buchen (RS-9027/B1)	Kleinzecher (RS-9027/ B2)	Unzhurst (RS-9027/E1)	Rohrbach (RS-9027/E2)	Herrngiersdorf (RS-9027/G1)	Wang-Inzkofen (RS-9027/G2)
Application date	11.04.1990	10.08.1990	03.05.1990	25.07.1990	08.05.1990	02.07.1990
Application equipment	Hand held CO_2 pressurised sprayer equipped with a 2.5 m boom					
Spray pressure (bar at boom)	2	2	3.6	3.6	3.0	3.0
Mean application volume actual (L/plot)	2.4	2.6	2.2	2.3	2.1	2.0
Nominal application rate (kg/ha)	4.8	4.8	4.8	4.8	4.8	4.8
Actual application rate (kg a.s./ha)	5.2	5.7	4.8	5.0	4.6	4.8
Mean air temperature (°C)	11	17	19	11	20	18
Mean wind speed (m/s)	calm	2	1-3	1	1	-
Wind direction	calm	NW	NE	NE	West	No wind
Relative air humidity (%)	80	80	low	medium	55	55
Cloud cover (%)	30	30	0	0	30	100
Ground cover (%)	0	0	0	0	0	0
Wetness of soil surface	dry	moist	dry	dry	dry, crumbly	dry, crumbly

3. Sampling

Samples of untreated soil were taken from each site (30 cm cores with 2.3 cm internal diameter). Treated soil was sampled directly after application, using 10 cm cores with a 5 cm internal diameter. At subsequent intervals, up to approximately 19 months, soil was sampled using a 30 cm x 2.3 cm internal diameter corer. For each trial, at each interval, 20 cores were taken (usually five per sub-plot) in order to obtain a representative sample. All soil samples were taken using a zero contamination corer with plastic inserts.

4. Specimen handling and preparation

All soil samples were frozen in dry ice within two hours of sampling and transferred to a deep freezer within 16 hours of sampling. The samples were maintained frozen at <-20 °C and shipped frozen to Jealotts Hill Research Station for analysis.

For the day 0 samples, where a nominal depth of 10 cm was sampled, the twenty cores were bulked together for analysis. For the pre-application samples and all other time intervals, soil was sampled to a depth of 30 cm. These cores were sectioned into three horizons: 0-10 cm, 10-20 cm and 20-30 cm. Soil from each horizon, from each of the twenty cores was then bulked together for analysis. Control soil taken from the untreated plot was sectioned into profiles and bulked as indicated above. All soil was then air-dried for approximately 24 hours, sieved and then stones and debris were removed.

5. Analytical procedures

Soil samples were analysed between January 1992 and June 1992 for residues of trimesium (trimethylsulphonium cation) (TMS+) using ICI Americas Residue Analytical Method RRC 85-33. The method is summarised below.

Samples were extracted by agitation with 10 % aqueous potassium hydroxide solution. After centrifugation, an aliquot was taken and treated with solid potassium hydroxide pellets at 100 °C to dealkylate the TMS+ and to form dimethyl sulphide (DMS) which was collected into toluene. Final quantitative determination of DMS was by gas-liquid chromatography using flame- photometric detection in the sulphur mode. Residues were quantified by external standardisation and were corrected for recovery values generated by analysis of fortified control samples.

The samples from trials Unzhurst (RS-9027/E1) and Wang-Inzkofen (RS-9027/G2) contained a background level of natural DMS. Prior to dealkylation an attempt was made to remove the natural DMS contaminant by treating the samples as follows. An aliquot of the extract was adjusted to a 20 % concentration with potassium hydroxide pellets and heated with toluene. In both cases the toluene was discarded. By following these procedures the natural DMS in the control was reduced 0.11 mg/kg (10 % of applied) in trial Unzhurst (RS-9027/E1) and 0.06 mg/kg (7 % of applied) in trial Wang-Inzkofen (RS-9027/G2).

Samples were analysed between February 1992 and May 1992 for residues of glyphosate (N phosphonomethylglycine (PMG)), derived from glyphosate-trimesium and also the metabolite AMPA (aminomethylphosphonic acid) using ICI Americas Residue Analytical Method WRC 85-34. The method is summarised below:

Glyphosate (PMG) and AMPA were extracted from soil samples using 0.5 M ammonium hydroxide. After centrifugation, an aliquot of the supernatant was filtered and taken to dryness using a rotary evaporator. After re-dissolving the residue in 0.05 M borate buffer the glyphosate (PMG) and AMPA were then derivatised with 9-fluorenylmethyl chloroformate. The derivatives were determined by HPLC using an S5-SAX column and fluorescence detection.

The extraction solution was modified to 0.25 M ammonium hydroxide + 0.10 M monobasic potassium phosphate for trial Rohrbach (RS-9027/E2).

Residues were quantified by external standardisation and were corrected for recovery values generated by analysis of fortified control samples.

The conditions for high-performance liquid chromatographic (HPLC) determination of glyphosate (PMG) and AMPA residues were optimised for the soil matrix.

The limit of detection for TMS+, PMG and AMPA was validated by use of untreated controls fortified at 0.05 mg/kg.

Recoveries from fortified untreated soil with trimesium (TMS+), glyphosate (PMG) and AMPA during the course of analysis reported in this study were as follows. Recoveries from soil fortified between 0.10 and 1.0 mg/kg (n = 55) of trimesium (TMS+), ranged from 62 to 122 %; the mean was 87 %, and the coefficient of variation (CV) was 16 %. Recoveries from soil fortified between 0.05 and 2.5 mg/kg (n = 39) of glyphosate (PMG) ranged from 63 to 94 %; the mean was 81 %, and the coefficient of variation was 12 %. Recoveries from soil fortified between 0.05 and 2.5 mg/kg (n = 39) of AMPA ranged from 53 to 111 %, the mean was 89 %, and the coefficient of variation was 15 %.

II. RESULTS AND DISCUSSION

A. DATA

Table 7.1.2.2.1-164 to Table 7.1.2.2.1-169 summarise the residues of soil samples for all soil layers for trimesium (TMS+), glyphosate (PMG) and AMPA over up to 19 months.

Table 7.1.2.2.1-164: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Buchen test site (RS-9027/B1)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD [2]	< 5	< LOD [2]	< 2	≤ 0.06 [2]
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
0	0 – 10	1.2 [2]	90	2.5 [2]	85	0.12 [2]
7	0 – 10	1.2	100	2.2	86	0.13
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
14	0 – 10	1.3	110	1.9	77	0.20
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
28	0 – 10	0.53	46	1.5	59	0.23
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
61	0 – 10	< LOD	< 5	0.75	30	0.30
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
91	0 – 10	< LOD	< 5	0.60	24	0.51
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
121	0 – 10	< LOD	< 5	0.23	10	0.18
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
182	0 – 10	< LOD	< 5	0.27	11	0.38
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
240	0 – 10	< LOD	< 5	0.18	7	0.31
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
322	0 – 10	< LOD	< 5	0.16	6	0.20
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
475	0 – 10	< LOD	< 5	0.15	6	0.33
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD

n.a. = Not analysed
[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than once

Table 7.1.2.2.1-165: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Kleinzecher test site (RS-9027/B2)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD [2]	< 5	< LOD [2]	< 2	< LOD [2]
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
0	0 – 10	1.3 [2]	97	2.0 [2]	67	0.12 [2]
7	0 – 10	0.98	83	1.9	74	0.25
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
14	0 – 10	0.36	31	1.4	55	0.28
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
28	0 – 10	0.14	12	1.0	40	0.29
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
61	0 – 10	0.08 [2]	13	0.82	32	0.37
	10 - 20	< LOD	< 5	< LOD	< 2	0.07
91	0 – 10	≤ 0.05 [2]	≤ 5	0.45	18	0.25
	10 - 20	< LOD	< 5	< LOD	< 2	0.09
119	0 – 10	0.05	5	0.54	22	0.31
	10 - 20	< LOD	< 5	< LOD	< 2	0.06
201	0 – 10	0.06	5	0.44	18	0.41
	10 - 20	0.06	5	< LOD	< 2	0.05
244	0 – 10	0.06	5	0.39	15	0.39
	10 - 20	< LOD	< 5	< LOD	< 2	0.06
298	0 – 10	< LOD	< 5	0.16	7	0.30
	10 - 20	< LOD	< 5	< LOD	< 2	0.06
479	0 – 10	< LOD	< 5	0.08	3	0.33
	10 - 20	< LOD	< 5	< LOD	< 2	0.09
567	0 – 10	< LOD	< 5	< LOD	< 2	0.24
	10 - 20	< LOD	< 5	< LOD	< 2	0.07

[1] DAA = Days after application
[2] Mean , where a sample has been analysed more than once

Table 7.1.2.2.1-166: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Unzhurst test site (RS-9027/E1)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	0.11 [2,3]	10	< LOD [2]	< 2	< LOD [2]
	10 - 20	0.10	10	< LOD	< 2	< LOD
0	0 – 10	1.6 [2]	110	3.2 [2]	100	0.07 [2]
7	0 – 10	1.1	110	1.8	76	0.14
	10 - 20	0.06 [3]	6	< LOD	< 2	< LOD
13	0 – 10	1.0	93	1.8	73	0.20
	10 - 20	0.09 [3]	9	< LOD	< 2	< LOD
27	0 – 10	0.79	70	1.4	55	0.17
	10 - 20	0.08 [3]	8	< LOD	< 2	< LOD
57	0 – 10	0.13	12	0.48	20	0.36
	10 - 20	0.07 [3]	7	< LOD	< 2	< LOD
90	0 – 10	0.10 [3]	9	0.34	15	0.40
	10 - 20	0.07 [3]	7	< LOD	< 2	< LOD
117	0 – 10	0.12 [3]	11	0.22	9	0.36

Table 7.1.2.2.1-166: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Unzhurst test site (RS-9027/E1)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
	10 - 20	0.08 [3]	8	< LOD	< 2	< LOD
187	0 – 10	0.12 [3]	11	0.15	6	0.35
	10 - 20	0.08 [3]	8	< LOD	< 2	< LOD
251	0 – 10	0.11 [3]	10	0.14	6	0.40
	10 - 20	0.09 [3]	9	< LOD	< 2	< LOD
314	0 – 10	0.12 [3]	12	0.12	5	0.35
	10 - 20	0.07 [3]	7	< LOD	< 2	< LOD
418	0 – 10	0.11 [3]	11	0.07	3	0.26
	10 - 20	0.09 [3]	9	< LOD	< 2	< LOD
564	0 – 10	n.a.	-	n.a.	-	n.a.
	10 - 20	n.a.	-	n.a.	-	n.a.

n.a. = Defrosted on arrival, therefore not analysed
[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than once
[3] Contaminant of natural DMS

Table 7.1.2.2.1-167: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Rohrbach test site (RS-9027/E2)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD [2]	< 5	< LOD	< 2	< LOD
	10 - 20	< LOD	< 5	≤ LOD	< 2	< LOD
0	0 – 10	0.98 [2]	67	2.1 [2]	65	≤ 0.05 [2]
7	0 – 10	1.2	97	2.0	78	0.22
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
14	0 – 10	1.3	110	1.5	58	0.31
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
28	0 – 10	1.2	100	1.0	39	0.30
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
56	0 – 10	0.32 [2]	28	0.29	12	0.45
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
85	0 – 10	0.08 [2]	7	0.11	5	0.42
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
231	0 – 10	< LOD	< 5	< LOD	< 2	0.37
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
282	0 – 10	< LOD	< 5	< LOD	< 2	0.35
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
418	0 – 10	< LOD	< 5	< LOD	< 2	0.17
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD
582	0 – 10	< LOD	< 5	< LOD	< 2	0.13
	10 - 20	< LOD	< 5	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than once

Table 7.1.2.2.1-168: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Herrngiersdorf test site (RS-9027/G1)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD [2]	< 6	< LOD	< 3	< LOD
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
0	0 – 10	1.2 [2]	84	1.9 [2]	62	0.05 [2]
6	0 – 10	1.0 [2]	105	1.3	61	0.21
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
13	0 – 10	1.1 [2]	115	0.94	46	0.16
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
28	0 – 10	1.2 [2]	130	0.90	45	0.23
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
58	0 – 10	0.10 [2]	11	0.27	14	0.23
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
90	0 – 10	≤ 0.05 [2]	7	0.16	8	0.23
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
125	0 – 10	< LOD	< 6	0.09	4	0.22
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
168	0 – 10	< LOD	< 6	< LOD	< 3	0.14
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
330	0 – 10	< LOD	< 6	< LOD	< 3	0.15
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
464	0 – 10	< LOD	< 6	< LOD	< 3	0.06
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD
541	0 – 10	< LOD	< 6	< LOD	< 3	< LOD
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD

[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than once

Table 7.1.2.2.1-169: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Wang-Inzkofen test site (RS-9027/G2)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	0.06 [2,3]	7	< LOD [2]	< 3	0.07 [2]
	10 - 20	< LOD	< 6	< LOD	< 3	< LOD [2]
0	0 – 10	1.2 [2]	91 [2]	2.3 [2]	78 [2]	0.21 [2]
7	0 – 10	1.0	120	1.2	62	0.30
	10 - 20	0.06 [3]	7	< LOD	< 3	< LOD
15	0 – 10	0.79	84	0.87	42	0.38
	10 - 20	0.06 [3]	7	< LOD	< 3	< LOD
29	0 – 10	0.83	90	0.81	40	0.46
	10 - 20	0.06 [3]	7	< LOD	< 3	< LOD
58	0 – 10	0.36	39	0.39	19	0.36
	10 - 20	0.06 [3]	7	< LOD	< 3	< LOD
94	0 – 10	0.14	14	0.23	11	0.39
	10 - 20	0.07 [3]	8	< LOD	< 3	< LOD
114	0 – 10	0.12	12	0.21	10	0.41
	10 - 20	0.06 [3]	7	< LOD	< 3	< LOD
275	0 – 10	0.08 [3]	9	0.11	6	0.32
	10 - 20	< LOD [3]	< 6	< LOD	< 3	< LOD

Table 7.1.2.2.1-169: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 4.8 kg/ha Sulfosate 48 % SL at the Wang-Inzkofen test site (RS-9027/G2)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
414	0 – 10	0.07 [3]	7	0.06	3	0.26
	10 - 20	0.06 [3]	7	< LOD	< 3	< LOD
549	0 – 10	0.07 [3]	7	< LOD	< 3	0.19
	10 - 20	0.06 [3]	7	< LOD	< 3	< LOD

[1] DAA = Days after application
[2] Mean, where a sample has been analysed more than once
[3] Contaminant of natural DMS

B. CHARACTERISATION OF RESIDUES

No residues of glyphosate (PMG) were measured in any trial, on any occasion, in the soil horizon below 10 cm. For all trials except Unzhurst (RS-9027/E1) and Wang-Inzkofen (RS-9027G2) the residues of trimesium (TMS+) measured in the soil horizon below 10 cm were always at or less than 0.06 mk/kg. For the other two trials a background level of natural DMS was present. This could not be totally removed before dealkylation of the TMS+ residue (to DMS). Background levels of 0.10 mg/kg and 0.07 mg/kg for trials Unzhurst (RS-9027/E1) and Wang-Inzkofen (RS-9027G2), respectively, were found. These were equivalent to 10 % and 7 % of the applied chemical. No residues of AMPA above the LOD were measured in any trial, on any occasion, in the soil horizon below 10 cm except for trial Kleinzecher (RS-9027/B2) where no residues were measured up until the 28 day sample and then after this time, the residues were not greater than 0.09 mg/kg.

Initial residues of trimesium (TMS+) measured in the soil at day 0 were greater than 84 % of the applied chemical for all the trials except trial Rohrbach (RS-9027/E2) where the recovery was 67 %. There is no reason evident from the field or weather data for the lower recovery found. This result is, however, also substantiated by the day 0 recovery (65 %) of glyphosate (PMG).

Trimesium (TMS+) degrades fairly rapidly and falls below 0.05 mg/kg (5 % of applied chemical) (LOD) in all trials within 300 days. In trials Unzhurst (RS-9027/E1) and Wang-Inzkofen (RS-9027G2) a background level of approximately 0.1 mg/kg remained in the samples and controls.

Initial residues of glyphosate (PMG) measured in the soil at day 0 were greater than 78 % for three out of the six trials. The other three trials gave recoveries of 62, 65 and 67 %. There is no reason evident from the field or weather data for the lower recoveries found and only one recovery (65 %, Rohrbach, RS-9027/E2) was substantiated by the day 0 recovery of TMS+ (67 %).
Glyphosate (PMG) degrades fairly rapidly and falls below 0.05 mg/kg by the end of four trials. In the other two trials, Unzhurst (RS-9027/E1) and Wang-Inzkofen (RS-9027G2), the residue values measured at the end of the trials were 0.15 mg/kg (< 7 % of applied) and 0.07 mg/kg (< 4 % of applied), respectively.

For all trials in this study, residues of AMPA in the soil increased as the glyphosate (PMG) residue decreased and then declined again to between 0.06 mg/kg and 0.33 mg/kg at the end of the trials.

C. KINETICS

New kinetic calculations based on more recent guidance are necessary, therefore the information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/001).

III. CONCLUSIONS

From the data it can be concluded that for the typical climatic conditions and soil types studied, glyphosate-trimesium dissipates fairly rapidly. Only for trimesium natural contamination was detected in two of six trials in the 10 to 20 cm layer. Since no residue greater than 0.10 mg/kg of trimesium (TMS+), glyphosate (PMG) or AMPA was determined in any sample taken from a depth greater than 10 cm it can be concluded that neither glyphosate-trimesium or its metabolite, leach or therefore present any potential groundwater contamination risk.

For all trials in this study, residues of AMPA in the soil increased as the glyphosate (PMG) residue decreased and then declined again to between 0.06 mg/kg and 0.33 mg/kg at the end of the trials.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides valuable information on the dissipation behavior of glyphosate under field conditions from a variety of different test site conditions. As the representative formulation of the current submission does not contain the trimesium cation, the trimesium findings were neglected for further consideration. The study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/014
Report author	[redacted]
Report year	1992
Report title	Glyphosate-trimesium: Soil Dissipation Study (Canada, 1988-1990)
Report No	RJ1225B
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No verification transport and processing
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
A study was carried out in Canada during 1988-1990 to monitor the rate of dissipation of glyphosate-trimesium in soil following a single application at a nominal rate of 5.76 kg/ha. Trials were carried out at five locations in the provinces of Saskatchewan, Manitoba, Ontario and Alberta.

Overall, residues in the soil degraded fairly rapidly. Evidence for some initial degradation was seen on most sites, but with temperatures falling below 0°C in October and November 1988, degradation appeared to slow or even cease until the temperature had started to rise again in the spring of the following year.

Samples were analysed for residues of trimesium (TMS+) (trimethylsulfonium cation), glyphosate (PMG) (n-Phosphomethylglycine) and AMPA (aminomethylphosphonic acid).

Residues of glyphosate (PMG) in the top layer ranged from 1.6 to 3.4 mg/kg at day 0 and decreased to <LOD in three trials after 297 to 615 days. The Grandora trial was terminated after 250 days, due to adverse weather conditions, causing soil erosion and flooding. Thus 0.91 mg/kg PMG remained in the top layer in the last sampling at day 212. In the Alberta trial 0.2 mg/kg PMG remained after 575 days, 67 % of the initial residue).

Residues of trimesium (TMS+) in the top layer ranged from 0.88 to 1.6 mg/kg at day 0 and decreased to <LOD in three trials after 212 to 391 days. Since the Grandora trial was terminated after 250 days in the last sampling at day 212, 1.3 mg/kg TMS+ remained in the top layer. In the Brooks trial 0.24 mg/kg TMS+ remained after 575 days.

Residues of AMPA, did not exceed 0.50 mg/kg at any interval in any trial and dissipated rapidly during the study period.

No residue greater than 0.06 mg/kg of glyphosate (PMG), AMPA or glyphosate-trimesium (TMS+), were determined in the second depth profile (10-20 cm) at any sampling interval, in any trial.

I. MATERIAL AND METHODS

A. MATERIALS

1.Test Material:

Identification:	Glyphosate as glyphosate-trimesium (ICIA 0224)
Product:	Sulfosate (TF 1242 or YF7712A), 48 % SL formulation
Lot No.:	WHD0401
Nominal concentration:	48 % glyphosate-trimesium

B. STUDY DESIGN

1. Test sites

Prior to application, each of the trial sites was cultivated, the surface levelled and the surface trash removed, by hand raking. At each of the trial sites the area was divided into two plots, a treated plot and an untreated (or control) plot, separated by a buffer zone. The treated plots were generally (in three out of five trials) 15 m x 15 m square and the smallest plot area was 9 m x 12 m. Control plots were generally smaller, ranging from 36 m^2 (3 m x 12 m) up to 180 m^2 (12 m x 15 m). Each of the treated plot areas was subdivided into at least three sub-plots from which a total of 30 core samples (generally 10 from each subplot) were taken. Pesticide use history over three years prior to the study were reported within the field data.

At each of the trial sites at least one soil pit was dug and samples were taken from at least two horizons to a depth of greater than 22.5 cm in all cases. Between 0.5 and 1 kg of soil was then bulked from each horizon and sent to Jealott's Hill Research Station, Bracknell, UK for physico-chemical characterisation.

An overview of the soil characterization is given in Table 7.1.2.2.1-170 to Table 7.1.2.2.1-174.

Table 7.1.2.2.1-170: Soil characteristics of the St. Davids, Ontario (CA-SD-88-01) test site

Parameter		Horizon		
Soil depth (cm)		**0-30**	**30-50**	**50 +**
Cation Exchange Capacity (meq/100g)		15.8	25.3	12.0
Particle Size Analysis (USDA) (%)	sand	11	1	14
	silt	49	19	46
	clay	41	80	40
Soil Type		Silty clay	Clay	Silty clay loam
Organic matter (%)		4.3	3.8	0.8
Organic carbon (%) [1]		2.494	2.204	0.464
Soil pH [2]		7.9	7.9	7.7
Field capacity (% soil moisture at 1/3 bar)		30.63	43.44	26.77

[1] Calculated from organic matter according to OC = OM x 0.58

[2] Medium not stated

Table 7.1.2.2.1-171: Soil characteristics of the Carman, Manitoba (CA-SD-88-02) test site

Parameter		Horizon	
Soil depth (cm)		**0-15**	**15-30**
Cation Exchange Capacity (meq/100g)		10.8	10.4
Particle Size Analysis (USDA) (%)	sand	80	81
	silt	10	9
	clay	10	10
Soil Type		Loamy sand	
Organic matter (%)		2.9	2.6
Organic carbon (%) [1]		1.682	1.508
Soil pH		7.8	8.1
Field capacity (% soil moisture at 1/3 bar)		10.26	10.34

[1] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-172: Soil characteristics of the Grandora, Saskatchewan (CA-SD-88-03) test site

Parameter		Horizon		
Soil depth (cm)		**0-30**	**30-50**	**50 +**
Cation Exchange Capacity (meq/100g)		15.3	15.5	15.8
Particle Size Analysis (USDA) (%)	sand	42	36	45
	silt	30	34	29
	clay	28	30	27
Soil Type		Clay loam		
Organic matter (%)		3.3	2.0	1.0
Organic carbon (%) [1]		1.914	1.160	0.580
Soil pH		7.1	7.9	8.6
Field capacity (% soil moisture at 1/3 bar)		21.44	22.51	20.01

[1] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-173: Soil characteristics of the Speers, Saskatchewan (CA-SD-88-04) test site

Parameter		Horizon		
Soil depth (cm)		**0-12**	**12-24**	**24 +**
Cation Exchange Capacity (meq/100g)		22.0	16.7	17.5
Particle Size Analysis (USDA) (%)	sand	12	7	8
	silt	55	60	59
	clay	34	33	33
Soil Type		Silty clay loam		
Organic matter (%)		9.1	2.0	0.9
Organic carbon (%) [1]		5.278	1.160	0.522
Soil pH		7.1	7.8	8.2
Field capacity (% soil moisture at 1/3 bar)		34.71	24.68	24.49

[1] Calculated from organic matter according to OC = OM × 0.58

Table 7.1.2.2.1-174: Soil characteristics of the Brooks, Alberta (CA-SD-88-05) test site

Parameter		Horizon	
Soil depth (cm)		**0-15**	**15-30**
Cation Exchange Capacity (meq/100g)		13.2	13.6
Particle Size Analysis (USDA) (%)	sand	36	39
	silt	42	38
	clay	22	23
Soil Type		Loam	
Organic matter (%)		1.7	1.7
Organic carbon (%) [1]		0.986	0.986
Soil pH		7.6	7.3
Field capacity (% soil moisture at 1/3 bar)		18.84	18.56

[1] Calculated from organic matter according to OC = OM × 0.58

Meteorological records were obtained from local stations close to the trial sites. Air temperature and precipitation were measured. Copies of these daily weather records for the study period are stored in the ICI Agrochemicals GLP Archives, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY under Study No: 88JH140.

Examination of these weather records showed that no extraordinary conditions were experienced during the dissipation period at each site.

Details on weather data are presented in the table below.

Table 7.1.2.2.1-175: Weather station and reporting time

Test site	Reporting time	Weather station
St. Davids, Ontario	September 1988 – July 1989	5 km from trial site
Carman, Manitoba	From July 1988 onwards	Environment Canada, climate reference station located at Morden, Manitoba, approximately 30 km from test site
Grandora and Speers, Saskatchewan	From July 1988 onwards	Saskatchewan Research Council, climate reference station located at Saskatoon Airport, approximately 12 km from Grandora test site
Brooks, Alberta	Not available in study report	Not available in study report

2. Application

Glyphosate-trimesium was applied as sulfosate (TF 1242 or YF7712) as 48 % SL formulation to each trial, at a nominal application rate of 5.76 kg a.s./ha. Actual application rates are detailed in the table below. One batch of spray solution was mixed to cover the entire plot, then divided into three or 4 portions. The application was made in all cases using a hand-held CO^2 pressurised sprayer equipped with a 3 m boom. Depending on the site size 3 to 5 passes were necessary for the application of the test compound. The sprayers were calibrated before use, unsprayed solution was collected and a sample was stored frozen for analysis. Conditions during application are detailed in the table below.

Table 7.1.2.2.1-176: Conditions during application

Treatment No.	St. Davids, Ontario	Carman, Manitoba	Grandora, Saskatchewan	Speers, Saskatchewan	Brooks, Alberta
Application date	30.09.1988	23.09.1988	23.09.1988	20.09.1988	26.09.1988
Application equipment	hand held CO^2 pressurised sprayer equipped with a 3 m boom				
Nozzle type	Teejet 8003, flat fan	Teejet 8002, flat fan	Teejet 8001, flat fan	Teejet 8001, flat fan	Teejet 8001 LP, flat fan
Spray pressure	35 PSI	275 kPa	276 kPa	275 kPa	245 kPa
Number of passes	4	3	5	5	5
Actual application volume (mL) per test site	5008	1362	1720	1660	2475
Nominal application rate (kg a.s./ha)	5.76	5.76	5.76	5.76	5.76
Actual application rate (kg a.s./ha)	6.41	6.48	7.50	7.24	5.76
Mean air temperature (°C)	12-15	15	-1	7	5
Mean wind speed (m/s)	0-1	1.4	0.8	1.4	1.3
Wind direction	SW-NE	W/E	SW	E/SE	N-S
Relative air humidity (%)	70-80	55	82	85	85
Cloud cover (%)	0	0	30	100	95
Ground cover (%)	0	0	0	0	0
Wetness of soil surface	moist	dry	dry	dry	dry
Soil surface description	uniform, slightly crusted	fine	fine	slightly cloddy	granular

3. Sampling

Prior to application, samples of soil were taken from each site (30 cm cores with 2.5 cm internal diameter). Treated soil was sampled at day 0 and one day and three days after application, using 10 cm cores with a 5 cm internal diameter. At subsequent intervals, up to approximately 20 months, soil was sampled using a 30 cm x 2.5 cm internal diameter corer. For each trial at each interval, 30 cores were taken (usually 10 cores per sub-plot), in order to obtain a representative sample. All soil samples were taken using a zero contamination corer with plastic inserts.

4. Specimen handling and preparation

All soil samples were frozen within two to four hours of sampling. The samples were maintained frozen at <-15 °C and shipped frozen to Jealotts Hill Research Station for analysis.

The samples were received deep frozen at Jealott's Hill between October 1988 and October 1990 and were stored at <-15°C in the Residue and Environmental Chemistry Laboratory deep freeze until required for analysis.

For the 0, 1 and 3 day samples, where a nominal depth of 10 cm was sampled, the cores taken from the sub-plots in the treated plot were bulked separately for analysis. In detail, 10 cores from three sub-plots each, were bulked for the trials in Manitoba (Carman), Saskatchewan (Grandora and Speers) and Alberta (Brooks). In Ontario (St. Davids) where there were four treated sub-plots, 6 to 8 cores were bulked per sub-plot. For the pre-application samples and all other time intervals, soil was sampled to a depth of 30 cm. These cores were sectioned into three horizons 0-10 cm, 10-20 cm and 20-30 cm. Soil from each horizon was then bulked from the sub-plots as indicated above. Control soil taken from the untreated plot was sectioned into profiles as indicated above. All soil was then air-dried for up to 48 hours, sieved and then stones and debris removed.

5. Analytical procedures

Soil samples were analysed for residues of trimethylsulphonium cation (TMS+) using ICI Americas Residue Analytical Method RRC 85-33. The method is summarised below:

Samples were extracted by agitation with 10 % aqueous potassium hydroxide. After centrifugation, an aliquot was taken and treated with solid potassium hydroxide pellets at 100 °C to dealkylate the TMS+ and to form dimethyl sulphide (DMS) which was collected into toluene. Final quantitative determination of DMS was by gas-liquid chromatography using flame- photometric detection in the sulphur mode.

Samples were analysed for residues of glyphosate (N-phosphonomethylglycine (PMG)), and the metabolite AMPA (aminomethylphosphonic acid) using ICI Americas Residue Analytical Method WRC 85-34. The method is summarised below:

Glyphosate and AMPA were extracted from soil samples using 0.5 M ammonium hydroxide. After centrifugation, an aliquot of the supernatant was filtered and taken to dryness using a rotary evaporator. After re-dissolving the residue in 0.05 M borate buffer the glyphosate and AMPA were then derivatised with 9-fluorenylmethyl chloroformate. The derivatives were determined by HPLC using an S5-SAX column and fluorescence detection.

The conditions for high-performance liquid chromatographic (HPLC) determination of glyphosate and AMPA residues were optimised for the soil matrix.

Residues were quantified by external standardisation and were corrected for recovery values generated by analysis of fortified control samples. The limit of determination for the trimesium cation, glyphosate and AMPA was 0.05 mg/kg.

Some preliminary analysis of spray solutions for each trial was carried out for trimesium (TMS+) (using a method based on the analytical procedures described earlier). Since the data were semiquantitative only, the full data were not reported and the data were used only in order to confirm the application rates. The investigations made, suggested that > 80 % of nominal applied was recovered for all trials with the exception of Speers. It was not possible to obtain a representative result from the Speers tank-mix sample.

Recoveries from fortified untreated soil with trimesium (TMS+), glyphosate (PMG) and AMPA were done for each trial separately and are as follows. Recoveries from soil fortified with trimesium (TMS+), ranged from 71 to 87 %; the coefficients of variation (CV) ranged from 8 to16 %. Recoveries from soil fortified with glyphosate (PMG) ranged from 71 to 93 %; the coefficients of variation ranged from 14 to 22 %. Recoveries from soil fortified with AMPA ranged from 82 to 90 %, the coefficients of variation ranged from 14 to 19 %.

II. RESULTS AND DISCUSSION

A. DATA

Table 7.1.2.2.1-177 to Table 7.1.2.2.1-181 summarise the residues of soil samples from all soil layers for trimesium (TMS+), glyphosate (PMG) and AMPA over up to 20 months.

Table 7.1.2.2.1-177: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 5.76 kg/ha TE 1242, 48 % SL at the St. Davids test site (CA-SD-88-01)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 4	< LOD	< 2	< LOD
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
0 [3]	0 – 10	1.4 [2] (1.2/1.2/ 1.5/1.6)	77 [2] (69/71/ 86/83)	2.2 [2] (1.5/1.7/ 2.6/3.2)	56 [2] (38/43/ 66/76)	0.14 [2] (0.13/0.13/ 0.19/0.11)
1 [3]	0 – 10	1.4 [2] (1.1/1.2/ 1.3/1.8)	75 [2] (65/69/ 72/95)	2.0 [2] (1.7/1.9/ 1.8/2.5)	50 [2] (47/48/ 47/60)	0.15 [2] (0.13/0.15/ 0.21/0.12)
3 [3]	0 – 10	1.3 [2] (1.0/1.5/ 1.4/1.3)	74 [2] (59/84/ 83/72)	0.83 [2] (0.78/0.84/ 0.86/n.a.)	22 [2] (21/21/ 23/-)	0.34 [2] (0.32/0.37/ 0.32/n.a.)
7	0 – 10	1.5	84	0.73	19	0.28
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
14	0 – 10	1.1	63	0.61	16	0.41
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
31	0 – 10	0.32	18	0.33	9	0.31
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
60	0 – 10	0.19	12	0.19	5	0.31
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
207	0 – 10	0.08	5	0.15	4	0.27
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
297	0 – 10	0.05	3	< LOD	< 2	0.06
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
391	0 – 10	< LOD	< 4	< LOD	< 2	0.06
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
577	0 – 10	< LOD	< 4	< LOD	< 2	< LOD
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD

n.a. = Sample not available, not analysed
[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)

Table 7.1.2.2.1-178: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Carman test site (CA-SD-88-02)

DAA [1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 4	< LOD	< 2	< LOD
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
0 [3]	0 – 10	0.88 [2] (0.86/0.93/ 0.85)	53 [2] (52/56/ 52)	1.6 [2] (1.8/1.6/ 1.5)	45 [2] (49/43/ 43)	0.10 [2] (0.09/0.10/ 0.12)
1 [3]	0 – 10	0.95 [2] (0.98/1.0/ 0.81)	48 [2] (59/64/ 49)	1.6 [2] (1.7/1.7/ 1.4)	45 [2] (48/47/ 40)	0.10 [2] (0.10/0.13/ 0.07)
3 [3]	0 – 10	0.78 [2] (0.78/0.73/ 0.84)	48 [2] (47/45/ 51)	1.4 [2] 1.2/1.2/ 1.8)	38 [2] (33/32/ 50)	0.08 [2] (0.07/0.06 0.10)
7	0 – 10	1.1	59	1.6	39	0.07
	10 - 20	< LOD	< 4	< LOD	< 2.0	< LOD
14	0 – 10	0.98	54	1.5	37	0.10
	10 - 20	0.06	< 4	< LOD	< 2	< LOD
215	0 – 10	0.64	36	0.37	10	0.26
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
308	0 – 10	< LOD	< 4	< LOD	< 2	0.08
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
360	0 – 10	< LOD	< 4	< LOD	< 2	0.05
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
577	0 – 10	< LOD	< 4	< LOD	< 2	0.07
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)

Table 7.1.2.2.1-179: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Grandora test site (CA-SD-88-03)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 4	< LOD	< 2	< LOD
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
0 [3]	0 – 10	1.6 [2] (1.6/1.4/ 1.7)	81 [2] 77/69/ 98)	2.9 [2] (2.8/2.5/ 3.4)	69 [2] (61/58/ 89)	0.1 [2] (0.09/0.10/ 0.13)
1 [3]	0 – 10	1.1 [2] (1.1/1.0/ 1.1)	58 [2] (59/53/ 61)	2.5 [2] (2.5/2.6/ 2.4)	63 [2] (62/63/ 63)	0.14 [2] (0.13/0.15/ 0.14)
3 [3]	0 – 10	1.2 [2] (1.2/1.4/ 1.1)	71 [2] (67/82/ 63)	2.9 [2] (2.7/2.9/ 3.0)	76 [2] (70/77/ 79)	0.10 [2] (0.09/0.10/ 0.10)
7	0 – 10	1.4	69	1.5	35	0.17
	10 - 20	< LOD	< 4.0	< LOD	< 2.0	< LOD
11	0 – 10	1.3	70	1.5	37	0.20
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
27	0 – 10	1.2	65	1.5	35	0.25
	10 - 20	< LOD	< 4	< LOD	< 2.	< LOD

Table 7.1.2.2.1-179: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Grandora test site (CA-SD-88-03)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
212	0 – 10	1 3	76	0.91	25	0.36
	10 - 20	< LOD	< 4	< LOD	< 2.	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)

Table 7.1.2.2.1-180: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Speers test site (CA-SD-88-04)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 4	< LOD	< 2	< LOD
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
0 [3]	0 – 10	1.8 [2] (1.8/1.8/ 2.0)	85 [2] (80/81 94)	3.4 [2] (2.9/3.2/ 4.1)	72 [2] (62/68 88)	< 0.08 [2] (<0.06/<0.06/ 0.12)
1 [3]	0 – 10	2.2 [2] (2.7/1.8/ 2.0)	98 [2] (120/80/ 98)	2.2 [2] (3.2/1.6/ 2.5)	50 [2] (63/33/ 56)	< 0.06 [2] (0.07/<LOD/ <LOD)
3 [3]	0 – 10	1.9 [2] (1.9/2.4/ 1.4)	82 [2] (76/100/ 65)	3.5 [2] (3.6/3.6/ 3.4)	70 [2] (67/71/ 70)	0.15 [2] (0.14/0.15/ 0.15)
9	0 – 10	1.5	67	3.2	68	0.27
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
14	0 – 10	1.5	66	2.3	47	0.24
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
30	0 – 10	1.4	57	2.0	39	0.22
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
232	0 – 10	0.95	45	1.2	26	0.46
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
308	0 – 10	< LOD	< 4	0.21	4	0.38
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
359	0 – 10	< LOD	< 4	< LOD	< 2	0.29
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
615	0 – 10	< LOD	< 4	< LOD	< 2	0.32
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)

Table 7.1.2.2.1-181: Summary of residues (mg/kg) and (% of applied) for trimesium (TMS+), glyphosate (PMG) and AMPA after application of 5.76 kg/ha TF 1242, 48 % SL at the Brooks test site (CA-SD-88-05)

DAA[1]	Soil depth (cm)	TMS+ (mg/kg)	TMS+ (% of applied)	PMG (mg/kg)	PMG (% of applied)	AMPA (mg/kg)
Pre-spray	0 – 10	< LOD	< 4	< LOD	< 2	< LOD
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
0	0 – 10	1.4 [2] (1.4/1.4/ 1.3)	104 [2] (100/97/ 120)	3.0 [2] (2.9/3.1/ 3.2)	106 [2] (90/98/ 130)	0.07 [2] (0.07/0.07/ 0.07)
1 [3]	0 – 10	1.2 [2] (1.4/1.3/ 1.1)	90 [2] (92/86/ 92)	2.2 [2] (2.5/2.3/ 1.9)	74 [2] (77/70/ 73)	0.06 [2] (0.06/0.06/ <LOD)
3 [3]	0 – 10	1.2 [2] (1.5/1.2/ 1.1)	89 [2] (97/79/ 90)	1.9 [2] (2.3/2.0/ 1.5)	63 [2] (70/61/ 56)	0.06 [2] (0.07/0.07/ <LOD)
7 [3]	0 – 10	0.89	60	1.9	60	0.10
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
16	0 – 10	1.4	97	2.2	72	0.20
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
29	0 – 10	0.97	68	1.4	45	0.17
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
218	0 – 10	1.4	100	1.1	36	0.33
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
323	0 – 10	1.2	80	0.65	20	0.42
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
361	0 – 10	0.38	27	0.21	7	0.34
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD
575	0 – 10	0.24	17	0.20	6	0.53
	10 - 20	< LOD	< 4	< LOD	< 2	< LOD

[1] DAA = Days after application
[2] Mean values
[3] Values in brackets refer to analyses of replicate samples, (bulked for each subplot separately)

B. CHARACTERISATION OF RESIDUES

Degradation of trimesium (TMS+) and glyphosate (PMG) at all of the sites appeared to show some dependence on temperature. Applications were not made on any of the sites until on or following the 20th September. Evidence for some initial degradation was then seen on most sites, but with temperatures falling below 0 °C in October and November 1988, degradation appeared to slow or even cease until the temperature had started to rise again in the spring of the following year. No residues of trimesium, glyphosate or AMPA greater than 0.06 mg/kg were found in any trial, on any occasion, in the soil horizon below 10 cm.

Residues of trimesium (TMS+) in the top layer ranged from 0.88 to 1.6 mg/kg at day 0, corresponding to >75 % of the applied chemical for all of the trials studied except at the Carman trial in Manitoba (53 % recovery), and decreased to <LOD in three trials after 212 to 391 days. The Grandora trial was terminated after 250 days, due to adverse weather conditions causing soil erosion and flooding. In the last sampling at day 212, 1.3 mg/kg TMS+ remained in the top layer. In the Brooks trial 0.24 mg/kg trimesium remained after 575 days.

Residues of glyphosate (PMG) in the top layer ranged from 1.6 to 3.4 mg/kg at day 0 and decreased to <LOD in three trials after 297 to 615 days. Since the Grandora trial was terminated after 250 days, in the last sampling at day 212, 0.91 mg/kg glyphosate remained in the top layer. In the Brooks trial 0.2 mg/kg glyphosate remained after 575 days.

Residues of AMPA (0.36 rng/kg and 0.32 mg/kg, i.e. < 15 % of the original day 0 glyphosate residues) remained at the termination of the Grandora and Speers trials respectively. In the Brooks trial similarly to glyphosate, the AMPA residue in the soil had not dissipated by the termination of the trial. 0.53 mg/kg of AMPA remained (equivalent to < 20 % of the day 0 PMG concentration). At St. Davids and Carman, AMPA had declined to < LOD and 0.07 mg/kg respectively at study termination.

C. KINETICS

An Ecoregion Crosswalk exercise was performed ([redacted] 2020, CA 7.1.2.2.1/002), and depending on its outcome, new kinetic calculations based on more recent guidance becomes necessary, therefore the kinetic information included in this study is not considered relevant. Evaluation of the rate of degradation is reported in [redacted] (2020, CA 7.1.2.2.1/003).

III. CONCLUSIONS

From the data it can be concluded that for the typical climatic conditions and soil types studied, glyphosate-trimesium dissipates fairly rapidly. Since no residue greater than 0.06 mg/kg of trimesium (TMS+), glyphosate (PMG) or AMPA was determined in any sample taken from a depth greater than 10 cm it can be concluded that neither glyphosate-trimesium or its metabolite, leach or therefore present any potential groundwater contamination risk.

For all of the trials, residues of AMPA in the soil increased as the glyphosate (PMG) residue decreased and then declined again to between 0.07 mg/kg and 0.53 mg/kg at the end of the trials. No residue significantly greater than the limit of determination was found at the end of the trial.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides valuable information on the dissipation behavior of glyphosate from a variety of different test site conditions. As the representative formulation of the current submission does not contain trimesium cation, the trimesium findings were neglected for further consideration.
The study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/015
Report author	[redacted]
Report year	1990
Report title	Dissipation of Glyphosate and Aminomethylphosphonic acid in forestry sites
Report No	MSL-9940
Document No	
Guidelines followed in study	None
GLP	Yes
Previous submission	Not accepted in RAR (2015)

Short description of study design and observations:

Study type: terrestrial field dissipation
Test item: Roundup

Test sites: Three sites in USA, forestry use,
- Chassell: harvested, foreseen for conifers.
- Corvallis: mixed conifers and hardwoods
- Cuthbert: blank, foreseen for planting of conifers

Soil types (0 – 15 cm depth):
- Chassell: sandy loam, pH 4.8, OM 2.5 %
- Corvallis: sandy clay loam, pH 5.8, OM 7.2 %
- Cuthbert: sandy loam, pH 5.4, OM 0.8 %

Application rate: 4.2 kg a.s./ha, single application
Application method: Aerial application by helicopter, over the forests
Application timing: late August – mid of September
Sampling periods and considered compartments:
- One month for water (flowing water, non-flowing pond)
- One year for sediment
- One year for exposed soil and soil under litter
- One year for litter
- One year for foliar and herbaceous vegetation

Sampling times: -9, -1, 0, 1, 3, 7, 14, 28/30, 58-63, 120-122, 180-187, 321-346, 365, (398-409) DAT
Sampling method:
- plant material by gloved hands
- soil samples by core sampler
- water: grab sampling (plastic bottles)
- sediment: soil core sampler

Sampling depth (soil): 0 - 15.2 cm depth and 15.2 – 30.4 cm depth

Sample storage: frozen directly after sampling and kept frozen until sample preparation.
Workup and analysis:
<u>Soil and plant material:</u>
- Grounding when frozen with dry ice, thawing overnight, mixing
- Extraction with chloroform and HCl.
- Elution through Chelex column chromatography
- Anion exchange chromatography
- Analysis by HPLC
- LOD = 0.05 mg/kg for soil, foliage, vegetation (both, glyphosate and AMPA)
- LOD = 0.1 mg/kg for leaf litter (both, glyphosate and AMPA)

<u>Sediment:</u>
- Same procedure as for soil, extraction is different:
- Extraction with KOH.

<u>Water:</u>
- Thawing of frozen water samples
- Concentration and drying of samples
- Mixing with HPLC buffer and EDTA.
- Filtering through a membrane filter
- Analysis by HPLC, LOD = 0.001 µg/L (both, glyphosate and AMPA)

	Recovery in fortified samples: Glyphosate / AMPA Pond water: 97.08 % / 94.72 % Stream water: 105.10 % / 100.23 % Pond sediment: 51.05 – 93.66 % / 59.52 – 85.05 % Stream sediment: 79.64 – 89.96 % / 79.12 – 86.34 % Soil: 72.89 – 91.41 % / 70.99 – 89.60 % Foliage: 92.77 % / 86.73 % Vegetation: 94.09 % / 90.67 % Leaf litter: 84.36 % / 86.59 %
Short description of results:	**Residues** Glyphosate: Chassell site: – Pond water: 1.678 µg/L (0 DAT), <0.001 µg/L (30 DAT0) – Stream water: 1.237 µg/L (0 DAT) , <0.001 µg/L (30 DAT) – Pond sediment: max. 1.92 mg/kg (60 DAT), 0.99 mg/kg (398 DAT) – Stream sediment: max. 0.69 mg/kg (DAT 7), <0.05 mg/kg (335 DAT) – Foliage: 1272.62 mg/kg (0 DAT), 0.24 mg/kg (335 DAT) – Vegetation: 628.63 mg/kg (0 DAT), <0.05 mg/kg (335 DAT) – Leaf litter: 322.4 mg/kg (0 DAT), 0.11 mg/kg (398 DAT) – Exposed soil (0 – 15 cm): max. 4.67 mg/kg (14 DAT), <0.05 mg/kg (398 DAT) – Exposed soil (15 – 30 cm): always <0.05 mg/kg – Soil under litter (0 – 15 cm): max. 1.4 mg/kg (7 DAT), 0.1 mg/kg (398 DAT) – Soil under litter (15 – 30 cm): max. 0.09 mg/kg (60 DAT), <0.05 mg/kg (398 DAT) Corvallis site: – Pond water: 0.09 µg/L (0 DAT, 0.002 µg/L (28 DAT) – Stream water: 0.035 µg/L (0 DAT), 0.001 µg/L (28 DAT) – Pond sediment: max. 19.42 mg/kg (28 DAT), 1.21 mg/kg (409 DAT) – Stream sediment: max. 0.11 mg/kg (DAT 180), <0.05 mg/kg (346 DAT) – Foliage: 652.19 mg/kg (0 DAT), 13.42 mg/kg (63 DAT) – Vegetation: 47.37 mg/kg (7 DAT), 0.44 mg/kg (346 DAT) – Leaf litter: 590.07 mg/kg (63 DAT), 0.19 mg/kg (409 DAT) – Exposed soil (0 – 15 cm): max. 0.15 mg/kg (122/180 DAT), <0.05 mg/kg (409 DAT) Soil under litter (0 – 15 cm): max. 0.07 mg/kg (63 DAT), <0.05 mg/kg (409 DAT) – Soil (both, 15 – 30 cm): always <0.05 mg/kg, except 346 DAT sample of exposed soil (0.07 mg/kg). Cuthbert site: – Pond water: 0.983 µg/L (0 DAT), 0.001 µg/L (30 DAT) – Stream water: 0.031 µg/L (0 DAT) , <0.001 µg/L (30 DAT) – Pond sediment: max. 0.26 mg/kg (0 DAT), <0.05 mg/kg (400 DAT) – Stream sediment: max. 0.18 mg/kg (1 DAT), 0.07 mg/kg (181 DAT) – Foliage: 760.01 mg/kg (0 DAT), <0.05 mg/kg (321 DAT) – Vegetation: 360.5 mg/kg (0 DAT), <0.05 mg/kg (321 DAT) – Leaf litter: max. 262.11 mg/kg (30 DAT), 8.41 mg/kg (120 DAT) – Exposed soil (0 – 15 cm): max. 1.87 mg/kg (3/7 DAT), <0.05 mg/kg (400 DAT)

	– Soil under litter (0 – 15 cm): max. 0.14 mg/kg (30 DAT),<0.05 mg/kg (400 DAT) – Soil (both, 15 – 30 cm): always <0.05 mg/kg. AMPA: Chassell site: – Pond water: 0.035 µg/L (3 DAT), <0.001 µg/L (30 DAT) – Stream water: 0.003 µg/L (1 DAT) , <0.001 µg/L (30 DAT) – Pond sediment: max. 1.37 mg/kg (30 DAT), 1.09 mg/kg (398 DAT) – Stream sediment: max. 0.38 mg/kg (14 DAT), <0.05 mg/kg (335 DAT) – Foliage: max. 2.65 mg/kg (0 DAT), <0.05 mg/kg (335 DAT) – Vegetation: max. 2.21 mg/kg (0 DAT), <0.05 mg/kg (335 DAT) – Leaf litter: max. 17.5 mg/kg (3 DAT), 0.13 mg/kg (398 DAT) – Exposed soil (0 – 15 cm): max. 0.51 mg/kg (187 DAT), <0.05 mg/kg (398 DAT) – Soil under litter (0 – 15 cm): max. 0.68 mg/kg (30 DAT), 0.12 mg/kg (398 DAT) – Soil (both, 15 – 30 cm): always <0.05 mg/kg Corvallis site: – Pond water: 0.002 µg/L (0/14 DAT), <0.001 µg/L (28 DAT) – Stream water: 0.002 µg/L (1 DAT) , <0.001 µg/L (28 DAT) – Pond sediment: max. 1.85 mg/kg (28 DAT), 0.56 mg/kg (409 DAT) – Stream sediment: max. 0.18 mg/kg (63 DAT), 0.11 mg/kg (346 DAT) – Foliage: max. 2.16 mg/kg (7 DAT), 0.64 mg/kg (63 DAT) – Vegetation: max. 0.2 mg/kg (63 DAT), <0.05 mg/kg (346 DAT) – Leaf litter: max. 4.4 mg/kg (63 DAT), 0.19 mg/kg (409 DAT) – Exposed soil (0 – 15 cm): max. 0.32 mg/kg (346 DAT), <0.05 mg/kg (409 DAT) – Soil under litter (0 – 15 cm): max. 0.14 mg/kg (346 DAT), 0.07 mg/kg (409 DAT) – Soil (both, 15 – 30 cm): always <0.05 mg/kg, except 346 DAT sample of exposed soil (0.11 mg/kg). Cuthbert site: – Pond water: 0.014 µg/L (0 DAT), 0.002 µg/L (30 DAT) – Stream water: always <0.001 µg/L – Pond sediment: max. 0.13 mg/kg (321 DAT), <0.05 mg/kg (400 DAT) – Stream sediment: always <0.05 mg/kg – Foliage: max. 1.66 mg/kg (7 DAT), <0.05 mg/kg (321 DAT) – Vegetation: max. 1.06 mg/kg (7 DAT), <0.05 mg/kg (321 DAT) – Leaf litter: max. 9.09 mg/kg (3 DAT), 1.79 mg/kg (120 DAT) – Exposed soil (0 – 15 cm): max. 0.18 mg/kg (400 DAT) – Soil under litter (0 – 15 cm): max. 0.23 mg/kg (181 DAT), 0.13 mg/kg (400 DAT) – Soil (both, 15 – 30 cm): always <0.05 mg/kg Half-life times: not calculated No evidence for leaching of glyphosate into the 15 – 30 cm soil horizon.
Reasons why the study is not considered	The study is considered invalid due to the following deficiencies: – Forestry sites

relevant/reliable or not considered as key study:	– Aerial application – Application to vegetation – high variance and uncertainty in dissipation data (falling leaves etc.). – Disturbance of sites by forest management. – Daily weather data only provided for nearest available weather stations, not for the site itself.
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.2.2.1/016
Report author	[redacted]
Report year	1989
Report title	ICIA 0224 – Field Dissipation Study for Terrestrial Uses, California, 1987-1988, Residue Data to Support Registration of TOUCHDOWN
Report No	WRC 89-37
Document No	
Guidelines followed in study	U.S. EPA 164-1 None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No information on site management and pesticide use history - Treated area was not divided into subplots - Verification of sample transport and processing was not conducted
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
The objective of this study was to determine the dissipation rates and mobility of glyphosate-trimesium (ICIA 0224) residues in a sandy loam soil. A total rate of 1 x 4.48 kg a.s./ha (4.0 lb a.s./acre) glyphosate-trimesium was applied to bare fallow ground on 7 July 1987 to the test plot near Orange Cove, California. Soil samples were taken at the following depths: 0-7.6 cm, 7.6-15.2 cm, 15.2-22.9 cm, 22.9-30.5 cm, 30.5-61.0 cm, 61.0-91.4 cm and 91.4-121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 1, 3, 7, 14, 31, 59, 205, and 366 after application.

The test site received one application with TOUCHDOWN 4-LC (batch WCH 1105) containing 40.1 % of active ingredient. The active ingredient in Touchdown 4-LC is glyphosate-trimesium (ICIA 0224).

Samples were analysed for residues of ICIA 0224, which included analyses for trimesium (TMS) (trimethylsulfonium cation), glyphosate (CMP) (carboxymethylaminomethyl-phosphonic acid anion) and AMPA (aminomethylphosphonic acid anions).

There was no evidence of leaching of ICIA 0224 residues. Residues were not found in soil below 0 – 7.6 cm (0 to 3 inch), except for a 0.12 mg/kg TMS residue at day 3 and 0.21 mg/kg TMS and 0.20 mg/kg CMP residues at day 7 after application in the 7.6 -15.2 cm (3 to 6 inch) soil depth.

Residues for trimesium (TMS) in the 0-7.6 cm soil layer decreased from 2.2 mg/kg (day 0) to 0.058 mg/kg (day 31). Glyphosate (CMP) amounted to 2.7 mg/kg on the day of application and decreased to 0.08 mg/kg on day 59; thereafter no residues > LOQ were encountered. AMPA residues of 0.075 mg/kg were found on day 0, increasing to 0.35 mg/kg (day 31) and decreasing below LOD after 1 year.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate as glyphosate-trimesium (ICIA 0224)
Tested formulation: Touchdown 4-LC
Lot No.: WCH 1105
Nominal concentration: 41.8 wt. % or 479 g/L (4 lb per gallon) glyphosate-trimesium
Measured concentrations: 40.1 wt. % Glyphosate-trimesium
27.5 wt. % Glyphosate (CMP)
14.2 wt. % Trimesium (TMS)

B. STUDY DESIGN

1. Test sites

The test site was located near Orange Cove, California, which is near ICI´s America´s Western Research Station at Visalia, California, about 30 km (20 miles) from the local climate weather station in Fresno, California (Lattitude 36 °, 46 min North, Longitude 119 °, 43 min West, Elevation: 1072 m (327 feet)). The non-replicated treatment plot had a size of 26 x 6 m, 158 m^2 (85 by 20 feet), containing one treated and one control plot.

An overview of the soil characterization is given in Table 7.1.2.2.1-182.

Table 7.1.2.2.1-182: Soil characteristics of the Californian test site

Parameter		Horizon			
Soil depth (cm)		**0-30.5**	**30.5-61.0**	**61.0-91.4**	**91.4-121.9**
Soil depth (inch)		**0-12**	**12-24**	**24-36**	**36-48**
Cation Exchange Capacity (meq/100g)		7.0	6.8	7.7	7.9
Particle Size Analysis (USDA) (%) [1]	sand	66	68	60	64
	silt	21	19	29	25
	clay	13	13	11	11
Soil Type		Sandy loam			
Organic matter (%)		0.6	0.2	0.2	0.1
Organic carbon (%) [2]		0.348	0.116	0.116	0.058
Soil pH [3]		7.1	7.7	7.7	7.6
Soil Bulk Density (g/L) b)		Not indicated in the study report			
Field capacity (% soil moisture at 1/3 bar)		10.85	10.64	11.17	11.57

[1] Due to rounding differences the sum may not correspond to 100 %
[2] Calculated from organic matter according to OC = OM × 0.58
[3] Medium not stated

Long-term daily air temperatures and precipitation data as well as annual average air temperatures and total annual precipitation was provided from the weather station in Fresno, California. Reported daily parameters include minimum, maximum and mean air temperatures, total daily precipitation, average wind speed and

direction, sky cover (sunrise – sunset) and peak wind. Additionally, monthly average soil (at 20.3 cm depth) and air temperature data are available from Madera, approximately 16-32 km (10-20 miles) away from the test site. Irrigation was applied and recorded prior to application and at weekly intervals throughout the test period in amounts typical for the area. In areas of natural rainfall, historical weekly rainfall records were obtained from the nearest weather station. If necessary, irrigation was applied to bring the total (rainfall plus irrigation) to 110 % of the historical weekly average.

2. Application

The test site received one application with TOUCHDOWN 4-LC (batch WCH 1105) containing 4.48 kg (4 lb/gallon) of active ingredient glyphosate. Application was conducted on 7 July 1987 with a tractor mounted boom sprayer to bare soil, consisting of fine clods. The formulation was not incorporated. The plots were not cultivated or fertilized before application. During application the air temperature was 29.4 °C (85 °F), soil temperature was 26.6 °C (80 °F), relative humidity was 53 %, and the air movement was 8 km/h (5 mph).

3. Sampling

Soil samples were taken at the following depths: 0 to 7.6 cm, 7.6 to 15.2 cm, 15.2 to 22.9 cm, 22.9 to 30.5 cm, 30.5 to 61.0 cm, 61.0 to 91.4 cm and 91.4 to 121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 1, 3, 7, 14, 31, 59, 205 and 366 after application.

Five separate field samples were randomly collected from both check (untreated control) and treated areas at each soil sampling time. A 10.2 cm length (4 inch) of 7.6 cm (3 inch) diameter aluminium tube was inserted into the ground to a 7.6 cm (3 inch) depth. The 0 to 7.6 cm (0 to 3 inch) depth soil sample was collected by removal of the soil inside the aluminium tube. The 7.6 to 121.9 cm (3 to 48 inch) soil sample was collected with a 2.54 cm (1 inch) diameter hydraulic soil probe; the probe contained an acetate liner to prevent contamination of the soil.

4. Specimen handling and preparation

Soil samples were chilled at the time of collection, transported to the Research Station and frozen. Frozen samples were shipped via overnight express courier or commercial refrigerated truck to ICI America's Western Research Center (WRC) analytical laboratory and arrived frozen. Samples were subdivided into the various appropriate lengths and stored at -20 °C until analysis. Lower depths increments (7.6 to 15.2 cm and below) were mixed to one combined sample, 0 to 7.6 cm samples were kept separately.

Storage stability in soil was assessed in a separate study (RRC 86-61 "Frozen Storage Stability of Touchdown in Soil"), while the results are summarised within the present report. The data indicate that ICIA 0224 residues (TMS, CMP and AMPA) in sandy loam soil (from Orange Cove, California) are stable, under the frozen storage conditions for at least two years. During this study no field-treated sample was stored in excess of 366 days (12 months).

5. Analytical procedures

The detection of TMS (trimesium) in the soil samples was performed by gas chromatography, using method RCC 85-33 ("Determination of SC-0224 Cation Residues in Crops, Water, and Soil by Gas Chromatographie"). The method is described briefly in the following. Soil samples were extracted with 10 % aqueous potassium hydroxide. An aliquot of the extract was made strongly alkaline with potassium hydroxide and heated. TMS was dealkylated and formed dimethylsulfide. The amount of dimethylsulfide was determined by gas chromatographie using flamephotometric detection in the sulfur mode, using a 390 nm bandpass filter.

CMP (glyphosate) and AMPA were analysed by liquid chromatography using RCC method 85-34 ("Determination of SC-0224 Anion Residues in Crops, Water, and Soil by Liquid Chromatographie"). The method is described briefly in the following. Soil samples were extracted with 0.5 M ammonium hydroxide. The extracts were purified by using a cation-exchange column. The CMP and its metabolite AMPA were eluted from the column, derivatised with 9-fluorenylmethylchloroformate, and determined by using an HPLC equipped with an anion-exchange column and a fluorescence detector.

For every set of field samples extracted, one untreated control sample and one fortified control sample were concurrently extracted. If the set was composed of more than ten samples, one control and one fortified control were concurrently extracted for each subset of ten field samples. Untreated control samples contained no residues above the 0.05 mg/kg detection limit for soil.

The limit of detection for TMS, CMP and AMPA was validated by use of untreated controls fortified at 0.05 mg/kg.

Additional recovery data for method validation are contained in the residue method reports (RCC reports No. 85-33 and 85-34), included in the present study report.

Recoveries from fortified untreated soil with TMS, CMP and AMPA during the course of analysis reported in this study as follows. Recoveries from soil fortified between 0.05 and 2.0 mg/kg of TMS ranged from 85 to 116 %; the mean was 101 %, and the coefficient of variation (CV) was 11 %. Recoveries from soil fortified between 0.05 and 0.5 mg/kg of CMP ranged from 70 to 118 %; the mean was 89 %, and the coefficient of variation was 15 %. Recoveries from soil fortified between 0.05 and 0.5 mg/kg of AMPA ranged from 64 to 120 %; the mean was 90 %, and the coefficient of variation was 17 %.

II. RESULTS AND DISCUSSION

A. DATA

Table 7.1.2.2.1-183 summarises the mean residues of soil samples from the 0 to 7.6 cm depth (0 to 3 inch) for trimesium (TMS), glyphosate (CMP) and AMPA over one year. The coefficients of variations of the replicate analyses were calculated over 14 days to assess uniform application of the test compound.

Table 7.1.2.2.1-183: Mean residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA and coefficient of variations (%) in the top layer (0 to 7.6 cm)

DAA	Mean residue (mg/kg)			Coefficient of variation (%)		
	TMS	CMP	AMPA	TMS	CMP	AMPA
0	2.2	2.7	0.075	17	17	32
1	1.9	2.5	0.070	23	26	12
3	1.9	1.7	0.14	14	11	9
7	1.4	1.04	0.25	12	20	30
14	0.81	0.71	0.21	43	37	9
31	0.058	0.28	0.35	n.c.	n.c.	n.c.
59	< LOD	0.08	0.23	n.r.	n.c.	25
205	< LOD	< LOD	0.10	n.r.	n r.	n.c.
366	< LOD	< LOD	< LOD	n.r.	n r.	n.r.
Mean	-			21		

n.c. = not calculated
n.r. = not relevant

A summary of the residues for trimesium (TMS), glyphosate (CMP) and AMPA for all soil layers is presented in Table 7.1.2.2.1-184.

Table 7.1.2.2.1-184: Summary of residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a.s,/ha

DAA[1]	Soil depth (cm)	TMS	CMP	AMPA
0	0 – 7.6	1.6/1.7 [2]	2.1/2.2 [2]	0.06/0.06 [2]
		2.4	2.5	0.10
		2.0	2.6.	0.05
		2.5	3.3	0.09
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
1	0 – 7.6	1.8/1.8 [2]	3.2/3.2 [2]	0.08/0.08 [2]
		2.4	2.2	0.07
		2.1	2.8	0.07
		1.4	1.7	0.06
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	n.a.	n.a.
3	0 – 7.6	1.8/1.8 [2]	1.8/1.7 [2]	0.15/0.15 [2]
		2.1	1.6	0.14
		1.6	1.5	0.15
		1.7	1.7	0.12
		2.2	2.0	0.15
	7.6 – 15.2	0.12	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
7	0 – 7.6	1.6/1.8 [2]	1.1/1.3 [2]	0.13/0.15 [2]
		1.3	1.3	0.20
		1.4	0.9	0.30
		1.3	1.0	0.30
		1.5	0.8	0.30
	7.6 – 15.2	0.21	0.2	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
14	0 – 7.6	0.78/0.82 [2]	1.00/1.10 [2]	0.19/0.21 [2]
		1.28	0.34	0.26
		0.30	0.81	0.18
		0.86	0.64	0.17
		0.80	0.72	0.24
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
	91.4 – 121.9	< LOD	< LOD	< LOD
31	0 – 7.6	0.056/0.060 [2]	0.29/0.27 [2]	0.36/0.34 [2]
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD	< LOD

Table 7.1.2.2.1-184: Summary of residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a.s,/ha

DAA[1]	Soil depth (cm)	TMS	CMP	AMPA
59	0 – 7.6	< LOD	< LOD/< LOD [2]	0.13/0.15 [2]
		< LOD	0.060	0.24
		< LOD	0.130	0.24
		< LOD	0.090	0.21
		< LOD	< LOD	0.30
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
	91.4 – 121.9	< LOD	< LOD	< LOD
205	0 – 7.6	< LOD/< LOD [2]	< LOD/< LOD [2]	0.10/0.10 [2]
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
366	0 – 7.6	n.a.	< LOD/< LOD [2]	< LOD/< LOD [2]
	7.6 – 15.2	n.a.	< LOD	< LOD
	15.2 – 22.9	n.a.	< LOD	< LOD
	22.9 – 30.5	n.a.	< LOD	< LOD
	30.5 – 61.0	n.a.	< LOD	< LOD

[1] DAA = Days after application
[2] Analysed in duplicate
n.a. = Not analysed

B. CHARACTERISATION OF RESIDUES

Residues for trimesium (TMS) in the top layer decrease from 2.2 mg/kg (day 0) to 0.058 mg/kg (day 31). Only one 0.12 mg/kg and one 0.21 mg/kg residue of TMS were found at day 3 and day 7, respectively, at 7.6 to 15.2 cm depth (3 to 6 inch). No residue was found in the lower soil depths and no residue above the 0.05 mg/kg detection limit was found in the sample fraction from the 59 to 205 days samplings.

Glyphosate (CMP) amounted to 2.7 mg/kg on the day of application and decreased to 0.08 mg/kg on day 59; thereafter no residues > LOQ were encountered. A 0.20 mg/kg CMP anion residue value was found on day 7 at 7.6-15.2 cm depth (3 to 6 inch). Significant amounts of residues were found only at the 0 to 7.6 cm soil depth (0 to 3 inch); these residues completely dissipated by day 203.

It can be concluded that AMPA is formed following the application of TOUCHDOWN 4-LC. Residue levels increased to about 0.35 mg/kg after 31 days and began to decline during the remaining period. AMPA is a very small, highly polar molecule, capable of binding tightly to soil. The 0.10 to 0.35 mg/kg residuals at 0 to 7.6 cm (0 to 3 inch) at days 31 to 205, may represent AMPA that is tightly bound to the soil and not capable of undergoing rapid dissipation. AMPA residues were not detected in the 366 day soi1 samples. AMPA residues were found only in the upper 0 to 7.6 cm (0 to 3 inch) layer, thus, it can be concluded that AMPA does not leach.

C. KINETICS

An Ecoregion Crosswalk exercise was performed (see [redacted] 2020, CA 7.1.2.2.1/002). The trial in California was found to be representative for European conditions and included in kinetic evaluation ([redacted], 2020, CA 7.1.2.2.1/003).

III. CONCLUSIONS

ICIA 0224 (as measured by trimesium and glyphosate residues) dissipated rapidly in sandy loam soil in California after application of TOUCHDOWN 4-LC formulation.

ICIA 0224 did not leach or migrate prior to its environmental degradation. Except for one 0.20 mg/kg glyphosate residue at 7 days and 0.12 mg/kg and 0.21 mg/kg trimesium residues at 3 and 7 days, respectively, in the 7.6 to 15.2 cm soil depth (3 to 6 inch), all residues were found in the 0 to 7.6 cm (0 to 3 inch) soil depth samples.

AMPA was formed as an intermediate degradate in the course of carbon recyclisation/mineralisation of glyphosate. From an initial 2.7 mg/kg glyphosate residue, the maximum amount of AMPA residue found was 0.35 mg/kg. It appeared that most of the AMPA was rapidly further degraded, but a small amount (0.1 mg/kg) became bound to the soil and unavailable for rapid degradation. AMPA was not found below the 0 to 7.6 cm (0 to 3 inch) soil depth sampled. AMPA was not detected in the 366 day soil samples.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed according to the respective guideline in force in 1987. There are minor deviations to current guideline requirements. Nevertheless, the study provides valuable information on the dissipation behavior of glyphosate under field conditions. As the representative formulation of the current submission does not contain trimesium cation, the trimesium findings were neglected for further consideration.
The study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/017
Report author	[redacted]
Report year	1989
Report title	ICIA 0224 – Field Dissipation Study for Terrestrial Uses, Mississippi, 1987-1988, Residue Data to Support Registration of TOUCHDOWN
Report No	WRC 89-40
Document No	
Guidelines followed in study	U.S. EPA 164-1 None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No information on site management and pesticide use history - Treated area was not divided into subplots - Verification of sample transport and processing was not conducted
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary

The objective of this study was to determine the dissipation rates and mobility of glyphosate-trimesium (ICIA 0224) residues in a silt loam soil. A total rate of 1 x 4.48 kg a.s./ha (4.0 lb a.s./acre) glyphosate-trimesium was applied to bare fallow ground on 7 July 1987 to the test plot in Leland, Mississippi. Soil samples were taken at the following depths: 0-7.6 cm, 7.6-15.2 cm, 15.2-22.9 cm, 22.9-30.5 cm, 30.5-61.0 cm, 61.0-91.4 cm and 91.4-121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 1, 3, 7, 14, 28, 57, 199, 380 and 542 after application.

The test site received one application with TOUCHDOWN 4-LC (batch WCL 1402) containing 41.4 % of active ingredient. The active ingredient in Touchdown 4-LC is glyphosate-trimesium (ICIA 0224).

Samples were analysed for residues of ICIA 0224, which included analyses for trimesium (TMS) (trimethylsulfonium cation), glyphosate (CMP) (carboxymethylaminomethyl-phosphonic acid anion) and AMPA (aminomethylphosphonic acid anions).

There was no evidence of leaching of ICIA 0224 residues. Residues were not found in soil below 0 – 7.6 cm (0 to 3 inch), except for a 0.05 mg/kg TMS residue at day 57 after application in the 7.6-15.2 cm (3 to 6 inch) soil depth.

Residues for trimesium (TMS) in the 0-7.6 cm soil layer decreased from 1.8 mg/kg (day 0) to 0.06 mg/kg (day 57). Glyphosate (CMP) amounted to 2.7 mg/kg on the day of application and decreased to 0.55 mg/kg on day 14; thereafter no residues > LOQ were encountered. AMPA residues of 0.09 mg/kg were found on day 0, increasing to 0.32 mg/kg (day 14) and decreasing to 0.06 mg/kg after 542 days.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate as glyphosate-trimesium (ICIA 0224)
Tested formulation:	Touchdown 4-LC
Lot No.:	WCL 1402
Nominal concentration:	41.8 wt. % or 479 g/L (4 lb per gallon) glyphosate-trimesium
Measured concentration:	41.4 wt. % glyphosate-trimesium

B. STUDY DESIGN

1. Test sites

The test site was located in Leland, Mississippi, which is near ICI´s America´s Southern Research Station at Leland, Mississippi. The local climate weather station (mid-south Agricultural Weather service Center) is located at Stoneville, Mississippi. The non-replicated treatment plot had a size of 12 x 15 m, 186 m^2 (40 by 50 feet), containing one treated and one control plot.

An overview of the soil characterization is given in Table 7.1.2.2.1-185.

Table 7.1.2.2.1-185: Soil characteristics of the Mississippi test site

Parameter		Horizon			
Soil depth (cm)		**0-30.5**	**30.5-61.0**	**61.0-91.4**	**91.4-121.9**
Soil depth (inch)		**0-12**	**12-24**	**24-36**	**36-48**
Cation Exchange Capacity (meq/100g)		7.1	10.6	9.6	13.5
Particle Size Analysis (USDA) (%) [1]	sand	23	12	22	12
	silt	62	67	59	56
	clay	15	21	19	32
Soil Type		silt loam	silt loam	silt loam	silty clay loam
Organic matter (%)		0.7	0.6	0.3	0.7
Organic carbon (%) [2]		0.406	0.348	0.174	0.406
Soil pH [3]		6.9	7.0	7.1	7.2
Soil Bulk Density (g/L) [b)]		Not indicated in the study report			
Field capacity (% soil moisture at 1/3 bar)		21.5	22.4	25.0	31.2

[1] Due to rounding differences the sum may not correspond to 100 %
[2] Calculated from organic matter according to OC = OM x 0.58
[3] Medium not stated

Long-term daily air temperatures and precipitation data as well as soil temperature and wind speed was provided from the weather service Center at Stoneville, Mississippi. Reported daily parameters include minimum and maximum air temperatures, minimum and maximum soil temperatures (at depths of 5.1, 10.2, 20.3 and 50.8 cm (2, 4, 8 and 20 inches), total daily precipitation, evaporation and wind speed. Daily rainfall was measured and irrigation was applied and recorded at 14-day intervals throughout the test period to bring the total (rainfall plus irrigation) to 110 % of the historical weekly average.

2. Application

The test site received one application with TOUCHDOWN 4-LC (batch WCL 1402) containing 4.48 kg (4 lb/gallon) of active ingredient. Application was conducted on 7 July 1987 with a tractor mounted boom sprayer to bare soil, consisting of dry small clods. The formulation was not incorporated. The plots were not cultivated or fertilized before application. During application the air temperature was 34.4 °C (94 °F), soil temperature was 30.0 °C (86 °F), relative humidity was 45 %, and the air movement was 3.2 km/h (2 mph) from southwest.

3. Sampling

Soil samples were taken at the following depths: 0 to 7.6 cm, 7.6 to 15.2 cm, 15.2 to 22.9 cm, 22.9 to 30.5 cm, 30.5 to 61.0 cm, 61.0 to 91.4 cm and 91.4 to 121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 1, 3, 7, 14, 28, 57, 199, 380 and 542 after application.

Five separate field samples were randomly collected from both check (untreated control) and treated areas at each soil sampling time. A 10.2 cm length (4 inch) of 7.6 cm (3 inch) diameter aluminium tube was inserted into the ground to a 7.6 cm (3 inch) depth. The 0 to 7.6 cm (0 to 3 inch) depth soil sample was collected by removal of the soil inside the aluminium tube. The 7.6 to 121.9 cm (3 to 48 inch) soil sample was collected with a 2.54 cm (1 inch) diameter hydraulic soil probe; the probe contained an acetate liner to prevent contamination of the soil.

4. Specimen handling and preparation

Soil samples were chilled at the time of collection, transported to the Research Station and frozen. Frozen samples were shipped via overnight express courier or commercial refrigerated truck to ICI America's Western Research Center (WRC) analytical laboratory and arrived frozen. Samples were subdivided into the various appropriate lengths and stored at -20 °C until analysis. Lower depths increments (7.6 to 15.2 cm and below) were mixed to one combined sample, 0 to 7.6 cm samples were kept separately.

Storage stability in soil was assessed in a separate study (RRC 86-61 "Frozen Storage Stability of Touchdown in Soil"), while the results are summarised within the present report. The data indicate that ICIA 0224 residues (TMS, CMP, and AMPA) in silty clay loam soil (from Leland, Mississippi) are stable, under the frozen storage conditions for at least two years. During this study no field-treated sample was stored in excess of 170 days (5.7 months).

5. Analytical procedures

The detection of TMS (trimesium) in the soil samples was performed by gas chromatography using method RCC 85-33 ("Determination of SC-0224 Cation Residues in Crops, Water, and Soil by Gas Chromatographie"). The method is described briefly in the following. Soil samples were extracted with 10 % aqueous potassium hydroxide. An aliquot of the extract was made strongly alkaline with potassium hydroxide and heated. TMS was dealkylated and formed dimethylsulfide. The amount of dimethylsulfide was determined by gas chromatographie using flamephotometric detection in the sulfur mode, using a 390 nm bandpass filter.

CMP (glyphosate) and AMPA were analysed by liquid chromatography using RCC method 85-34 ("Determination of SC-0224 Anion Residues in Crops, Water, and Soil by Liquid Chromatographie"). The method is described briefly in the following. Soil samples were extracted with 0.5 M ammonium hydroxide. The extracts were purified by using a cation-exchange column. The CMP and its metabolite AMPA were eluted from the column, derivatised with 9-fluorenylmethylchloroformate, and determined by using an HPLC equipped with an anion-exchange column and a fluorescence detector.

For every set of field samples extracted, one untreated control sample and one fortified control sample were concurrently extracted. If the set was composed of more than ten samples, one control and one fortified control were concurrently extracted for each subset of ten field samples. Untreated control samples contained no residues of above the 0.05 mg/kg detection limit for soil.

The limit of detection for trimesium, glyphosate and AMPA was validated by use of untreated controls fortified at 0.05 mg/kg.
Additional recovery data for method validation are contained in the residue method reports (RCC reports No. 85-33 and 85-34), included in the present study report.

Recoveries from fortified untreated soil with trimesium, glyphosate and AMPA during the course of analysis reported in this study are as follows. Recoveries from soil fortified between 0.05 and 2.0 mg/kg of TMS ranged from 84 to 117 %; the mean was 99 %, and the coefficient of variation (CV) was 11 %. Recoveries from soil fortified between 0.05 and 0.5 mg/kg of CMP ranged from 60 to 94 %; the mean was 79 %, and the coefficient of variation was 16 %. Recoveries from soil fortified between 0.05 and 0.5 mg/kg of AMPA ranged from 74 to 108 %; the mean was 93 %, and the coefficient of variation was 9 %.

II. RESULTS AND DISCUSSION

A. DATA

Table 7.1.2.2.1-186 summarises the mean residues of soil samples from the 0 to 7.6 cm depth (0 to 3 inch) for trimesium (TMS), glyphosate (CMP) and AMPA over one year. The coefficients of variations of the replicate analyses were calculated over 14 days to assess uniform application of the test compound.

Table 7.1.2.2.1-186: Mean residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA and coefficient of variations (%) in the top layer (0 to 7.6 cm)

DAA	Mean residue (mg/kg)			Coefficient of variation (%)		
	TMS	CMP	AMPA	TMS	CMP	AMPA
0	1.8	2.7	0.09	23	18	8
1	1.6	2.4	0.11	16	18	14
3	1.4	2.0	0.22	12	26	17
7	0.50	1.11	0.27	68	7	11
14	0.11	0.55	0.32	24	38	18
28	0.051	< LOD	< LOD	n.c.	n r.	n.r.
57	0.061	< LOD	0.12	n.c.	n r.	n.c.
199	< LOD	< LOD	0.068	n.r.	n r.	n.c.
380	n.a.	< LOD	0.09	n.r.	n r.	n.c.
542	n.a.	< LOD	0.058	n.r.	n.r.	n.c.
Mean	-			21		

n.a. = Not analysed
n.c. = Not calculated
n.r. = Not relevant

A summary of the residues for trimesium (TMS), glyphosate (CMP) and AMPA for all soil layers is presented in Table 7.1.2.2.1-187.

Table 7.1.2.2.1-187: Summary of residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a.s,/ha

DAA[1]	Soil depth (cm)	TMS	CMP	AMPA
0	0 – 7.6	1.2/2.0 [2]	3.1/3.2 [2]	0.09/0.09 [2]
		1.3	2.2	0.08
		1.7	2.5	0.09
		1.9	2.5	0.09
		2.4	3.3	0.10
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
1	0 – 7.6	1.2/1.2 [2]	1.7/1.8 [2]	0.08/0.09 [2]
		1.8	3.0	0.12
		1.5	2.3	0.11
		1.8	2.6	0.13
		1.7	2.3	0.12
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
3	0 – 7.6	1.39/1.43 [2]	2.0/1.5 [2]	0.24/0.21 [2]
		1.51	2.6	0.26
		1.47	2.4	0.25
		1.08	1.6	0.18
		1.34	1.4	0.17
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD

Table 7.1.2.2.1-187: Summary of residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a.s,/ha

DAA[1]	Soil depth (cm)	TMS	CMP	AMPA
7	0 – 7.6	0.56/0.54 [2]	1.4/0.90 [2]	0.32/0.25 [2]
		0.19	1.10	0.26
		1.05	1.20	0.26
		0.23	1.00	0.24
		0.46	1.10	0.29
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
14	0 – 7.6	0.09/0.09 [2]	0.55/0.45 [2]	0.35/0.32 [2]
		0.11	0.77	0.41
		0.13	0.40	0.27
		0.13	0.78	0.32
		0.07	0.31	0.27
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
	91.4 – 121.9	< LOD	< LOD	< LOD
28	0 – 7.6	< LOD/0.051 [2]	< LOD/< LOD [2]	< LOD/< LOD [2]
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD	< LOD
57	0 – 7.6	0.059/0.062 [2]	< LOD/< LOD [2]	0.12/0.12 [2]
	7.6 – 15.2	0.051	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD	< LOD
199	0 – 7.6	< LOD/< LOD [2]	< LOD/< LOD [2]	0.068/0.078 [2]
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
380	0 – 7.6	n.a.	< LOD/< LOD [2]	0.09/< LOD [2]
	15.2 – 22.9	n.a.	< LOD	< LOD
	22.9 – 30.5	n.a.	< LOD	< LOD
	30.5 – 61.0	n.a.	< LOD	< LOD
542	0 – 7.6	n.a.	< LOD	0.058
	15.2 – 22.9	n.a.	< LOD	< LOD
	22.9 – 30.5	n.a.	< LOD	< LOD
	30.5 – 61.0	n.a.	< LOD	< LOD
	61.0 – 91.4	n.a.	< LOD	< LOD
	91.4– 121.9	n.a.	< LOD	< LOD

[1] DAA = Days after application
[2] Analysed in duplicate
n.a. = Not analysed

B. CHARACTERISATION OF RESIDUES

Residues for trimesium (TMS) in the top layer decreased from 1.8 mg/kg (day 0) to 0.06 mg/kg (day 57). Only one 0.05 mg/kg residue of TMS was found at day 57 at 7.6 to 15.2 cm depth (3 to 6 inch). No residue was found in the lower soil depths and no residue above the 0.05 mg/kg detection limit was found in the sample fraction from the 199 day samplings.

Glyphosate (CMP) amounted to 2.7 mg/kg on the day of application and decreased to 0.55 mg/kg on day 14; thereafter no residues > LOQ were encountered. No residues were found in the lower soil depths of 7.6 to 121.9 cm (3 to 48 inch).

It can be concluded that AMPA is formed following the application of TOUCHDOWN 4-LC. Residue levels increased to about 0.32 mg/kg after 14 days and began to decline during the remaining period. AMPA is a very small, highly polar molecule, capable of binding tightly to soil. AMPA residues were not detected in the 28 day soi1 samples. The 0.12 to 0.06 mg/kg residuals at 0 to 7.6 cm (0 to 3 inch) at days 57 to 542 may represent AMPA that is tightly bound to the soil and not capable of undergoing rapid dissipation. AMPA residues were found only in the upper 0 to 7.6 cm (0 to 3 inch) layer, thus, it can be concluded that AMPA does not leach.

C. KINETICS

An Ecoregion Crosswalk exercise was performed [redacted], 2020, CA 7.1.2.2.1/002) and the trial is not considered reprentative for European conditions. Therefore, a new kinetic evaluation of the data is not performed.

III. CONCLUSIONS

ICIA 0224 (as measured by trimesium and glyphosate residues) dissipated rapidly in silty loam soil in Mississippi after application of TOUCHDOWN 4-LC formulation.

ICIA 0224 did not leach or migrate prior to its environmental degradation. Except for one 0.05 mg/kg trimesium residue at day 57, in the 7.6 to 15.2 cm soil depth (3 to 6 inch), all residues were found in the 0 to 7.6 cm (0 to 3 inch) soil depth samples.

AMPA was formed as an intermediate degradate in the course of carbon recyclisation/mineralisation of glyphosate. From an initial 2.7 mg/kg glyphosate residue, the maximum amount of AMPA residue found was 0.32 mg/kg. It appeared that most of the AMPA was rapidly further degraded, but a small amount (0.1 mg/kg) became bound to the soil and unavailable for rapid degradation. AMPA was not found below the 0 to 7.6 cm (0 to 3 inch) soil depth sampled.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was performed according to the respective guideline in force in 1987. There are minor deviations to current guideline requirements. Nevertheless, the study provides valuable information on the dissipation behavior of glyphosate under field conditions. As the representative formulation of the current submission does not contain trimesium cation, the trimesium findings were neglected for further consideration.

Since the trial is not considered representative for European conditions, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/018
Report author	[redacted]
Report year	1989
Report title	ICIA 0224 – Field Dissipation Study for Terrestrial Uses, Georgia, 1987-1988, Residue Data to Support Registration of TOUCHDOWN
Report No	WRC 89-23, Protocol No. RP-87-27
Document No	
Guidelines followed in study	U.S. EPA 164-1 None
Deviations from current test guideline	From OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, March 2016: - No information on site management and pesticide use history is missing - Treated area was not divided into subplots - Verification of sample transport and processing was not conducted
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary

The objective of this study was to determine the dissipation rates and mobility of glyphosate-trimesium (ICIA 0224) residues in a sandy loam soil. A total rate of 1 x 4.48 kg a.s./ha (4.0 lb a.s./acre) glyphosate-trimesium was applied to bare fallow ground on 12 August 1987 to the test plot near Donalsonville, Georgia. Soil samples were taken at the following depths: 0-7.6 cm, 7.6-15.2 cm, 15.2-22.9 cm, 22.9-30.5 cm, 30.5-61.0 cm, 61.0-91.4 cm and 91.4-121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 2, 5, 7, 14, 33, 58, 182, and 369 after application.

The test site received one application with TOUCHDOWN 4-LC (batch WCL 1402) containing 41.4 % of active ingredient. The active ingredient in Touchdown 4-LC is glyphosate-trimesium (ICIA 0224).

Samples were analysed for residues of ICIA 0224, which included analyses for trimesium (TMS) (trimethylsulfonium cation), glyphosate (CMP) (carboxymethylaminomethyl-phosphonic acid anion) and AMPA (aminomethylphosphonic acid anions).

There was no evidence of leaching of ICIA 0224 residues. Residues were not found in soil below 0 – 7.6 cm (0 to 3 inch), except for a 0.12 mg/kg residue for both TMS and CMP at day 0 in the 7.6 -15.2 cm (3 to 6 inch) soil depth, attributed to inadvertent contamination.

Residues for trimesium (TMS) in the 0-7.6 cm soil layer decreased from 1.15 mg/kg (day 0) to 0.11 mg/kg (day 33). Glyphosate (CMP) amounted to 1.2 mg/kg on the day of application and decreased to 0.08 mg/kg on day 14; thereafter no residues > LOQ were encountered. AMPA residues of 0.09 mg/kg were found on day 0, increasing to 0.42 mg/kg (day 7) and decreasing below LOD after 1 year.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:
Identification: Glyphosate as glyphosate-trimesium (ICIA 0224)
Tested formulation: Touchdown 4-LC
Lot No.: WCL 1402
Nominal concentration: 41.8 wt. % or 479 g/L (4 lb per gallon) glyphosate-trimesium
Measured concentration: 41.4 wt. % glyphosate-trimesium

B. STUDY DESIGN

1. Test sites
The test site was located near Donalsonville, Georgia. The non-replicated plot had a size of 22 x 5 m, 120 m^2 (72 by 18 feet), containing one treated and one control plot.

An overview of the soil characterization is given in Table 7.1.2.2.1-188.

Table 7.1.2.2.1-188: Soil characteristics of the Georgia test site

Parameter		Horizon			
Soil depth (cm)		**0-30.5**	**30.5-61.0**	**61.0-91.4**	**91.4-121.9**
Soil depth (inch)		**0-12**	**12-24**	**24-36**	**36-48**
Cation Exchange Capacity (meq/100g)		2.9	1.6	1.3	3.1
Particle Size Analysis (USDA) (%) [1]	sand	86	88	88	72
	silt	9	7	7	4
	clay	5	5	5	24
Soil Type		Loamy sand	Sand	Sand	Sandy clay loam
Organic matter (%)		1.2	0.4	0.1	0.1
Organic carbon (%) [2]		0.696	0.232	0.058	0.058
Soil pH [3]		6.5	5.1	5.3	5.5
Soil Bulk Density (g/L) [b)]		Not indicated in the study report			
Field capacity (% soil moisture at 1/3 bar)		3.8	4.1	3.4	9.2

[1] Due to rounding differences the sum may not correspond to 100 %
[2] Calculated from organic matter according to OC = OM × 0.58
[3] Medium not stated

Long-term daily air temperatures and precipitation data was provided from the Southern Agricultural Research Inc. in Donalsonville, Georgia. Reported daily parameters include minimum and maximum air temperatures, minimum and maximum soil temperatures at 7.6 cm below ground level (3 inches), total daily precipitation, evaporation and relative humidity. Irrigation was applied and recorded prior to application and at weekly intervals throughout the test period in amounts typical for the area. If necessary, irrigation was applied to bring the total (rainfall plus irrigation) to 110 % of the historical weekly average.

Prior to the test, the site was cultivated with bahiagrass and bermudagrass pasture. To maintain bare soil in the plots, a herbicide mixture with residual soil activity was applied (Atrazine + Dual) in order to prevent grass re-establishing in plots. The tankmix was applied after 3 passes with disk (to destroy "turf") and prior to final two diskings.

2. Application
The test site received one application with TOUCHDOWN 4-LC (batch WCL 1402) containing 4.48 kg (4 lb/gallon) of active ingredient. Application was conducted on 12 August 1987 with a carbon dioxide-charged backpack sprayer with a four-nozzle boom to bare dry soil, previously in bahiagrass and

bermudagrass pasture. The formulation was not incorporated. The plots were not cultivated or fertilized before application. During application the air temperature was 30.6 °C (87 °F), soil temperature was 35.6 °C (96 °F), relative humidity was 59 %, and the atmospheric condition was calm.

3. Sampling

Soil samples were taken at the following depths: 0 to 7.6 cm, 7.6 to 15.2 cm, 15.2 to 22.9 cm, 22.9 to 30.5 cm, 30.5 to 61.0 cm, 61.0 to 91.4 cm and 91.4 to 121.9 cm (0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 inches) and on days 0, 2, 5, 7, 14, 33, 58, 182, and 369 after application.

Three separate field samples were randomly collected from both check (untreated control) and treated areas at each soil sampling time. A 10.2 cm (4 inch) length of 15.2 cm (6 inch) diameter aluminium tube was inserted into the ground to a 7.6 cm (3 inch) depth. The 0 to 7.6 cm (0 to 3 inch) depth soil sample was collected by removal of the soil inside the aluminium tube. The 7.6 to 121.9 cm (3 to 48 inch) soil sample was collected with a 2.54 cm (1 inch) diameter hydraulic soil probe; the probe contained an acetate liner to prevent contamination of the soil. On day 5 sample collection was only possible from the top layer, soil probe samples below the 0 to 7.6 cm (0 to 3 inch) horizon could not be physically collected because of the saturated soil after cumulative rainfall (89 mm (3.5 inches) over 4 days).

4. Specimen handling and preparation

Soil samples were chilled at the time of collection, transported to a freezer within two hours of sampling and frozen. Frozen samples were shipped via overnight express courier or commercial refrigerated truck to ICI America's Western Research Center (WRC) analytical laboratory and arrived frozen. Samples were subdivided into the various appropriate lengths and stored at -20 °C until analysis.

Storage stability in soil was assessed in a separate study (RRC 86-61 "Frozen Storage Stability of Touchdown in Soil"), while the results are summarised within the present report. The data indicate that ICIA 0224 residues (TMS, CMP, and AMPA) in a fine sand soil are stable, under the frozen storage conditions for at least two years. During this study no field-treated sample was stored in excess of 133 days (4.4 months).

5. Analytical procedures

The detection of TMS (trimesium) in the soil samples was performed by gas chromatography, using method RCC 85-33 (“Determination of SC-0224 Cation Residues in Crops, Water, and Soil by Gas Chromatographie”). The method is described briefly in the following. Soil samples were extracted with 10 % aqueous potassium hydroxide. An aliquot of the extract was made strongly alkaline with potassium hydroxide and heated. TMS was dealkylated and formed dimethylsulfide. The amount of dimethylsulfide was determined by gas chromatographie using flamephotometric detection in the sulfur mode, using a 390 nm bandpass filter.

CMP (glyphosate) and AMPA were analysed by liquid chromatography using RCC method 85-34 (“Determination of SC-0224 Anion Residues in Crops, Water, and Soil by Liquid Chromatographie”). The method is described briefly in the following. Soil samples were extracted with 0.5 M ammonium hydroxide. The extracts were purified by using a cation-exchange column. The CMP and its metabolite AMPA were eluted from the column, derivatised with 9-fluorenylmethylchloroformate, and determined by using an HPLC equipped with an anion-exchange column and a fluorescence detector.

For every set of field samples extracted, one untreated control sample and one fortified control sample were concurrently extracted. If the set was composed of more than ten samples, one control and one fortified control were concurrently extracted for each subset of ten field samples. Untreated control samples contained background equivalent to 0.01-0.04 mg/kg of TMS and 0.01-0.08 mg/kg AMPA. There was no background interference for CMP analysis.

The limit of detection for trimesium, glyphosate and AMPA was validated by use of untreated controls fortified at 0.05 mg/kg.

Additional recovery data for method validation are contained in the residue method reports (RCC reports No. 85-33 and 85-34), included in the present study report.

Recoveries from fortified untreated soil with trimesium, glyphosate and AMPA in the course of analysis reported in this study are as follows. Recoveries from soil fortified between 0.05 and 1.0 mg/kg of TMS ranged from 80 to 124 %; the mean was 106 %, and the coefficient of variation (CV) was 12 %.

Recoveries from soil fortified between 0.05 and 2.0 mg/kg of CMP ranged from 68 to 115 %; the mean was 81 %, and the coefficient of variation was 14 %. Recoveries from soil fortified between 0.05 and 2.0 mg/kg of AMPA ranged from 70 to 118 %; the mean was 82 %, and the coefficient of variation was 15 %.

II. RESULTS AND DISCUSSION

A. DATA

Table 7.1.2.2.1-189 summarises the mean residues of soil samples from the 0 to 7.6 cm depth (0 to 3 inch) for trimesium (TMS), glyphosate (CMP) and AMPA over one year. The coefficients of variations of the replicate analyses were calculated over 58 days to assess uniform application of the test compound. The coefficient of variation reflects the less then optimal application achieved by use of a backpack sprayer as contrasted to a tractor-mounted boom.

Table 7.1.2.2.1-189: Mean residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA and coefficient of variations (%) in the top layer (0 to 7.6 cm)

DAA	Mean residue (mg/kg)			Coefficient of variation (%)		
	TMS	CMP	AMPA	TMS	CMP	AMPA
0	1.15	1.2	0.09	29	13	0
2	0.85	0.55	0.26	64	82	62
5	0.59	0.27	0.37	47	56	30
7	1.01	0.19	0.42	28	17	7
14	0.51	0.08 [1]	0.21	90	n.c.	n.c.
33	0.11	< LOD	0.29	40	n r.	34
58	0.02 [1]	< LOD	0.083	n.r.	n r.	35
182	< LOD	< LOD	0.02 [1]	n.r.	n r.	n.c.
369	n.a.	< LOD	< LOD	n.r.	n r.	n.r.
Mean	-			40		

n.a. = Not analysed
n.c. = Not calculated
n.r. = Not relevant
[1] Only one of three samples was > LOD

A summary of the residues for trimesium (TMS), glyphosate (CMP) and AMPA for all soil layers is presented in Table 7.1.2.2.1-190.

Table 7.1.2.2.1-190: Summary of residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a i,/ha

DAA[1]	Soil depth (cm)	TMS	CMP	AMPA
0	0 – 7.6	1.28/1.01 [2]	1.4/1.1 [2]	0.10/0.08 [2]
		0.82	1.0	0.09
		1.47	1.2	0.09
	7.6 – 15.2	0.26	0.25	< LOD
		< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD

Table 7.1.2.2.1-190: Summary of residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a i,/ha

DAA[1]	Soil depth (cm)	TMS	CMP	AMPA
2	0 – 7.6	0.34/0.34 [2]	0.25/0.23 [2]	0.16/0.14 [2]
		1.42	1.06	0.44
		0.78	0.35	0.19
	7.6 – 15.2	< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
5 [3]	0 – 7.6	0.29/0.39 [2]	0.12/0.12 [2]	0.24/0.26 [2]
		0.89	0.41	0.47
		0.54	0.28	0.40
7	0 – 7.6	0.91/0.93 [2]	0.18/0.16 [2]	0.40/0.50 [2]
		0.65	0.18	0.40
		1.25/1.20 [2]	0.23	0.40
	7.6 – 15.2	< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
	15.2 – 22.9	n.a.	n.a	< LOD
		< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
14 [4]	0 – 7.6	0.26/0.19 [2]	< LOD/< LOD [2]	0.16/0.17 [2]
		1.04	n.a.	n.a.
		0.25	0.13	0.25
	7.6 – 15.2	< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
	91.4 – 121.9	< LOD	< LOD	< LOD
33	0 – 7.6	0.08/0.08 [2]	< LOD/< LOD [2]	0.25/0.25 [2]
		0.09	< LOD	0.22
		0.16	< LOD	0.41
	7.6 – 15.2	< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
		< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
		0.05	< LOD	< LOD
		< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
	91.4– 121.9	< LOD	< LOD	< LOD
58	0 – 7.6	< LOD/0.05 [2]	< LOD/< LOD [2]	0.10/0.10 [2]
		< LOD	< LOD	0.10
		< LOD	< LOD	0.05
	7.6 – 15.2	< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
	30.5 – 61.0	< LOD	< LOD	< LOD
	61.0 – 91.4	< LOD	< LOD	< LOD
182	0 – 7.6	< LOD/< LOD [2]	< LOD/< LOD [2]	< LOD/< LOD [2]

Table 7.1.2.2.1-190: Summary of residues (mg/kg) for trimesium (TMS), glyphosate (CMP) and AMPA after application of TOUCHDOWN 4-LC at 4.48 kg a i,/ha

DAA[1]	Soil depth (cm)	TMS	CMP	AMPA
		< LOD	< LOD	0.07
		< LOD	< LOD	< LOD
	15.2 – 22.9	< LOD	< LOD	< LOD
	22.9 – 30.5	< LOD	< LOD	< LOD
369	0 – 7.6	n.a.	< LOD/< LOD [2]	< LOD/< LOD [2]
	15.2 – 22.9	n.a.	< LOD	< LOD
	22.9 – 30.5	n.a.	< LOD	< LOD
	30.5 – 61.0	n.a.	< LOD	< LOD
	61.0 – 91.4	n.a.	< LOD	< LOD

[1] DAA = Days after application
[2] Analysed in duplicate
[3] Soil probe samples below the 0 to 7.6 cm (0 to 3 inch) horizon could not be collected because of the saturated soil after cumulative rainfall (89 mm (3.5 inches) over 4 days)
[4] Horizons below 3 inch were sampled at day 15 after application
n.a. = Not analysed

B. CHARACTERISATION OF RESIDUES

Residues for trimesium (TMS) in the top layer decreased from 1.15 mg/kg (day 0) to 0.11 mg/kg (day 33). Only one 0.12 mg/kg residue of TMS was found at day 0, attributed to inadvertent contamination, at 7.6 to 15.2 cm depth (3 to 6 inch). No residue was found in the lower soil depths and no residue above the 0.05 mg/kg detection limit was found in the sample fractions from the 58 to 182 days samplings.

Glyphosate (CMP) amounted to 1.2 mg/kg on the day of application and decreased to 0.08 mg/kg on day 14; thereafter no residues > LOQ were encountered. A 0.12 mg/kg CMP anion residue value was found on day 0, attributed to inadvertrant contamination, at 7.6 to 15.2 cm depth (3 to 6 inch). Significant amounts of residues were found only at the 0 to 7.6 cm soil depth (0 to 3 inch); these residues completely dissipated by day 33.

It can be concluded that AMPA is formed following the application of TOUCHDOWN 4-LC. Residue levels increased to about 0.42 mg/kg after 7 days and began to decline during the remaining period. AMPA is a very small, highly polar molecule, capable of binding tightly to soil. The 0.21 to 0.06 mg/kg residuals at 0 to 7.6 cm (0 to 3 inch) at days 14 to 182 may represent AMPA that is tightly bound to the soil and not capable of undergoing rapid dissipation. AMPA residues were not detected in the 369 day soi1 samples. AMPA residues were found only in the upper 0 to 7.6 cm (0 to 3 inch) layer, thus, it can be concluded that AMPA does not leach.

C. KINETICS

An Ecoregion Crosswalk exercise was performed [redacted] 2020, CA 7.1.2.2.1/002) and the trial is not considered reprentative for European conditions. Therefore, a new kinetic evaluation of the data is not performed.

III. CONCLUSIONS

ICIA 0224 (as measured by trimesium and glyphosate residues) dissipated rapidly in Lucy loamy sand in Georgia after application of TOUCHDOWN 4-LC formulation.

ICIA 0224 did not leach or migrate prior to its environmental degradation. Except for one 0.12 mg/kg residue of both TMS and CMP at the day of application in the 7.6 to 15.2 cm soil depth (3 to 6 inch), attributed to inadvertent contamination, all residues were found in the 0 to 7.6 cm (0 to 3 inch) soil depth samples.

AMPA was formed as an intermediate degradate in the course of carbon recyclisation/mineralisation of glyphosate. From an initial 1.2 mg/kg glyphosate residue, the maximum amount of AMPA residue found was 0.42 mg/kg. It appeared that most of the AMPA was rapidly further degraded, but a small amount (0.1 mg/kg) became bound to the soil and unavailable for rapid degradation. AMPA was not found below the 0 to 7.6 cm (0 to 3 inch) soil depth sampled. AMPA was not detected in the 369 day soil samples.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed according to the respective guideline in force in 1987. There are minor deviations to current guideline requirements. Nevertheless, the study provides valuable information on the dissipation behavior of glyphosate under field conditions. As the representative formulation of the current submission does not contain the trimesium cation, the trimesium findings were neglected for further consideration.
Since the trial is not considered representative for European conditions, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/019
Report author	[redacted]
Report year	1986
Report title	Frozen storage stability of SC-0224 in soil
Report No	RRC 86-61
Document No	
Guidelines followed in study	None
Deviations from current test guideline	Not relevant
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
The determination of storage stability of Touchdown residues was investigated in soil when stored at -20 °C (April 1984 to April 1986). Separate analyses were done to delineate the stability of the Touchdown trimethylsulfonium cation (trimesium, TMS), glyphosate (carboxamethyl aminomethyl phosphonate (CMP)) and aminomethyl phosphonic acid (AMPA), a metabolite.

Current data show that AMPA, glyphosate and trimesium residues are stable for two years when stored at -20 °C.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate as glyphosate-trimesium (ICIA 0224)
Tested formulation: Touchdown 4-LC

2. Soil:

Test substances are field treated samples containing Touchdown residues. Test commodities are soils of three different types: St. Johns fine sand, sandy loam and silty clay loam.

Table 7.1.2.2.1-191: Characteristics of test soils

Parameter	Results		
Soil	St. Johns fine sand	Sandy loam	Silty clay loam
Test site location	Sanford, Florida	Orange cove, California	Libon, Iowa
pH [1]	5.4	6.9	n.i.
Organic matter (%)	0.5	2.2	6.0
Organic carbon (%) [2]	0.29	1.28	3.48

[1] Medium not stated
[2] Calculated from organic matter according to OC=OM x 0.58
n.i. = Not indicated

B. STUDY DESIGN

1. Experimental conditions

The three test soils were field-treated with Touchdown 4-LC at a rate of 6.72 kg a.s./ha (6.0 lb/acre) applied in water via mechanical sprayers at post-emergence tests sites in Sandford, Florida (St. Johns Fine sand), Orange cove, California (Sandy loam) and Libon, Iowa (Silty clay loam). Several 2.54 cm (1 inch) core samples were taken, composited and then frozen until time of analysis. Untreated controls and untreated controls fortified at the time of extraction were analysed to obtain recovery data. Control and fortified samples were prepared for each soil type. Samples were prepared for each soil type and analysed annually in triplicate for each test compound: AMPA, glyphosate (CMP) and trimesium (TMS).

2. Sampling

Field treated samples were stored in freezers at -20 °C inside sealed plastic bags. Subsamples were taken as needed from the composited soil stored in the plastic bag (0 days, 1 and 2 years after application).

3. Analytical procedures

Four different analytical test methods are described as indicated in the following table. Test methods for determination of residues for glyphosate and AMPA in soil are the same for RRC 83-44 and RRC 85-34. Test method RRC 83-44 describes additional clean-up steps at different pH values. Both RRC 83-45 and RRC 85-33 use the same methods to determine trimesium residues. However, methods RRC 83-44 and RRC 83-45 were not used for the storage stability test; therefore no details on these methods are given. Further, while the used methods describe analysis also in other commodities, this summary only describes the relevant methods for analysis in soil.

Table 7.1.2.2.1-192: Summary of test methods used for determination of residues in soil

Test method	Title	Limit of detection in soil
RRC 83-44	Determination of SC-0224 Anion Residues in Crops and Soils by Liquid Chromatography	CMP and AMPA: 0.06 to 0.1 mg/kg
RRC 85-34	Determination of SC-0224 Anion Residues in Crops Soil, and Water by Liquid Chromatography	CMP and AMPA: 0.05 mg/kg
RRC 83-45	Determination of SC-0224 Cation Residues in Crop sand Soils by Liquid Chromatography	TMS: 0.1 mg/kg
RRC 85-33	Determination of SC-0224 Cation Residues in Crops Water, and Soil by Gas Chromatography	TMS: 0.05 mg/kg

RRC 85-34
In the study RRC 85-33 soils were fortified with trimesium between 0.05 and 1.0 mg/kg. Recoveries for trimesium ranged from 74 to 115 %.

Soil samples are extracted with 10 % aqueous potassium hydroxide. An aliquot of the extract is treated with base to dealkylate the trimesium and form dimethyl sulfide (DMS). The amount of DMS formed is determined by gas chromatography using flame photometric detection in the sulfur mode.

RRC 85-34
In the study RRC 85-34 soils were fortified with glyphosate and AMPA between 0.2 and 0.5 mg/kg. Recoveries for AMPA ranged from 79 to 98 % and recoveries for glyphosate ranged from 61 to 111 %. Background concentrations were measured between 0.01 and 0.03 mg/kg which is <10 % of the fortification amount.

The glyphosate and AMPA are extracted from soil with 0.5 M NH_4OH. The extracts are cleaned up using a cation exchange column. Glyphosate and AMPA are collected separately, converted to fluorescing derivatives with 9-fluorenylmethyl chloroformate, and determined by HPLC using an anion exchange column and a fluorescence detector.

II. RESULTS AND DISCUSSION

A. DATA

A summary of residues at day 0 and after 1 and 2 years for the frozen field samples is presented below.

Table 7.1.2.2.1-193: Summary of residues of (mg/kg) glyphosate, trimesium and AMPA in soil after application of 6.72 kg/ha Touchdown

Analyte	Storage interval	Residues (mg/kg)				
		Silty clay loam	Sandy loam			St. Johns Fine Sand
		A-23178-2	A-22338-2	A-22338-4	A-22338-7	A-19647-3
CMP	0 Days	1.3	15.7	6.1	5.3	1.6
	1 Year	1.4	7.6	-	-	1.7
	2 Years	0.7	3.6	6.4	6.0	2.0
TMS	0 Days	0.8	8.2	-	-	1.0
	1 Year	0.8	8.7	-	-	0.8
	2 Years	0.6	8.0	-	-	1.3
AMPA	0 Days	2.3	4.5	2.2	1.9	0.7
	1 Year	2.8	6.3	-	-	0.6
	2 Years	2.3	6.9	2.3	2.0	0.8

B. CHARACTERISATION OF RESIDUES

Results for field treated samples reflect the higher variability caused by field application. Glyphosate results decreased between the first and the second year of storage in the silty clay soil, but remain stable in the St. Johns fine sand soil. There is a high variability between the residues of the three subsamples in the sandy loam soil with a decrease for subsample-02, while residues for the other two subsamples are similar over the two year period. Residues of AMPA remain the same for the 0 day, 1 year and 2 year sample analysis.

Some variability is also evident for other soils, slight increases or decreases compared to day 0 data can be seen. All in all, field treated samples thus confirm the storage stability of trimesium (TMS), glyphosate (CMP) and AMPA.

3. Assessment and conclusion

Assessment and conclusion by applicant:
It was shown that AMPA, glyphosate and trimesium residues in soil are stable for two years when stored at -20 °C. The study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/020
Report author	[redacted]
Report year	1984
Report title	Dissipation of Glyphosate in U.S. field soils following multiple applications of Roundup herbicide
Report No	MSL-3352
Document No	
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: Terrestrial field dissipation Test item: Roundup Test sites: - 16 trials in orchards and vineyard sites at 9 locations in USA (Alabama, Florida, Virginia, New York, Washington, Michigan, 2x Oregon - Five locations with bare soil in USA (California, Florida, Illinois, New York, Wisconsin) Soil types: fine sand, gravel loam, sandy loam, sandy clay loam, silty loam, clay loam Information about pH of organic matter content not given Application: multiple applications (method not given) - Orchards & vineyards: total application of 6.7 to 134.5 kg Roundup/ha over 1 to 6 years, 1st application spring or autumn - Bare soil: 4 x 4.2 kg glyphosate/ha within 1 year, 1st application in autumn

	Sampling (method not given): - Orchards & vineyards: one or multiple samples per plot until 7 to 613 days after last application - Bare soil: multiple samples per plot until 159 to 412 days after last application; one trial with incomplete sampling was excluded from further assessment. Sampling depth: 0 - 15.2 cm depth and 15.2 - 30.4 cm depth (the latter not for all sites) Sample storage: frozen at day of sampling and kept frozen until sample preparation Workup and analysis: analysis was done for glyphosate, AMPA and N-nitrosoglyphosate (NNG) - Air drying, mixing and removing of stones and foreign matter, soil moisture adjusted to 10 - 20 % - Twofold extraction with 0.5 M ammonium hydroxide - Primary cleanup with anion exchange chromatography - Glyphosate and AMPA are quantified by HPLC - NNG is quantified with a Griess postcolumn reactor and an absorbance detector - LOD = 0.05 mg/kg for glyphosate and AMPA LOD = 0.02 mg/kg for NNG Recovery in fortified samples: Glyphosate: mean = 78 % AMPA: mean = 76 % NNG: mean = 75 % All results were corrected for average analytical recoveries.
Short description of results:	<u>Orchards and vineyards</u> (residues after 7 to 476 days after last application) Glyphosate: non-detectable to 10 % of total applied amount for most trials; up to 48 % of total applied amount for one location, assumed to be caused by unrecorded treatments or sampling deficiencies AMPA: 1.4 - 54 % of total applied glyphosate equivalents, but <20 % for 12 out of 16 plots NNG: not detected for 7 of 9 locations, up to 0.09 mg/kg for two locations (confirmed by secondary analytical method). <u>Bare soil</u> (residues at last sampling date, 159 to 412 days after last application) Glyphosate: <0.05 - 0.87 mg/kg AMPA: ≤0.16 - 0.52 mg/kg NNG: not detected Half-life times: not calculated

Reasons why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: - No soil characterization (only soil type) - No climate and weather data provided - No information on soil history provided - Multiple applications - No sampling documentation (only sampling protocol provided) - No information on sampling method - Number of sampling times insufficient - No day 0 samples taken for some locations
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.2.2.1/021
Report author	[redacted]
Report year	1983
Report title	Dissipation of Glyphosate in U.S. field soils following direct application of Roundup herbicide
Report No	MSL-3210
Document No	
Guidelines followed in study	Not stated
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Data point:	CA 7.1.2.2.1/0022
Report author	[redacted]
Report year	1988
Report title	Addendum to MSL 3210 - Dissipation of Glyphosate in U.S. field soils following direct application of Roundup herbicide
Report No	MSL-8081
Document No	
Guidelines followed in study	US EPA Guideline 164-1
GLP	No, but conducted in general accordance with the principles of good laboratory practice
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: Terrestrial field dissipation Test item: Roundup Test sites: 15 locations in USA (North Dakota, Illinois, 2x Colorado, Idaho, Indiana, Kentucky, Ohio, Oklahoma, Tennessee, Texas, California, North Carolina, Minnesota, Florida) Soil types: Four clay loam, one loamy sand, two silt loam, one silt clay loam, three sandy loam, one loam and three sandy clay loam; Soil pH: 5.25 - 8.15 (medium not stated) OM: 0.5 - 7 % Application rate: 2.2, 4.5 & 8.9 kg a.s./ha, single application Application method: CO_2 pressured sprayer; directly to the soil; at 8 locations, soil was tilled after application; no information about crops given

	Application timing: April/May for 9 locations, Jun to Aug for 4 locations, Sep/Oct for 2 locations Sampling times: 4 events between 0 and 100 DAT (4 locations); to 5 – 7 events between day 0 and 377 DAT (11 locations) Sampling method: core samplers or shovels (not stated at which locations) Sampling depth: 0 - 15.2 cm, only, for 1 - 3 months; later additionally 15.2 - 30.4 cm Sample storage: frozen directly after sampling and kept frozen until sample preparation Workup and analysis: analysis was done for glyphosate, AMPA and N-nitrosoglyphosate (NNG). - Pre-processing of samples: mixing and adjustment of soil moisture to 10-20 % - Twofold extraction with 0.5 M ammonium hydroxide solution - Primary cleanup with anion exchange chromatography - Glyphosate and AMPA are quantified by HPLC - NNG is quantified with a Griess postcolumn reactor and an absorbance detector - LOD = 0.05 mg/kg for glyphosate and AMPA LOD = 0.02 mg/kg for NNG Recovery in fortified samples: Glyphosate: mean = 85.5 % AMPA: mean = 80.2 % NNG: mean = 75.0 %
Short description of results:	Residues: Glyphosate: day-0 recovery (mg/kg): - <LOD – 3.7 (2.2 kg/ha applied) - <LOD – 2.43 (4.5 kg/ha applied) - <LOD – 12.6 (8.9 kg/ha applied) Time when 90 % dissipation was reached - 10 – 291 days (2.2 kg/ha applied) - 18 – 301 days (4.5 kg/ha applied) - 12 – 291 days (8.9 kg/ha applied) AMPA: highest residues in the range of 0.2 – 0.8 mg/kg, observed after one year after application in only 8 of the total 42 plots NNG: not detected in any soil sample Glyphosate and AMPA were infrequently observed in the lower soil layer, indicating that their presence was not from leaching but as an artefact of sampling. Half-life times: calculated considering a two-compartment-model and by regression analysis (bi-phasic): - 2 – 174 days, (independent of application rate) - mean of 34 days (2.2 kg/ha applied) - mean of 37 days (4.5 kg/ha applied) - mean of 44 days (8.9 kg/ha applied) The dissipation of glyphosate was not dependent on the application rate.

	In addendum, half-life times were re-calculated considering a log-transformation approach: - all locations: mean of 57 days, range: 13 - 159 days - excluding 3 sites due to consideration of outliers: mean of 45 days, range: 13 – 124 days
Reasons why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: - No soil characterization (only soil type, pH and OM) - Some test plots were tilled after application - 2 locations not sampled at day 0 - Number of sampling times insufficient for some locations - No sampling documentation (only sampling protocol provided) - Some locations sampled with shovels (not stated which)
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.2.2.1/023
Report author	[redacted]
Report year	1983
Report title	Roundup herbicide dissipation in cool climate forest soil and leaf litter
Report No	MSL-2950
Document No	
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: terrestrial field dissipation in forest soil and leaf litter Test item: Roundup Test site: one location in British Columbia, Canada, cool climate, forest soil (Douglas fir) Soil type: “red mineralized forest soil” Application rate: 1.7 and 3.4 kg a.s./ha, single application Application method: hand-held CO_2 pressurized sprayer Three replicate plots; appl. to leaf litter and to bare soil (without plant matter) and untreated control per plot. Application timing: 23 September 190 Sampling times: six events, at 0, 15, 28, 58, 247, 344 DAT Sampling method: manually from a 400 cm² area (0 DAT), pipe core sampler (all other samplings) Sampling depth: Soil: 0 – 6 cm depth, 7 – 12 cm depth Litter: 0 – 6 cm depth Sample storage: frozen with dry ice within 4 hours after collection and during shipment and storage Workup and analysis: - Soil: air drying, sieving (#8 mesh standard sieve), mixing in a mechanical mixer - Raw leaf litter: blending at high speed with dry ice

	- Samples frozen again after processing - Extraction of samples with ammonium hydroxide - Primary cleanup with anion exchange chromatography - Further cleanup and separation with cation exchange chromatography - Analysis by HPLC, limit of sensitivity: 0.05 mg/kg - Duplicate analysis of each sample Recovery in fortified samples: Soil: - Glyphosate: mean: 84 % - AMPA: mean: 72 % Litter: - Glyphosate: mean: 83 % - AMPA: mean: 71 %
Short description of results:	Residues: Glyphosate: 0 DAT recovery soil (mg/kg), average over 3 plots: 0-6 cm: - 21.2 (1.7 kg/ha appl. rate) - 31.5 (3.4 kg/ha appl. rate) 7-12 cm: - 1.06 (1.7 kg/ha appl. rate) - 1.17 (3.4 kg/ha appl. rate) litter (mg/kg), average over 3 plots: - 7.60 (1.7 kg/ha appl. rate) - 28.2 (3.4 kg/ha appl. rate) 344 DAT soil (mg/kg), average over 3 plots: 0-6 cm: - 1.48 (1.7 kg/ha appl. rate) - 8.63 (3.4 kg/ha appl. rate) 7-12 cm: - 0.16 (1.7 kg/ha appl. rate) - 0.83 (3.4 kg/ha appl. rate) litter (mg/kg), average over 3 plots: - 0.11 (1.7 kg/ha appl. rate) - 0.53 (3.4 kg/ha appl. rate) AMPA: soil: 0-6 cm: max. 0.89 mg/kg at 15 DAT 7-12 cm: max. 0.14 mg/kg at 344 DAT litter: max. 3.96 mg/kg, observed at 15 DAT Half-life times: no reliable half-life according SFO could be calculated but 50 % of the initial concentration dissipates within 2 months or faster. Glyphosate and AMPA remain mostly in the leaf litter or 0 – 6 cm soil layer.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: - Field trials conducted on forest soil - No soil characterization (only soil type and OM) - No soil management history - No climate and weather documentation - Evidence for not evenly sprayed products provided - Number of sampling times insufficient - Day 0 samples not immediately after application - Sampling depth only 12 cm - Overall: documentation is very poor/unreadable
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.2.2.1/024
Report author	[redacted]
Report year	1982
Report title	Dissipation of Glyphosate in field soils following minimum till application of Roundup alone or in tank mix combinations with Lasso ME, Atrazine, Dyanap or Metribuzin.
Report No	MSL-2422
Document No	
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: Terrestrial field dissipation Test item: Roundup (solo or in tank mix combinations with Lasso ME, Atrazine, Dyanap or Metribuzin) Test sites: 6 locations in USA: Holdenville (Oklahoma), Shawnee (Oklahoma), Tumbleton (Alabama), Mankato (Minnesota), Adel (Iowa), Olathe (Kansas); 2 experiments each (solo and tank mix) Soil types: Two loam, two silty clay loam, one silty clay, one sandy loam Soil pH not given OM 0.8 % - 6.5 % Application rate: 5 kg a.s./ha, single application, pre-emergence - Solo and in tank mix with Lasso ME and atrazine to corn (n = 2) - Solo and in tank mix with Lasso ME and Dyanap to peanuts (n = 3) - Solo and in tank mix with Lasso ME and metribuzin to soybeans (n = 1) Application method: CO_2 sprayer Application timing: Beginning to mid of May Sampling times: three to five events, between day 0 and 336 DAT; day 0 sampling at 0 or 1 DAT Sampling method: not reported Sampling depth: 0 - 15.2 cm and 15.2 – 30.4 cm

	Tillage: minimum tillage Sample storage: no information provided Workup and analysis: analysis was done for glyphosate, AMPA and N-nitrosoglyphosate (NNG), which could theoretically be formed from glyphosate in a nitrosating medium. - Pre-processing of samples: mixing and adjustment of soil moisture to 10-20 % - Threefold extraction with 0.5 M ammonium hydroxide solution - Primary cleanup with anion exchange chromatography - Further purification and separation with cation exchange chromatography. - Glyphosate and AMPA are quantified by GLC-FPD - NNG is quantified with liquid chromatograph equipped with a Partisil SAX analytical column, a postcolumn Griess reactor and a 546 nm absorbance detector - LOD = 0.05 mg/kg for glyphosate and AMPA LOD = 0.02 mg/kg for NNG Recovery of externally fortified samples: Glyphosate: 65.1 - 72.9 % AMPA: 69.0 – 72.1 % NNG: 66.0 – 75.5 % All residues data were corrected for recoveries of fortified samples but not for soil moisture content.
Short description of results:	Residues: Glyphosate: day-0 recovery at 0 - 15.2 cm: - 0.38 – 5.88 mg/kg for plots with application of Roundup solo (n = 6) - 0.08 – 4.67 mg/kg for tank mix plots (n = 6) AMPA: At 4 locations increasing to last sampling date, at 2 locations peak concentration at 43 and 92 DAT. Maximum concentration: 1.23 mg/kg NNG: not detected in any soil sample Half-life times (estimated with computer program “HALFLI”): - Mean of 38.6 days (27.3 – 55.5 days), for Roundup solo plots (n = 6) - Mean of 35.3 days (31.8 & 38.8 days), for Roundup + Lasso ME + Dyanap (n = 2) - Mean of 37.5 days (48.8 & 26.3 days), for Roundup + Lasso ME + atrazin (n = 2) - 32.5 days, for Roundup + Lasso ME + metribuzin (n = 1) Glyphosate and AMPA were occasionally observed in the 15 to 30 cm layer.

Reasons why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: - No soil characterization (only soil type and OM) - No climate and weather data provided - No information on soil history provided - The test plots were cropped - Tank mixtures applied - Insufficient number of sampling times - Residue data were corrected for recoveries of fortified samples but not for soil moisture - Day 0 samples not taken immediately after application - Sampling method and sample storage conditions not provided
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.2.2.1/025
Report author	[redacted]
Report year	1979
Report title	Field soil dissipation studies of Roundup conducted in Sweden and France
Report No	MLL30033
Document No	
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: terrestrial field dissipation Test item: Roundup, containing 360 g glyphosate /L Test sites: 4 locations in France, planted with vines (total 8 trials) 1 location in France, planted with maize (total 2 trials) 6 locations in Sweden, forestry cultivated (total 10 trials) <u>France:</u> Soil types: clay loam, sandy clay loam, clay sand, loamy sand; pH 6.7 – 8 Application rate: vines: 4.3 and 8.6 kg a.s./ha, maize: 2.15 & 4.3 kg a.s./ha, single application Application method: Knapsack sprayer; application on existing vegetation Application timing: end of May to begin of July Sampling times: six events, between 0 and 70 DAT Sampling method: core samplers, 10 cm depth, 3 replicates (pooled) <u>Sweden:</u> Soil types: clay loam, sandy gravel, brown soil, podsol; pH 4.6 – 6.6 Application rate: 2 and 4 kg a.s./ha, single application Application method: Knapsack sprayer; application on existing vegetation Application timing: end of July to mid of August Sampling times: six events, between 1 and 828 DAT Sampling method: core samplers, 5 cm depth, 3 replicates (pooled)

	<u>All locations:</u> Sample storage: -20 °C Workup and analysis: extraction with water, analysis by GC-FPD, LOD = 0.05 mg/kg Recovery in fortified samples: Glyphosate: mean: 73 %, range: 52 – 96 % AMPA: mean: 68 %, range: 33 – 99 %
Short description of results:	Residues: Glyphosate: France: day-0 recovery: 35 – 93 % of applied amount, final sampling (61 – 70 DAT): 2 – 16 % of applied amount Sweden: day-0 recovery: 10.3 – 81 % of applied amount, final sampling (818 – 827 DAT): ≤2 % of applied amount AMPA: maximum 13 % of applied amount, observed around 20 to 50 DAT with subsequent decline (except two trials in France) Half-life times: calculated according to SFO by regression analysis; France: 11.0 – 30.1 days, Sweden: 13.6 – 36.1 days (independent of application rate).
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: - Trials were conducted in existing cultures (vines, maize, forestry) - Application was made onto existing vegetation - Sampling depth was only 5 to 10 cm - No weather data is reported - Soils are characterized insufficiently (only type, pH, Corg) - Analytical procedure is described insufficiently
Category study in AIR 5 dossier (L docs)	Category 3b

Relevant articles from literature search

1. Information on the study

Data point:	CA 7.1.2.2.1/026
Report author	Passeport, E., *et al.*
Report year	2014
Report title	Dynamics and mitigation of six pesticides in a “Wet” forest buffer zone
Document No	DOI 10.1007/s11356-013-1724-8 E-ISSN 1614-7499
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Pesticide pollution is one of the main current threats on water quality. This paper presents the potential and functioning principles of a “Wet” forest buffer zone for reducing concentrations and loads of glyphosate,

isoproturon, metazachlor, azoxystrobin, epoxiconazole, and cyproconazole. A tracer injection experiment was conducted in the field in a forest buffer zone at Bray (France). A fine time-scale sampling enabled to illustrate that interactions between pesticides and forest buffer substrates (soil and organic rich litter layer), had a retarding effect on molecule transfer. Low concentrations were observed for all pesticides at the forest buffer outlet thus demonstrating the efficiency of "Wet" forest buffer zone for pesticide dissipation. Pesticide masses injected in the forest buffer inlet directly determined concentration peaks observed at the outlet. Rapid and partially reversible adsorption was likely the major process affecting pesticide transfer for short retention times (a few hours to a few days). Remobilization of metazachlor, isoproturon, desmethylisoproturon, and AMPA was observed when non-contaminated water flows passed through the forest buffer. Our data suggest that pesticide sorption properties alone could not explain the complex reaction mechanisms that affected pesticide transfer in the forest buffer. Nevertheless, the thick layer of organic matter litter on the top of the forest soil was a key parameter, which enhanced partially reversible sorption of pesticide, thus retarded their transfer, decreased concentration peaks, and likely increased degradation of the pesticides. Consequently, to limit pesticide pollution transported by surface water, the use of already existing forest areas as buffer zones should be equally considered as the most commonly implemented grass buffer strips.

Materials and Methods

The forest buffer zone is located at the outlet of a tile drained agricultural watershed at Bray (France).

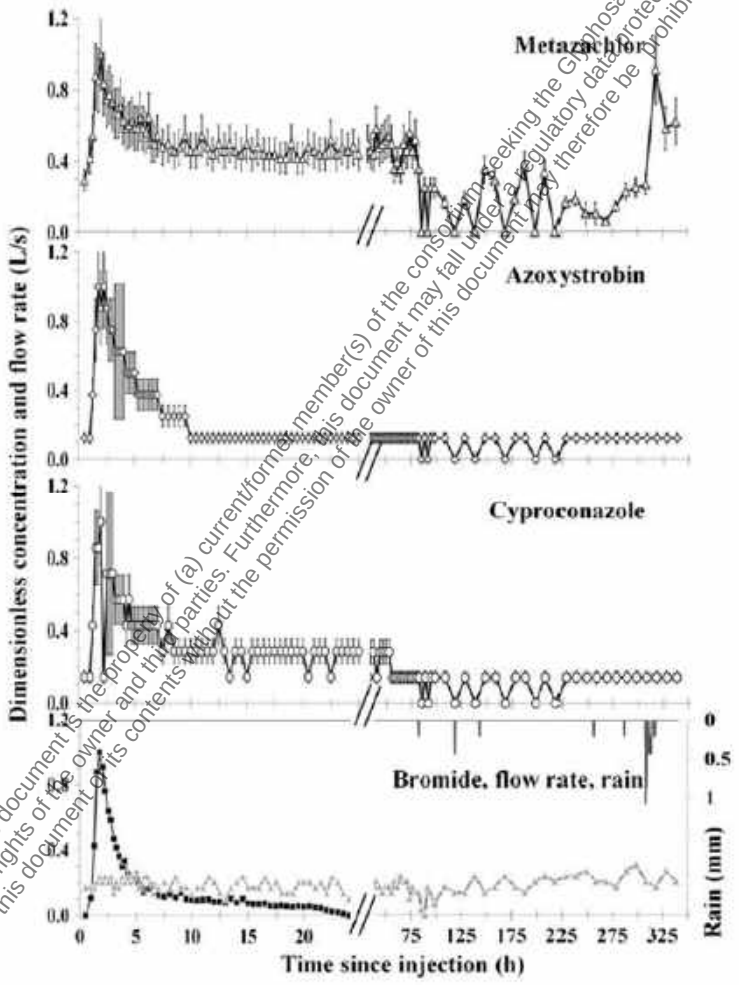
Chemicals

An injection solution was prepared with six pesticides and potassium bromide as a conservative tracer. Pesticides were provided by farmers and diluted in deionized water before injection. Commercial solutions that were used are indicated into parentheses: three herbicides, glyphosate (Glyphogan), isoproturon (Isoproturon), and metazachlor (Novall), and three fungicides, azoxystrobin (Priori Xtra), cyproconazole (Amistar Xtra), and epoxiconazole (Opus) were selected for their contrasting properties and wide use in agriculture.

Tracer Experiment

The forest buffer tracer experiment took place for a period of 14 days, from 19 February 2009, 10:50 to 5 March 2009, 13:20 in a reduced portion of the forest buffer, using watershed outlet flows as incoming flows into the forest buffer. The experimental plot was delimited with soil border levees leading to a 54 m^2 surface area (36 m×1.5 m). Only one significant rainfall event occurred on 308.5 h after the start of the experiment, with a cumulative rainfall depth of 9.94 mm, measured with the on-site tipping bucket rain gauge (R01 3030A Danae, Précis Mécanique, Bezons, France). Water temperature was 5.9±3.7 °C during the course of the experiment, and was close to or greater than monthly averages. The inlet flow rate was 0.32±0.08 L/s. At the outlet, a flow restriction helped manually measuring flow rates by frequently timing the filling of a container with a known volume. Water from the watershed was allowed to flow through the forest buffer experimental plot on 18 February 2009 at 15:50, in order to saturate the soil and ensure a permanent flow rate for the next day injection. Two peristaltic pumps (Eijkelkamp 12 V SDEC Reignac-sur-Indre, France) were used to ensure a 0.30 L/s injection flow rate during 78 s. Grab water samples or samples collected by means of a time-dependent automated sampler (ISCO 3700 Neotek, Trappes, France) were taken at the outlet of the experimental plot. The sampling frequency was modified along the course of the experiment: every 15 min for the first 7 h, every 30 min until 28.5 h after the start of the experiment, then every 3 h until 94 h since injection, and every 10 h from days 4 to 10 following the start of the experiment. Finally, five grab water samples were taken at forest buffer inlet to control pesticides' background concentrations coming from the artificially drained watershed.

Figure 7.1.2.2.1-2: **Flowrate at the forest outlet (gray triangle, bottom panel, in liter per second), and dimensionless (C/C_{max}) concentration pattern during the first 24 h (left panels) and the next 350 h (right panels) after injection, for molecules that exhibited the clearest transfer pattern: metazachlor (white triangles), azoxystrobin (white diamonds), cyproconazole (white circles), and bromide (black squares). The double slash bars (//) indicate a change in time step. C concentration at time t; C_{max} peak concentration measured 2 h (metazachlor, azoxystrobin, and cyproconazole) and 1.8 h (bromide) after injection. No rainfall event occurred during the first 24 h; rain beyond 24 h (bottom-most right-hand side panel) is plotted on the right hand vertical axis, in reverse order. Error bars correspond to dimensionless expanded uncertainties, i.e., expanded uncertainties on concentrations (U, coverage factor = 2), divided by C_{max}**



Analytical method

Water sample analysis

Subsamples were taken from water samples, filtered and analyzed for bromide with ion chromatography and an IonPac AS9-HC column. The limit of quantification (LQ) was 1 mg/L. Metazachlor, cyproconazole, epoxiconazole, azoxystrobin, isoproturon and two of its metabolites, desmethylisoproturon and 1-(4-isopropylphenyl)urea, were extracted by solid-phase extraction on pre-filtered samples, and then analyzed by high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (LCMS-MS). Limits of quantification were 0.02 µg/L for these seven pesticides and metabolites.

Glyphosate and its main metabolite, AMPA, were first derivatized with 9-fluorenylmethyl chloroformate (FMOC) before LC-MS-MS analysis (LQ = 0.1 µg/L for both glyphosate and AMPA).

Litter and soil sampling and analysis
Litter, and soil grab samples were taken in the forest experimental plot at the end of the tracer experiment. Another litter and soil samples were collected outside the experimental plot to compare with those collected inside the experimental plot. All samples were frozen before pesticide analysis. Glyphosate and AMPA were extracted by ultrasonic waves in water, then derivatized with FMOC and analyzed by LCMS-MS, whereas extraction for the other molecules from soil samples was carried out with ultrasonic waves in acetone. Extracts were analyzed by LC-MS-MS. Litter samples were treated with an internal procedure developed by the laboratory (Institut Pasteur de Lille). Limits of quantification were 0.01 mg/kg dry weight for each compound.

Data analysis
The hydraulic retention time was calculated based on the bromide conservative tracer using the moment theory on residence time distribution (see Passeport *et al.* (2010), Kadlec and Wallace (2008)).

Statistical analyses
Pearson correlation coefficients were determined with the R software to detect possible correlations among pesticide concentrations, injected masses, and pesticide physico-chemical properties.

Table 7.1.2.2.1-194: Forest buffer inlet concentrations

Molecule	Pesticide inlet concentrations (µg/L)				
	Time from injection (h)				
	LQ	0.58	6.75	24.17	339
Glyphosate	0.1	n.d.[b]	<LQ	<LQ	n.d.
AMPA	0.1	0.30	<LQ	<LQ	0.30
Isoproturon	0.02	1.60	1.20	1.30	1.40
Desmethylisoproturon	0.02	0.12	0.11	0.10	0.11
1-(4-isopropylphenyl)urea	0.02	<LQ	<LQ	<LQ	n.d.
Metazachlor	0.02	0.29	0.30	0.25	0.19
Epoxiconazole	0.02	<LQ	0.02	n.d.	n.d.
Azoxystrobin	0.02	<LQ	<LQ	n.d.	n.d.
Cyproconazole	0.02	<LQ	<LQ	n.d.	n.d.

[a] Limit of quantification
[b] n.d. is "not detected"

Results
Hydrology
Water ran off through the forest buffer experimental plot as a shallow sheet flow with an average outlet flow rate of 0.18± 0.11 L/s (average ± expanded uncertainty for 95 % confidence interval). Bromide started to be detected 1 h after injection and reached a concentration peak 1.8 h after injection (Figure 7.1.2.2.1-2). Bromide recovery rate and hydraulic residence time were 74 % and 6.3 h, respectively.

Inlet water quality
During the experiment, watershed tile-drain flows continuously entered the experimental plot at a controlled flow rate of 0.3 L/s. We determined that some of the studied pesticides also entered the experimental plot via watershed flows during the course of the experiment. Non-negligible concentrations of isoproturon, desmethylisoproturon, glyphosate, AMPA and metazachlor were measured (Table 7.1.2.2.1-194). Epoxiconazole was detected once (6.8 h after injection) but with a concentration at the limit of quantification. The most recent applications of glyphosate and metazachlor on the Bray watershed were approximately 16 months before the start of the experiment.

Table 7.1.2.2.1-195: Tracer experiment dynamics characteristics and mass recovery rates

Molecule	Peak conc ± U(C) (µg/L)	Peak conc time (h after injection)	Percent recovery (%)	Time for conc reaching < LQ (h after injection)
Bromide	1750	1.75	74	24.0
Glyphosate	0.05±0.03	NA[a]	NA	NA
AMPA	0.30±0.08	1.75	NA	NA
Isoproturon	1.70±0.41	2.50	NA	NA
Desmethylisoproturon	0.14±0.03	NA	NA	NA
1-(4-isopropylphenyl)urea	0.02±0.01	NA	NA	NA
Metazachlor	0.48±0.10	2.00	NA	4.5
Epoxiconazole	0.04±0.01	2.75	NA	NA
Azoxystrobin	0.08±0.02	2.00	22	10.0
Cyproconazole	0.07±0.02	2.00	45	8.5

[a]NA means Not Available, when peak concentration ("Peak Conc") time could not be clearly identified and mass balances could not be reasonably calculated due to a large portion of the concentration dataset below limits of quantifications

Pesticide dynamics description

Table 7.1.2.2.1-195 presents the main characteristics for pesticide concentration peaks, dynamics and mass balances. Apart from isoproturon, concentrations were lower than 0.50 µg/L for AMPA and metazachlor, and did not exceed 0.15 µg/L for the other pesticides (glyphosate, azoxystrobin, epoxiconazole, cyproconazole, desmethylisoproturon, and 1-(4-isopropylphenyl)urea). Only injections of metazachlor, azoxystrobin and cyproconazole resulted in a clear transfer pattern at the forest plot outlet (Figure 7.1.2.2.1-2). Two hours after injection, these pesticides exhibited concentration peaks of 0.48 ± 0.10, 0.08 ± 0.02, and 0.07 ± 0.02 µg/L for metazachlor, azoxystrobin, and cyproconazole, respectively. These concentration peaks were observed closely after that of the conservative tracer, which was recorded 1.8 h after injection (Table 7.1.2.2.1-195). For glyphosate, AMPA, epoxiconazole, and 1-(4-isopropylphenyl)urea, concentrations at the forest plot outlet were so low that only a qualitative assessment of the data can reasonably be performed. In addition, high background concentration levels of isoproturon and desmethylisoproturon hindered an accurate quantitative analysis of the data for these two molecules. In all water samples, glyphosate concentrations were below the LQ and those for AMPA never exceeded 0.30 ± 0.08 µg/L. No temporal variation was observed for these molecules, besides two small AMPA concentration rises, one after injection (between 1.8 and 3.8 h) and a second one after the rainfall event (between 318.5 and 328.5 h). Concentration peaks for the injected molecules were significantly correlated (p value=1.75×10^{-5}) with background concentrations, highlighting the strong influence that this artifact exerted on the results. The second strongest correlation (despite not significant at a $\alpha = 5$ % significance level) was between pesticide concentration peaks and injected masses. With this small dataset, no statistically significant correlations were found between the ratios and the pesticide sorption properties.

Discussion

Hydrology

The ratio between outlet and inlet flow rates (0.61), and the bromide recovery rate (74 %) are suggestive of some water losses outside the experimental plot, via infiltration, possibly due to poor soil levee compaction, earthworm burrows, and tree roots.

Forest buffer efficiency for pesticide removal

A key conclusion of our study relies on the fact that, for most pesticides, very low concentrations were measured at the forest outlet, thus demonstrating the efficiency of such buffer zones for pesticide removal.

Sorption as part of a complex set of removal processes

The high sorption coefficients of glyphosate, AMPA and epoxiconazole may partly explain their low concentrations measured at the forest outlet. Contrary to glyphosate and AMPA, epoxiconazole was detected on dead leaves at the forest plot inlet and middle zones 14 days after injection even after large rainfall events. This supports a possible strong adsorption of epoxiconazole onto the forest litter. Because

epoxiconazole was not detected in the soil below the litter layer, it is likely that the litter layer acted as a key sorption material that prevents strongly sorbing pesticides from leaching to deep soil horizons.

Degradation and remobilization of pesticides

Due to the moderately long half-lives of their parent molecules, glyphosate and isoproturon, the detection of AMPA and desmethylisoproturon at the beginning of the experiment can hardly be attributed to the injected parent molecules. It should be noted that AMPA, isoproturon, and desmethylisoproturon were detected at the forest plot inlet indicating that these molecules were also transferred to the experimental plot from the tile-drain watershed. Glyphosate and isoproturon were applied previously on the agricultural watershed and may have been partially degraded in the catchment and forest buffer soils thus generating these metabolites.

"Dry" vs. "Wet" buffer zone

In this study, the "Wet" forest buffer soil had a high clay content thus limiting downward infiltration. Even if water losses via infiltration might occur, it could not explain alone the observed pesticide removal. It is a fundamental difference with "Dry" buffer zones like grass areas, where infiltration plays a crucial role. The second major difference between grass and forest buffer zones lies in the presence of thick litter layer rich in organic matter in the latter. The litter provides many sorption sites for pesticides and is biologically active, thereby biodegrading retained pesticides. Consequently, when buffer zone soil is saturated, pesticide sorption and degradation should more easily occur in forested areas than in grass areas, provided that the contaminated water runs off through the litter layer as a shallow and slow water flow.

Conclusions

The objective of this experiment was to demonstrate at the field scale the potential of forest buffer zones to reduce the concentrations and loads of pesticides presenting a wide range of physico-chemical properties. Very low concentrations were measured at the forest outlet thus suggesting a potential of the forest buffer to effectively reduce the pollution with pesticides. Understanding processes, which govern the removal of pesticides through the forest buffer was beyond the scope of this study. However, the fine sampling frequency used in this study helped to provide some explanations about the observed dynamics of pesticide transfer through the forest buffer zone. At short time-scales (lower than a month), retention processes are suspected to dominate. Our results highlighted the dual role of organic matter. On the one hand, organic substrates enabled rapid adsorption of pesticides transported in highly contaminated flows. On the other hand, when fresher (i.e., less contaminated) flows crossed the forest buffer, previously adsorbed pesticides were shown to desorb thus being released back to the water column. Organic matter also plays an indirect role in this process as it supports growth of microbial populations. Any forested area adequately located in the landscape could be used as an efficient buffer zone for reducing pesticide pollution. Indeed, even old wood that were not necessarily well maintained could be good candidates for buffering pesticide contaminated flows provided a thick litter layer has had time to accumulate over time. At a short time scale (here approx. 350 h), highly organic material would therefore mainly act as a retarding factor that temporarily affect pesticide dynamics. For extended periods of water retention, degradation reactions leading to metabolites are likely to occur, however, more research is needed to confirm the extent of pesticide degradation that could be achieved. The results of this study are suggestive of a high potential of "Wet" forest buffer zone for the reduction of downstream pesticide concentrations and loads. Further research should investigate the efficiency of forest buffers for pesticide removal (1) under various climatic conditions, and for a wide range of forest buffer (2) sizes and shapes, and (3) locations in the watershed (headstream vs. downstream). Such results are needed to better understand pesticide fate and the role of the litter layer, and to establish guidelines to design forest buffer zones and incorporate them in land management strategies.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the mitigation of glyphosate among other pesticides by a wet forest buffer zone in France. Not all required parameters are reported to check validity of the study (e.g. information on test substance, analytical method, characterization of soil).
The article is classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.2.2.1/027
Report author	Todorovic, G. *et al.*
Report year	2014
Report title	Influence of soil tillage and erosion on the dispersion of glyphosate and aminomethylphosphonic acid in agricultural soils
Document No	DOI 10.2478/intag-2013-0031
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Erosion processes can strongly influence the dissipation of glyphosate and aminomethylphosphonic acid applied with Roundup Max in agricultural soils; in addition, the soil structure state shortly before erosive precipitations fall can be a key parameter for the distribution of glyphosate and its metabolite. Field rain simulation experiments showed that severe erosion processes immediately after application of Roundup Max can lead to serious unexpected glyphosate loss even in soils with a high presumed adsorption like the Cambisols, if their structure is unfavourable. In one of the no-tillage-plot of the Cambisol, up to 47 % of the applied glyphosate amount was dissipated with surface run-off. Moreover, at the Chernozem site with high erosion risk and lower adsorption potential, glyphosate could be found in collected percolation water transported far outside the 2x2 m experimental plots. Traces of glyphosate were found also outside the treated agricultural fields.

Materials and Methods
The experiments were carried out where following soil tillage systems were compared in 3 field replications:

– conventional tillage (CT) with plough with and without cover crop during winter period;
– no-tillage (NT) with cover crop during winter period.

The investigated soils were a Chernozem from loess at Pixendorf and a sandy stagnic Cambisol from tertiary carbonate free sediments at Kirchberg, Austria. In order to investigate the influence of erosion and tillage on glyphosate and AMPA, two rain simulation experiments were conducted in 3 field replications (1, 2, 3) within the CT and NT plots. For this, Roundup Max was applied onto rain simulation soil plots

according to the common agricultural practice (180 mg glyphosate/m^2). In both sites, the vegetation cover degree was typically higher in the NT-plots (80-100 % of weed cover) than in the CT-plots (only few yield residues of maize) and the application was carried out in sunny and not windy weather shortly before starting the rain simulation experiment (worst case scenario). The average slope in both sites was 12-15 % at the Cambisol and 10 % at the Chernozem. Both sites are known as rather erodible. The soil surface of the Chernozem immediately before the rain simulation was crumby; in turn, the cambisol had a crusted, dry, and cracky surface. The rain simulator was designed as a portable equipment, the spray pattern was generated by full jet nozzles, the rain fall intensity was controlled with intermittent spraying.

During 60 min of rain simulation with 30 mm, run-off fractions were collected at different time intervals at the Chernozem and averagely at the Cambisol and cooled in boxes. In the laboratory, the run-off samples were immediately centrifuged to separate the liquid from the solid phase. Immediately after the rain simulation, soil samples were collected within the simulation soil plots of 2x2 m at different depths (0-2, 2-5, 5-10, 10-15, and 15-20 cm at the Chernozem and at 0-2 and 2-5 cm at the Cambisol). Glyphosate and AMPA were analyzed according to Rampazzo *et al.*, 2013. All physical and chemical analyses on soil samples were carried out according to the standard methods.

Table 7.1.2.2.1-196: Fe-oxide distribution in the investigated soils

Site	Soil type (WRB)	Depth (cm)	Fe_o (mg kg^{-1})	Fe_d (mg kg^{-1})	Fe_o/Fe_d
Pixendorf	Chernozem	0-5	983	7 970	0.12
		5-20	1 040	8 378	0.12
Kirchberg	Cambisol	0-5	3 422	14 843	0.23
		5-20	3 726	15 032	0.25

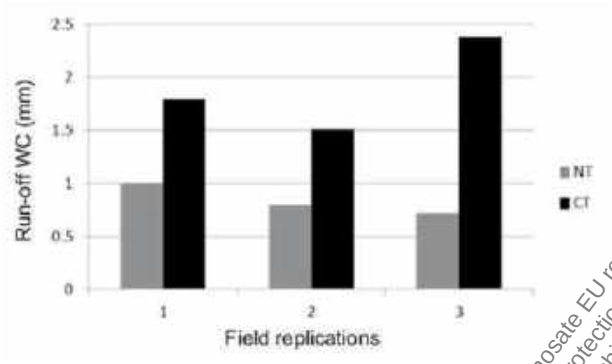
Fe_o – amorphous (weakly crystallized) Fe-oxides, oxalate-soluble; Fe_d – well crystallized Fe-oxides, dithionite-soluble.

Results and Discussion

The Chernozem shows the development from loess with typical silty texture (topsoil 0-20 cm, 12 % clay, 65 % silt, 23 % sand, pH 7.3, 15 % $CaCO_3$ and 3 % OM), whereas the Cambisol is a loamy sandy soil (topsoil 0-20 cm, 14 % clay, 33 % silt, 53 % sand, pH 5.7, no $CaCO_3$ and 3 % OM). The Chernozem exhibited a low content and the Cambisol a high content of Fe oxides and therefore the expected sorption capacity for glyphosate and AMPA was theoretically higher at the Cambisol (Table 7.1.2.2.1-196).

Figure 7.1.2.2.1-3 shows the amount of total (liquid and solid) run-off after the rain simulation experiments on the Chernozem. Before glyphosate and AMPA were analyzed, a separation of the solid and liquid run-off phase in the laboratory was carried out. The CT-plots produced the highest run-off amounts because of their lower protecting weed cover, causing a splash of the surface by the erosive precipitation with consequent loss of infiltrability. On the other hand, the amount of runoff at the Chernozem was 10 times lower than the Cambisol because of its crumby structure with a better infiltration rate during the rainfall simulation, whereas the soil surface of the Cambisol was compacted and crusty. The different amounts of run-off between the 3 field replications of the Chernozem (Figure 7.1.2.2.1-3) were due to the inhomogeneity of the field conditions. Consequently, the total amount of glyphosate washed out of the plots by liquid run-off at the Chernozem was much higher in the CT-plots than in the NT-plots (Figure 7.1.2.2.1-4).

Figure 7.1.2.2.1-3: Chernozem: total run-off of the conventional tillage (CT) and no-tillage (NT) plots in the 3 field replications, WC – water column



A fractionation of the time-dependent glyphosate contents in run-off-fractions of the Chernozem at time intervals of 15 min is shown in Figure 7.1.2.2.1-5a. As it was expected, the first fraction showed the highest contents in both variables CT and NT and then decreasing with time. The CT-plots showed again higher glyphosate contents than the NT-plots, which instead showed higher glyphosate concentration (less dilution) at the same time (Figure 7.1.2.2.1-5b).

According to Gjettermann *et al.,* 2011, desorption kinetics are important for evaluating the significance of dissolved and particle-facilitated transport of glyphosate. Consequently, the separation from water and solid phases should be done within a short time of minutes. We managed to do this within 30 min from field sampling. The contents of glyphosate and AMPA in the solid phase of run-off in the Chernozem are shown in Figure 7.1.2.2.1-5c,d. The glyphosate contents retained by the run-off sediment is an analogue to that in the total and fractionated runoff (Figure 7.1.2.2.1-4, Figure 7.1.2.2.1-5a), where the first collected fraction of run-off sediment contains the highest amounts of glyphosate which then generally decreases in the following fractions and the CT-plots shows higher amounts than the NT-plots. Analogous is the distribution of AMPA in the sediment (Figure 7.1.2.2.1-5d). Since the loss of glyphosate by run-off was higher in the CT-plots (Figure 7.1.2.2.1-5), the amount of glyphosate and AMPA adsorbed by the Chernozem immediately after the rain simulation experiments was consequently higher in the NT-plots (Figure 7.1.2.2.1-6). Moreover, there is a clear depth function of the adsorption of glyphosate and AMPA through the soil immediately after Roundup Max application and rainfall simulation at the Chernozem. The glyphosate and AMPA contents clearly decreased with soil depth.

Figure 7.1.2.2.1-4: Chernozem: total amounts of glyphosate in liquid run-off at the 3 field replication plots. Legend as in Figure 7.1.2.2.1-3

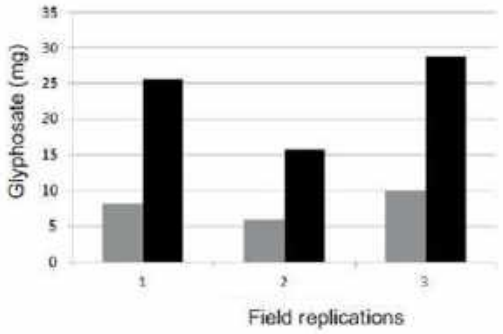


Figure 7.1.2.2.1-5: Chernozem: a – glyphosate amount, b – glyphosate concentrations in liquid, and c – glyphosate contents, d –AMPA contents in the solid phase of run-off-fractions at 15-min intervals (average of 3 field replications). Legend as in Figure 7.1.2.2.1-3

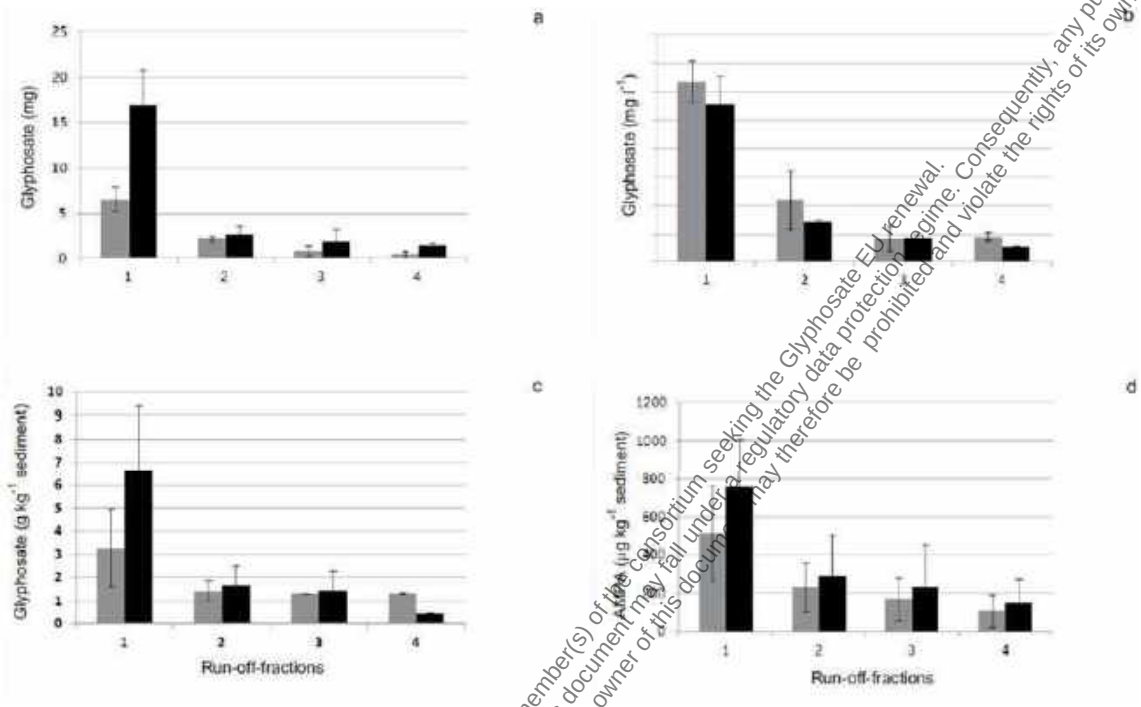
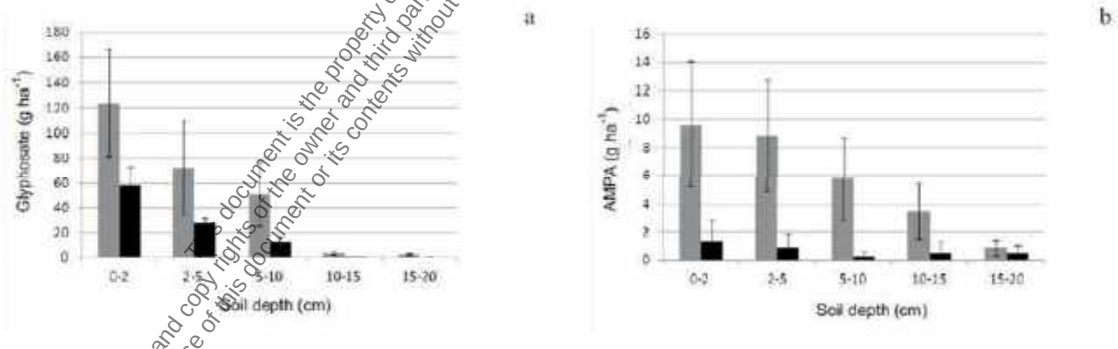


Figure 7.1.2.2.1-6: Chernozem: a – glyphosate contents, and b – AMPA contents in the soil within the rain simulation plots (average value from the 3 field replications). Legend as in Figure 7.1.2.2.1-3

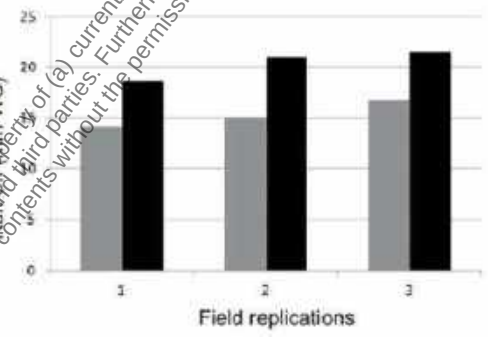


The Chernozem had a favourable crumby structure in the NT-plots, with no cracks, no preferential flow, and optimal conditions for water retention in the upper soil layers at the moment of the rainfall simulation experiment, so that more than 50 % of the adsorbed glyphosate was retained in the first 5 cm of the soil. The fact that AMPA could already be detected 1 h after the Roundup Max application underlines the quick glyphosate degradation in soil, as reported by Mamy *et al.* (2005) as well. The total (liquid and solid) amount of surface run-off in the Cambisol is shown in Figure 7.1.2.2.1-7. The Cambisol had a dry, crusty, and very deeply cracky soil surface of the CT-plots before starting the rainfall simulation and therefore the first amount of the precipitation quickly infiltrated in the cracks, but very soon a splash process and loss of infiltration took place due to the fine sandy texture and low surface protection by weeds. This led to a higher

surface run-off of the CT-plots than the NT-plots. Consequently, Figure 7.1.2.2.1-8 and Figure 7.1.2.2.1-9a show that the contents and concentrations of glyphosate in the liquid run-off of the NT-plots of the Cambisol were much higher than in the CT-plots. In the dry and cracky soil surface of the CT-plots, it took some time before run-off started and glyphosate could easily enter deeper into the soil; on the other hand, the NT-plots had a nearly 100 % weed cover, as reported also by Locke and Bryson (1997); consequently, this might buffer potential effects of glyphosate in the soil (Locke *et al.,* 2008).

In this study, most of the applied glyphosate adhered to the photosynthetically active plant organs (stem and leaves) immediately after application; consequently, glyphosate was literally washed out of the 2x2 m simulation plots with runoff and had less time to infiltrate the soil surface (Figure 7.1.2.2.1-10). Based on the high content of pedogenical Fe-oxides (15 000 mg Fe_d/kg, Table 7.1.2.2.1-196), high soil adsorption of glyphosate was expected for the Cambisol. The surprisingly high loss of glyphosate by surface run-off (in one of the 3 field replications about 47 % of the applied glyphosate) measured in this study confirmed the crucial effect of soil structure and preferential flow on the dissipation of glyphosate after heavy erosive precipitations, which were also be observed by other scientists. The contents of glyphosate and AMPA in the solid phase of run-off at the Cambisol are shown in Figure 7.1.2.2.1-9b, c, respectively. The concentrations of glyphosate and AMPA in the solid phase of run-off at the Cambisol are similarly distributed to the corresponding aqueous fractions of run-off; they are mostly higher in the NT-plots than in the CT-plots. Figure 7.1.2.2.1-10 shows the content of glyphosate and AMPA adsorbed by the soil immediately after the rain simulation experiments at the Cambisol. Immediately after the rain simulation experiment, a very clear distribution in the soil appears: glyphosate and AMPA are first adsorbed in the upper 0-2 cm of the soil and only a small amount reaches the next soil depth of 2-5 cm. In general, the NT-plots show a clearly lower content of glyphosate and AMPA as compared to the CT-plots. This is explained by the respectively higher glyphosate contents in run-off of NT-plots (Figure 7.1.2.2.1-8). The soil losses of the Chernozem and Cambisol through erosion processes are shown in Figure 7.1.2.2.1-11.

Figure 7.1.2.2.1-7: Cambisol: total run-off of the CT- and NT-plots in the 3 field replications, mm WC – mm water column. Legend as in Figure 7.1.2.2.1-3



At both sites, the soil loss from the CT-plots, measured as sediment in the surface run-off, was higher than from the NT-plots because of the much lower vegetation cover before the simulation experiment, splash, and reduction of infiltration. The loss of the Cambisol soil was 10 times higher than that of the Chernozem. The reason for this is that the two experimented soils had a completely different soil structure and surface conditions, before starting the rain simulation. The Chernozem had a very friable, crumby, permeable structure after the wheat yield. The Cambisol stood right after the corn yield, the soil surface was crusty and less permeable, except for shrinking cracks which swelled during the experiment.

Figure 7.1.2.2.1-8: Cambisol: total amounts of glyphosate in liquid run-off at the 3 field replication plots. Legend as in Figure 7.1.2.2.1-3

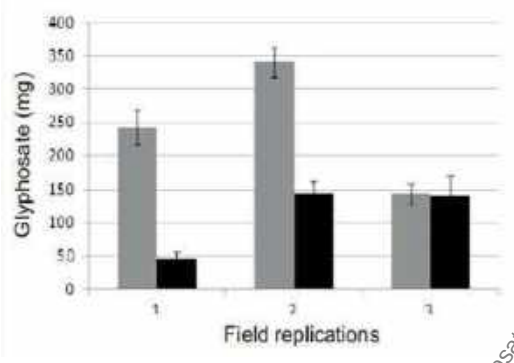
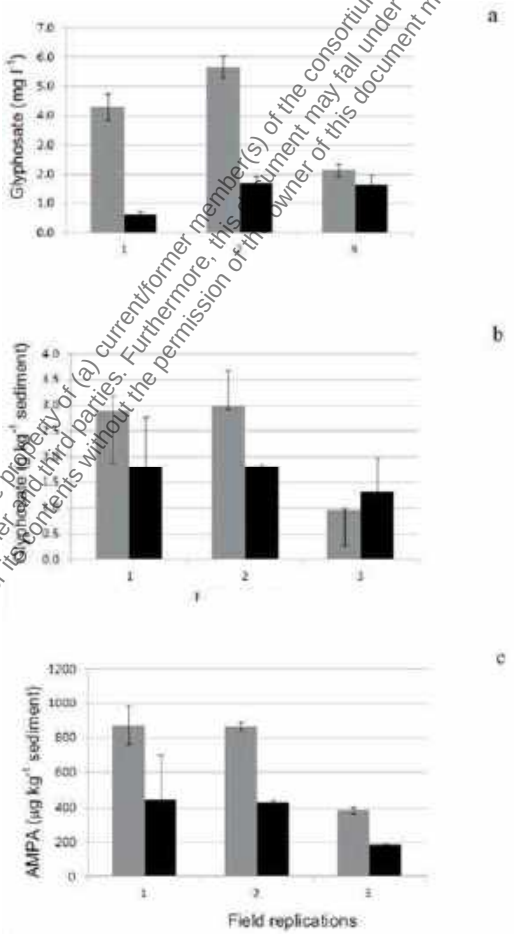


Figure 7.1.2.2.1-9: Cambisol: a – glyphosate concentrations in liquid, b – glyphosate, and c – AMPA contents in the solid phase of run-off at the 3 field replications (average of the 60 min rain simulation). Legend as in Figure 7.1.2.2.1-3



The Chernozem at Pixendorf and surroundings is generally known as a location with high erosion risk because of the high silt amount (>60 mass %) and especially with corn crop, where deep gully erosion forms. The erosion rills discharge downslope to an artificial run-off retention basin at the footslope of the

experimental field. This basin can run over and flow downwards on different paths and is collected through further toeslope retention basins. Water samples from both retention basins were analyzed and

Figure 7.1.2.2.1-10: Cambisol: a – glyphosate and b – AMPA contents in the soil within the rain simulation plots (average value from the 3 field replications). Legend as in Figure 7.1.2.2.1-3

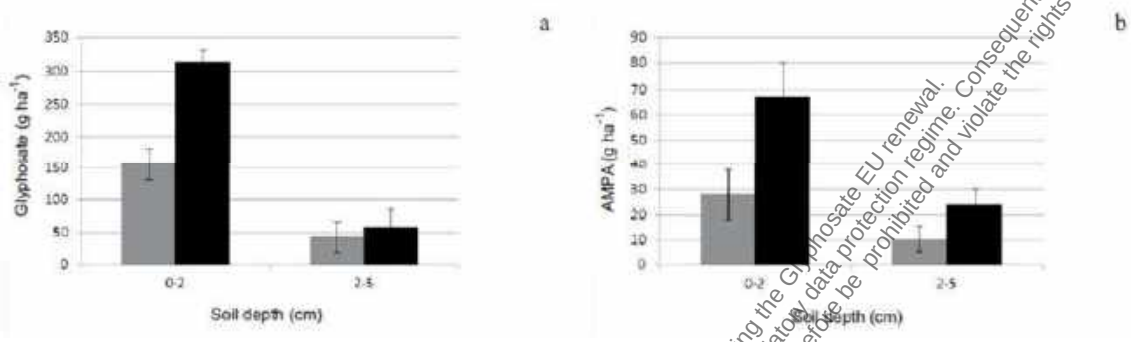


Figure 7.1.2.2.1-11: Total soil loss of the investigated soils after the rain simulation experiments (averages of 3 field replications). Legend as in Figure 7.1.2.2.1-3

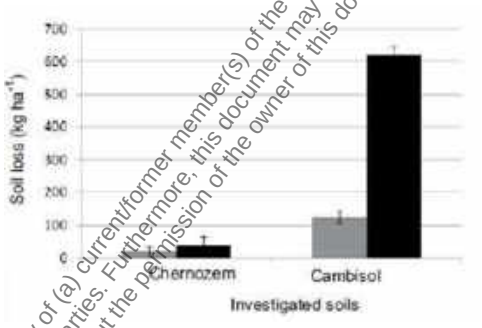


Figure 7.1.2.2.1-12: Glyphosate concentrations in natural run-off retention basins outside the experimental fields

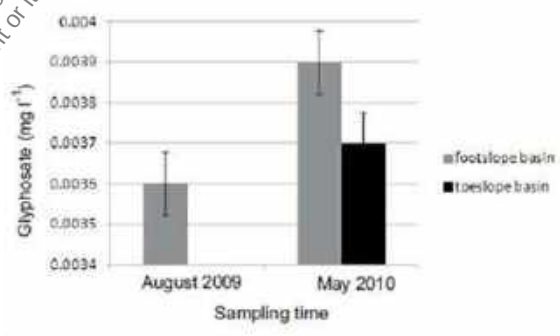
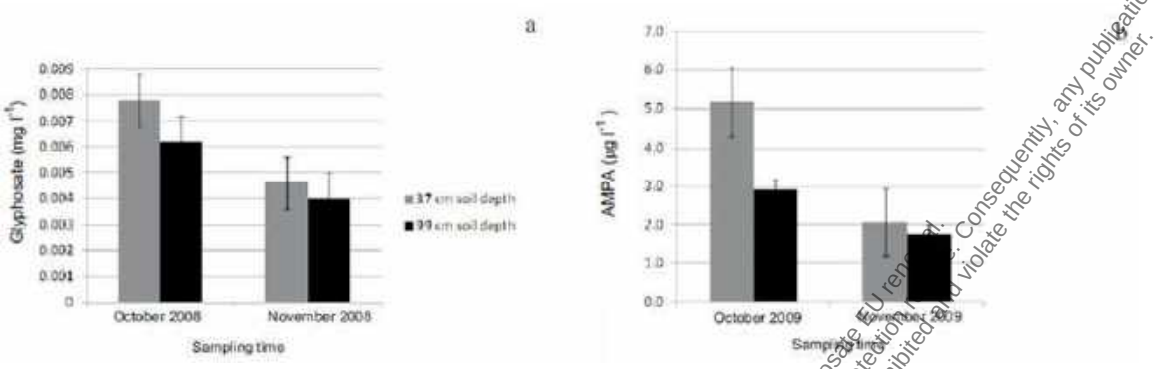


Figure 7.1.2.2.1-13: Concentrations of: a – glyphosate, and b – AMPA in percolation water at 2 different times and soil depths



Conclusions

1. The rain simulation experiments clearly showed that even in a potentially high glyphosate adsorbing soil like the Cambisol, erosion and surface run-off can lead to severe glyphosate loss if the soil structure state *eg* compaction degree, crusting, infiltrability, pore size distribution, in the case of erosive precipitations shortly after Roundup Max application, is unfavourable. In this study, in one of the NT-plot repetitions, up to 47 % of the applied glyphosate amount were dispersed with run-off.

2. Traces of glyphosate in collected percolation soil water at Pixendorf, probably from previous conventional field application of Roundup Max, confirmed the general low glyphosate adsorption capacity of Chernozems from Loess and the risk of transport towards groundwater.

3. Analysis of water from run-off retention basins in the landscape in the surroundings of the investigated Chernozem confirmed that through high erosion processes, especially in maize crop, glyphosate is partly transported outside the treated agricultural fields.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the runoff behavior of glyphosate and AMPA in two field experiments in two different European agricultural soils with artificial rainfall. No details on the description of the analytical method and of statistical analysis are provided.
In addition, water samples from percolation water and from two run-off retention basins were analyzed for glyphosate and AMPA but no details on experimental design, sampling or analytical method are given.
The article is therefore classified as reliable with restrictions for the runoff experiment while the results for percolation water and the run-off retention basins are considered not reliable.

Assessment and conclusion by RMS:

CA 7.1.2.2.2 Soil accumulation studies

Field accumulation studies are not required for glyphosate and have not been performed.

CA 7.1.3 Absorption and desorption in soil

The adsorption and desorption behaviour in soil of glyphosate (PMG) and its major soil degradation product aminomethylphosphonic acid (AMPA) was investigated in various soils in batch equilibrium experiments (glyphosate: [redacted], 2020, CA 7.1.3.1.1/001 incl. amendment CA 7.1.3.1.1/002 and additional report CA 7.1.3.1.1/030; AMPA: Bloss, 2020, CA 7.1.3.1.2/001; [redacted] 2002, CA 7.1.3.1.2/003 and [redacted] 1993, CA 7.1.3.1.2/006). Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data using the approach according to Freundlich. Furthermore, seven studies are considered as supportive and could provide additional information for glyphosate ([redacted] 1996, CA 7.1.3.1.1/004, [redacted] 1996, CA 7.1.3.1.1/005, [redacted] 1993, CA 7.1.3.1.1/007, [redacted] 1992, CA 7.1.3.1.1/008, [redacted] 1992, CA 7.1.3.1.1/009, [redacted] 1986, CA 7.1.3.1.1/011) and AMPA ([redacted] 1996, CA 7.1.3.1.2/004). For the latter studies adsorption data in the form of K_D values can be derived from single test concentrations.

The adsorption tests were evaluated in view of the EU OECD 106 Evaluators Checklist (EFSA, 2017. Technical report on the outcome of the pesticides peer review meeting on the OECD 106 evaluators checklist. EFSA supporting publication 2017:EN-1326. 18 pp. doi: 10.2903/sp.efsa.2017.EN-1326). The results of the evaluation are presented each with the respective summary.

For the active substance glyphosate the calculated adsorption coefficients $K_{F(ads)}$ of the Freundlich adsorption isotherms range from 18.1 to 166.4 mL/g (geometric mean: 54.2~~53.0~~ mL/g) and correspond to adsorption coefficients normalised to organic carbon content, $K_{F,OC(ads)}$, in the range from 1031 to 9615 mL/g (geometric mean: 4348~~4245~~ mL/g). The Freundlich exponents expressed as 1/n are in the range of 0.546 to 0.777 (arithmetic mean: 0.682~~0.695~~). The adsorption coefficients of glyphosate were assessed on pH dependency ~~was found to be not dependent on pH of soil~~ (see Table 7.1.3.1.1-86). For glyphosate, a significant correlation between soil pH and $K_{F(ads)}$ was found but not between soil pH and $K_{F, OC(ads)}$.

For metabolite AMPA the calculated adsorption coefficients $K_{F(ads)}$ of the Freundlich adsorption isotherms range from 15.7 to 189.7 mL/g (geometric mean: 40.5 mL/g) and the adsorption coefficients $K_{F, OC(ads)}$ (normalised to organic carbon content) range from 1160 to 8248 mL/g (geometric mean: 3167 mL/g). The Freundlich exponents 1/n are in the range of 0.551 to 0.791 (arithmetic mean: 0.690). Adsorption of AMPA was found to be not dependent on soil pH (see Table 7.1.3.1.2-109).

Within the actual review of scientific literature for glyphosate (2010-2019), 16 articles were identified in total to potentially provide relevant information to the data point. The outcome of the reliability assessment was that the articles are "reliable with restrictions", thus not to be considered in the risk assessment.

Overall, the published data reported results are in good agreement with the findings of the applicant studies. Sorption values are not always provided in the published data, but several articles report K_F or $K_{F, OC}$ values (Albers *et al.*, 2018, CA 7.1.3.1.1/014, Skeff *et al.*, 2018, CA 7.1.3.1.1/016, Gómez *et al.*, 2017, CA 7.1.3.1.1/017, Sidoli *et al.*, 2016, CA 7.1.3.1.1/023, Tévez & dos Santos Afonso, 2015, CA 7.1.3.1.1/026, Jodeh *et al.*, 2014, CA 7.1.3.1.1/027, Bergström *et al.*, 2011, CA 7.1.3.1.1/029) or Freundlich values 1/n (Gómez *et al.*, 2017, CA 7.1.3.1.1/017, Sidoli *et al.*, 2016, CA 7.1.3.1.1/023 Tévez & dos Santos Afonso, 2015, CA 7.1.3.1.1/026). All these data confirm the strong binding capacity of glyphosate and low 1/n values also found in the application information.

Many articles concentrate on the mechanisms and main factors driving glyphosate sorption and availability in soils. Some articles investigate the behaviour of glyphosate in presence of phosphate or phosphate fertilizers (Munira *et al.*, 2017, CA 7.1.3.1.1/019, Munira *et al.*, 2016, CA 7.1.3.1.1/022, Sidoli *et al.*, 2016, CA 7.1.3.1.1/023), others investigate several factors e.g. pH, organic carbon content, CEC and clay content (Dollinger *et al.*, 2015, CA 7.1.3.1.1/024), phosphorus, organic matter, pH and temperature (Jodeh *et al.*, 2014, CA 7.1.3.1.1/027) or pedotransfer functions (Sidoli *et al.*, 2016, CA 7.1.3.1.1/023, Dollinger *et al.*, 2015, CA 7.1.3.1.1/024 and Rampoldi *et al.*, 2014, CA 7.1.3.1.1/028).

Further articles investigate the effect of the presence of other pesticides (in this case MCPA) on glyphosate (Munira & Farenhorst, 2017, CA 7.1.3.1.1/018), biochar addition to soils (Zhelezova *et al.* 2017) or effects of pig manure or a mulch cover on sorption of glyphosate (Albers *et al.*, 2018, CA 7.1.3.1.1/014, Cassigneul

et al., 2016, CA 7.1.3.1.1/021). Behaviour of glyphosate in specific soils was investigated by Tévez & dos Santos Afonso (2015, CA 7.1.3.1.1/026) and Dollinger (2018, CA 7.1.3.1.1/015).

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

Table 7.1.3.1.1-1: Adsorption/desorption studies of glyphosate

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.3.1.1/001	[redacted], 2020	Batch adsorption	Glyphosate	Valid	
CA 7.1.3.1.1/002	[redacted] 2020	Batch adsorption	Glyphosate	Valid	Amendment to CA 7.1.3.1.1/001
CA 7.1.3.1.1/030	[redacted] 2020	Batch adsorption	Glyphosate	Valid	Additional report related to CA 7.1.3.1.1/001
CA 7.1.3.1.1/003	[redacted] 2001	Batch adsorption	Glyphosate	Invalid	
CA 7.1.3.1.1/004	[redacted], 1996	Batch adsorption	Glyphosate	Supportive	
CA 7.1.3.1.1/005	[redacted], 1996	Batch adsorption	Glyphosate	Supportive	
CA 7.1.3.1.1/006	[redacted] 1994	Batch adsorption	Glyphosate	Invalid	
CA 7.1.3.1.1/007	[redacted], 1993	Batch adsorption	Glyphosate isopropylamine salt	Supportive	
CA 7.1.3.1.1/008	[redacted] 1992	Batch adsorption	Glyphosate-Trimesium	Supportive	
CA 7.1.3.1.1/009	[redacted] 1992	Batch adsorption	Glyphosate	Supportive	
CA 7.1.3.1.1/010	[redacted] 1991	Batch adsorption	Glyphosate	Invalid	
CA 7.1.3.1.1/011	[redacted] 1986	Batch adsorption	Glyphosate	Supportive	
CA 7.1.3.1.1/012	[redacted] 1986	Batch adsorption	HOE 017411 (Carbendazim)	Unknown	Report not available (presumably erroneously listed in Monograph, 2000, as HOE 017411 is active substance carbendazim)
CA 7.1.3.1.1/013	[redacted] 1978	Batch adsorption	Glyphosate and Sodium sesquiglyphosate	Invalid	

Table 7.1.3.1.1-2: Adsorption/desorption – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.3.1.1/014	Albers *et al.*, 2018	Batch adsorption	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/015	Dollinger *et al.*, 2018	Batch adsorption	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/016	Skeff *et al.*, 2018	Batch adsorption	Glyphosate, AMPA	Reliable with restrictions	
CA 7.1.3.1.1/017	Gómez *et al.*, 2017	Batch adsorption	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/018	Munira & Farenhorst, 2017	Batch adsorption	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/019	Munira *et al.*, 2017	Batch adsorption	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/020	Zhelezova *et al.*, 2017	Batch adsorption	Glyphosate	Reliable with restrictions	Summary under CA 7.1.2.1.1/010
CA 7.1.3.1.1/021	Cassigneul *et al.*, 2016	Batch adsorption	Glyphosate	Reliable with restrictions	Summary under CA 7.1.2.1.1/011
CA 7.1.3.1.1/022	Munira *et al.*, 2016	Batch adsorption	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/023	Sidoli *et al.*, 2016	Batch adsorption	Glyphosate, AMPA	Reliable with restrictions	
CA 7.1.3.1.1/024	Dollinger *et al.*, 2015	Modelling study	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/025	Kanissery *et al.*, 2015	Batch adsorption	Glyphosate	Reliable with restrictions	Summary under CA 7.1.2.1.1/013
CA 7.1.3.1.1/026	Tévez & dos Santos Afonso, 2015	Batch adsorption	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/027	Jodeh *et al.*, 2014	Batch adsorption	Glyphosate	Reliable with restrictions	
CA 7.1.3.1.1/028	Rampoldi *et al.*, 2014	Batch adsorption	Glyphosate	Reliable with restrictions	Summary under CA 7.1.2.1.1/014
CA 7.1.3.1.1/029	Bergström *et al.*, 2011	Batch adsorption	Glyphosate	Reliable with restrictions	Summary under CA 7.1.2.1.1/017

Table 7.1.3.1.1-3: Summary of soil adsorption parameters for glyphosate

Study	Soil Type	OC (%)	pH ($CaCl_2$)	pH (H_2O)	K_D (mL/g)	$K_{D, OC}$ (mL/g)	K_F (mL/g)	$K_{F, OC}$ (mL/g)	1/n
[redacted] 2020, CA 7.1.3.1.1/001	Speyer 2.2, loamy sand	1.71	5.6	5.21	-	-	59.4434 ~~49.5629~~	3476.22 ~~2898.41~~	0.546 ~~0.595~~
	RefeSol 01-A, loamy sand	0.80	5.33	6.11	-	-	59.8046 ~~56.4215~~	7475.57 ~~7052.69~~	0.704 ~~0.760~~
	18 Acres, sandy clay loam	1.9	6.2	6.11	-	-	166.3529	8755.42	0.579
	M-SL-PF (Mutchler, US), sandy clay loam	1.9	6.1	6.44	-	-	152.4533	8023.86	0.546
	Speyer 2.3, sandy loam	0.67	5.9	7.02	-	-	52.8781	7892.25	0.751
	RefeSol 02-A, silt loam	0.92	6.19	6.98	-	-	88.4624	9615.48	0.658
	Gartenacker, loam	2.1	7.1	7.16	-	-	21.6447	1030.70	0.757
	Speyer 6S, clay	1.78	7.2	7.32	-	-	70.5279 ~~70.6584~~	3962.24 ~~3969.57~~	0.736 ~~0.760~~
	Speyer 5M, sandy loam	0.92	7.4	7.56	-	-	18.8542	2049.37	0.770
	LAD-SL-PF (Pavillion, US), sandy loam	0.87	8.1	8.11	-	-	18.1119	2081.83	0.777
Geometric mean (if not pH dependent) (n = 10)							**54.2** ~~**53.0**~~	**4348** ~~**4245**~~[1]	-
Arithmetic mean (if not pH dependent) (n = 10)							-	-	**0.682** ~~**0.695**~~[1]
pH dependence								No	

[1] For modelling, preliminary data with $K_{F, OC}$ = 4243 L/kg and 1/n = 0.697 was used as final report was not available at time of calculations.

Batch adsorption studies with glyphosate

1. Information on the study

Data point:	CA 7.1.3.1.1/001
Report author	[redacted]
Report year	2020
Report title	Glyphosate – Adsorption/Desorption of [14C]Glyphosate in Ten Soils
Report No	20190441
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Total material balance below 90% for some replicates at single test concentrations of three soils; the respective tests were repeated in CA 7.1.3.1.1/030
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

Data point:	CA 7.1.3.1.1/002
Report author	[redacted]
Report year	2020
Report title	Amendment
Report No	Not applicable
Document No	
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

Data point:	CA 7.1.3.1.1/030
Report author	[redacted]
Report year	2020
Report title	Glyphosate – Adsorption/Desorption of [14C]Glyphosate in Ten Soils, Experiments Supporting IES Study 20190441
Report No	20200276
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The adsorption behaviour of [glycine-1-^{14}C]Glyphosate was studied in ten soils under conditions of laboratory batch equilibrium tests in the dark at 20 ± 2 °C.

Soil	Origin	Texture (USDA)	pH[1]	OC [%]
1 (Speyer 2.2)	Hanhofen	Sandy loam	5.21 / 5.6	1.71
2 (RefeSol 01-A-05)	Schmallenberg	Loamy sand	6.11 / 5.33	0.80
3 (18 Acres)	Berkshire	Loamy sand	6.11 / 6.2	1.9
4 (M-SL-PF (Mutchler))	Grand Forks	Sandy clay loam	6.44 / 6.1	1.9
5 (Speyer 2.3)	Offenbach	Sandy loam	7.02 / 5.9	0.67
6 (RefeSol 02-A-06)	Schmallenberg	Silt loam	6.98 / 6.19	0.92
7 (Gartenacker)	Vouvry	Loam	7.16 / 7.1	2.1
8 (Speyer 6S)	Siebeldingen	Clay	7.32 / 7.2	1.78
9 (Speyer 5M)	Mechtersheim	Sandy loam	7.56 / 7.4	0.92
10 (LAD-SL-PF (Pavillion))	Fremont	Sandy loam	8.11 / 8.1	0.87

[1] in water / $CaCl_2$

For the definitive phase, the adsorption step was carried out for four hours at a soil-to-solution ratio of 1:200 (soils 1-6 and 8) or 1:50 (soils 7, 9 and 10) using pre-equilibrated samples of air-dried and sterilised soils. Nominal concentrations of glyphosate were 5.00, 1.61, 0.50, 0.16 and 0.05 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$.

For the definitive phase, mean material balances of radioactivity ranged from 92.9 ~~85.0~~ to 99.6 ~~95.0~~ % for soil 1, from 90.7 ~~88.2~~ to 98.7 ~~88.2~~ % for soil 2, from 97.5 to 103.9 % for soil 3, from 90.0 to 97.7 % for soil 4, from 92.8 to 99.1 % for soil 5, from 93.2 to 98.6 % for soil 6, from 92.6 to 97.5 % for soil 7, from 91.7 ~~72.0~~ to 102.0 ~~95.1~~ % for soil 8, from 98.0 to 101.2 % for soil 9 and from 96.5 to 101.6 % for soil 10.

The stability of the test item was investigated by chromatographic analysis of aqueous supernatants and following extraction of soil by HPLC. Stability had been demonstrated for at least 4 hours under the conditions of the test. Analysis for test item was additionally performed for supernatants and soil extracts in the definitive phase.

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated according to the indirect method and based on the Freundlich isotherms ranged from 18.11 to 166.35 mL/g for all soils. The Freundlich exponents 1/n

were in the range of 0.546 to 0.777. The corresponding, calculated $K_{F, OC(ads)}$ values varied from 1031 to 9615 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[glycine-1-^{14}C]Glyphosate

Batch No.	MXM 20013
Specific activity	5.81 MBq/mg
Radiochemical purity	>98 %
Chemical purity	Not reported

2. Test Soils

The soil was sampled from the upper 20 cm soil layer. All plots have not been treated with pesticides for at least four years. The soils were air-dried at ambient temperature and sieved through a 2-mm sieve. All soils were sterilised by X-ray irradiation before use. A description of the soils used is summarised in the tables below.

Table 7.1.3.1.1-4: Physico-chemical properties of test soils 1-5

Parameter	Results				
Soil	1	2	3	4	5
	Speyer 2.2	RefeSol 01-A-05	18 Acres	M-SL-PF (Mutchler)	Speyer 2.3
Horizon (cm)	0-20	0-20	0-20	0-20	0-20
Geographic Location					
City	Hanhofen	Schmallenberg	Berkshire	Grand Forks	Offenbach
State	Rhineland-Palatinate	North Rhine-Westphalia	South East England	North Dakota	Hesse
Country	Germany	Germany	UK	USA	Germany
Textural Class (USDA)	Sandy loam	Loamy sand	Loamy sand	Sandy clay loam	Sandy loam
Sand (5 µm – 2 mm) (%)	78.3	76.6	56	62	59.6
Silt (2 µm – 5 µm) (%)	13.7	17.7	24	17	33.6
Clay (< 2 µm) (%)	8.0	5.7	20	21	6.8
pH					
- in 0.01 M $CaCl_2$	5.6	5.33	6.2	6.1	5.9
- in water	5.21	6.11	6.11	6.44	7.02
Organic Carbon	1.71	0.80	1.9	1.9	0.67
Organic Matter	2.95	1.38	3.3	3.3	1.16
Cation Exchange Capacity (meq/100 g)	9.2	7.60[1]	14.3	16.9	7.6

USDA: United States Department of Agriculture
[1] units of mmol/100 g soil

Table 7.1.3.1.1-5: Physico-chemical properties of test soils 6-10

Parameter	Results				
Soil	6	7	8	9	10
	RefeSol 02-A-06	Gartenacker	Speyer 6S	Speyer 5M	LAD-SL-PF (Pavillion)
Horizon (cm)	0-20	0-20	0-20	0-20	0-20
Geographic Location					
City	Schmallenberg	Vouvry	Siebeldingen	Mechtersheim	Fremont
State	North Rhine-Westphalia	Wallis	Rhineland-Palatinate	Rhineland-Palatinate	Wyoming
Country	Germany	Switzerland	Germany	Germany	USA
Textural Class (USDA)	Silt loam	Loam	Clay	Sandy loam	Sandy loam
Sand (5 µm – 2 mm) (%)	4.1	46	24.1	57.8	76
Silt (2 µm – 5 µm) (%)	80.1	46	35.1	30.9	11
Clay (< 2 µm) (%)	15.8	8	40.8	11.3	13
pH					
- in 0.01 M $CaCl_2$	6.19	7.1	7.2	7.4	8.1
- in water	6.98	7.16	7.32	7.56	8.11
Organic Carbon	0.92	2.1	1.78	0.92	0.87
Organic Matter	1.59	3.6	3.07	1.59	1.50
Cation Exchange Capacity (meq/100 g)	5.91[1]	8.4	25.7	13.3	17.6

USDA: United States Department of Agriculture
[1] units of mmol/100 g soil

B. STUDY DESIGN

1. Experimental Conditions

Sealed Teflon tubes were used as test systems. The experiments were performed with duplicate soil samples. All experiments were performed at 20 ± 2 °C in the dark. Tubes were shaken to keep the soil in homogeneous suspension.

Soil samples were pre-equilibrated with at least 90 % of the target volume of 0.01 M $CaCl_2$ for approx. 16 hours at 20 ± 2 °C prior to application of the test item.

In the preliminary tests, the optimal soil-to-solution ratio, the appropriate adsorption equilibration time and adsorption of test item to test vessel surface in absence of soil was determined. The preliminary phase also included tests on extractability from soil and the stability of glyphosate in the presence of sterilised or non-sterilised soil for various contact times. The final test for test item stability was performed at the lowest test concentration of 0.055 µg/mL and an adsorption time of 4 hours to confirm the stability of the test item for all soils tested and the suitability of the analytical techniques.

The definitive phase was performed with sterilised soils. The adsorption step was carried out using pre-equilibrated samples at a soil-to-solution ratio of 1:200 (for soils 1-6 and 8) or 1:50 (for soils 7, 9 and 10). Glyphosate was applied at nominal concentrations of 5.00, 1.61, 0.50, 0.16 and 0.05 mg/L in aqueous 0.01 M $CaCl_2$ solution. For the adsorption step, a series of control samples without soil, but containing five test item concentration each (duplicates) were subjected to precisely the same steps as the test samples in order to check for the stability of the test item in $CaCl_2$ solution. Additionally, test item stability was investigated in aqueous supernatants ($CaCl_2$) and soil extracts for 0.05 mg/L samples for all soils and for all concentrations for soils 2, 3 and 4.

The definitive adsorption step was carried out for 4 hours in the dark at 20 ± 2 °C under continuous agitation. No desorption steps were performed.

2. Analytical Procedures

After the adsorption step, the aqueous supernatant was separated from the soil by centrifugation and the radioactivity content was analysed by liquid scintillation counting (LSC).

Within the preliminary stability tests soil samples were extracted three times at ambient temperature with 0.25 M ammonium hydroxide/0.1 M monopotassium phosphate following the adsorption phase. The extracts were combined for analysis. Aqueous $CaCl_2$ solutions and combined soil extracts were analysed by HPLC/radiodetection. Extracted soil samples were dried, combusted and analysed by LSC to determine non-extractable radioactivity.

Within the definitive phase soil samples were extracted following the adsorption step as described for the preliminary tests for the test concentration of 0.50 mg/L of all soils. Additionally, soils of all test concentrations were extracted for soils 2, 3 and 4. Aqueous $CaCl_2$ solutions and combined soil extracts were analysed by HPLC/radiodetection. The aqueous supernatants of the lowest test concentration were also analysed for test item by TLC.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation. The adsorption values of soil 4 were corrected by purity as determined by the chromatographic analysis in order to take into account the slight extent of degradation observed. For the other soils, Glyphosate was considered stable under the test conditions with no need for correction of the adsorption results.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

For the definitive phase, mean material balances ranged from 92.9 ~~85.0~~ to 99.6 ~~95.0~~ % of applied radioactivity (% AR) for soil 1, from 90.7 ~~88.2~~ to 98.7 ~~88.2~~ % AR for soil 2, from 97.5 to 103.9 % AR for soil 3, from 90.0 to 97.7 % AR for soil 4, from 92.8 to 99.1 % AR for soil 5, from 93.2 to 98.6 % AR for soil 6, from 92.6 to 97.5 % AR for soil 7, from 91.7 ~~72.0~~ to 102.0 ~~95.1~~ % AR for soil 8, from 98.0 to 101.2 % AR for soil 9 and from 96.5 to 101.6 % AR for soil 10.

B. STABILITY OF TEST ITEM

The test item was stable in aqueous 0.01 M $CaCl_2$ solution, i.e. in absence of soil and did not show adsorption to the surface of the test vessels. After incubation for 4 hours the test item was detected with ≥97.9 % AR.

The stability of the test item during the adsorption phase was investigated in preliminary tests by chromatographic analysis of aqueous supernatants and soil extracts. Stability of the test item was demonstrated by HPLC and TLC analysis in non-sterile and sterilised soil. As a result of analysis it was demonstrated that the test item was stable for at least for 4 hours in sterile soils under the test conditions.

C. FINDINGS

For the definitive tests, the percentage of radioactivity adsorbed to the soil ranged from 11.0 to 52.9 % in soil 1, in soil 2 from 14.0 to 45.5 ~~32.4~~ %, in soil 3 from 28.6 to 80.6 %, in soil 4 from 25.4 to 76.6 % in soil 5 from 14.6 to 34.3 %, in soil 6 from 19.6 to 55.2 %, in soil 7 from 21.3 to 47.6 %, in soil 8 from 17.8 to 46.0 ~~37.7~~ %, in soil 9 from 19.9 to 44.3 % and in soil 10 from 20.3 to 44.1 % (see table below).

Table 7.1.3.1.1-6: [^{14}C]Glyphosate: Percentage adsorbed to soil (mean values)

Soil	Test Concentration [mg/L]				
	5.00	1.61	0.50	0.16	0.05
1 (Speyer 2.2)	11.0	19.6	39.4 ~~26.3~~	51.8 ~~28.5~~	52.9
2 (RefeSol 01-A-05)	14.0	24.1	30.5 ~~23.8~~	39.3	45.5 ~~32.4~~
3 (18 Acres)	28.6	47.0	64.2	76.5	80.6
4 (M-SL-PF (Mutchler))	25.4	44.0	60.9	70.6	74.9
5 (Speyer 2.3)	14.6	19.8	23.4	32.6	34.3
6 (RefeSol 02-A-06)	19.6	30.4	38.0	50.1	55.2
7 (Gartenacker)	21.3	29.5	34.2	42.4	47.6
8 (Speyer 6S)	17.8	23.6	31.3	37.7	46.0 ~~30.1~~
9 (Speyer 5M)	19.9	26.9	32.3	38.9	44.3
10 (LAD-SL-PF (Pavillion))	20.3	28.6	31.7	40.6	44.1

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated according to the indirect method and based on the Freundlich isotherms ranged from 18.11 to 166.35 mL/g for all soils. The Freundlich exponents 1/n were in the range of 0.546 to 0.777. The corresponding, calculated $K_{F, OC(ads)}$ values varied from 1031 to 9615 mL/g. For details see table below.

Table 7.1.3.1.1-7: [^{14}C]Glyphosate: Adsorption parameters in soil at 20 °C

Soil	Adsorption			
	$K_{F(ads)}$	1/n	R^2	$K_{F, OC(ads)}$
1 (Speyer 2.2)	52.1434 ~~49.5629~~	0.546 ~~0.595~~	0.9578 ~~0.9813~~	3476.22 ~~2898.41~~
2 (RefeSol 01-A-05)	59.8046 ~~56.4215~~	0.704 ~~0.760~~	0.9890 ~~0.9894~~	7475.57 ~~7052.69~~
3 (18 Acres)	166.3529	0.579	0.9870	8755.42
4 (M-SL-PF (Mutchler))	152.4533	0.546	0.9810	8023.86
5 (Speyer 2.3)	52.8781	0.751	0.9955	7892.25
6 (RefeSol 02-A-06)	88.4624	0.658	0.9941	9615.48
7 (Gartenacker)	21.6447	0.757	0.9977	1030.70
8 (Speyer 6S)	70.5279 ~~70.6584~~	0.736 ~~0.760~~	0.9965 ~~0.9945~~	3962.24 ~~3969.57~~
9 (Speyer 5M)	18.8542	0.770	0.9989	2049.37
10 (LAD-SL-PF (Pavillion))	18.1119	0.777	0.9902	2081.83

The measured concentrations in the aqueous phase and extracts were used for the Freundlich evaluation. The linearised Freundlich isotherms and the corresponding plot of the residuals are shown in the figures below.

Figure 7.1.3.1.1-1: Definitive phase, soil 1 (Speyer 2.2): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

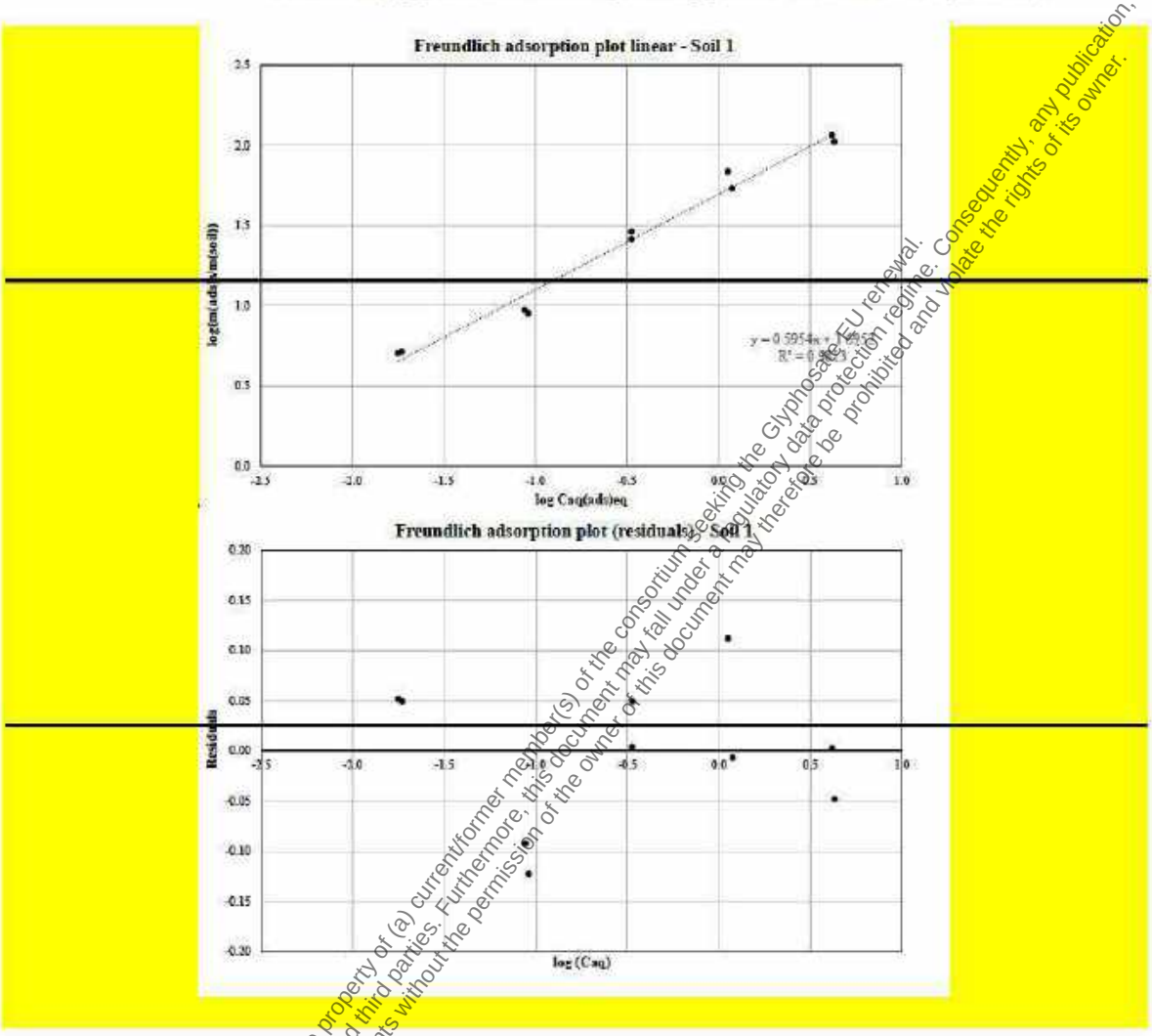


Figure 7.1.3.1.1-1: Definitive phase, soil 1 (Speyer 2.2): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

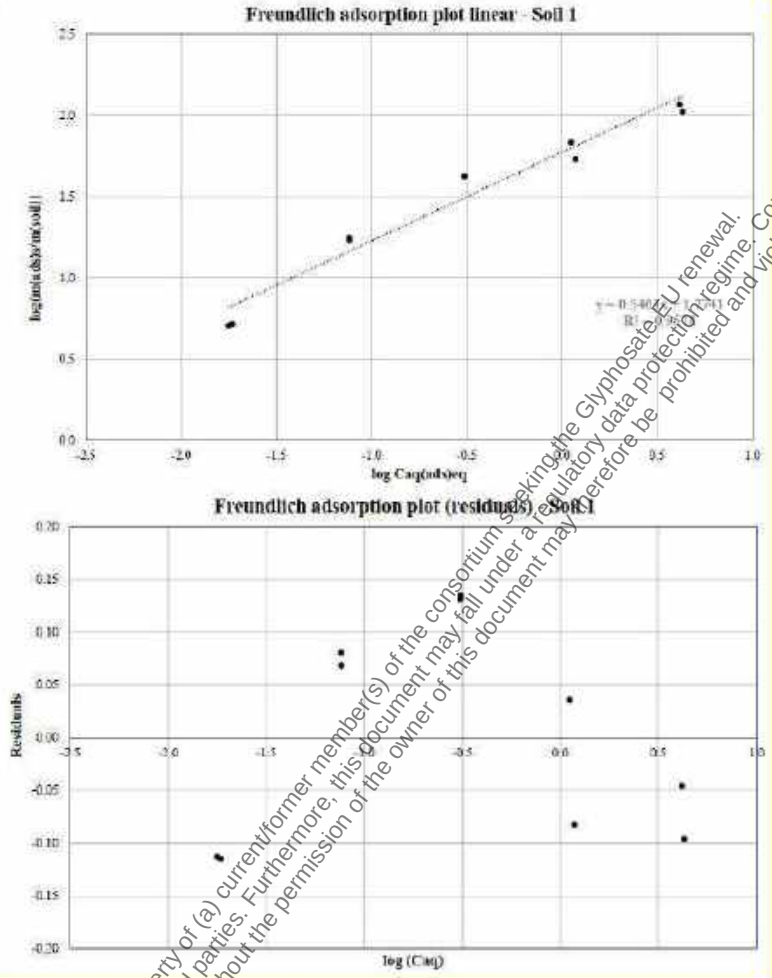


Figure 7.1.3.1.1-2: Definitive phase, soil 2 (RefeSol 01-A-05): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

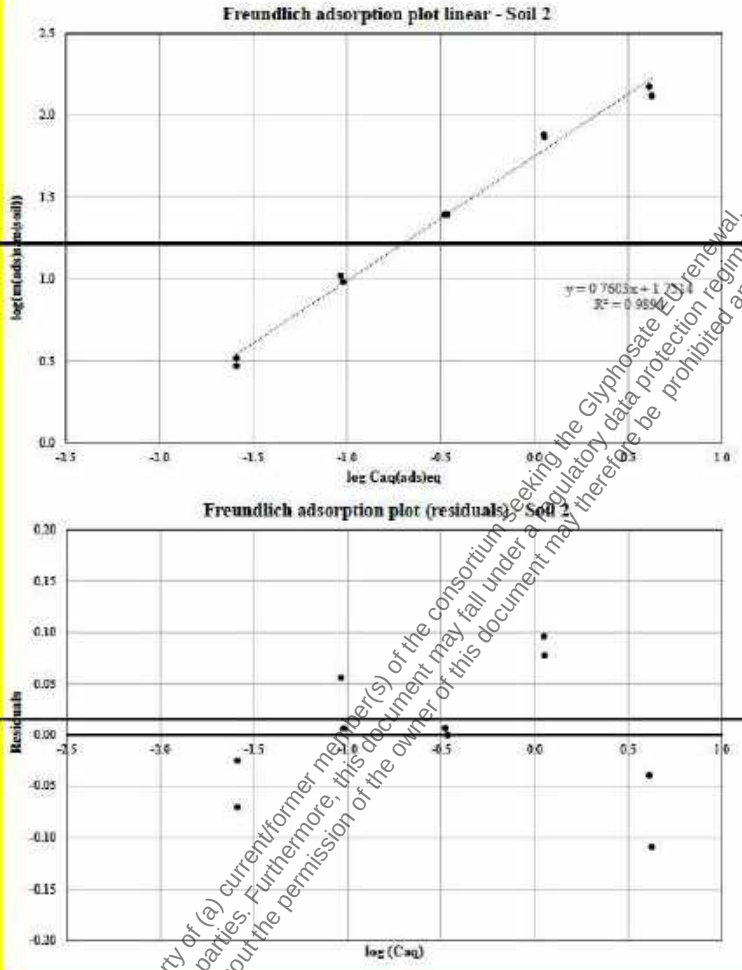


Figure 7.1.3.1.1-2: Definitive phase, soil 2 (RefeSol 01-A-05): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

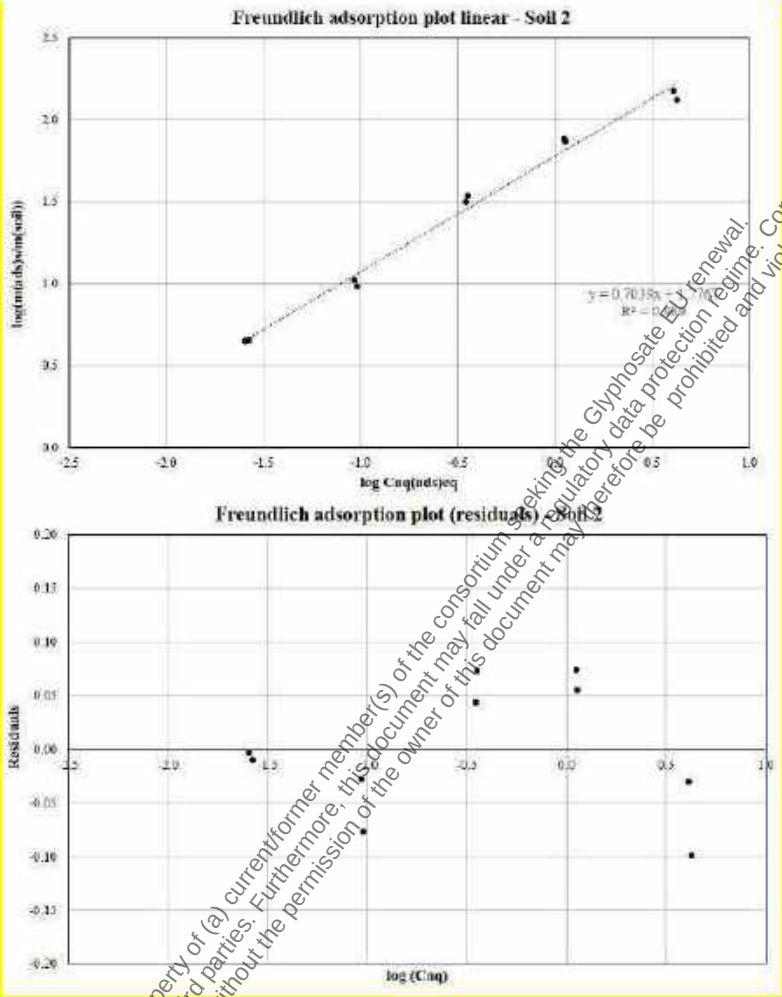


Figure 7.1.3.1.1-3: Definitive phase, soil 3 (18 Acres): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

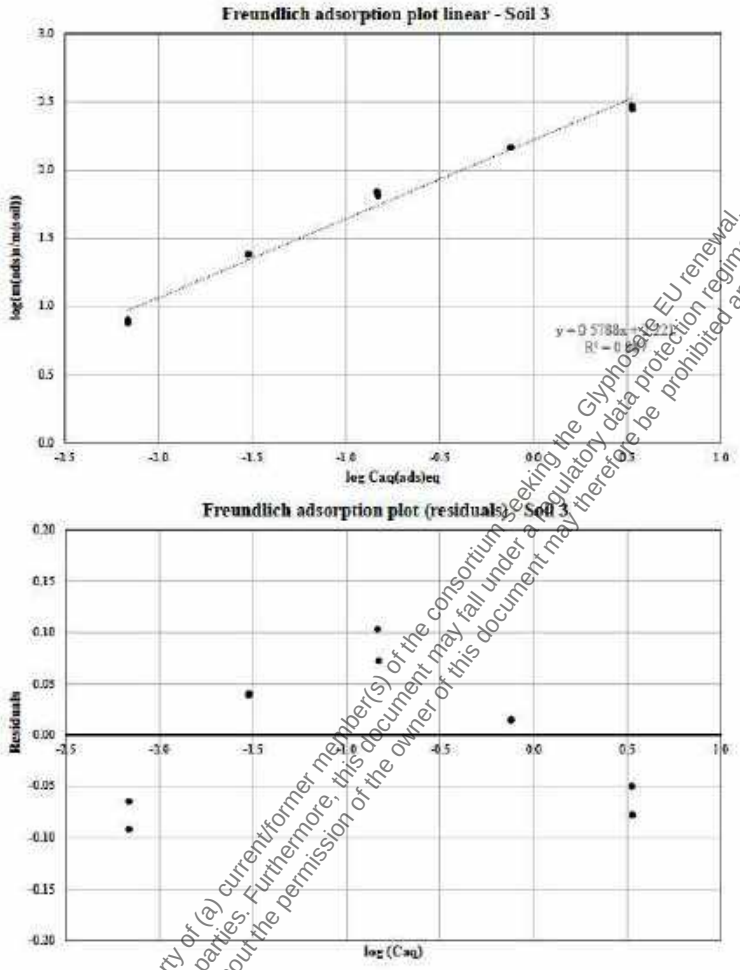


Figure 7.1.3.1.1-4: Definitive phase, soil 4 (M-SL-PF (Mutchler)): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

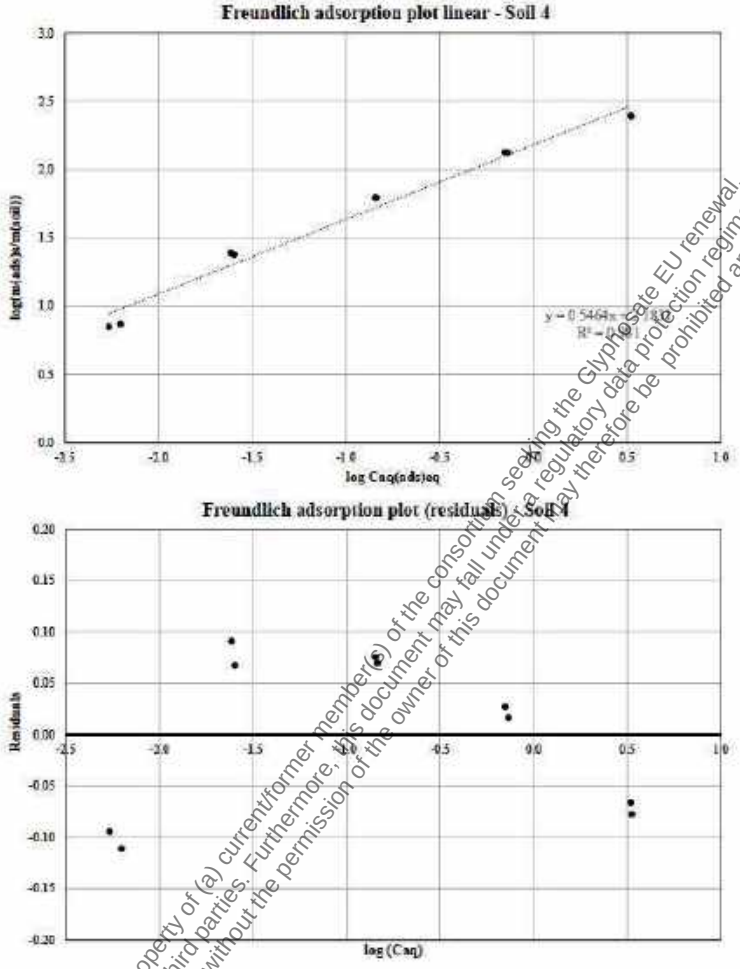


Figure 7.1.3.1.1-5: Definitive phase, soil 5 (Speyer 2.3): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

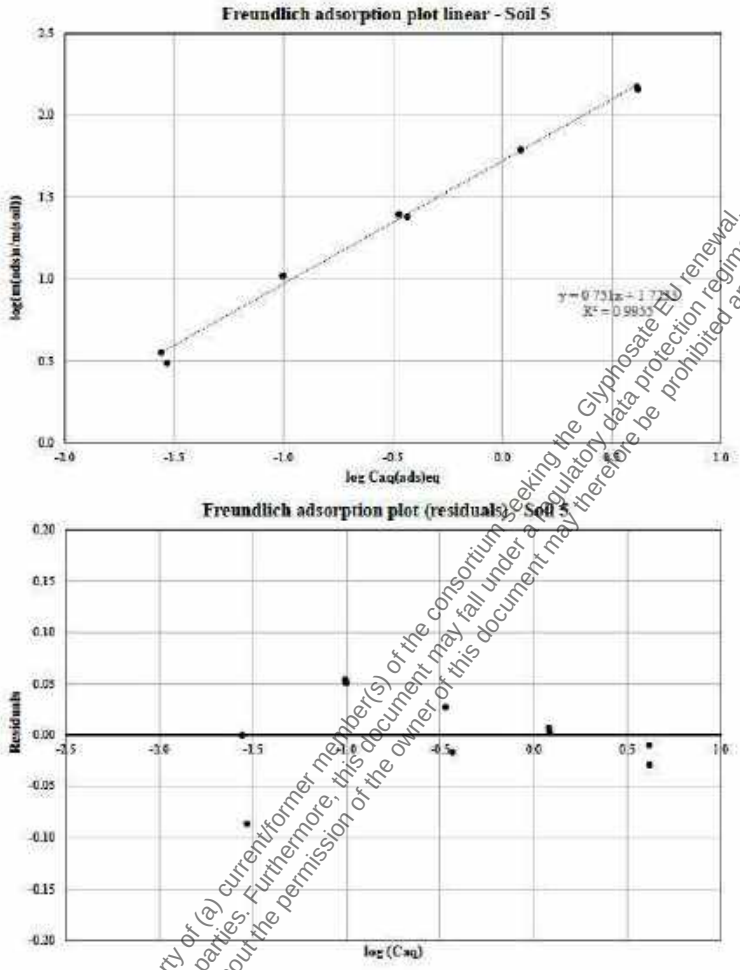


Figure 7.1.3.1.1-6: Definitive phase, soil 6 (RefeSol 02-A-06): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

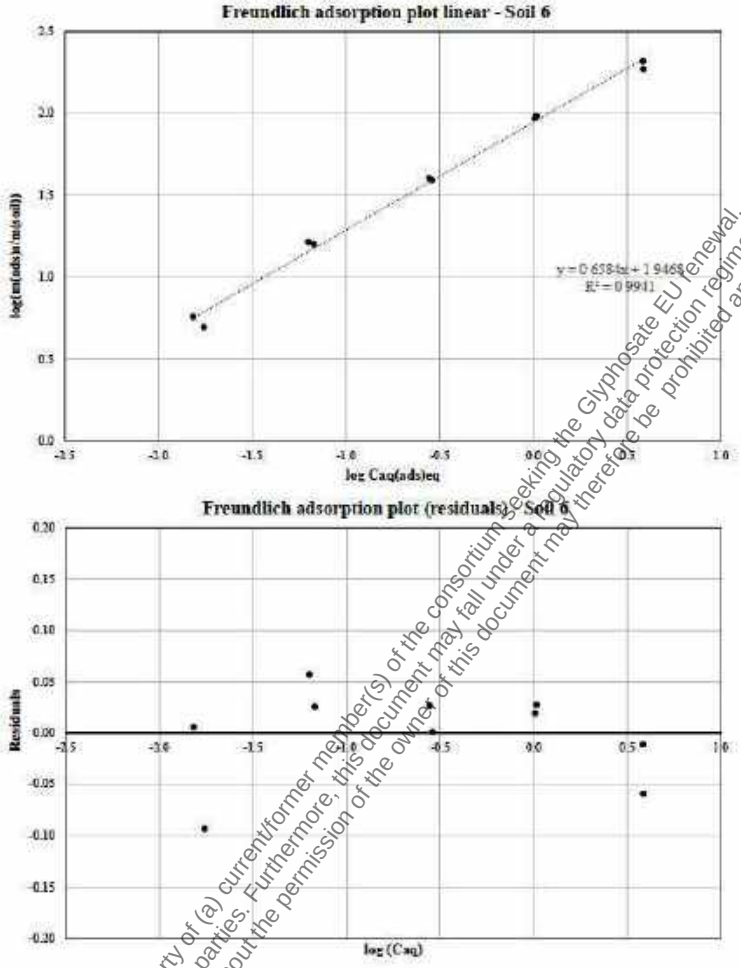


Figure 7.1.3.1.1-7: Definitive phase, soil 7 (Gartenacker): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

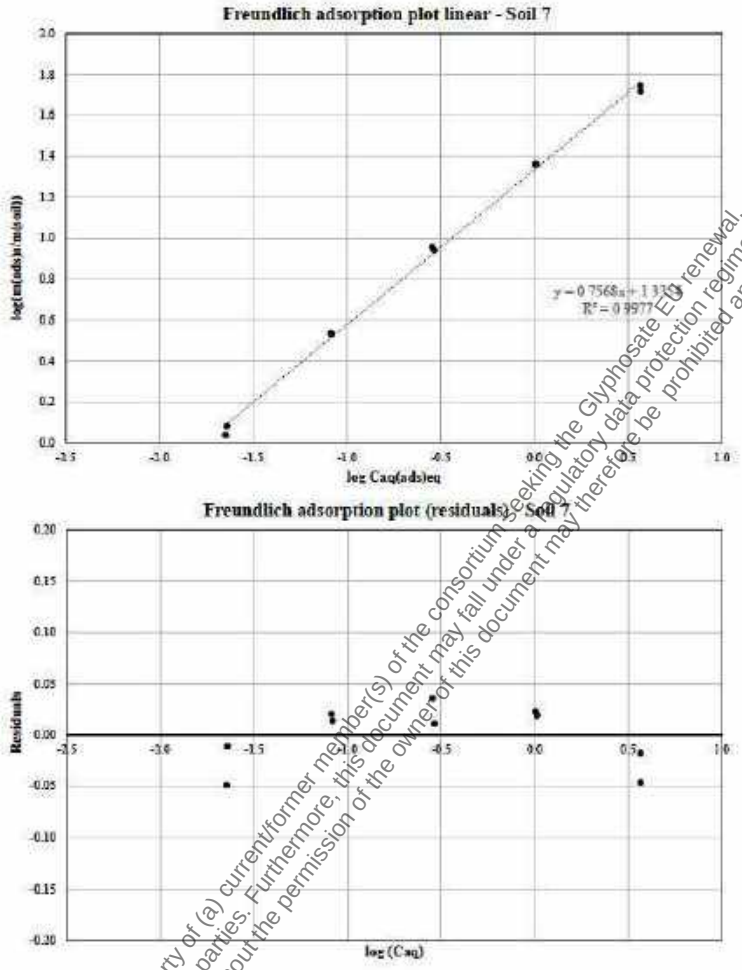


Figure 7.1.3.1.1-8: Definitive phase, soil 8 (Speyer 6S): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

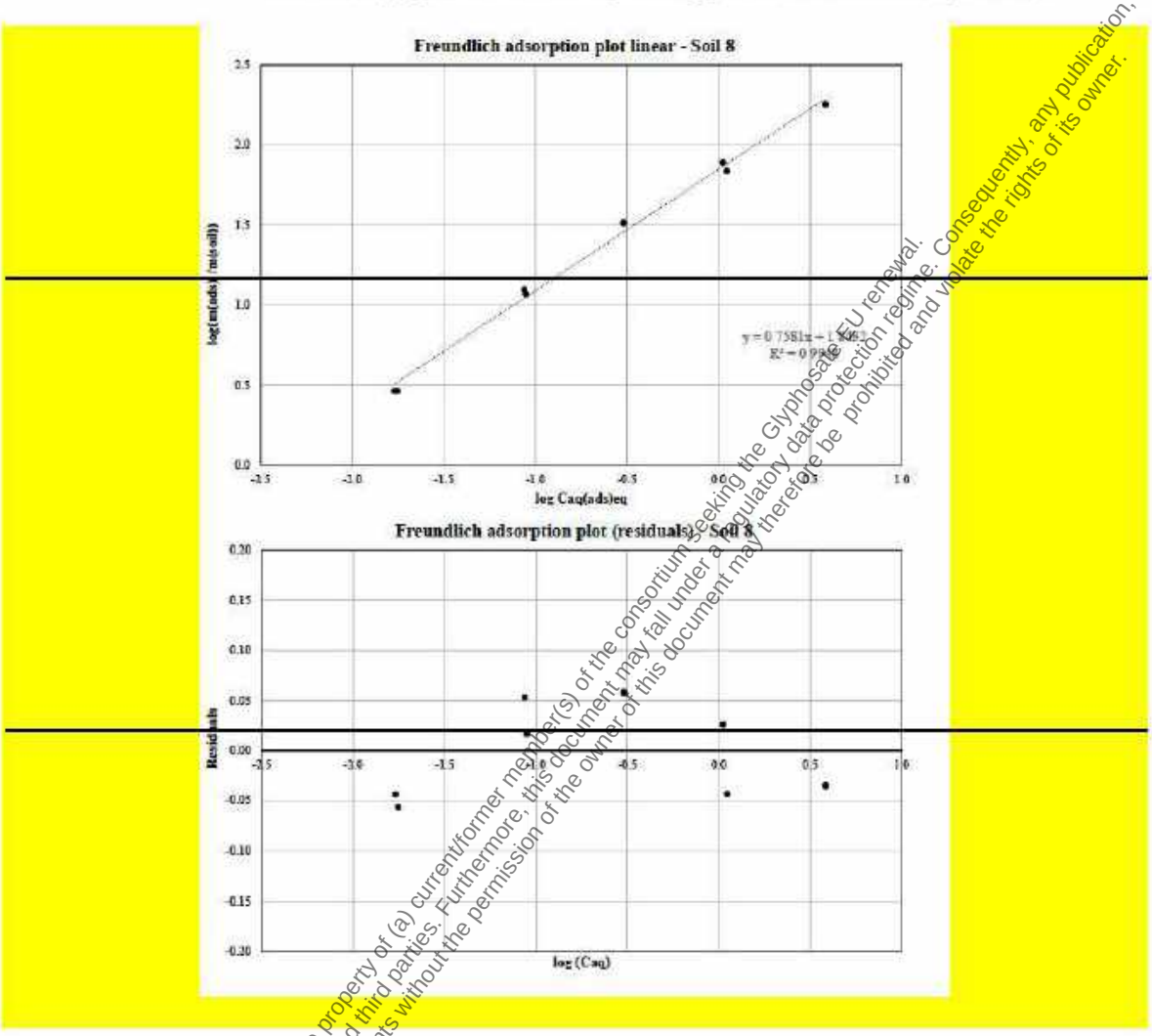


Figure 7.1.3.1.1-8: Definitive phase, soil 8 (Speyer 6S): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

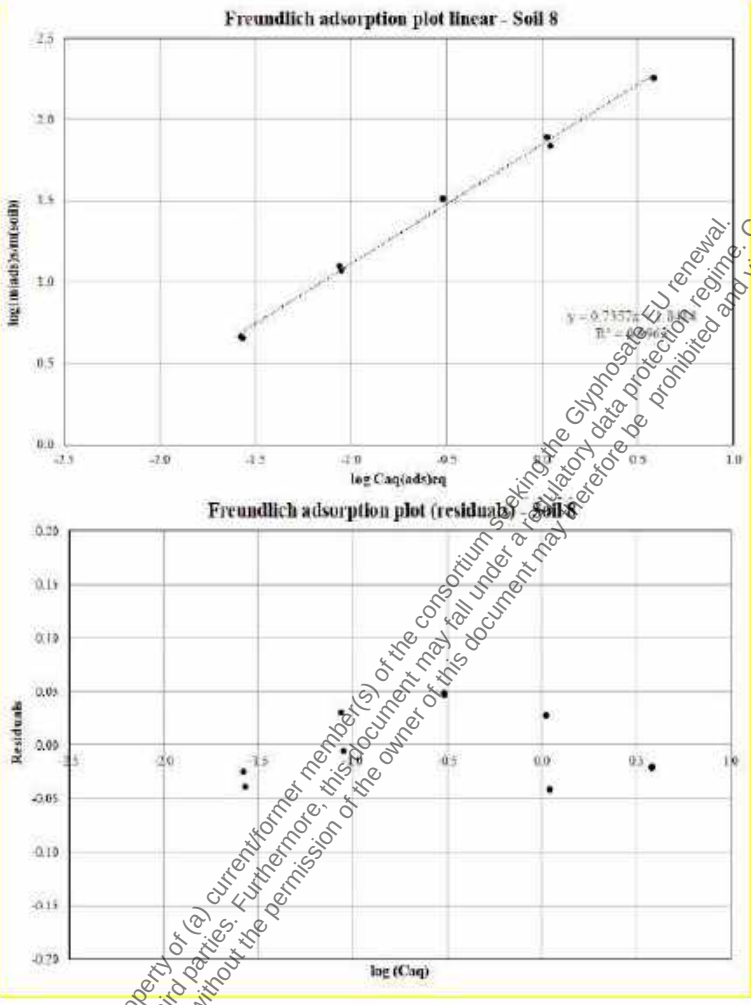


Figure 7.1.3.1.1-9: Definitive phase, soil 9 (Speyer 5M): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)

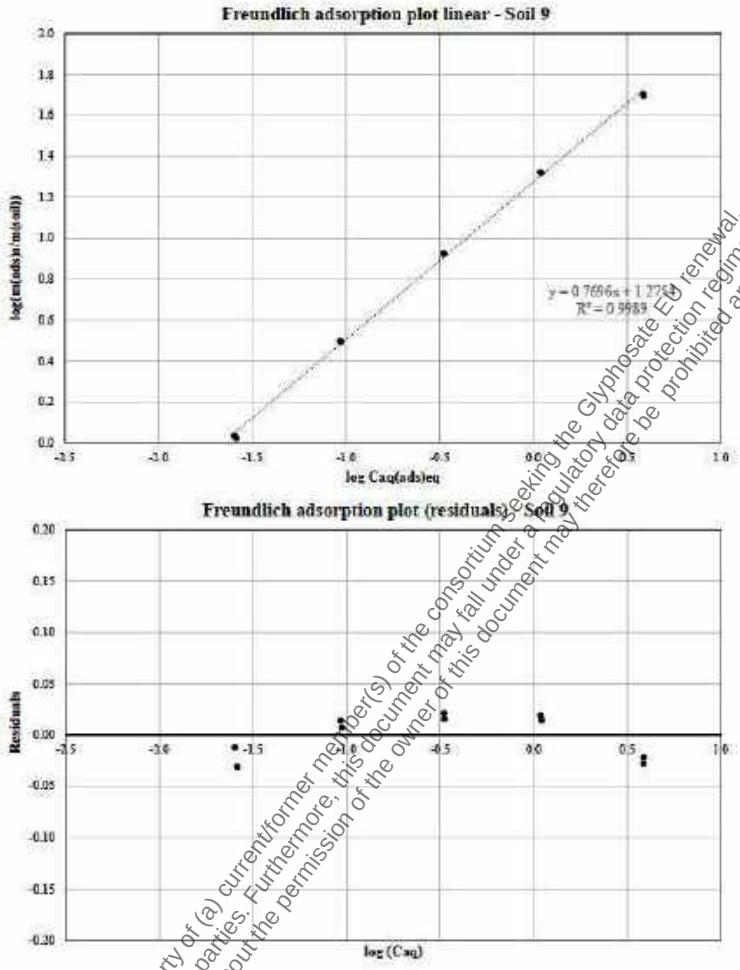
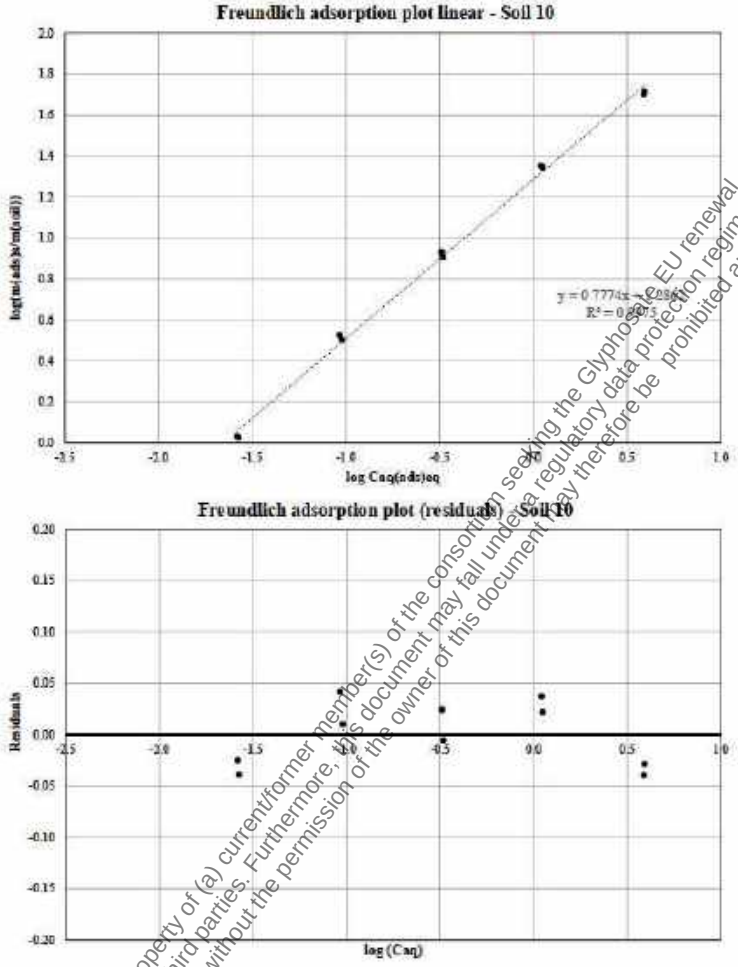


Figure 7.1.3.1.1-10: Definitive phase, soil 10 (LAD-SL-PF (Pavillion)): Linearised Freundlich adsorption isotherm (top) and the corresponding plot of the residuals (bottom)



III. CONCLUSIONS

The Freundlich adsorption coefficients $K_{F(ads)}$ of glyphosate as investigated for ten soils ranged from 18.11 to 166.35. The corresponding values normalised for organic carbon content of soil $K_{F\ OC(ads)}$ varied between 1030 and 9615.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is conducted consistent with the current guideline~~, showing minor deviations~~.
In the initial report (CA 7.1.3.1.1/001) total material balance were below 90 % for some replicates at single test concentrations of three soils. The respective tests were repeated in CA 7.1.3.1.1/030 with the respective material balances being above 90%. The results reported in CA 7.1.3.1.1/030 are considered to replace those in study 20190441. ~~In conclusion, the deviations do not influence the overall outcome of the study.~~

The study is considered acceptable to address this data point.

Assessment and conclusion by RMS:

All relevant quality checks following OECD 106 Evaluators Checklist were performed. Mass balances of radioactivity were from 94.9 to 99.0 % (5 mg/L) and percentage adsorption was from 14.9 to 89.6 % in the definitive test (see Table 7.1.3.1.1-8 and Table 7.1.3.1.1-9). Estimated K_{FE}/K_F values were rather variable from 1.1 to 3.6, dependent on soil and test concentration. The validity of the analytical method was confirmed over the entire range of concentrations measured (LOQ = 0.26 % AR and at least two orders of magnitude lower than lowest test concentration). K_D x soil/solution ratios were between 0.1 and 7.1 in all soils. The graphical fits of the Freundlich equation are presented below based on the standard linear regression form using log-log transformed data alongside the associated residual plots (see Figure 7.1.3.1.1-11 to Figure 7.1.3.1.1-20). The R^2 of the standard linear regressions ranged from 0.982 to 0.999.

Table 7.1.3.1.1-8: Glyphosate: Evaluation of result according to EU OECD 106 Evaluators Checklist

		Soil				
	Units	**1**	**2**	**3**	**4**	**5**
Adsorption method	-	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:200	1:200	1:200	1:200	1:200
Mass balance (at 5 mg/L)	% AR	95.0	96.8	97.5	95.8	97.6
Adsorbed percentage	%	14.9-62.5	16.1-50.0 ~~45.8~~	33.5-85.4	34.2-89.6	17.8-41.7
K_D x (soil:solution ratio)		0.1-1.4	0.2-0.9 ~~0.6~~	0.4-5.7	0.4-3.1	0.2-0.6
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	59.428 (47.852-73.805) ~~49.555 (42.700-57.511)~~	59.795 52.619-67.949 ~~56.386 (49.193-64.631)~~	166.375 (141.732-195.302)	152.395 (127.581-182.036)	52.888 (48.681-57.458)
$^{ads}1/n$ (95 % confidence interval)	-	0.547 (0.452-0.642) ~~0.597 (0.531-0.664)~~	0.704 (0.643-0.765) ~~0.762 (0.697-0.827)~~	0.580 (0.525-0.635)	0.542 (0.484-0.601)	0.751 (0.711-0.791)
$^{ads}R^2$	-	0.957 ~~0.982~~	0.989	0.987	0.983	0.996
$^{ads}K_{F,OC}$	L/kg OC	3475 ~~2898~~	7474 ~~7048~~	8757	8021	7894
K_{FE} / K_F (5 mg/L)	-	1.42/1.55	1.23/1.26	1.18/1.18	1.24/1.25~~1.27/1.27~~	1.25/1.24

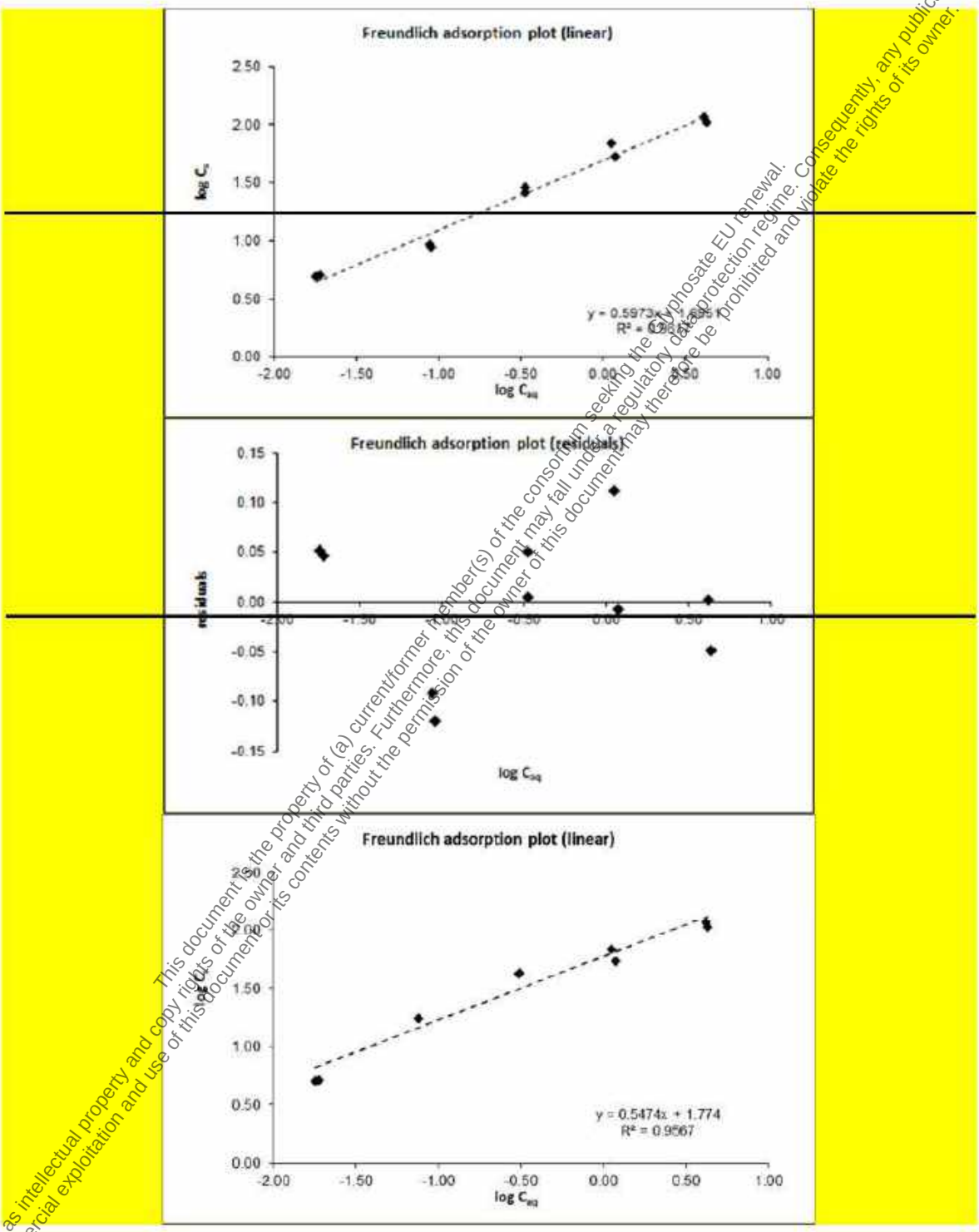
Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

Table 7.1.3.1.1-9: Glyphosate: Evaluation of result according to EU OECD 106 Evaluators Checklist (continued)

		Soil				
	Units	**6**	**7**	**8**	**9**	**10**
Adsorption method	-	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:200	1:50	1:200	1:50	1:50
Mass balance (at 5 mg/L)	% AR	97.0	95.9	94.9	98.0	99.0
Adsorbed percentage	%	24.0-68.8	27.2-52.1	24.3-48.0 ~~62.5~~	23.7-47.6	22.8-45.8
K_D x (soil:solution ratio)		0.2-1.9	0.3-1.1	0.2-0.9 ~~0.8~~	0.3-0.9	0.3-0.8
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	88.447 (80.480-97.201)	21.644 (20.230-23.157)	70.533 (65.517-75.934) ~~70.641 (63.469-78.623)~~	18.851 (18.047-19.691)	18.111 (15.868-20.670)
$^{ads}1/n$ (95 % confidence interval)	-	0.656 (0.616-0.696)	0.758 (0.727-0.789)	0.735 (0.700-0.770) ~~0.762 (0.715-0.810)~~	0.769 (0.748-0.790)	0.777 (0.714-0.840)
$^{ads}R^2$	-	0.994	0.997	0.997 ~~0.994~~	0.999	0.990
$^{ads}K_{F,OC}$	L/kg OC	9614	1033	3963 ~~3969~~	2049	2082
K_{FE} / K_F (5 mg/L)	-	1.24/1.23	1.28/1.28	1.37/1.37	1.20/1.20	1.15/1.14

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.

Figure 7.1.3.1.1-11: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 1



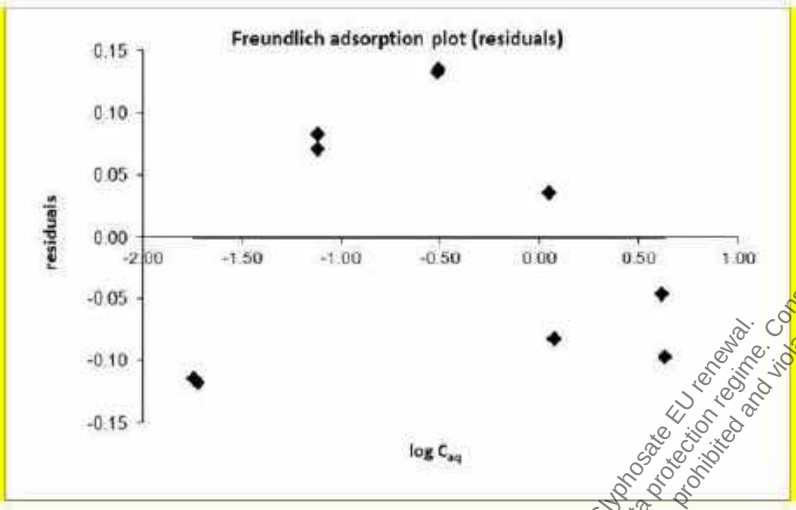
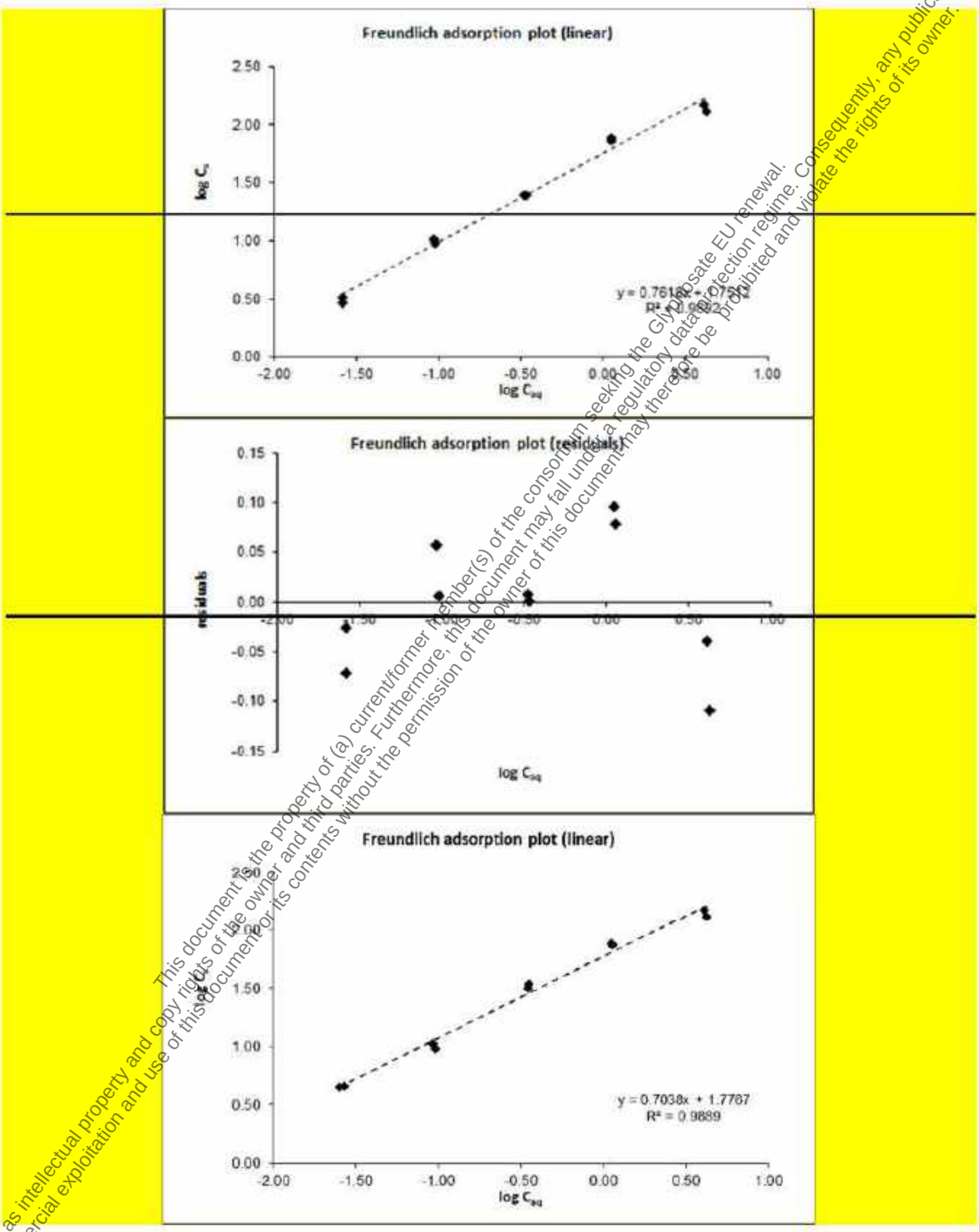


Figure 7.1.3.1.1-12: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 2



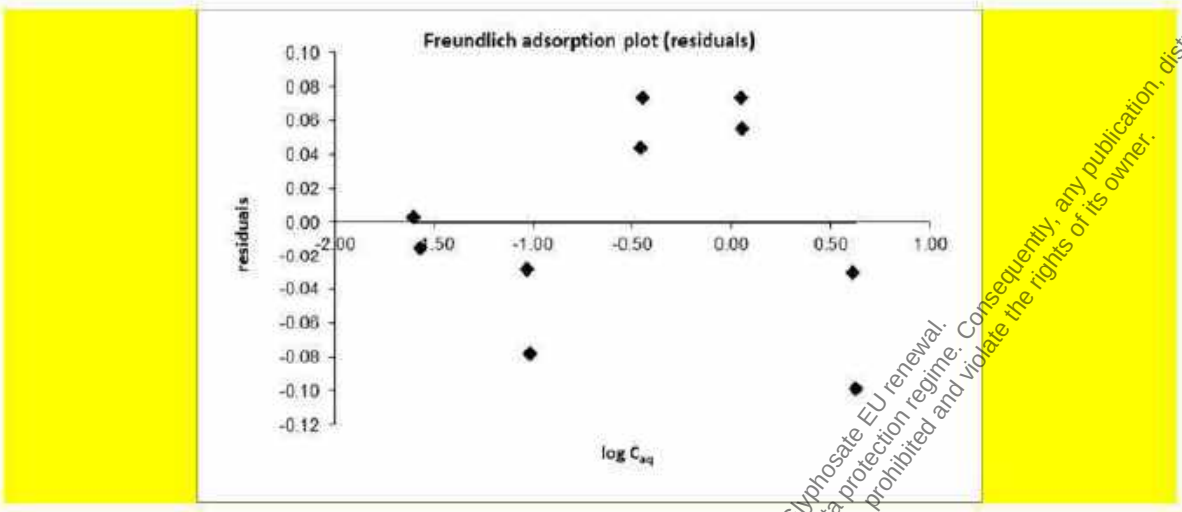


Figure 7.1.3.1.1-13: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 3

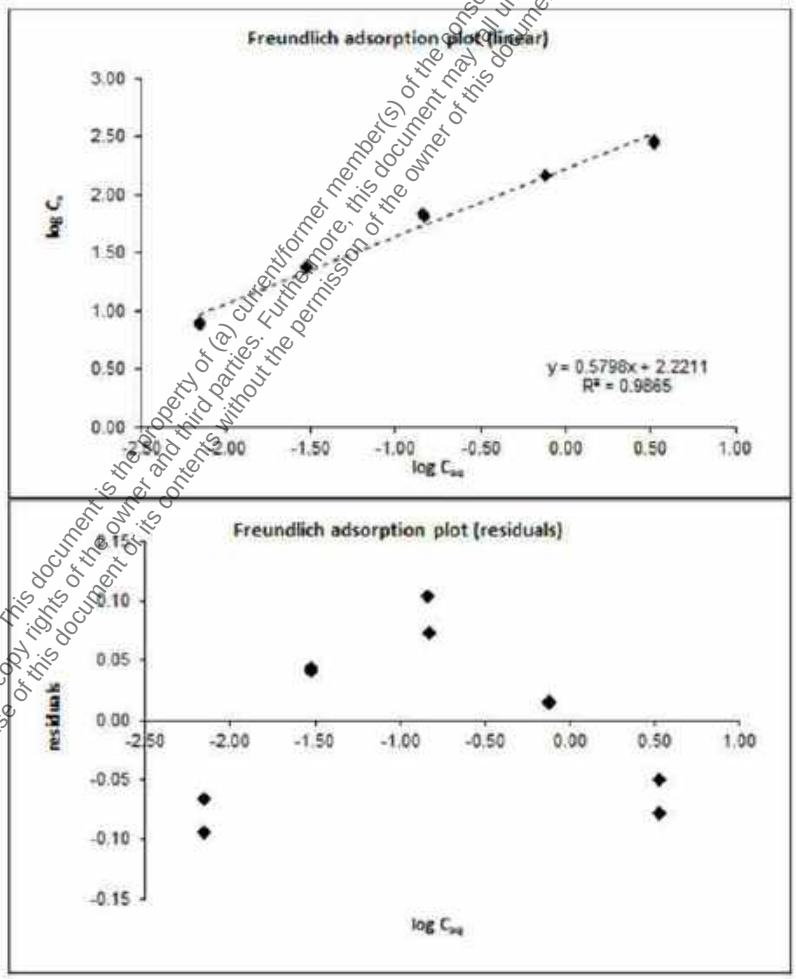


Figure 7.1.3.1.1-14: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 4

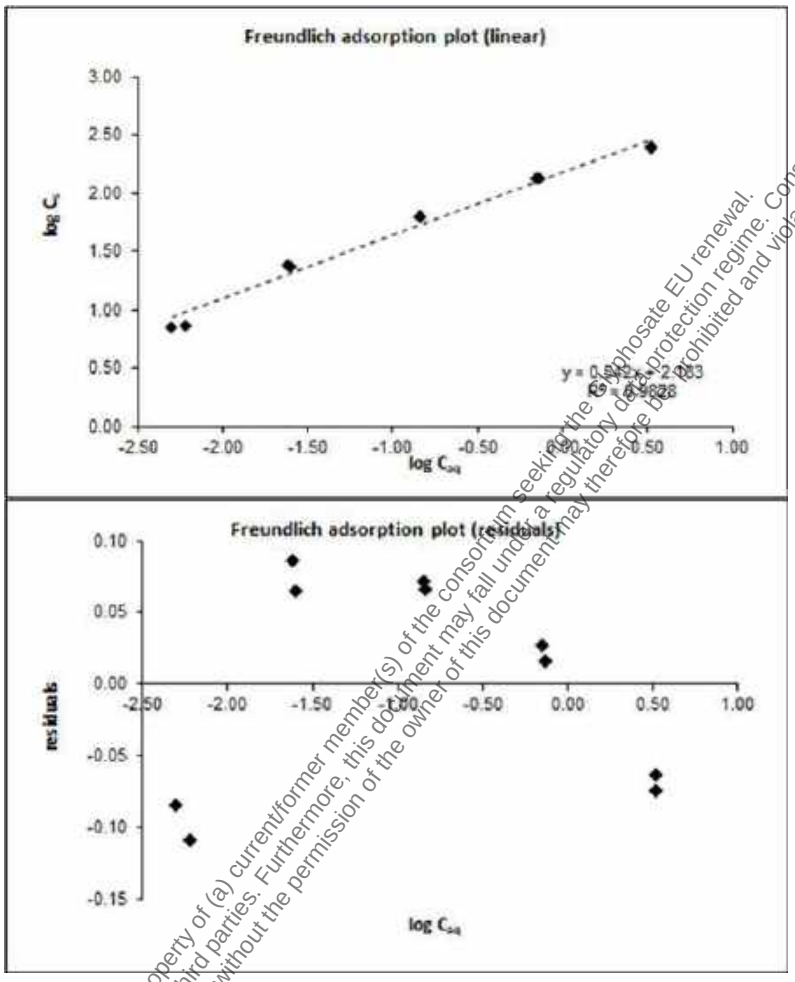


Figure 7.1.3.1.1-15: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 5

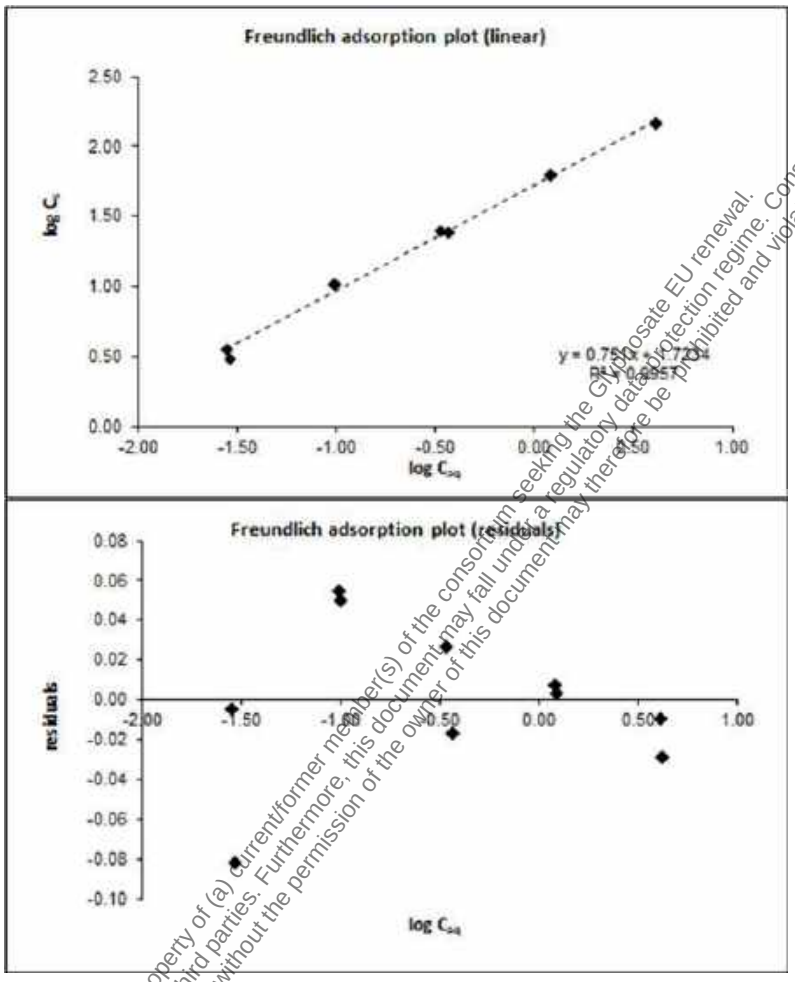


Figure 7.1.3.1.1-16: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 6

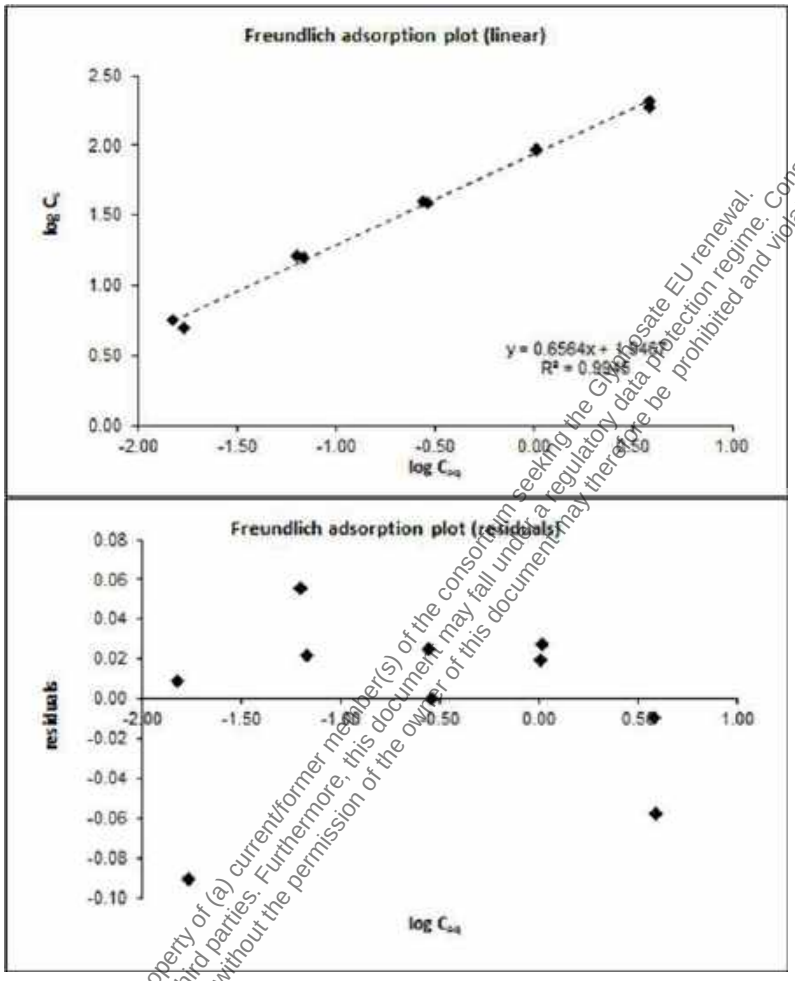


Figure 7.1.3.1.1-17: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 7

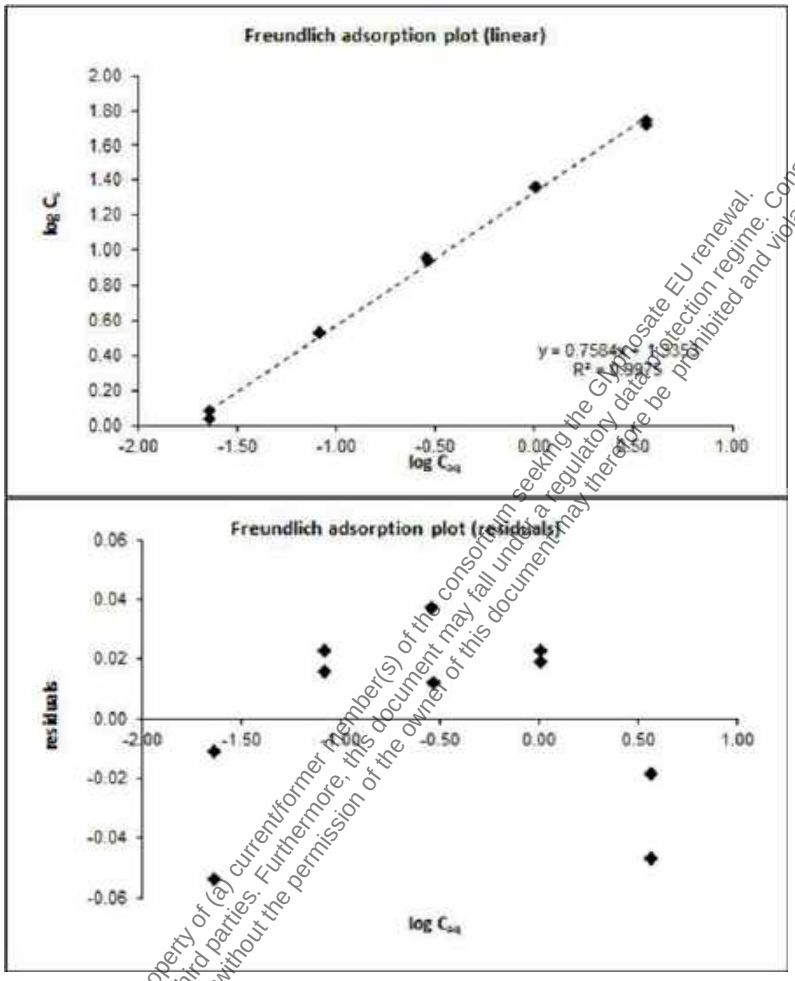
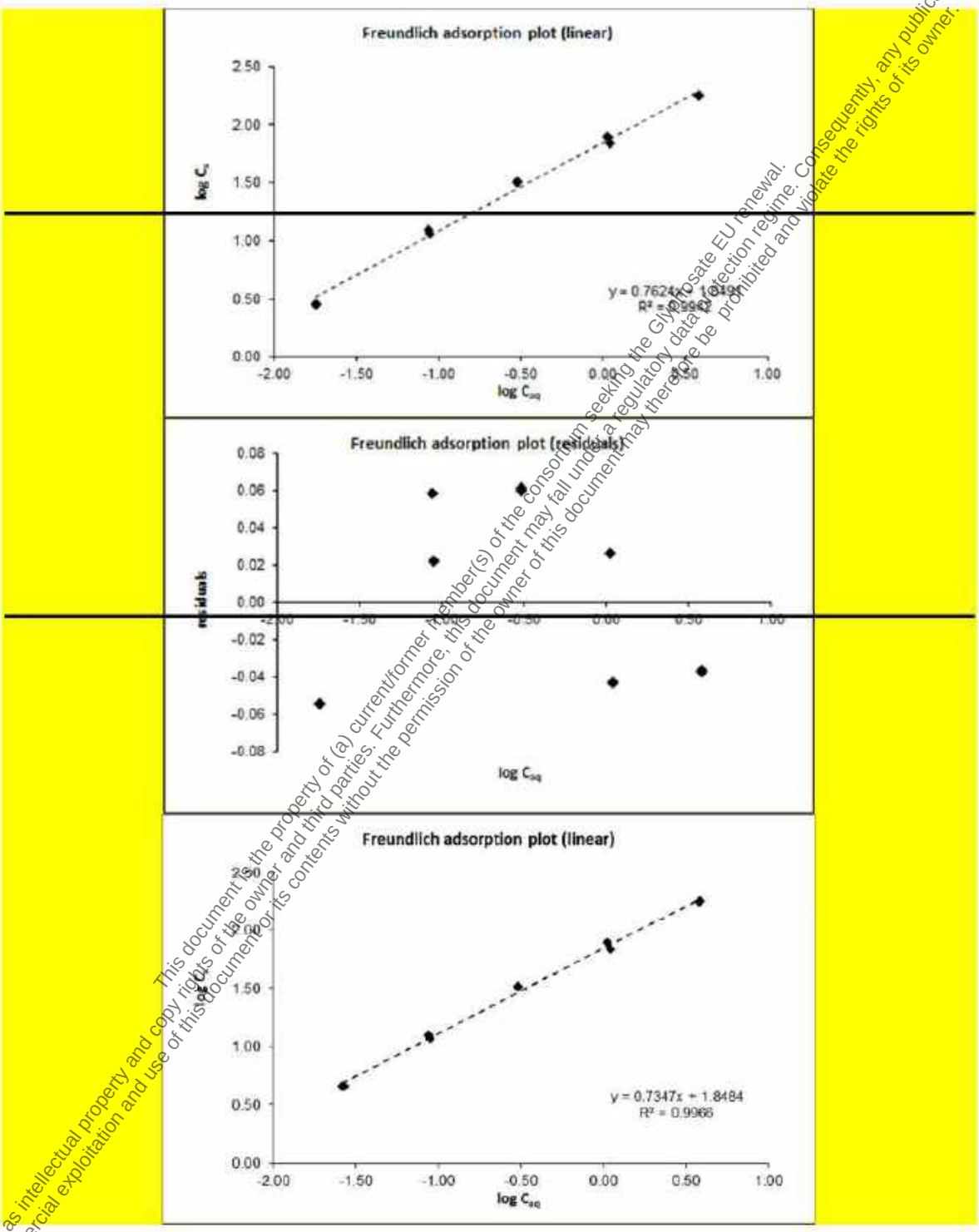


Figure 7.1.3.1.1-18: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 8



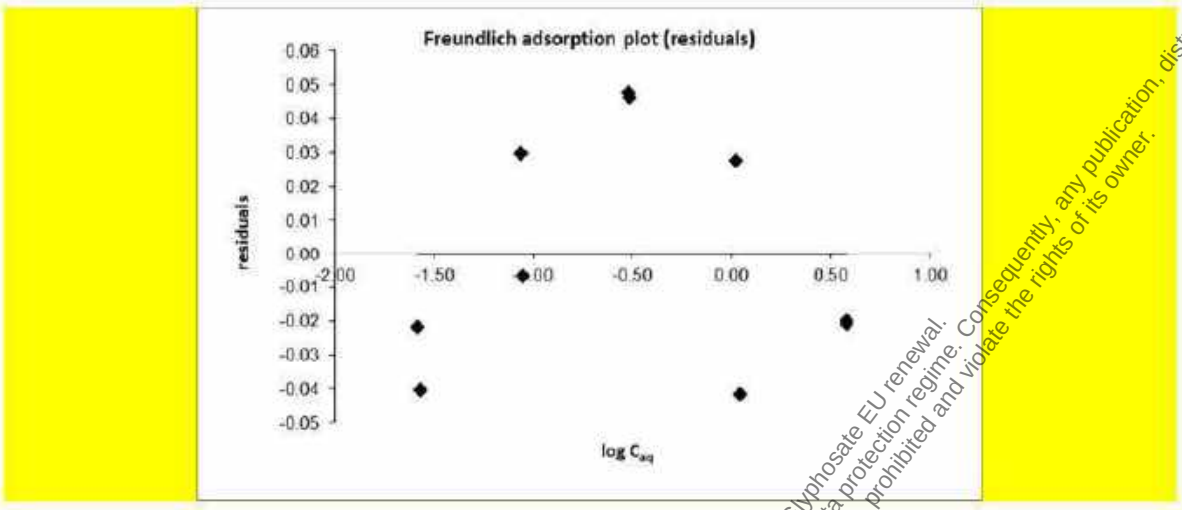


Figure 7.1.3.1.1-19: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 9

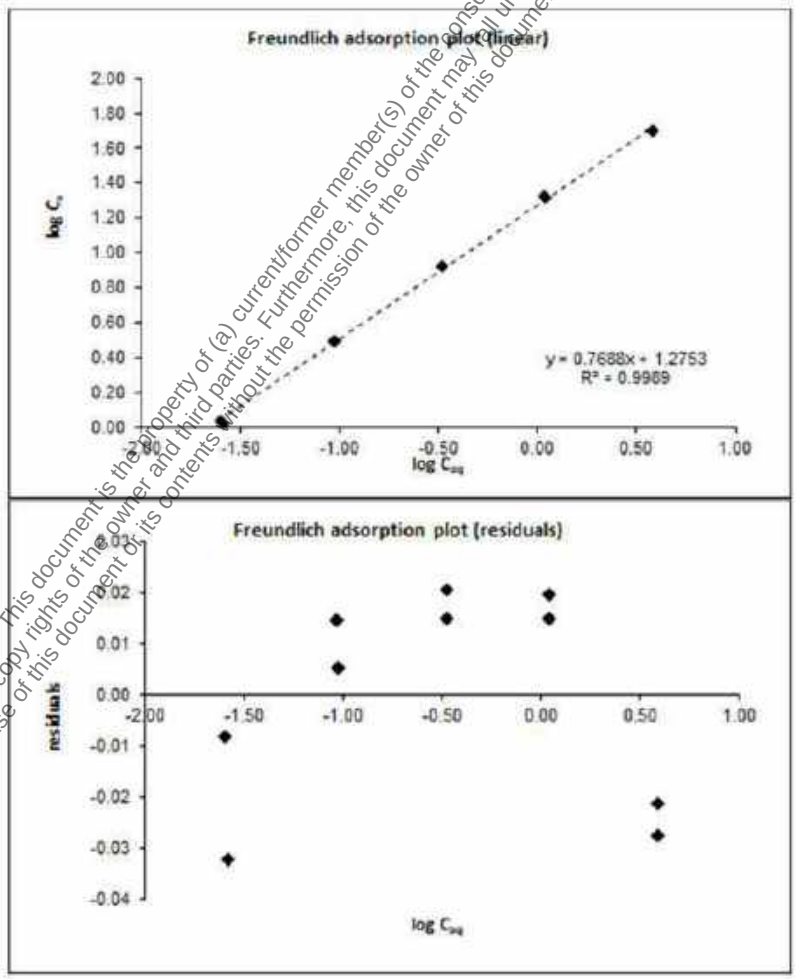
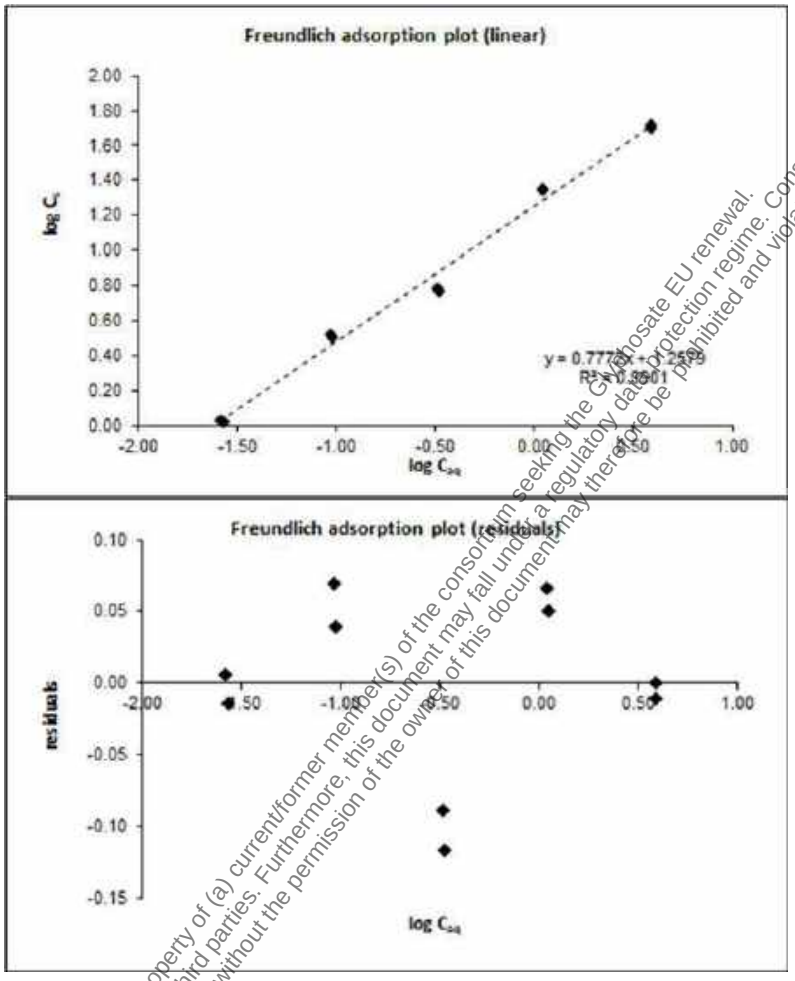


Figure 7.1.3.1.1-20: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 10



1. Information on the study

Data point:	CA 7.1.3.1.1/003
Report author	[redacted]
Report year	2001
Report title	Adsorption/desorption of glyphosate on soil
Report No	320164
Document No	
Guidelines followed in study	OECD Guideline 106 US EPA OPPTS 835.1220 SETAC Procedures of Assessing the Environmental Fate and Ecotoxicity, Part 1, Section 4
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No parental mass balance established since no extraction of soils performed. Stability of test item investigated in aqueous supernatants only. - Recovery of radioactivity >90 % not given for all samples
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The adsorption/desorption behaviour of [^{14}C]glyphosate was studied in four soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 2 °C using the indirect method.

Soil	Origin	Texture (USDA)	pH	OC [%]
Speyer 2.1	Rheinzabern, Germany	Sand	6.0 [1]	0.56
Cranfield 115	Eversham, United Kingdom	Clay loam	7.4 [2]	1.7
Cranfield 164	Buxton, United Kingdom	Silt loam	6.5 [2]	3.0
Cranfield 243	Stoneleigh, United Kingdom	Sandy loam	4.3 [2]	1.1

[1] pH values were derived from aqueous $CaCl_2$ suspension
[2] pH values were derived from aqueous KCl suspension

For the definitive phase, the adsorption step was carried out at a soil to solution ratio of 1:100 for 24 hours using pre-equilibrated samples. Nominal test concentrations of glyphosate were 5.0, 2.0, 1.0, 0.2, and 0.04 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$.

The desorption phase was conducted using each soil and each treatment rate with one desorption cycle.

Mean material balances after 48 h of equilibration ranged from 80.5 to 90.3 % for Speyer 2.1, from 78.3 to 84.6 % for Cranfield 115, from 88.8 to 92.1 % for Cranfield 164, and from 90.4 to 93.7 % for Cranfield 243.

Stability of test item was investigated only in aqueous supernatants after adsorption and desorption. Recovery of glyphosate after 24 h of adsorption was 97 % for Speyer 2.1, 80 % for Cranfield 115, 91 % for Cranfield 164, and 95 % for Cranfield 243. After desorption, recovery of glyphosate was 84 % for Speyer 2.1, 16 % for Cranfield 115, 63 % for Cranfield 164, and 73 % for Cranfield 243. From these

results, it appears that glyphosate was not stable during the test and degraded in the presence of soil, primarily during the desorption phase of the isotherms experiment.

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms ranged from 57.4 to 56.9 mL/g for Speyer 2.1, 224 to 208 mL/g for Cranfield 115, 894 to 900 mL/g for Cranfield 164, and 222 to 223 mL/g for Cranfield 243. The Freundlich exponents 1/n were in the range of 0.59 to 0.73 across all soils. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 10 and 30 x 10^3 mL/g.

The desorption coefficients $K_{F(des)}$ of glyphosate calculated based on the Freundlich isotherms ranged from 139 to 148 mL/g for Speyer 2.1, 352 to 408 mL/g for Cranfield 115, 1460 to 1530 mL/g for Cranfield 164, and 362 to 366 mL/g for Cranfield 243. The Freundlich exponents 1/n were in the range of 0.62 to 0.72 across all soils. The corresponding, calculated $K_{F,OC(des)}$ values varied between 21 and 51 x 10^3 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate (PMG label)

Lot No.	3415135
Specific activity	1.89 GBq/mmol (56.5 mCi/mmol by mass spectral analysis)
Radiochemical purity	99 %
Chemical purity	99 %

2. Test Soils

The soils were collected before study fresh start from the upper soil horizon (0 to 22 cm), sieved to a particle size of ≤2 mm and air-dried prior to use. The soils history was known for the previous five years. A description of the soils used is summarised in the table below.

Table 7.1.3.1.1-10: Physico-chemical properties of test soils

Parameter	Results			
Soil Designation	Speyer 2.1	Cranfield 115	Cranfield 164	Cranfield 243
Horizon (cm)	20	0-10	15-22	5-15
Geographic Location				
City	Rheinzabern	Netherton, Evesham	Chelmorton, Buxton	Stoneleigh
State	Rheinland-Pfalz	Worcester	Derbyshire	Warwickshire
Country	Germany	United Kingdom	United Kingdom	United Kingdom
Textural Class (USDA)	Sand	Clay loam	Silt loam	Sandy loam
Sand (53 µm – 2 mm) (%)	90.2	43.74	15.95	71.93
Silt (2 µm – 53 µm) (%)	8.2	23.50	72.91	15.97
Clay (< 2 µm) (%)	1.7	32.76	11.14	12.10
pH				
- in $CaCl_2$	6.0	--	--	--
- in water	--	7.9	7.1	5.4
- in KCl	--	7.4	6.5	4.3
Organic Carbon	0.56	1.7	3.0	1.1
Organic Matter	0.97	2.9	5.2	1.9
Cation Exchange Capacity (meq/100 g)	4	19.6	18.1	3.3
Water Holding Capacity (%)	29	55.3	72.8	51.1
Moisture at 1/3 bar (%)	--	30.4	41.2	22.7

USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Polypropylene centrifuge tubes were used as test systems. The experiments were performed with duplicate soil samples.

In preliminary tests, the adsorption of glyphosate to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times, and the stability of glyphosate were determined.

For the definitive phase, the adsorption step was carried out using pre-equilibrated samples from air-dried soils in aqueous 0.01 M $CaCl_2$ solution at a soil-to-solution ratio of 1:100. Glyphosate was applied at nominal concentrations of 5.0, 2.0, 1.0, 0.2, and 0.04 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 24 hours in the dark at 20 ± 2 °C under continuous agitation.

The desorption step was performed by supplying pre-absorbed soil samples with fresh aqueous 0.01 M $CaCl_2$ solution. The resultant samples were re-equilibrated for 24 hours in the dark at 20 ± 2 °C under continuous agitation.

2. Analytical Procedures

After the adsorption step and desorption step, the aqueous supernatant was separated from the soil by centrifugation and the radioactivity in the supernatants was determined by liquid scintillation counting (LSC).

In the preliminary mass balance test, the aqueous supernatants were analysed by LSC and high performance liquid chromatography (HPLC) to determine the stability of glyphosate in aqueous supernatants. Soil samples were combusted followed by quantitation using LSC.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

For the definitive phase, mean material balances after 48 h of equilibration ranged from 80.5 to 90.3 % for Speyer 2.1, from 78.3 to 84.6 % for Cranfield 115, from 88.8 to 92.1 % for Cranfield 164, and from 90.4 to 93.7 % for Cranfield 243.

B. STABILITY OF TEST ITEM

Following adsorption and desorption steps of the definitive phase, stability of test item was investigated in single aqueous supernatants by chromatographic analysis. The recovery of glyphosate in aqueous supernatants of samples after 24 h of adsorption was 97 % for Speyer 2.1, 80 % for Cranfield 115, 91 % for Cranfield 164, and 95 % for Cranfield 243. After desorption, recovery of glyphosate in aqueous supernatants was 84 % for Speyer 2.1, 16 % for Cranfield 115, 63 % for Cranfield 164, and 73 % for Cranfield 243. From these results, it appears that glyphosate was not stable during the test and degraded in the presence of soil, primarily during the desorption phase of the isotherms experiment.

C. FINDINGS

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms ranged from 57.4 to 56.9 mL/g for Speyer 2.1, 224 to 208 mL/g for Cranfield 115, 894 to 900 mL/g for Cranfield 164, and 222 to 223 mL/g for Cranfield 243. The Freundlich exponents 1/n were in the range of 0.59 to 0.73 across all soils. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 10 and 30 x 10^3 mL/g.

The desorption coefficients $K_{F(des)}$ of glyphosate calculated based on the Freundlich isotherms ranged from 139 to 148 mL/g for Speyer 2.1, 352 to 408 mL/g for Cranfield 115, 1460 to 1530 mL/g for Cranfield 164, and 362 to 366 mL/g for Cranfield 243. The Freundlich exponents 1/n were in the range of 0.62 to 0.72 across all soils. The corresponding, calculated $K_{F, OC(des)}$ values varied between 21 and 51 x 10^3 mL/g.

Table 7.1.3.1.1-11: [^{14}C]Glyphosate: Adsorption parameters in soil at 20 °C

Soil	Replicate	Adsorption			
		K_F [10^2 mL/g]	1/n	R^2	$K_{F, OC}$ [10^3 mL/g]
Speyer 2.1	A	0.574	0.60	0.9879	10
	B	0.569	0.60	0.9840	10
Cranfield 115	A	2.24	0.67	0.9898	13
	B	2.08	0.64	0.9925	12
Cranfield 164	A	8.94	0.72	0.9925	30
	B	9.00	0.73	0.9952	30
Cranfield 243	A	2.22	0.59	0.9886	20
	B	2.23	0.59	0.9895	20

Table 7.1.3.1.1-12: [^{14}C]Glyphosate: Desorption parameters in soil at 20 °C

Soil	Replicate	Desorption			
		K_F [10^3 mL/g]	1/n	R^2	$K_{F, OC}$ [10^3 mL/g]
Speyer 2.1	A	0.139	0.71	0.9967	25
	B	0.148	0.72	0.9974	26
Cranfield 115	A	0.408	0.70	0.9897	24
	B	0.352	0.67	0.9893	21
Cranfield 164	A	1.53	0.72	0.9936	51
	B	1.46	0.71	0.9953	48
Cranfield 243	A	0.366	0.62	0.9934	33
	B	0.362	0.62	0.9937	33

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms ranged from 0.574 to 9.00 × 10^2 mL/g across all soils. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 10 and 30 × 10^3 mL/g.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study had been assessed as invalid during AIR2 – following the citation in the RAR, 2015 (Outcome of the discussions in the Pesticides Peer Review Meeting 126, February 2015): "It was also noted that desorbed glyphosate was degrading in soil solution within the equilibrium time of batch experiments, though it was noted that the Van Noorloos & Slangen experiment the equilibrium time was longer and more degradation of glyphosate was apparent. On balance the experts considered that the results of the Van Noorloos & Slangen experiments should be excluded from the dataset as the longer batch equilibrium time (compared to other investigations or investigations where soils were sterilised) meant that degradation of glyphosate that occurred during the study resulted in lower confidence in these data." In light of the requirements of the EU Evaluators Checklist, the applicant agrees with this assessment.

A further evaluation of the results is therefore regarded as not necessary.

Assessment and conclusion by RMS:

Though the study does not fulfil the requirements as set out in the EU Evaluators Checklist, the results of the study were summarised formally below.

Table 7.1.3.1.1-13: Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Speyer 2.1	Cranfield 115	Cranfield 164	Cranfield 243
Adsorption method	-	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:100	1:100	1:100	1:100
Parental mass balance (at highest conc.)	%	- [1]	- [1]	- [1]	- [1]
Adsorbed percentage	%	21.9-76.3	57.5-93.5	89.4-98.3	57.1-96.3
K_D x (soil:solution ratio)		0.3-3.1	1.4-14.6	8.8-57.2	1.4-26.0
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	57.153 (49.374-66.158)	215.760 (181.947-255.857)	902.915 (726.288-1122.497)	222.843 (184.679-268.894)
$^{ads}1/n$ (95 % confidence interval)	-	0.603 (0.545-0.662)	0.656 (0.604-0.707)	0.729 (0.680-0.779)	0.593 (0.541-0.644)
$^{ads}R^2$	-	0.986	0.991	0.993	0.989
$^{ads}K_{F,OC}$	L/kg OC	10206	12692	30097	20259
K_{FE} / K_F	-	- [2]	- [2]	- [2]	- [2]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] A parental mass balance was not established in the course of the study.
[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to a missing parental mass balance providing the f-factor necessary for the calculations.

Figure 7.1.3.1.1-21: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Speyer 2.1

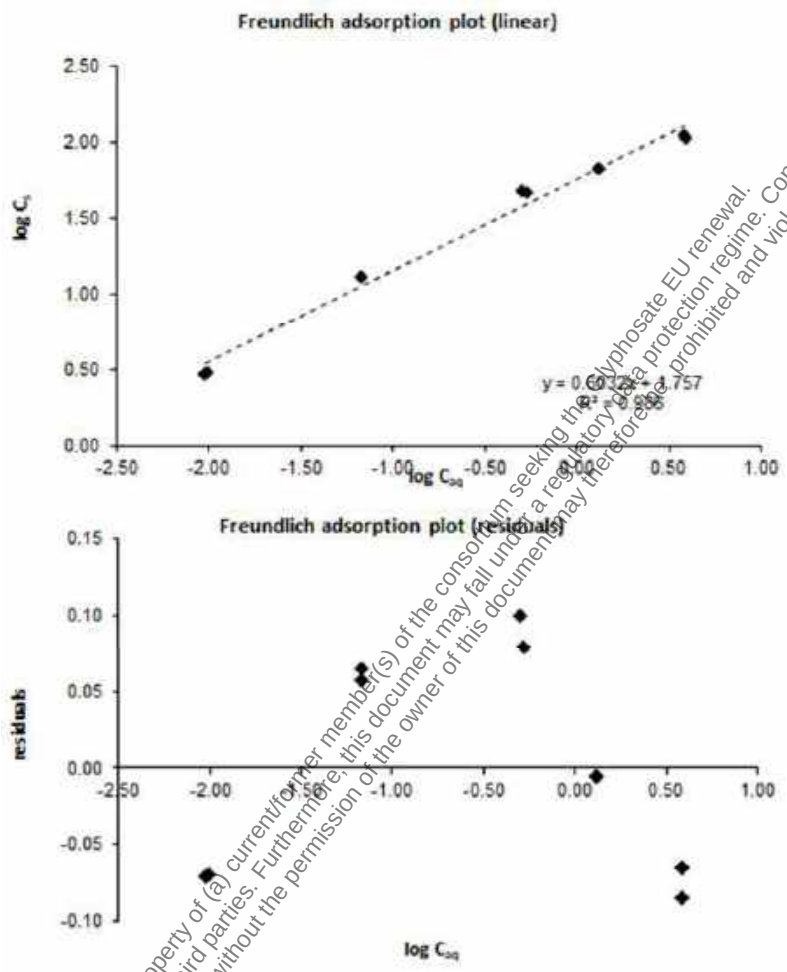


Figure 7.1.3.1.1-22: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Cranfield 115

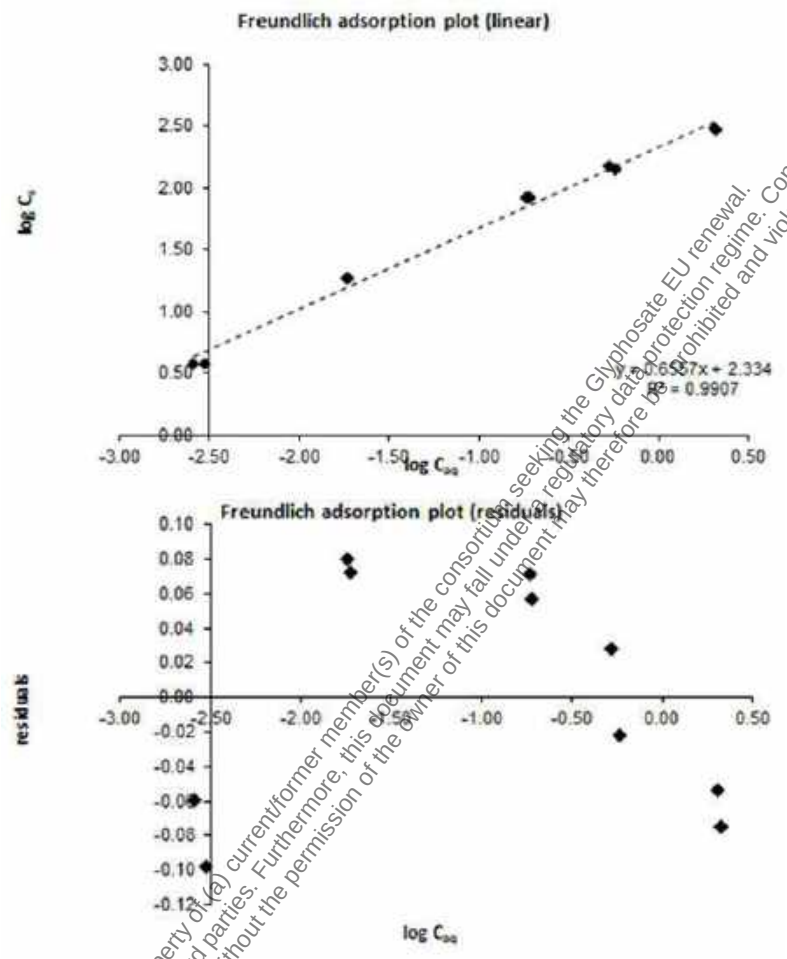


Figure 7.1.3.1.1-23: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Cranfield 164

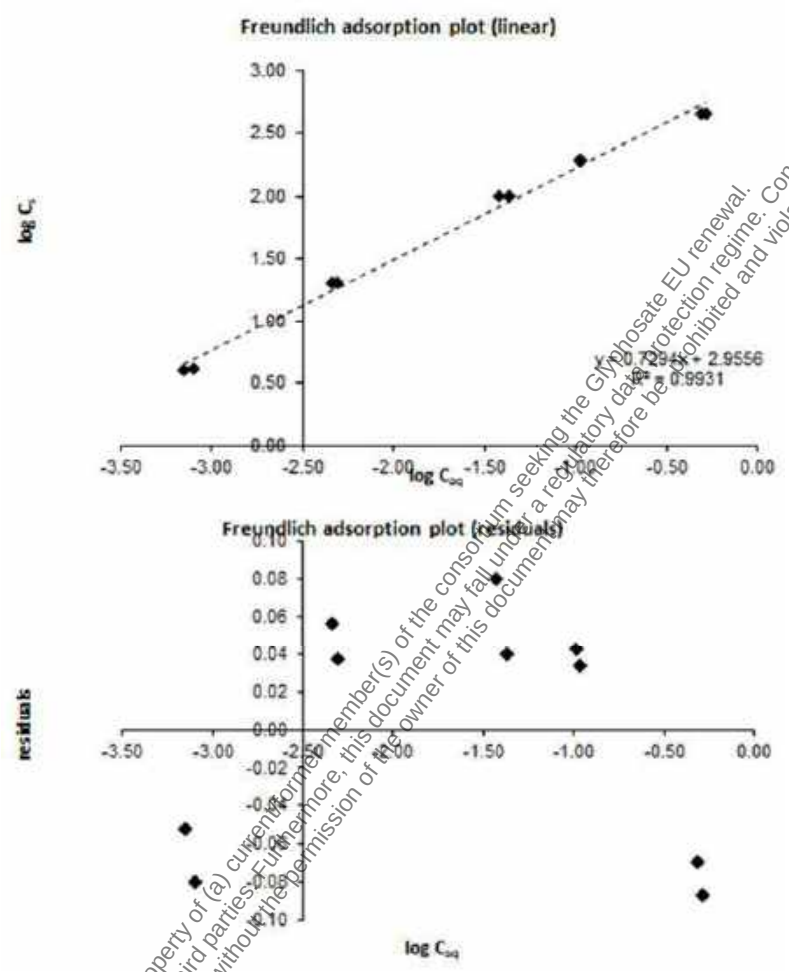
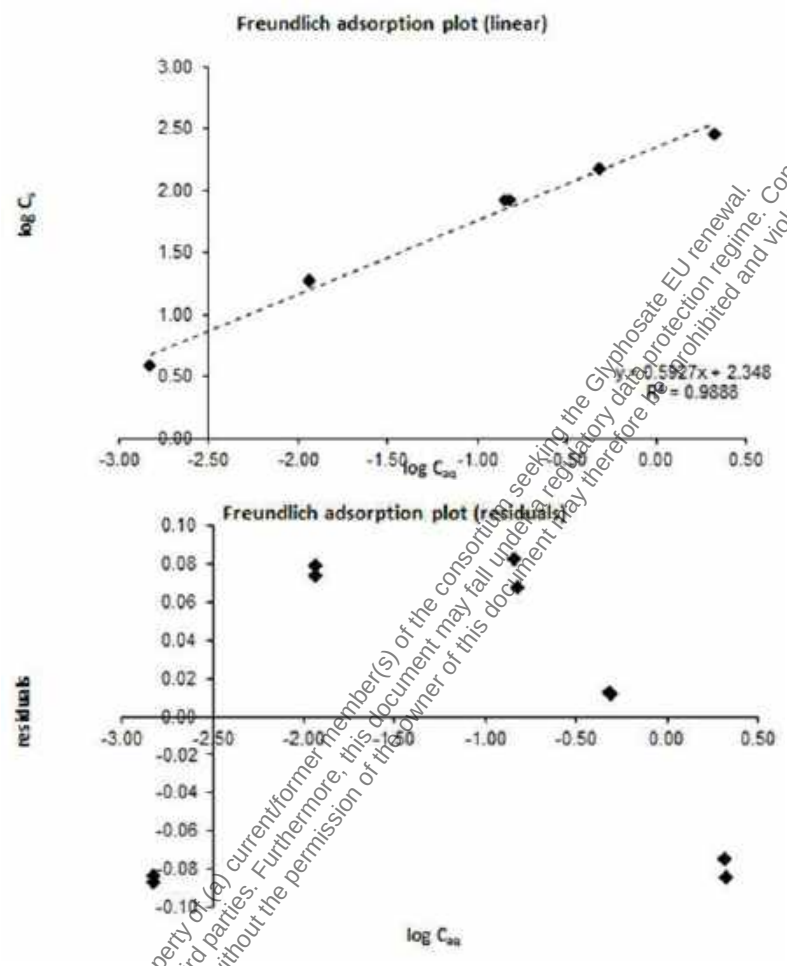


Figure 7.1.3.1.1-24: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Cranfield 243



1. Information on the study

Data point:	CA 7.1.3.1.1/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: adsorption and desorption properties in 5 soils
Report No	RJ2152B
Document No	
Guidelines followed in study	OECD Guideline 106 US EPA 163-1
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Indications that test item was not stable to clearly fulfil parental mass balance criterion (degradation >10 % AR in supernatant and soil extracts for respective fraction reported) - Results of parental mass balance not reported in detail thus test cannot be evaluated following the indirect method approach
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The adsorption/desorption behaviour of [^{14}C]glyphosate was studied in five soils in batch equilibrium experiments in the laboratory at 20 ± 2 °C using the indirect method.

Soil	Origin	Texture (USDA)	pH [1]	OM [%]
Lilly Field	Churt, Surrey, England	Sand	5.7	0.5
Visalia	Visalia, CA, USA	Sandy loam	8.4	1.0
Wisborough Green	Wisborough, Sussex, England	Silty clay loam	5.7	3.9
Champaign	Champaign, IL, USA	Silty clay loam	6.2	3.7
18 Acres	Bracknell, Berkshire, England	Sandy loam	7.4	3.1

[1] pH values were derived from soil:water (1:2) suspension

For the definitive phase, the adsorption step was carried out at a soil to solution ratio of 1:20 for 4 hours using sterilized, pre-equilibrated air-dried soils. Nominal concentrations of glyphosate were 2.0, 1.0, 0.2, 0.1, and 0.05 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$. The desorption phase was conducted by supplying pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ for each soil and concentration with one desorption cycle for 21 hours.

Material balances were established for all concentrations tested for soil Visalia only. For all other soils material balances were established for one test concentration (0.2 µg/mL) only. After adsorption/desorption recovery of radioactivity ranged from 84 to 105 % for soil Visalia. For the remaining soils material balances (duplicates of 0.2 µg/mL samples) were 97 and 98 % for soil Champaign, 87 to 95 % for soil Wisborough Green, 96 to 97 % for soil 18 Acres soil and 88 to 93 % for soil Lilly Field.

The TLC analysis of aqueous supernatants and soil extracts showed a single major metabolite aminomethylphosphonic acid (AMPA) in addition to parent glyphosate. Only relative amounts of glyphosate and AMPA in aqueous supernatants and soil extracts were reported. Results show that recovery of parent glyphosate was always <90 % of the radioactivity in aqueous adsorption supernatant and soil extract. The only exception was aqueous adsorption supernatant of the 1.0 µg/mL sample of soil Visalia with 94 % relative glyphosate recovery. However, glyphosate in the soil extract of this sample amounted to 67 % only with 9.9 % AMPA formed.

The percentage of glyphosate adsorbed onto the soil ranged from 78 to 93 % (mean 87 %) in soil Lilly Field from 31 to 54 % (mean 44 %), in soil Visalia from 97 to 98 % (mean 97 %), in soil Wisborough Green, in soil Champaign from 97 to 98 % (mean 98 %) and in soil 18 Acres from 85 to 94 % (mean 91 %).

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the four test soils ranged from 9.4 to 700 mL/g. The Freundlich exponents 1/n were in the range of 0.72 to 0.94, demonstrating a decrease in adsorption with increasing rate of application, there was however no saturation of adsorption sites at the highest rate of application. The corresponding, calculated $K_{F, OC(ads)}$ values ranged from 1600 to 33000 mL/g.

The desorption coefficients corrected for organic carbon, $K_{F, OC(des)}$, ranged from 3000 to 56000 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate (PMG label)	
Lot No.	Not provided
Specific activity	1.67 GBq/mmol
Radiochemical purity	95 %
Chemical purity	Not provided

2. Test Soils

The soils were sieved to a particle size of ≤2 mm and were air-dried prior to use. The soils were gamma irradiated with between 25 and 40 kGy before application. A description of the soils used is in the table below.

Table 7.1.3.1.1-14: Physico-chemical properties of test soils

Parameter	Results				
Soil Designation	Lilly Field	Visalia	Wisborough Green	Champaign	18 Acres
Geographic Location					
City	Churt	Visalia	Wisborough Green	Champaign	Warfield, Bracknell
State	Surrey	California	Sussex	Illinois	Berkshire
Country	England	United States	England	United States	England
Textural Class (USDA)	Sand	Sandy loam	Silty clay loam	Silty clay loam	Sandy loam
Sand (50 µm – 2 mm) (%)	92	69	8	12	58
Silt (2 µm – 50 µm) (%)	4	18	60	52	23
Clay (< 2 µm) (%)	4	13	32	36	19
pH in soil:water (1:2)	5.7	8.4	5.7	6.2	7.4
Organic Carbon (%) [1]	0.29	0.58	2.27	2.15	1.80
Organic Matter (%)	0.5	1.0	3.9	3.7	3.1

Table 7.1.3.1.1-14: Physico-chemical properties of test soils

Cation Exchange Capacity (meq/100 g)	1.8	7.3	11.9	28.3	14.4
Water Holding Capacity					
at 1/3 bar (%)	3.1	10.4	30.9	22.7	17.1
at 15 bar (%)	1.1	4.8	19.8	13.5	10.4

[1] Calculated using the conversion factor as follows: % organic carbon = % organic matter / 1.72
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Teflon® centrifuge tubes with self-sealing caps were used as test systems. The experiments were performed in duplicate.

In preliminary tests, the appropriate adsorption and desorption equilibration times were determined.

For the definitive phase, the adsorption step was carried out using sterile air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:20. Glyphosate was applied at nominal concentrations of 2.0, 1.0, 0.2, 0.1, and 0.05 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 4 hours at 20 ± 2 °C under continuous agitation.

The desorption phase was performed by supplying pre-absorbed soil samples with fresh aqueous 0.01 M $CaCl_2$ solution. The resultant samples were re-equilibrated for 21 hours at 20 ± 5 °C under continuous agitation.

2. Analytical Procedures

After the adsorption step and desorption step, the aqueous supernatant was separated from the soil by centrifugation and radioactivity in the supernatants was determined by liquid scintillation counting (LSC).

In the mass balance test, two additional samples at each concentration for Visalia soil and two additional samples at 0.2 mg/L for the other soils were analysed for test substance by LSC after the adsorption step. After transferring the supernatant, the wet soil was extracted with phosphate buffer followed by two acetone washes. Glyphosate in the extracts was quantified by LSC. Soil samples were combusted followed by quantitation using LSC.

After the adsorption step and desorption step, aliquots of aqueous supernatants and soil extracts were analysed by thin layer chromatography (TLC) and radiodetection for degradates.
Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Material balances were established for all concentrations tested for soil Visalia only. For all other soils material balances were established for one test concentration (0.2 µg/mL) only. After adsorption/desorption recovery of radioactivity ranged from 84 to 105 % for soil Visalia. For the remaining soils material balances (duplicates of 0.2 µg/mL samples) were 97 and 98 % for soil Champaign, 87 to 95 % for soil Wisborough Green, 96 to 97 % for soil 18 Acres soil and 88 to 93 % for soil Lilly Field.

B. STABILITY OF TEST ITEM

The TLC analysis of aqueous and soil extracts showed a single major metabolite aminomethylphosphonic acid (AMPA) in addition to parent glyphosate. Only relative amounts of glyphosate and AMPA in aqueous supernatant and soil extracts were reported. Results show that recovery of parent glyphosate was always <90 % of the radioactivity in aqueous adsorption supernatant and soil extract. The only exception was

aqueous adsorption supernatant of the 1.0 µg/mL sample of soil Visalia with 94 % relative glyphosate recovery. However, glyphosate in the soil extract of this sample amounted to 67 % only with 9.9 % AMPA formed.

C. FINDINGS

The percentage of glyphosate adsorbed onto the soil ranged from 78 to 93 % (mean 87 %) in soil Lilly Field from 31 to 54 % (mean 44 %), in soil Visalia from 97 to 98 % (mean 97 %), in soil Wisborough Green, in soil Champaign from 97 to 98 % (mean 98 %) and in soil 18 Acres from 85 to 94 % (mean 91 %).

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the four test soils ranged from 9.4 to 700 mL/g. The Freundlich exponents 1/n were in the range of 0.72 to 0.94, demonstrating a decrease in adsorption with increasing rate of application, there was however no saturation of adsorption sites at the highest rate of application. The corresponding, calculated $K_{F, OC(ads)}$ values ranged from 1600 to 33000 mL/g.

The desorption coefficients corrected for organic carbon, $K_{F, OC(des)}$, ranged from 3000 to 56000 mL/g.

Table 7.1.3.1.1-15: [^{14}C]Glyphosate: Percentage adsorbed to soil (mean values)

Soil	Test Concentration [mg/L]				
	2.0	1.0	0.2	0.1	0.05
Lilly Field	78	85	90	93	92
Visalia	31	31	53	51	54
Wisborough Green	97	97	97	97	98
Champaign	97	98	98	98	98
18 Acres	85	88	93	94	94

Table 7.1.3.1.1-16: [^{14}C]Glyphosate: Adsorption and desorption parameters in soil at 20 °C

Soil	Adsorption				Desorption
	K_F	1/n	R^2	$K_{F, OC}$	$K_{F, OC}$
Lilly Field	64	0.75	0.99	22000	50000
Visalia	9.4	0.72	0.99	1600	3000
Wisborough Green	470	0.93	1.00	21000	21000
Champaign	700	0.94	0.98	33000	56000
18 Acres	90	0.76	1.00	5000	6600

III. CONCLUSIONS

Glyphosate was strongly adsorbed in the five soils tested. The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the four test soils ranged from 9.4 to 700 mL/g. The Freundlich exponents 1/n were in the range of 0.72 to 0.94, demonstrating a decrease in adsorption with increasing rate of application, there was however no saturation of adsorption sites at the highest rate of application. The corresponding, calculated $K_{F, OC(ads)}$ values ranged from 1600 to 33000 mL/g.

The desorption coefficients corrected for organic carbon, $K_{F, OC(des)}$, ranged from 3000 to 56000 mL/g.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The assessment of data in the test was performed using the indirect method to calculate adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, the established PMB was insufficient with regard to test item stability to fulfil this criterion. Although, only relative amounts of glyphosate and AMPA in aqueous supernatants and soil extracts are reported it can be stated that degradation of test item glyphosate was >10 % since the relative recoveries were with only one single exception below 90 %.

The data of the study are therefore considered as supportive infomration. It is noted that the raw data of the study possibly could provide additional information (i.e. chromatographic results of soil extracts) to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

A further evaluation of results according to the EU OECD 106 Evaluators Checklist is presented for information.

Assessment and conclusion by RMS:

Though the study does not fulfil the requirements as set out in the EU OECD 106 Evaluators Checklist, the results of the study were summarised formally below.

Table 7.1.3.1.1-17: Glyphosate: Results of evaluation of data according to EU OECD 106 Evaluators Checklist

	Units	Lillyfield	Visalia	Wisborough Green	Champaign	18 Acres [3]
Adsorption method	-	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:20	1:20	1:20	1:20	1:20
Parental mass balance (at highest conc.)	%	<90 [1]	<90 [1]	<90 [1]	<90[1]	<90 [1]
Adsorbed percentage	%	76.5-92.3	27.0-52.8	96.4-97.4	97.2-98.3	84.3-94.2
K_D x (soil:solution ratio)		3.5-12.3	0.4-1.2	28.3-38.9	35.9-58.6	5.7-16.8
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	64.547 (39.825-104.618)	9.426 (6.376-13.936)	470.551 (251.947-878.828)	708.663 (227.856-2204.044)	89.272 (74.195-107.414)
$^{ads}1/n$ (95 % confidence interval)	-	0.746 (0.619-0.873)	0.725 (0.562-0.888)	0.935 (0.808-1.061)	0.938 (0.725-1.151)	0.762 (0.717-0.807)
$^{ads}R^2$	-	0.992	0.985	0.995	0.985	0.999
$^{ads}K_{F,OC}$	L/kg OC	21516	1571	20459	33746	4960
K_{FE} / K_F	-	- [2]	- [2]	- [2]	- [2]	- [2]

Table 7.1.3.1.1-17: Glyphosate: Results of evaluation of data according to EU OECD 106 Evaluators Checklist

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] Values of parental mass balance not reported. However, degradation of glyphosate reported to be >10 %.
[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to a missing results of the parental mass balance providing the f-factor necessary for the calculations.
[3] Typo in Table 11 p.44 for concentration in aq. solution resulting in different results if used in the checklist as reported. Correct value should be 0.0156 µg/L instead of 0.156 µg/L.

Figure 7.1.3.1.1-25: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Lillyfield

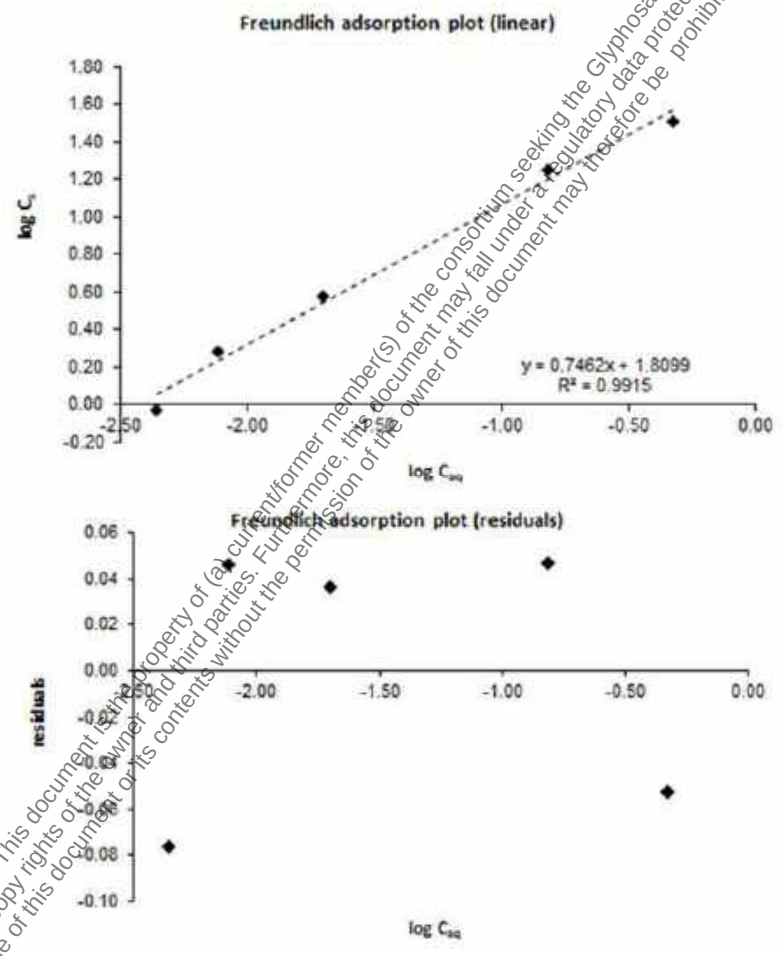


Figure 7.1.3.1.1-26: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Visalia

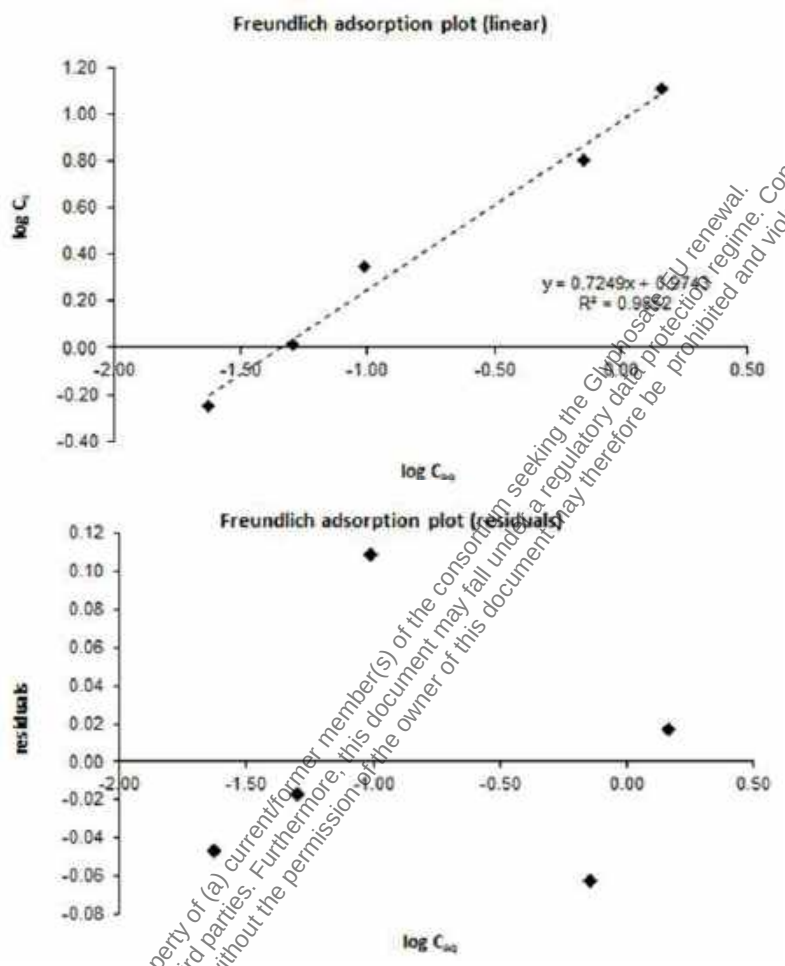


Figure 7.1.3.1.1-27: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Wisborough Green

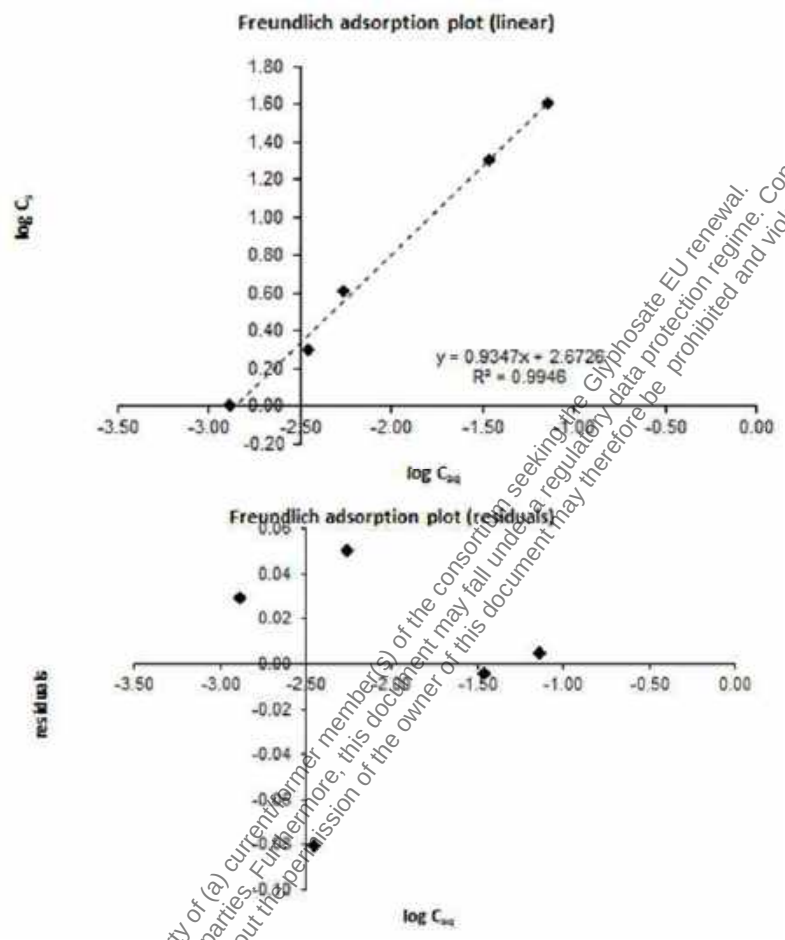


Figure 7.1.3.1.1-28: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Champaign

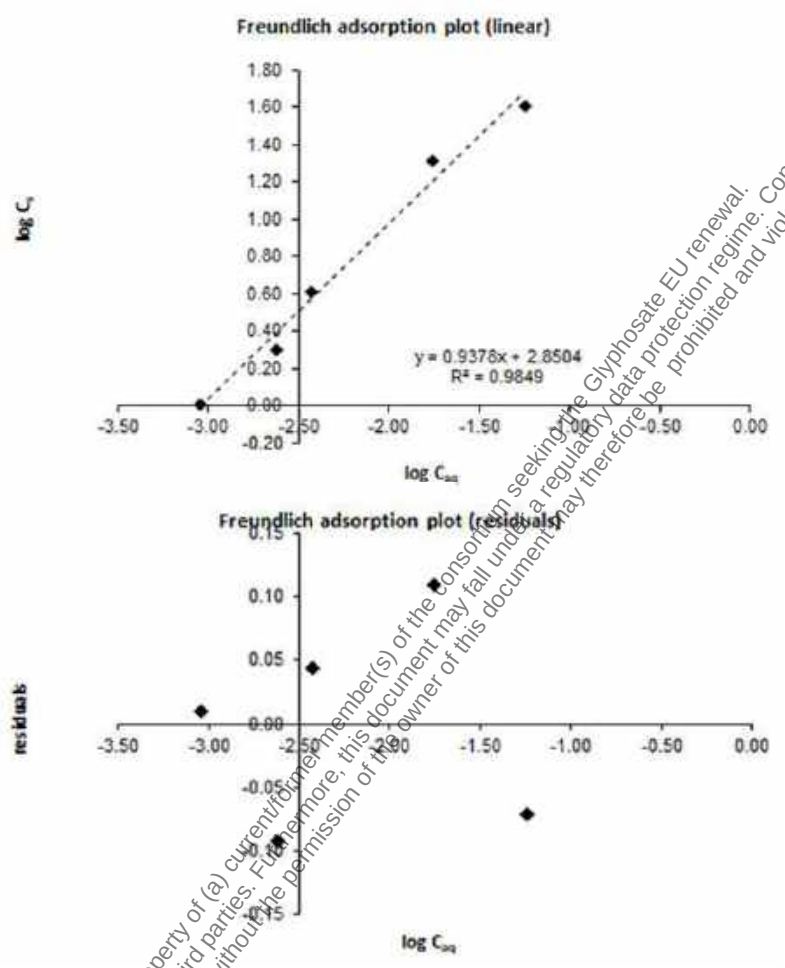
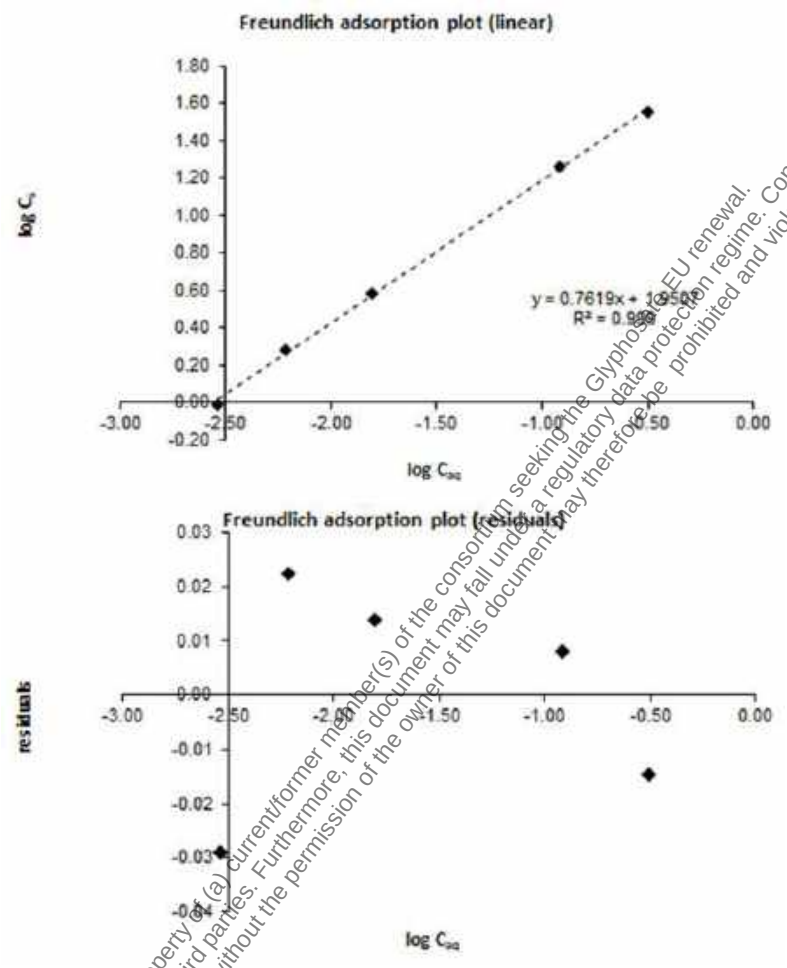


Figure 7.1.3.1.1-29: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 18 Acres



1. Information on the study

Data point:	CA 7.1.3.1.1/005
Report author	[redacted]
Report year	1996
Report title	Glyphosate: determination of adsorption and desorption properties based on the OECD method 106
Report No	95-111-1020
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): – Stability of test item not demonstrated sufficiently – Extraction steps performed but no results of chromatographic analysis presented – Chromatographic results only of second desorption step shown, residues in soil after desorption > 10 % – Results of parental mass balance not given in detail – No pre-equilibration of soils
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The adsorption/desorption behaviour of [^{14}C]glyphosate was studied in three soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 1 °C using the indirect method.

Soil	Origin	Texture (DIN 4220)	pH [1]	OC [%]
Speyer 2.1	Germany	Sand	5.9	0.62
Speyer 2.2	Germany	Loamy sand	5.6	2.32
Speyer 2.3	Germany	Loamy sand	6.4	1.22

[1] pH values were derived from aqueous 0.01 M $CaCl_2$ suspensions

For the definitive phase, the adsorption step was carried out at a soil-to-solution ratio of 1:5 for 5 hours using air-dried soils conditioned in aqueous 0.01 M $CaCl_2$ solution prior to application. Nominal concentrations of glyphosate were 4.66, 0.98, 0.19, and 0.04 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$.

The desorption study was conducted using each soil and each concentration of glyphosate with two desorption cycles.

Mean material balances after 5 hours of equilibration were 95.3 % AR for soil Speyer 2.1, 96.0 % AR for soil Speyer 2.2 and 95.8 % AR for soil Speyer 2.3.

The percentage of glyphosate adsorbed onto the soil ranged from 84.3 to 92.9 % for soil Speyer 2.1, from 93.7 to 97.3 % for soil Speyer 2.2 and from 87.6 to 94.7 % for soil Speyer 2.3.

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the three test soils ranged from 29.52 to 71.72 mL/g. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 3091 and 4762 mL/g.

The desorption coefficients $K_{F(des)}$ of glyphosate after the first desorption step calculated based on the Freundlich isotherms of the three test soils ranged from 39.59 to 118.07 mL/g. The corresponding, calculated $K_{F, OC(des)}$ values varied between 3245 and 8178 mL/g. The desorption coefficients $K_{F(des)}$ of glyphosate after the second desorption calculated based on the Freundlich isotherms of the three test soils ranged from 51.72 to 123.6 mL/g. The corresponding, calculated $K_{F, OC(des)}$ values varied between 4240 and 9401 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate (PMG label)

Lot No.	D1
Specific activity	11.7 MBq/mg (316 µCi/mg)
Radiochemical purity	99.6 %
Chemical purity	Not provided

2. Test Soils

The soils were collected prior to study start (depth of ≈ 20 cm) and sieved to a particle size of 2 mm. The soils were air-dried, the moisture content was adjusted, and soils were conditioned in aqueous 0.01 M $CaCl_2$ solution before application. A description of the soils used is in the table below.

Table 7.1.3.1.1-18: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	Speyer 2.1	Speyer 2.2	Speyer 2.3
Geographic Location			
City	Not provided	Not provided	Not provided
State	Not provided	Not provided	Not provided
Country	Germany	Germany	Germany
Textural Class (DIN 4220)	Sand	Loamy sand	Loamy sand
Sand (> 63 µm) (%)	88.4	81.2	60.9
Silt (2 µm – 63 µm) (%)	9.8	13.4	29.6
Clay (< 2 µm) (%)	1.9	5.5	9.5
pH			
- in $CaCl_2$	5.9	5.6	6.4
Organic Carbon (%)	0.62	2.32	1.22
Organic Matter (%) [1]	1.07	3.99	2.10
Cation Exchange Capacity (meq/100 g)	5.0	10.9	10.2
Water Holding Capacity maximum (g/100 g dry soil)	31	48	39

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon × 1.72
DIN: Deutsches Institut für Normung

B. STUDY DESIGN

1. Experimental Conditions

Teflon® centrifuge tubes with Teflon® screw caps were used as test systems. The experiments were performed in triplicate.

For the preliminary phase, tests on glyphosate adsorption to the surface of the test vessels at all test concentrations and the appropriate adsorption equilibration times at the highest test concentration (5 mg/L) were performed. Two additional samples per soil were prepared at the highest test concentration (5 mg/L) for the material balance test.

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:5 (5 g soil/ 25 mL solution). ^{14}C-Glyphosate was applied at nominal concentrations of 4.66, 0.98, 0.19, and 0.04 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 5 hours in the dark at 20 ± 1 °C under continuous agitation.

In each desorption phase, the supernatant was removed and fresh aqueous 0.01 M $CaCl_2$ solution was added to the tubes. The resultant samples were re-equilibrated for 24 hours at 20 ± 1 °C under continuous agitation.

2. Analytical Procedures

After the adsorption step and each desorption step, the aqueous supernatant was separated from the soil by centrifugation and the amount of glyphosate in the supernatants was analysed by LSC.

In the mass balance test, two additional samples per soil were analysed by LSC after the adsorption step. After transferring the supernatant, the wet soil was extracted three times with phosphoric acid in $CaCl_2$. Glyphosate in the extracts was quantified by LSC. Unextractable radioactivity in the soil samples was determined by combustion followed by quantitation using LSC.

After the adsorption step and each desorption step, aliquots of the supernatant of the 4.66 mg/L test solution were analysed by high-performance liquid chromatography (HPLC) for degradates.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Mean material balances after 5 hours of equilibration were 95.3 % of applied radioactivity (% AR) for soil Speyer 2.1, 96.0 % AR for soil Speyer 2.2 and 95.8 % AR for soil Speyer 2.3.

B. STABILITY OF TEST ITEM

HPLC analysis of the supernatant of the 4.66 mg/L test solutions showed that after the first desorption step 65.9 % of the radioactivity present in the Speyer 2.3 sample consisted of degradates (mainly AMPA). After the second desorption step, 71.8 % of the radioactivity present in the Speyer 2.3 sample consisted of degradates. No degradates were found for the Speyer 2.1 and Speyer 2.2 soils. Results of the chromatographic analyses of aqueous supernatants and soil extracts after the adsorption step were not reported.

C. FINDINGS

The percentage of glyphosate adsorbed onto the soil ranged from 84.3 to 92.9 % for soil Speyer 2.1, from 93.7 to 97.3 % for soil Speyer 2.2 and from 87.6 to 94.7 % for soil Speyer 2.3. Results are presented in the table below:

Table 7.1.3.1.1-19: [^{14}C]Glyphosate: Percentage adsorbed to soil (mean values)

	Test Concentration [mg/L]			
	4.66	**0.98**	**0.19**	**0.04**
Speyer 2.1	84.3	89.9	91.9	92.9
Speyer 2.2	93.7	96.0	96.9	97.3
Speyer 2.3	87.6	92.2	93.8	94.7

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the three test soils ranged from 29.52 to 71.72 mL/g. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 3091 and 4762 mL/g. Results are presented in the table below:

Table 7.1.3.1.1-20: [^{14}C]Glyphosate: Adsorption parameters in soil at 20 °C

Soil	Adsorption			
	$K_{F(ads)}$ [mL/g]	**1/n**	**R^2**	**$K_{F(ads), OC}$ [mL/g]**
Speyer 2.1	29.52	0.843	0.997	4762
Speyer 2.2	71.72	0.840	0.997	3091
Speyer 2.3	37.72	0.837	0.997	3092

The desorption coefficients $K_{F(des)}$ of glyphosate after the first desorption calculated based on the Freundlich isotherms of the three test soils ranged from 39.59 to 118.07 mL/g. The corresponding, calculated $K_{F, OC(des)}$ values varied between 3245 and 8178 mL/g.

The desorption coefficients $K_{F(des)}$ of glyphosate after the second desorption calculated based on the Freundlich isotherms of the three test soils ranged from 51.72 to 123.6 mL/g. The corresponding, calculated $K_{F, OC(des)}$ values varied between 4240 and 9401 mL/g. Results are presented in the table below:

Table 7.1.3.1.1-21: [^{14}C]Glyphosate: Desorption parameters in soil at 20 °C

Soil	First desorption				Second desorption			
	$K_{F (des)}$ [mL/g]	**1/n**	**R^2**	**$K_{F, OC(des)}$ [mL/g]**	**$K_{F (des)}$ [mL/g]**	**1/n**	**R^2**	**$K_{F, OC (des)}$ [mL/g]**
Speyer 2.1	50.70	0.910	0.999	8178	58.29	0.883	0.999	9401
Speyer 2.2	118.07	0.878	0.999	5089	123.6	0.844	0.999	5327
Speyer 2.3	39.59	0.872	0.999	3245	51.72	0.899	0.999	4240

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 29.52 to 71.72 mL/g. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 3091 and 4762 mL/g.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The assessment of data in the test was performed using the indirect method to calculate adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, the data reported do not allow for the conclusion that the test substance was stable.

The data of the study are therefore considered as supportive infomration. It is noted that the raw data of the study possibly could provide additional information (i.e. chromatographic results of soil extracts) to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

A further evaluation of results according to the EU OECD 106 Evaluators Checklist is presented for information.

Assessment and conclusion by RMS:

Though the study does not fulfil the requirements as set out in the EU OECD 106 Evaluators Checklist, the results of the study were summarised formally below.

Table 7.1.3.1.1-22: Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Speyer 2.1	Speyer 2.2 [3]	Speyer 2.3
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%		- [1]	- [1]
Adsorbed percentage	%	84.8-93.3	94.1-97.7	88.3-95.1
K_D x (soil:solution ratio)		5.6-14.0	15.9-42.7	7.5-19.7
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	29.608 (26.468-33.119)	71.570 (62.714-81.676)	37.733 (33.615-42.355)
$^{ads}1/n$ (95 % confidence interval)		0.845 (0.815-0.874)	0.839 (0.811-0.867)	0.838 (0.809-0.866)
$^{ads}R^2$	-	0.998	0.998	0.998
$^{ads}K_{F,OC}$	L/kg OC	4775	3085	3093
K_{FE} / K_F	-	- [2]	- [2]	- [2]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] Values of parental mass balance not reported. No information on NER. After 2nd desorption step >80 % remain adsorbed.
[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to a missing results of the parental mass balance providing the f-factor necessary for the calculations.
[3] Typo in Table 4 p.39 for concentration in aq. solution of third replicate of lowest test concentration resulting in different results if used in the checklist as reported. Correct value should be 0.00100 µg/L instead of 0.00010 µg/L.

Figure 7.1.3.1.1-30: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Speyer 2.1

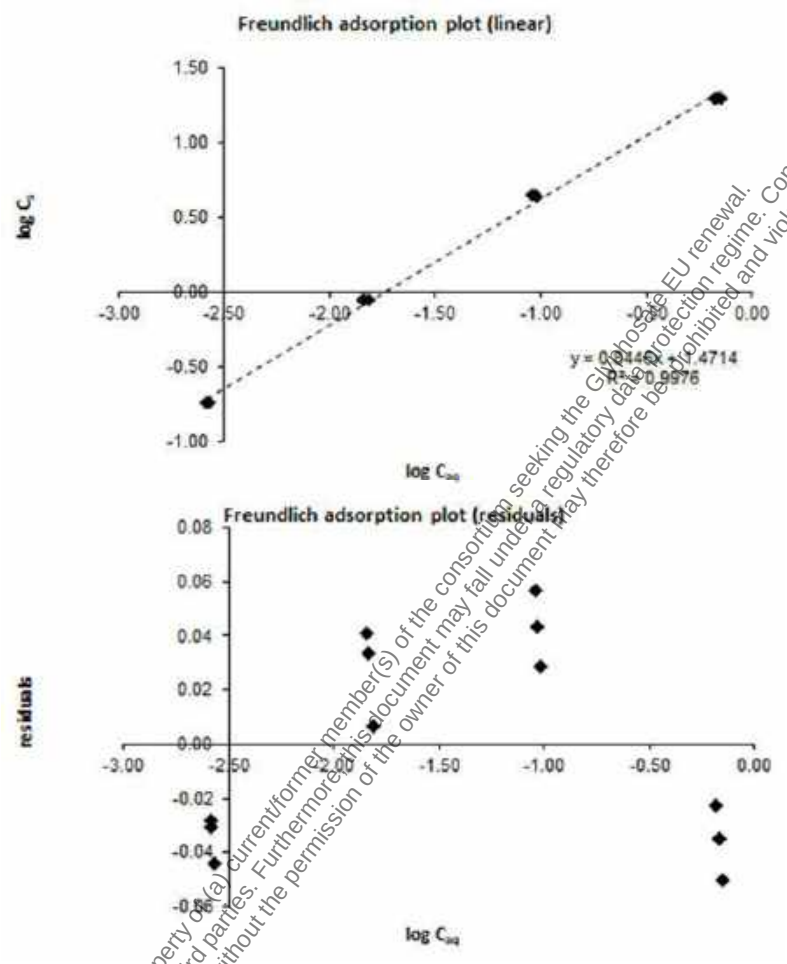


Figure 7.1.3.1.1-31: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Speyer 2.2

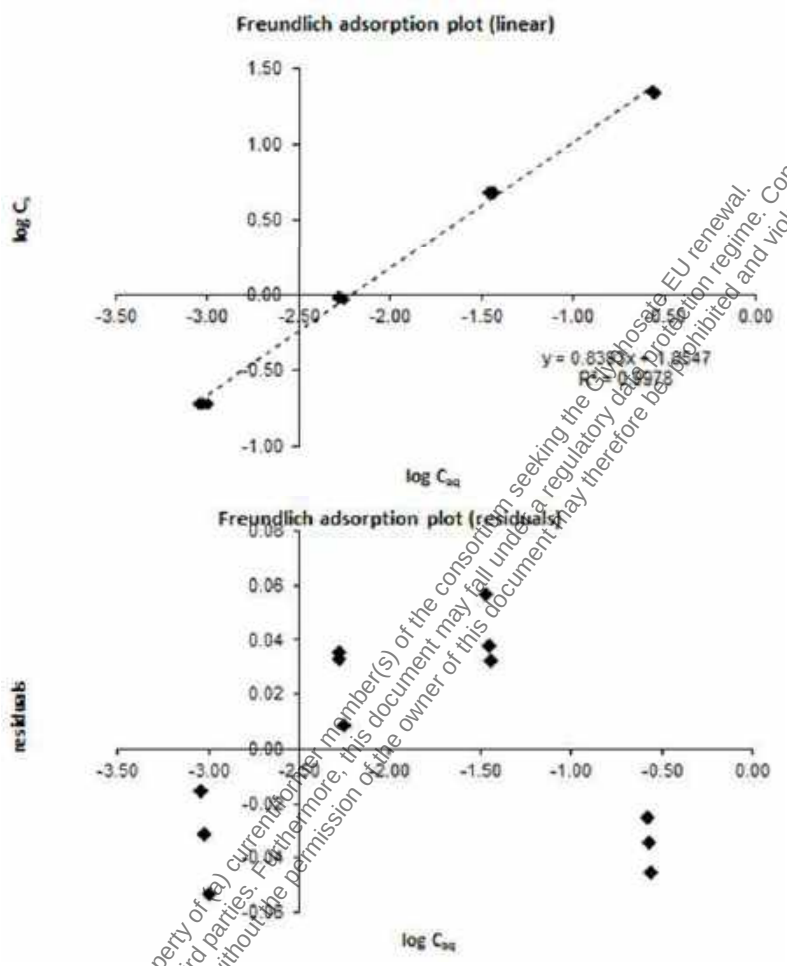
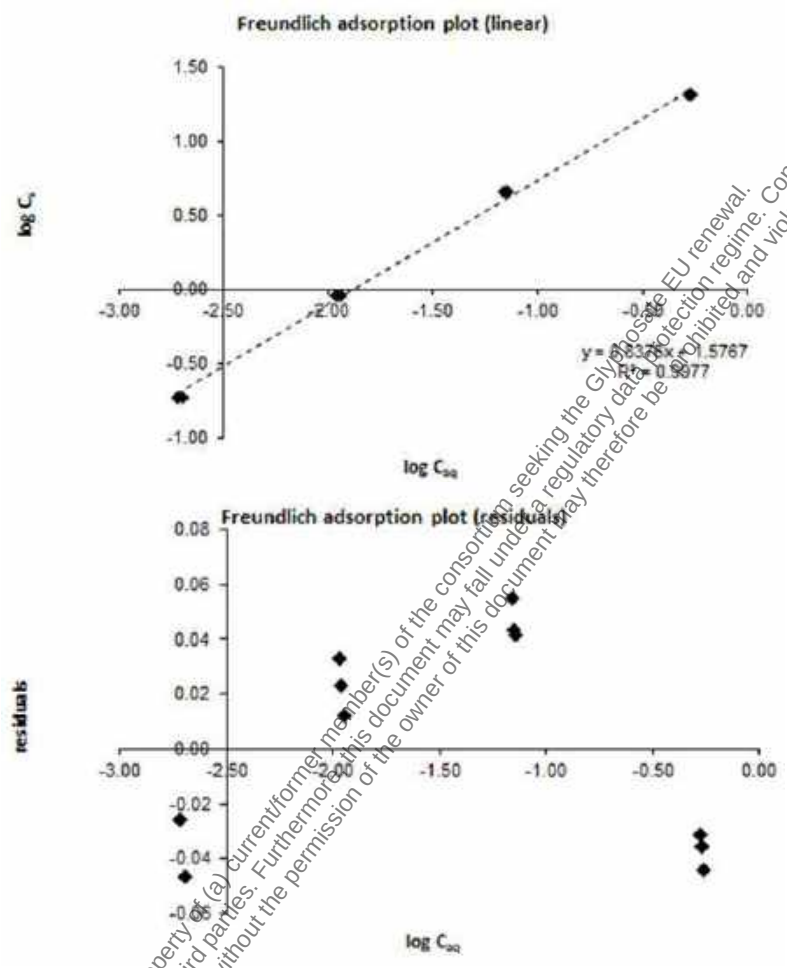


Figure 7.1.3.1.1-32: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Speyer 2.3



1. Information on the study

Data point:	CA 7.1.3.1.1/006
Report author	[redacted]
Report year	1994
Report title	Adsorption and desorption of glyphosate on three types of soil
Report No	ALK-AA-15001
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - KCl instead of $CaCl_2$ solution used - No pre-tests for soil:solution ratio, equilibration time, test item stability and adsorption of test item to test vessel surface - No parental mass balance established - No pre-equilibration of samples - Adsorption isotherm with three concentrations covering one order of magnitude
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The adsorption/desorption behaviour of non-labelled glyphosate was studied in three sterilized soils in batch equilibrium experiments in the laboratory at ambient temperature using the indirect method.

Soil/Sediment	Origin	Texture [1]	pH [2]	Organic matter [%]
Sand	Hungary	Sand	5.27	0.84
Loam	Hungary	Sandy loam	7.64	1.88
Clay	Hungary	Clayey loam	4.42	2.36

[1] Classification system not reported
[2] pH values were derived in KCl

The equilibration solution used was 0.01 M aqueous KCl. For the definitive phase, the adsorption step was carried out at a soil to solution ratio of approximately 1:10 for 24 hours. The nominal test concentrations of glyphosate were 30, 100 and 300 mg/L. The desorption phase was conducted by diluting the equilibrium solution of the pre-adsorbed soil specimens with fresh 0.01 M aqueous KCl for the highest test concentration of each soil with three desorption cycles each for 24 hours.

Values for the Freundlich adsorption coefficient $K_{F(ads)}$ of glyphosate ranged from 0.0047 to 0.3595 mL/g for the three soils tested. Values of the Freundlich exponent 1/n were in the range of 0.68282 to 1.1777. Values for the Freundlich desorption coefficient $K_{F(des)}$ of glyphosate ranged from 0.1435 to 46.6874 mL/g for the three soils tested. Values of the Freundlich exponent 1/n were in the range of -0.2651 to 0.10188 for desorption.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Identification:	Glyphosate isopropylamine salt (non-labelled)
Batch No.:	451760292
Chemical purity:	99.3 %
Content referring to glyphosate acid	72.7 %

2. Test Soils

The soils were sampled from the field, air dried, sieved to 2 mm and sterilized. The characterisation of test soils used is summarised in the table below.

Table 7.1.3.1.1-23: Physico-chemical properties of test soils

Parameter		**Results**		
Soil Designation		Sand	Loam	Clay
Geographic Location				
City		Nyíregyháza	Nyíregyháza	Tiszaadony
State		-	-	-
Country		Hungary	Hungary	Hungary
Textural Class		Sand	Sandy loam	Clayey loam
Sand	(50 µm – 2 mm) (%)	-	-	-
Silt	(2 µm – 50 µm) (%)	-	-	-
Clay	(< 2 µm) [(%)	4.79	22.69	35.60
pH in KCl		5.27	7.64	4.42
Organic matter [%]		0.84	1.88	2.36
Cation Exchange S		14.63	30.93	28.20

B. STUDY DESIGN

1. Experimental Conditions

Centrifuge tubes were used as test vessels. The experiments were performed in triplicate.

Preliminary tests were not performed. Soils were sterilised prior to application.

For the definitive phase the adsorption step was carried out at a soil-to-solution ratio of approximately 1:10 (i.e. 1 g soil and 10 mL 0.01 M KCl solution). Glyphosate was applied each at nominal concentrations of 30, 100 and 300 mg/L in aqueous 0.01 M KCl solution. The adsorption step was carried out for 24 hours under continuous agitation.

For each of the three successive desorption steps in total, 4 mL fresh aqueous 0.01 M KCl solution was added to pre-adsorbed soil samples of the highest concentration (still containing approx. 6 mL of adsorption supernatant) and the resultant samples were re-equilibrated for 24 hours under continuous agitation. The procedure was repeated for two further desorption steps.

2. Analytical Procedures

After each adsorption and desorption step, the aqueous supernatant was separated from the soil by centrifugation and the amount of glyphosate in the supernatants only was analysed by gas chromatography coupled with a thermionic ionization detector following derivatization of the samples. A method validation was not.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE
An overall recovery of test item in water and soil was not investigated.

B. STABILITY OF TEST ITEM
Stability of glyphosate was not demonstrated.

C. FINDINGS
The calculated concentrations in adsorption and desorption supernatants are shown in the tables below:

Table 7.1.3.1.1-24: Glyphosate: Concentration at start and after adsorption in supernatants (mean values of triplicates)

Soil	Initial concentration [µM/L]	Equilibrium concentration [µM/L]
Sand	177.42	85.12
	592.16	267.37
	1773.38	690.76
Loam	177.42	40.72
	592.16	195.69
	1773.38	477.34
Clay	177.42	12.34
	592.16	34.68
	1773.38	275.25

Table 7.1.3.1.1-25: Glyphosate: Concentration in desorption supernatants of samples of highest test concentration (mean values of triplicates)

Soil	Desorption time [h]	Initial concentration [µM/L]	Equilibrium concentration [µM/L]
Sand	24	1497.08	460.73
	48	1312.78	332.39
	72	1179.71	200.93
Loam	24	1588.35	475.79
	48	1398.03	639.49
	72	1142.23	337.44
Clay	24	1663.28	253.84
	48	1617.03	211.62
	72	1552.38	150.07

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated on the three test soils ranged from 0.0047 to 0.3595 mL/g and the desorption coefficient $K_{F(des)}$ values ranged from 0.1435 to 46.6874 mL/g. Results are presented in the table below:

Table 7.1.3.1.1-26: Glyphosate: Adsorption and desorption parameters in different soils

Soil	Adsorption			Desorption		
	$K_{F(ads)}$ [mL/g]	1/n	r^2	$K_{F(des)}$ [mL/g]	1/n	r^2
Sand	0.0047	1.1777	0.9992	0.1435	0.06264	0.8092
Loam	0.0466	0.88725	0.9823	46.6874	-0.2651	0.4400
Clay	0.3595	0.68282	0.9675	8.2565	0.10188	0.9125

III. CONCLUSIONS

Values for the Freundlich adsorption coefficient $K_{F(ads)}$ of glyphosate ranged from 0.0047 to 0.3595 mL/g for the three soils tested. Values of the Freundlich exponent 1/n were in the range of 0.68282 to 1.1777. Values for the Freundlich desorption coefficient $K_{F(des)}$ of glyphosate ranged from 0.1435 to 46.6874 mL/g for the three soils tested. Values of the Freundlich exponent 1/n were in the range of -0.2651 to 0.10188 for desorption.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The test was performed using the indirect method for determination of adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, no PMB was determined in this test to fulfil this criterion. Furthermore, potassium chloride solution was used instead of calcium chloride as aqueous phase and method validation is missing. The study is thus considered as invalid.

A further evaluation of the results in view of the EU OECD 106 Evaluators Checklist is therefore regarded as not necessary.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/007
Report author	[redacted]
Report year	1993
Report title	Glyphosate isopropylaminesalt adsorption/desorption
Report No	PR93/017
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No pre-tests on soil:solution ratio and adequate equilibration time - No pre-equilibration of soils - No extraction of soil after the adsorption step (parental mass balance established after the desorption phase) - Low recovery of test item

Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The adsorption / desorption behaviour of glyphosate isopropylamine salt was studied in three soils in batch equilibrium experiments in the laboratory using the indirect method.

Soil	Origin	Texture (USDA)	pH	OC [%]
2.1	Not provided	Not provided	5.9	0.70
2.3	Not provided	Not provided	6.3	1.34
F3, 341	Not provided	Not provided	7.3	1.20

For the definitive phase, the adsorption step was carried out at a soil-to-solution ratio of 1:5 for 16 hours. The nominal amount of glyphosate applied as isopropylamine salt was 5 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$. The desorption phase was conducted by supplying the pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ for each soil with two desorption cycles each for 16 hours.

The total recoveries of test item in terms of parental mass balances following two desorption steps were 74.8 to 75.2 % for soil 2.1, 62.0 to 63.0 % for soil 2.3, and 32.6 to 34.4 % for soil F3, 341.

Most of the test item (89.9 to 94.6 %) was adsorbed to the soil and 6.3 to 7.4 % was desorbed following two desorption cycles.

The adsorption coefficients $K_{D(ads)}$ of glyphosate isopropylamine salt calculated on the three test soils ranged from 54.4 to 76.5 mL/g and the corresponding $K_{D, OC(ads)}$ values ranged from 4533 to 9486 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Glyphosate isopropylamine salt (non-labelled)
Lot No. 10819
Chemical purity 98 %

2. Test Soils
The characteristics of test soils is summarised in the table below.

Table 7.1.3.1.1-27: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	2.1 (#14292)	2.3 (#3101)	F3, 341 (F331)
Geographic Location			
City	Not provided	Not provided	Not provided
State	Not provided	Not provided	Not provided
Country	Germany	Germany	Germany
Textural Class			
(630 µm – 2 mm) (%)	4.5 ± 0.6	3.2 ± 0.6	1.1 ± 0.2
(200 µm – 630 µm) (%)	62.9 ± 2.4	32.5 ± 3.2	13.5 ± 0.3
(63 µm – 200 µm) (%)	20.0 ± 2.8	28.4 ± 2.9	25.2 ± 0.7
(20 µm – 63 µm) (%)	4.7 ± 2.0	16.4 ± 3.3	30.3 ± 0.5
(6 µm – 20 µm) (%)	2.5 ± 0.7	7.5 ± 1.2	10.0 ± 0.4
(2 µm – 6 µm) (%)	1.9 ± 0.8	3.9 ± 0.5	4.7 ± 0.3
(< 2 µm) (%)	3.5 ± 1.6	8.3 ± 1.4	15.2 ± 0.4
pH			
- in water	5.9	6.3	7.3
Organic Carbon	0.70 ± 0.07	1.34 ± 0.14	1.20 ± 0.07
Organic Matter [1]	1.20	2.30	2.06
Cation Exchange Capacity (mval/100 g)	4.9 ± 0.8	9.5 ± 0.9	13 ± 0.0
Water Holding Capacity maximum (g/100 g soil DW)	26.1	34.9 ± 1.6	45.7

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon × 1.72
DW: dry weight

B. STUDY DESIGN

1. Experimental Conditions

No preliminary tests were performed. For the definitive phase, the adsorption step was carried out at a soil-to-solution ratio of 1:5 (2 g soil/10 mL solution). Test item was applied at a nominal concentration of 5 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 16 hours under continuous agitation. In parallel control samples were prepared without soil and test solution only.

For each of the two successive desorption steps, fresh aqueous 0.01 M $CaCl_2$ solution was added to the pre-adsorbed soil samples the tubes. The resultant samples were re-equilibrated for 16 hours under continuous agitation followed by centrifugation.

2. Analytical Procedures

The aqueous supernatant after adsorption was separated by centrifugation and the glyphosate isopropylamine salt residues in the supernatant were analysed by gas chromatography (GC). The aqueous supernatant after each desorption step was separated by centrifugation. HPLC-clean up of the supernatant was performed to collect two fractions (fraction 1: AMPA, fraction 2: Glyphosate) and fractions were analysed by gas chromatography (GC). The limit of detection in tap water (method not validated for 0.01 M $CaCl_2$) was 0.0.2 µg/L for glyphosate and 0.06 µg/L for AMPA. For soil the limit of detection was 20 µg/kg for glyphosate and AMPA.

To determine the recovery of the test item, glyphosate isopropylamine salt was extracted from the soil with water and phosphoric acid following the desorption phase. Soil extracts were analysed by GC.

II. RESULTS AND DISCUSSION

A. STABILITY OF TEST ITEM

Total recoveries of test item in aqueous adsorption and desorption supernatants and soil extract following the desorption phase were 74.8 and 75.2 % for soil 2.1, 62.0 and 63.0 % for soil 2.3, and 32.6 and 34.4 % for soil F3, 341. The reason for parental mass balances ≤75 % was the formation of non-extractable residues following various extraction steps.

B. FINDINGS

Most of the glyphosate isopropylaminesalt (89.9 to 94.6 %) was adsorbed to the soil and 6.3 to 7.4 % was desorbed following two desorption cycles. Results are summarised in the table below:

Table 7.1.3.1.1-28: Glyphosate isopropylamine salt: Recovery in supernatant

Soil	Replicate	Percentage[1]	
		Adsorption	Desorption
2.1	1	9.3	7.4
	2	5.6	6.9
2.3	1	7.0	7.4
	2	5.4	6.3
F3, 341	1	10.1	6.3
	2	7.2	6.8

[1] Mean values expressed as percentage of applied glyphosate

The adsorption coefficients $K_{D(ads)}$ of glyphosate isopropylamine salt calculated on the three test soils ranged from 54.4 to 76.5 mL/g and the corresponding $K_{D, OC(ads)}$ values ranged from 4533 to 9486 mL/g. Results are presented in the table below:

Table 7.1.3.1.1-29: Glyphosate isopropylamine salt: Adsorption parameters in soil

Soil	Adsorption	
	$K_{D(ads)}$ [mL/g]	$K_{D, OC(ads)}$ [mL/g]
2.1	66.4	9486
2.3	76.5	5709
F3, 341	54.4	4533

III. CONCLUSIONS

The adsorption coefficients $K_{D(ads)}$ of glyphosate isopropylamine salt calculated on the three test soils ranged from 54.4 to 76.5 mL/g and the corresponding $K_{D, OC(ads)}$ values ranged from 4533 to 9486 mL/g.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The test was performed using the indirect method for determination of adsorption to soil since the concentration of the test item was determined in aqueous adsorption supernatant only and not in the soil phase (i.e. soil extracts). Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, no sufficient parental mass balance was established since the recovery of the test item was investigated following the desorption phase. Soil extraction and analysis of extracts for test item following the adsorption step was not performed.
The data are therefore regarded as supporting. It is noted that the raw data of the study possibly could provide additional information (i.e. chromatographic results of soil extracts) to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

A further evaluation of results according to the Evaluators Checklist is presented for information only.

Assessment and conclusion by RMS:

Though the study does not fulfil the requirements as set out in the EU Evaluators Checklist, the results of the study were summarised formally in the table below. No graphical and statistical evaluation according to Freundlich and the EU Evaluators Checklist is possible.

Table 7.1.3.1.1-30: Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Soil 2.1	Soil 2.2	Soil 2.3
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	75.0 [1]	62.5 [1]	33.5 [1]
Adsorbed percentage	%	90.8-94.4	93.0-94.6	90.0-92.8
K_D x (soil:solution ratio)		9.9-16.9	13.3-17.5	9.0-12.9
$^{ads}K_F$ [2] (95 % confidence interval)	L/kg dw	66.8	77.0	54.7
$^{ads}1/n$ (95 % confidence interval)	-	- [3]	- [3]	- [3]
$^{ads}R^2$	-	- [3]	- [3]	- [3]
$^{ads}K_{F,OC}$ [2]	L/kg OC	9545	5747	4560
K_{FE} / K_F	-	1.4	1.7	>3.5

Note: Values derived from the EU OECD 106 evaluators checklist may vary from those in the study reports due to rounding errors.
[1] Large amounts of NER formed.
[2] Only K_D because of one test concentration only
[3] Not applicable because of one test concentration only

1. Information on the study

Data point:	CA 7.1.3.1.1/008
Report author	[redacted]
Report year	1992
Report title	[14C-PMG] Glyphosate-Trimesium: Adsorption/desorption in four soils
Report No	RR92-016B
Document No	
Guidelines followed in study	US EPA-FIFRA N-163-1 40 CFR, Sec. 158.130 and 158.50
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Recovery of radioactivity <90 % for at least two test concentrations for all soils - No detailed parental mass balance reported - No pre-equilibration of soils performed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary

The adsorption / desorption behaviour of [^{14}C]glyphosate-trimesium was studied in four soils in batch equilibrium experiments in the laboratory in the dark at 25 ± 1 °C.

Soil	Texture (USDA)	pH	OC [%] [1]
Atterbery Silt Loam	Silt loam	5.6	1.5
Sorrento Loam	Loam	6.8	2.0
Visalia Sandy Loam	Sandy loam	7.4	0.4
Biggs Clay	Clay	6.1	1.2

[1] Calculated as: OC [%] = OM [%] / 2.0 (calculated within report)

For the definitive phase, the adsorption step was carried out at a soil to solution ratio of 1:5 for 4 hours. Nominal concentrations of glyphosate-trimesium were 12.4, 1.24, 0.124, and 0.0124 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$.

The desorption phase was conducted using each soil with one desorption cycle.

Mean material balances corrected for test vessel adsorption were 95.0 % AR for soil Atterbery Silt Loam (range from 83.0 to 111 % AR), 87.0 % AR for soil Sorrento Loam (range from 74.6 to 93.7 % AR), 92.3 % AR for soil Visalia Sandy Loam (range from 79.7 to 114 % AR), and 90.1 % AR for soil Biggs Clay (range from 71.9 to 103 % AR).

The relative amount of the test item in the soil extracts after the desorption phase accounted to 98.5 % for soil Atterbery Silt Loam, 98.4 % for soil Sorrento Loam, 97.1 % for soil Visalia Sandy Loam and 99.3 % for soil Biggs Clay. Aqueous supernatants after adsorption and desorption were not analysed.

The adsorption coefficients $K_{F(ads)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the four test soils ranged from 31.5 to 2060 mL/g. The Freundlich exponents n were in the range of 0.909 to 1.14. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 2860 and 179000 mL/g.

The desorption coefficients $K_{F(des)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the three test soils ranged from 40.4 to 3230 mL/g. The Freundlich exponents n were in the range of 0.901 to 1.09. The corresponding, calculated $K_{F, OC(des)}$ values varied between 3030 and 281000 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate-trimesium (^{14}C-methyl-glycine)	
Lot No.	PMS-363; 88J30
Specific activity	55.95 mCi/mmol
Radiochemical purity	95.0 ± 2.2 %
Chemical purity	Not provided

2. Test Soils

The characterisation of the test soils is summarised in table below.

Table 7.1.3.1.1-31: Physico-chemical properties of test soils

Parameter	Results			
Soil Designation	Atterbery Silt Loam	Sorrento Loam	Visalia Sandy Loam	Biggs Clay
Geographic Location				
City	Not provided	Not provided	Not provided	Not provided
State	Not provided	Not provided	Not provided	Not provided
Country	Not provided	Not provided	Not provided	Not provided
Textural Class (USDA)	Silt Loam	Loam	Sandy Loam	Clay
Sand (%)	16	38	57	21
Silt (%)	55	41	34	30
Clay (%)	28	21	9	49
pH (medium not reported)	5.6	6.8	7.4	6.1
Organic Carbon (%) [1]	1.5	2.0	0.4	1.2
Organic Matter (%)	3.0	3.9	0.8	2.3
Cation Exchange Capacity (meq (100/g))	19.2	17.3	7.6	31.5
Water Holding Capacity at 1/3 bar (%)	29.48	22.52	15.70	32.44

[1] Calculated as: OC [%] = OM [%] / 2.0 (calculated within report)
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Teflon® centrifuge tubes were used as test systems. The experiments were performed in duplicate.

In preliminary tests, the adsorption of glyphosate to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times and test item stability in soil extracts

following the desorption phase (at highest test concentration) were determined. Additionally, a material balance test using sterile soils was performed at the highest test concentration including an adsorption and desorption step.

For the definitive phase the adsorption step was carried out using soils with a soil-to-solution ratio of 1:5. Glyphosate-trimesium was applied at nominal concentrations of 12.4, 1.24, 0.124, and 0.0124 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 4 hours in the dark at 25 ± 1 °C under continuous agitation.

For the desorption step, pre-adsorbed soil was supplied with fresh aqueous 0.01 M $CaCl_2$ solution was. The resultant samples were agitated for 8 hours at 25 ± 1 °C under continuous agitation.

2. Analytical Procedures

After the adsorption step and desorption step, the aqueous supernatant was separated from the soil by centrifugation and the radioactivity in the supernatants was determined by liquid scintillation counting (LSC). After desorption the radioactivity content in the soils was determined by combustion/LSC to establish full material balances of radioactivity.

For investigation of test item stability soils were extracted in an additional test after the adsorption and desorption phase using of 3 N aqueous hydrochloric acid (HCl) at 25 °C. Soil extracts were analysed by thin layer chromatography (TLC).

Adsorption isotherms were calculated by evaluation of the adsorption data via the indirect method according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Mean material balances corrected for test vessel adsorption were 95.0 % AR for soil Atterbery Silt Loam (range from 83.0 to 111 % AR), 87.0 % AR for soil Sorrento Loam (range from 74.6 to 93.7 % AR), 92.3 % AR for soil Visalia Sandy Loam (range from 79.7 to 114 % AR), and 90.1 % AR for soil Biggs Clay (range from 71.9 to 103 % AR). Material balances for sterile soils were 99.0 % for soil Atterbery Silt Loam, 95.5 % for soil Sorrento Loam, 97.5 % for soil Visalia Sandy Loam and 77.0 % AR for soil Biggs Clay. For details see table below:

Table 7.1.3.1.1-32: Material balance at different test concentrations

		Test Concentration [mg/L]			
		12.4	**1.24**	**0.124**	**0.0124**
Atterbery Silt Loam	I	111	98.5	83.0	84.2
	II	107	101	88.3	87.3
Sorrento Loam	I	89.1	93.7	93.7	79.1
	II	88.8	88.7	74.6	87.9
Visalia Sandy Loam	I	92.0	92.0	82.8	79.7
	II	89.9	107	114	81.0
Biggs Clay	I	95.1	86.6	95.1	71.9
	II	103	91.8	90.8	86.3

B. STABILITY OF TEST ITEM

The relative amount of the test item in the soil extracts after the desorption phase accounted to 98.5 % for soil Atterbery Silt Loam, 98.4 % for soil Sorrento Loam, 97.1 % for soil Visalia Sandy Loam and 99.3 % for soil Biggs Clay. The mean extraction efficiencies were 93.9 % for soil Atterbery Silt Loam, 78.1 % for soil Sorrento Loam, 96.9 % for soil Visalia Sandy Loam and 71.5 % for soil Biggs Clay. Aqueous supernatants after adsorption and desorption were not analysed in the study.

C. FINDINGS

The adsorption coefficients $K_{F(ads)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the four test soils ranged from 31.5 to 2060 mL/g. The Freundlich exponents n were in the range of 0.909 to 1.14. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 2860 and 179000 mL/g.

The desorption coefficients $K_{F(des)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the three test soils ranged from 40.4 to 3230 mL/g. The Freundlich exponents n were in the range of 0.901 to 1.09. The corresponding, calculated $K_{F, OC(des)}$ values varied between 3030 and 281000 mL/g.

Table 7.1.3.1.1-33: [^{14}C]Glyphosate-trimesium: Percentage adsorbed / desorbed in soil

Soil	Replicate	Test Concentration [mg/L]							
		Adsorption [1]				Desorption [2]			
		12.4	1.24	0.124	0.0124	12.4	1.24	0.124	0.0124
Atterbery Silt Loam	I	98.7	98.6	99.0	98.7	0.64	0.83	0.62	0.79
	II	98.6	99.0	99.0	98.7	0.93	0.70	0.61	1.01
Sorrento Loam	I	90.7	94.5	94.7	94.4	7.24	5.60	5.97	4.91
	II	90.4	94.3	94.6	94.6	7.65	5.58	5.02	5.25
Visalia Sandy Loam	I	81.9	92.4	93.0	91.4	10.8	7.57	6.56	5.36
	II	81.9	91.8	94.0	91.8	11.1	6.01	8.46	6.80
Biggs Clay	I	99.6	99.7	99.6	99.2	0.19	0.14	0.29	0.66
	II	99.6	99.5	99.6	99.2	0.30	0.19	0.20	0.44

[1] End of adsorption phase, expressed as percentage of applied radioactivity
[2] End of desorption phase, expressed as percentage of applied radioactivity

Table 7.1.3.1.1-34: [^{14}C]Glyphosate-trimesium: Adsorption and desorption parameters in soil at 25 ± 1 °C

Soil	Adsorption				Desorption			
	K_F	n	R^2	$K_{F, OC}$	K_F	n	R^2	$K_{F, OC}$
Atterbery Silt Loam	376	1.02	0.9972	25100	550	1.00	0.9957	36700
Sorrento Loam	55.7	1.08	0.9968	2860	59.1	1.06	0.9986	3030
Visalia Sandy Loam	31.5	1.14	0.9879	7880	40.4	1.09	0.9962	10100
Biggs Clay	2060	0.909	0.9904	179000	3230	0.901	0.9782	281000

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the four test soils ranged from 31.5 to 2060 mL/g. The Freundlich exponents n were in the range of 0.909 to 1.14. The corresponding, calculated $K_{F, OC(ads)}$ values ranged from 2860 to 179000 mL/g. The desorption coefficients $K_{F(des)}$ of glyphosate-trimesium calculated based on the Freundlich isotherms of the three test soils ranged from 40.4 to 3230 mL/g. The Freundlich exponents n were in the range of 0.901 to 1.09. The corresponding, calculated $K_{F, OC(des)}$ values ranged from 3030 to 281000 mL/g.

For soils Atterbery and Visalia relative test item recovery (regarding soil extracts only) was >90 % following the desorption phase. In combination with an extraction efficiency of >90 % for both soils total test item stability could be sufficient (i.e. >90 %).

For soils Sorrento Loam and Biggs Clay it is noted that the extraction efficiencies following the desorption phase were low (78.1 % AR for soil Sorrento Loam and 71.5 % AR for soil Biggs Clay) resulting in formation of non-extractable residues (NER) of >20 % AR for both soils. Since NER are considered as degradation products of the parent test item the test item is considered unstable in the course of the study for soils Sorrento Loam and Biggs Clay.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The test was performed using the indirect method for determination of adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. The results of the stability test (parental mass balance) are not reported in detail while the recovered amounts of the test item are reported for soil extracts following the desorption phase.
The data are therefore regarded as supportive information. It is noted that the raw data of the study possibly could provide additional information to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

Assessment and conclusion by RMS:

An evaluation following EFSA Evaluators Checklist was not performed due to the concluded supportive character of the study and the missing data necessary for a complete evaluation.

1. Information on the study

Data point:	CA 7.1.3.1.1/009
Report author	[redacted]
Report year	1992
Report title	(14C)-Glyphosate : Adsorption/desorption in soil
Report No	7180
Document No	
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision N: Chemistry: Environmental Fate Section 163-1 (October, 1982)
Deviations from current test guideline	From OECD Guideline 106 (January 2000): • No parental mass balance established • No pre-equilibration of soils
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The adsorption/desorption behaviour of [^{14}C]glyphosate was studied in five sterilized soils and one sterilized sediment in batch equilibrium experiments in the laboratory at 21-26 °C using the indirect method.

Soil/Sediment	Origin	Texture (USDA)	pH [1]	OC [%]
Greenan sand	Scotland	Sand	5.7	0.8
Auchincruive	Scotland	Sandy loam	7.1	1.6
Headley Hall	England	Sandy clay loam	7.8	1.4
Californian sandy soil	United States	Loamy sand	8.3	0.6
Les Evouettes II	Switzerland	Silt loam	6.1	1.4
Darnconner sediment	Scotland	Loam	7.1	3.0

[1] pH values were derived in water

The equilibration solution used was 0.01 M aqueous $CaCl_2$. For the definitive phase, the adsorption step was carried out at a soil to solution ratio of 1:5 for 16 hours. The nominal test concentration of glyphosate was 5 mg/L. The desorption phase was conducted by supplying the pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ for each soil and sediment with two desorption cycles each for 16 hours.

Following 48 h of equilibration, mean material balances were 89.74 % for Greenan sand, 88.02 % for Auchincruive sandy loam, 92.99 % for Headley Hall sandy clay loam, 92.19 % for the Californian loamy sand, 94.60 % for Les Evouettes II silt loam and 96.75 % for Darnconner loam sediment, each in terms of applied radioactivity (% AR).

Following the adsorption step, stability of glyphosate was investigated in aqueous supernatants of soils Headley Hall, Californian sandy soil, and Les Evouettes II only. Supernatants. For all investigated supernatants (for three soils only) the majority of the radioactivity (82 to 94 %) in the supernatant consisted of glyphosate as demonstrated by co-chromatography with authentic standard.

With the exception of Californian sandy soil >89 % AR was adsorbed to soil following equilibration for 16 hours. The total radioactivity desorbed was <6 % after two successive desorption steps of 16 hours each. In Californian sandy soil, approximately 50 % AR was adsorbed after 16 hours and approximately 19 % was desorbed after two 16 hour desorption steps.

The adsorption coefficients $K_{D(ads)}$ of glyphosate based on a single test concentration of 5 mg/L ranged from 5 to 811 mL/g and corresponding $K_{D, OC(ads)}$ values ranged from 884 to 50660 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate (PMG label)	
Lot No.	CFQ.6228 and CFQ.6647
Specific activity	11.2 MBq/mg (304 µCi/mg) and 11.1 MBq/mg (299 µCi/mg)
Radiochemical purity	99.2 % and 99.4 %
Chemical purity	Not provided

2. Test Soils

The soils were stored covered under outdoor conditions. Soils were moistened with deionised water and the excess water was allowed to evaporate. The soils were kept moist by the addition of deionised water before application. The characterisation of test soils used is summarised in the table below.

Table 7.1.3.1.1-35: Physico-chemical properties of test soils and sediment

Parameter	Results					
Soil Designation	Greenan sand	Auchincruive	Headley Hall	Californian sandy soil	Les Evouettes II	Darnconner sediment
Geographic Location						
City	-	-	Leeds	-	-	-
State	-	-	West Yorkshire	-	-	-
Country	Scotland	Scotland	England	United States	Switzerland	Scotland
Textural Class (USDA)	Sand	Sandy loam	Sandy clay loam	Loamy sand	Silt loam	Loam
Sand (50 µm – 2 mm) (%)	95	75	47	83	38.0	39
Silt (2 µm – 50 µm) (%)	2	12	21	11	50.7	40
Clay (< 2 µm) (%)	3	13	32	6	11.3	21
pH						
- in water	5.7	7.1	7.8	8.3	6.1	7.1
- in KCl	4.7	6.1	7.1	7.6	5.3	6.0
Organic Carbon	0.8	1.6	1.4	0.6	1.4	3.0
Organic Matter (%) [1]	1.38	2.75	2.41	1.03	2.41	5.16
Cation Exchange Capacity (meq/100 g))	5.0	12.0	13.0	7.0	15.5	17.0
Water Holding Capacity						
at 33KPa (%)	8.1	18.1	23.5	11.9	29.7	NA
Bulk Density (disturbed) (g/mL)	1.38	1.05	1.09	1.44	0.88	1.14

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon x 1.72
USDA: United States Department of Agriculture; NA: Not applicable

B. STUDY DESIGN

1. Experimental Conditions

Culture glass tubes with screw caps were used as test vessels. The experiments were performed in duplicate.

The preliminary phase consisted of tests on adsorption of glyphosate to the test vessels, test item stability and the appropriate adsorption and desorption equilibration times.

For the definitive phase, the adsorption test was carried out at a soil-to-solution ratio of 1:5. Soils and sediment were sterilised by gamma irradiation (25 kgy) prior to application. Glyphosate was applied at a nominal concentration of 5 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 16 hours under continuous agitation.

For each desorption step, fresh aqueous 0.01 M $CaCl_2$ solution was added to pre-adsorbed soil samples and the resultant samples were re-equilibrated for 16 hours under continuous agitation.

2. Analytical Procedures

After the adsorption step and each desorption step, the aqueous supernatant was separated from the soil by centrifugation and the amount of glyphosate in the supernatants was analysed by liquid scintillation counting (LSC).

Triplicate aliquots of soil samples were combusted followed by quantitation using radioassay.

Aliquots of Headley Hall, Californian sandy soil, and Les Evouettes II supernatants were analysed by thin layer chromatography (TLC) for glyphosate and degradation products.

Adsorption coefficients of glyphosate were calculated by analysis of the adsorption data.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Following 48 h of equilibration, mean material balances were 89.74 % AR for Greenan sand, 88.02 % AR for Auchincruive sandy loam, 92.99 % AR for Headley Hall sandy clay loam, 92.19 % AR for the Californian loamy sand, 94.60 % AR for Les Evouettes II silt loam and 96.75 % AR for Darnconner loam sediment.

B. STABILITY OF TEST ITEM

Stability of glyphosate was investigated in aqueous $CaCl_2$ supernatants following the adsorption step only. Headley Hall, Californian sandy soil, and Les Evouettes II supernatants were analysed and in all cases the majority of the sample radioactivity (82 to 94 %) co-chromatographed with the glyphosate standard.

C. FINDINGS

In all soil/sediment types with the exception of Californian sandy soil, >89 % AR was adsorbed after 16 hours equilibration and <6 % was desorbed after two 16 hour desorption steps. In Californian sandy soil, approximately 50 % AR was adsorbed after 16 hours and approximately 19 % was desorbed after two 16 hour desorption steps. Results are presented in the table below:

Table 7.1.3.1.1-36: [^{14}C]Glyphosate: Percentage adsorbed and desorbed (mean values)

Soil	% AR adsorbed	% AR desorbed
Greenan sand	98.04	0.99
Auchincruive	99.35	0.37
Headley Hall	90.50	4.43
Californian sandy soil	50.11	19.39
Les Evouettes II	89.88	5.32
Darnconner sediment	98.86	3.40

The adsorption coefficients $K_{D(ads)}$ of glyphosate calculated on the five test soils and one sediment ranged from 5 to 811 mL/g and the corresponding $K_{DOC(ads)}$ values ranged from 884 to 50660 mL/g. Results are presented in the table below:

Table 7.1.3.1.1-37: [^{14}C]Glyphosate: Adsorption coefficients in soil and sediment at a single test concentration of 5 mg/L

Soil/Sediment	Adsorption	
	$K_{D(ads)}$ [mL/g]	$K_{D, OC(ads)}$ [mL/g]
Greenan sand	263	32838
Auchincruive	811	50660
Headley Hall	50	3598
Californian sandy soil	5	884
Les Evouettes II	48	3404
Darnconner sediment	510	17010

III. CONCLUSIONS

The adsorption coefficients $K_{D(ads)}$ of glyphosate calculated on the five test soils and one sediment ranged from 5 to 811 mL/g and the corresponding $K_{D, OC(ads)}$ values ranged from 884 to 50660 mL/g.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The test was performed using the indirect method for determination of adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, no PMB was determined in this test to fulfil this criterion.

The results of the study are thus considered as supportive information. Since the test was performed at one test concentration only, the results cannot be evaluated according to the EU Evaluators Checklist.

Assessment and conclusion by RMS:

Though the study does not fulfil actual data requirements, the results of the study were summarised formally in the table below. No graphical and statistical evaluation according to Freundlich and the EU Evaluators Checklist is possible.

Table 7.1.3.1.1-38: Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Greenan sand	Auchin-cruive	Headley Hall	Californian sandy soil	Les Evouettes II	Darnconner sediment
Adsorption method	-	indirect	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:5	1:5	1:5	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	- [1]	- [1]	- [1]	- [1]	- [1]	- [1]
Adsorbed percentage	%	98.1-98.2	99.3-99.4	90.9-91.1	52.4-53.2	90.5-90.7	98.6-99.3
K_D x (soil:solution ratio)		51.1-54.0	150.5-171.4	10.0-10.3	1.1	9.5-9.7	68.4-134.1
$^{ads}K_F$ [1] (95 % confidence interval)	L/kg dw	262.6	804.8	50.7	5.6	48.0	506.5
$^{ads}1/n$ (95 % confidence interval)	-	- [3]	- [3]	- [3]	- [3]	- [3]	- [3]
$^{ads}R^2$	-	- [3]	- [3]	- [3]	- [3]	- [3]	- [3]
$^{ads}K_{F,OC}$ [2]	L/kg OC	32821	50301	3621	931	3431	16882
K_{FE} / K_F	-	- [4]	- [4]	- [4]	- [4]	- [4]	- [4]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] PMB was not established. Only aq. supernatants analysed by chromatographic methods (≥ 30 % NER).
[2] Only K_D because of one test concentration only
[3] Not applicable because of one test concentration only
[4] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to missing parental mass balance providing the f-factor necessary for the calculations.

1. Information on the study

Data point:	CA 7.1.3.1.1/010
Report author	[redacted]
Report year	1991
Report title	Behaviour of Glyphosate in water and soil, Part 2 Adsorption/desorption on soil.
Report No	PR90/002
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No adsorption coefficients reported - No concentrations in aqueous supernatants reported - Recovery of test item >110 % or <50 % for two soils - Method validation not reported (no LOD/LOQ available) - No pre-tests for determination of soil-to-solution ratio and equilibration time performed - No pre-equilibration of soils performed
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The adsorption/desorption behaviour of glyphosate was studied in three soils in batch equilibrium experiments in the laboratory.

Soil	Origin	Texture (USDA)	pH	OC [%]
F3, 341	Not provided	Not provided	7.3	1.20
WO-41	Not provided	Not provided	3.8	2.76
2.1	Not provided	Not provided	6.1	0.70

For the definitive phase, the adsorption step was carried out at a soil to solution ratio of 1:5 for 16 hours. The nominal test concentration of glyphosate was 1 mg/L in 0.01 M aqueous $CaCl_2$. For the desorption step fresh 0.01 M aqueous $CaCl_2$ solution was added to the soils.

The desorption study was conducted using each soil with two desorption cycles.

Total mean recoveries of glyphosate in soil extracts after the desorption phase were 117, 102, and 47 % for soil F3, 341, WO-41, and 2.1, respectively.

More than 90 % of glyphosate was adsorbed at the soil phase and 2 % or less was desorbed following two desorption cycles.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Glyphosate (non-labelled)	
Lot No.	00516
Chemical purity	99 %

2. Test Soils

A description of the soils used is in the table below.

Table 7.1.3.1.1-39: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	F3, 341	WO-41	2.1
Geographic Location			
City	Not provided	Not provided	Not provided
State	Not provided	Not provided	Not provided
Country	Not provided	Not provided	Not provided
Textural Class			
(630 µm – 2 mm) (%)	1.1	3.6	4.5
(200 µm – 630 µm) (%)	13.5	51.9	62.9
(63 µm – 200 µm) (%)	25.2	27.7	20.0
(20 µm – 63 µm) (%)	30.3	8.2	4.7
(6 µm – 20 µm) (%)	10.0	7.4	2.5
(2 µm – 6 µm) (%)	4.7	0.5	1.9
(< 2 µm) (%)	15.2	0.7	3.5
pH in water	7.3	3.8	6.1
Organic Carbon	1.20	2.76	0.70
Organic Matter (%) [1]	2.06	4.75	1.20
Cation Exchange Capacity (mval/100 g)	13	8	4.9
Water Holding Capacity			
maximum (g (100 g soil DW^{-1}))	45.7	--	31.9
Bulk density (g/1000 mL)			1365

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon × 1.72
DW: dry weight

B. STUDY DESIGN

1. Experimental Conditions

The tests were performed with duplicate soil samples.

For the definitive phase the adsorption step was carried out in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:5. Nominal amount of glyphosate used 1 mg/L. The adsorption step was carried out for 16 hours under continuous agitation.

The desorption step was conducted using each soil with two desorption cycles. In each of the two desorption phases, pre-adsorbed soil specimens were supplied with fresh aqueous 0.01 M $CaCl_2$ solution. The resultant samples were re-equilibrated for 16 hours under continuous agitation followed by centrifugation.

2. Analytical Procedures
The aqueous supernatant after adsorption and after desorption was separated by centrifugation. Chromatographic analysis for glyphosate residues was reported to follow method iCD033E.
To determine the recovery of the test item, glyphosate was extracted from the soil with water and phosphoric acid following the desorption phase.

II. RESULTS AND DISCUSSION

A. STABILITY OF TEST ITEM
Total mean recoveries of glyphosate in soil extracts after the desorption phase were 117, 102, and 47 % for soil F3, 341, WO-41, and 2.1, respectively.

B. FINDINGS
More than 90 % of glyphosate was adsorbed at the soil phase and 2 % or less was desorbed following two desorption cycles.

Table 7.1.3.1.1-40: Glyphosate: Recovery in supernatant (mean values)

Soil	Percentage [1]		
	Adsorption	Desorption Step 1	Desorption Step 2
F3, 341	6	2	1
WO-41	5.5	1	0.6
2.1	0	1.5	-

[1] Mean values expressed as percentage of applied glyphosate

III. CONCLUSIONS

More than 90 % of glyphosate was adsorbed at the soil phase and 2 % or less was desorbed following two desorption cycles. An evaluation according to EFSA Evaluators Checklist was not possible due to missing data (no concentrations reported).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is considered as invalid due to various significant deviations from current OECD Guideline 106. No pre-tests for determination of soil-to-solution ratio and equilibration time were performed. For the adsorption step soils were not pre-equilibrated and the test item was applied together with the 0.01 M $CaCl_2$ solution. Furthermore, recovery of test item was >110 % or <50 % for two soils (F3, 341 and 2.1) and therefore outside of the acceptable range.

Finally, an evaluation according to EFSA Evaluators Checklist cannot be performed due to missing data of test item concentrations in the aqueous adsorption supernatants. Therefore, the results remain uncertain.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/011
Report author	[redacted]
Report year	1986
Report title	Australian notification base testing requirements for N- (Phosphonomethyl) Iminodiacetic Acid (Glyphosate Intermediate), Part II: Adsorption/Desorption Data.
Report No	MSL-5393
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): – No complete material balance (recovery: approx. 70 % AR), possibly due to loss of CO_2 – No parental mass balance established, chromatographic analysis of adsorption supernatants only – Glyphosate reported to be not stable in supernatant (≤59 % test item) – No pre-equilibration of soils
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The adsorption/desorption behaviour of [^{14}C]glyphosate was studied in three soils in batch equilibrium experiments in the laboratory at 24-26 °C using the indirect method.

Soil	Origin	Texture (USDA)	pH [1]	OC [%]
Drummer	Decatur, IL, USA	Silty clam loam	6.5	1.45
Dupo	St. Charles, MO, USA	Silt loam	7.4	0.87
Spinks	East Lansing, MI, USA	Loamy sand	5.2	1.10

[1] pH values were derived from a 1:1 soil:water suspension

For the definitive phase, the adsorption step was carried out at a soil to solution ratio of 1:5 for 24 hours using pre-equilibrated soil samples. Nominal test concentrations of glyphosate were 5.0, 1.0, 0.2 and 0.04 mg/L in 0.01 M aqueous $CaCl_2$. The desorption step was conducted by applying fresh 0.01 M aqueous $CaCl_2$ solution to the soil samples from the adsorption step by performing two successive desorption cycles for each soil and each test concentration.

Recovered radioactivity determined in a material balance test accounted for 71 % of applied radioactivity (% AR) for soil Drummer, 67 % AR for soil Dupo and 67 % AR for soil Spinks.

In the aqueous supernatants of the adsorption phase glyphosate was found at relative amounts of 50 % for soil Drummer, 48 % for soil Dupo and 59 % for soil Spinks. The metabolite aminomethylphosphonic acid (AMPA) was found at relative amounts of 14 % for soil Drummer, 47 % for soil Dupo and 32 % for soil Spinks.

The percentage of adsorbed radioactivity onto the soil ranged from 97.9 to 99.0 % AR for soil Drummer, from 87.8 to 93.0 % for soil Dupo and from 96.9 to 98.8 % for soil Spinks.

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the three test soils ranged from 33 to 660 mL/g. The Freundlich exponents 1/n were in the range of 0.80 to 1.16. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 3800 and 60000 mL/g.

After the desorption phase, radioactivity desorbed from the soil ranged from 0.9 to 2.3 % AR for soil Drummer, from 6.3 to 7.9 % for soil Dupo and from 0.7 to 2.2 % for soil Spinks.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]glyphosate (label position not reported)	
Lot No.	C-927.3A
Specific activity	8.79 mCi/mmol
Radiochemical purity	98.0 %
Chemical purity	Not provided

2. Test Soils

The soils were sieved to a particle size of ≤425 µm. The soils were air-dried before application. A description of the soils used is in the table below.

Table 7.1.3.1.1-41: **Physico-chemical properties of test soils**

Parameter	Results		
Soil Designation	Drummer	Dupo	Spinks
Geographic Location			
City	Decatur	St. Charles	East Lansing
State	Illinois	Missouri	Michigan
Country	USA	USA	USA
Textural Class	Silty clam loam	Silt loam	Loamy sand
Sand [%]	16.0	18.0	74.0
Silt [%]	56.4	68.0	22.4
Clay [%]	27.6	14.0	3.6
pH			
- in water	6.5	7.4	5.2
Organic Carbon [%]	1.45	0.87	1.10
Organic Matter [%] [1]	2.49	1.50	1.89
Cation Exchange Capacity [meq/100 g]	20.2	8.7	5.8

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon x 1.72

B. STUDY DESIGN

1. Experimental Conditions

Glass centrifuge tubes (either 25 or 50 mL) were used as test systems. The tests were performed with triplicate soil samples.

In preliminary tests, the appropriate adsorption and desorption equilibration times and the stability of the test item were determined. Samples with a concentration of 5 mg/L were prepared to establish a material balance.

For the definitive phase the adsorption phase was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution at a soil-to-solution ratio of 1:5. The nominal test concentrations were 5.0, 1.0, 0.2 and 0.04 mg/L. The adsorption step was carried out for 21 to 24 hours (details regarding not reported) under continuous agitation at 24-26 °C.

In the first desorption step, fresh aqueous 0.01 M $CaCl_2$ solution was added to pre-adsorbed soil specimens for all test concentrations. The resultant samples were re-equilibrated for 17 to 26 hours under continuous agitation. In the second desorption step, the procedure was repeated. The resultant samples were re-equilibrated for 21 to 23 hours under continuous agitation.

2. Analytical Procedures

After the adsorption step and each desorption step, the aqueous supernatant was separated by centrifugation and the radioactivity in the supernatant was determined by liquid scintillation counting (LSC).

Radioactivity in adsorption solutions of the highest test concentration was characterized by high performance liquid chromatography (HPLC).

For the material balance test radioactivity adsorbed to soil was determined by combustion of aliquots of samples and determination by LSC following the adsorption phase. The remaining soil was extracted using 0.5 M NH_4OH and radioactivity of the soil extracts was determined by LSC. Chromatographic analyses of soil extracts were not performed.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Recovered radioactivity determined in a material balance test accounted for 71 % of applied radioactivity (% AR) for soil Drummer, 67 % AR for soil Dupo and 67 % AR for soil Spinks.

B. STABILITY OF TEST ITEM

In the aqueous supernatants of the adsorption phase glyphosate was found at relative amounts of 50 % for soil Drummer, 48 % for soil Dupo and 59 % for soil Spinks. The metabolite aminomethylphosphonic acid (AMPA) was found at relative amounts of 14 % for soil Drummer, 47 % for soil Dupo and 32 % for soil Spinks.

B. FINDINGS

At the end of the adsorption phase 97.86-99.00 %, 87.84-93.05 %, and 96.92-98.78 % of the applied test material were adsorbed to soils Drummer, Dupo, and Spinks, respectively. The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the three test soils ranged from 33 to 660 mL/g. The Freundlich exponents 1/n were in the range of 0.80 to 1.16. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 3800 and 60000 mL/g.

After the desorption phase, between 0.70 and 7.88 % of the initially adsorbed radioactivity was desorbed from the respective soils.

Table 7.1.3.1.1-42: **[^{14}C]Glyphosate: Percentage adsorbed to soil (mean values of triplicates)**

	Test Concentration [mg/L]			
	5	**1**	**0.2**	**0.04**
Drummer	98.68	99.00	98.55	97.86
Dupo	87.84	91.84	93.05	91.98
Spinks	98.78	98.47	98.22	96.92

Table 7.1.3.1.1-43: [^{14}C]Glyphosate: Percentage desorbed from soil (mean values)

Soil	**Test Concentration [mg/L]**			
	Desorption [1]			
	5	**1**	**0.2**	**0.04**
Drummer	1.56	0.93	1.36	2.30
Dupo	7.26	6.34	6.26	7.88
Spinks	0.90	0.70	1.27	2.17

[1] End of desorption phase, mean values expressed as percentage of applied radioactivity

Table 7.1.3.1.1-44: [^{14}C]Glyphosate: Adsorption parameters in soil

Soil	**Adsorption**			
	$K_{F(ads)}$	**1/n**	**R^2**	**$K_{F, OC(ads)}$**
Drummer	324	0.92	0.9985	22300
Dupo	33	0.80	0.9999	3800
Spinks	660	1.16	0.9969	60000

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of glyphosate calculated based on the Freundlich isotherms of the three test soils ranged from 33 to 660 mL/g. The respective $K_{F\ oc(ads)}$ values were in the range of from 3800 and 60000 mL/g. The Freundlich exponents 1/n were in the range of 0.80 to 1.16.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The assessment of data in the test was performed using the indirect method to calculate adsorption to soil. Following the current EU OECD 106 Evaluators Checklist the use of the indirect method requires the demonstration of stability as documented by the parental mass balance (PMB) for the test substance. However, the data reported do not allow for the conclusion that the test substance was stable since no parental mass balance was established covering the soil phase following the adsorption step.

The data are therefore regarded as supportive information. It is noted that the raw data of the study possibly could provide additional information (i.e. chromatographic results of soil extracts) to derive K_D for the concentration tested in the parental mass balance test by applying the direct method.

An evaluation of information in study according to the EU OECD 106 Evaluators Checklist is presented for information only.

Assessment and conclusion by RMS:

Though the study does not fulfil the actual EU requirements, the results of the study were summarised formally below.

Table 7.1.3.1.1-45: Glyphosate: Evaluation of results according to EU OECD 106 Evaluators Checklist

	Units	Spinks	Dupo	Drummer
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw mL	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	- [1]	- [1]	- [1]
Adsorbed percentage	%	96.9-98.8	87.9-93.2	97.8-99.0
K_D x (soil:solution ratio)		31.5-81.4	7.3-13.8	44.6-99.0
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	828.5 (219.3-3128.7)	38.8 (16.4-91.6)	645.5 (71.4-5839.0)
$^{ads}1/n$ (95 % confidence interval)	-	1.227 (0.9639-1.490)	0.903 (0.677-1.130)	1.123 (0.708-1.538)
$^{ads}R^2$	-	0.995	0.993	0.985
$^{ads}K_{F,OC}$	L/kg OC	75315	4459	44518
K_{FE} / K_F	-	- [2]	- [2]	- [2]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] PMB was not established. Only aq. supernatants analysed by chromatographic methods (glyphosate recovery ≤59 %).
[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to missing parental mass balance providing the f-factor necessary for the calculations.

Figure 7.1.3.1.1-33: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Spinks

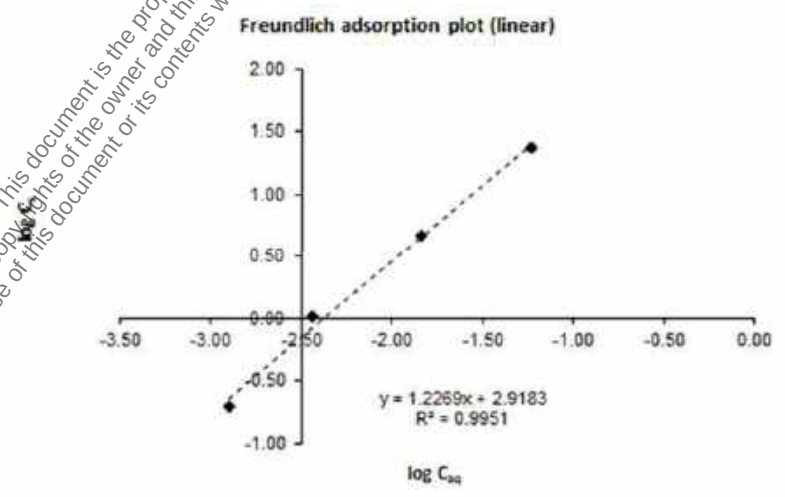


Figure 7.1.3.1.1-33: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Spinks

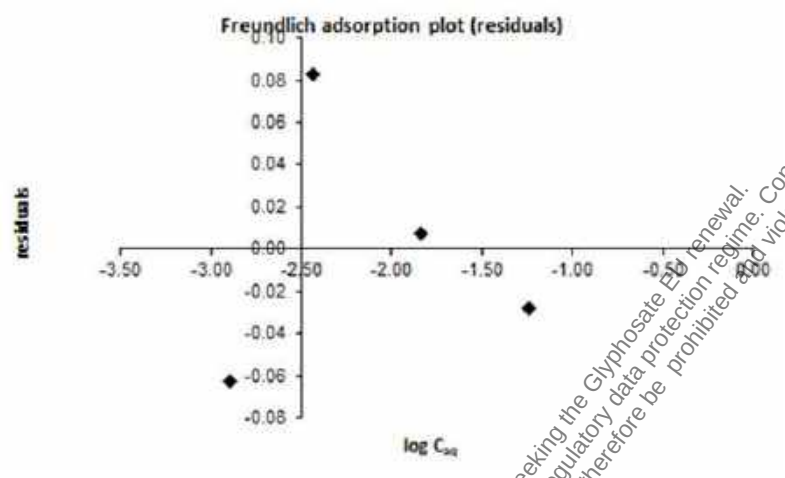


Figure 7.1.3.1.1-34: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Dupo

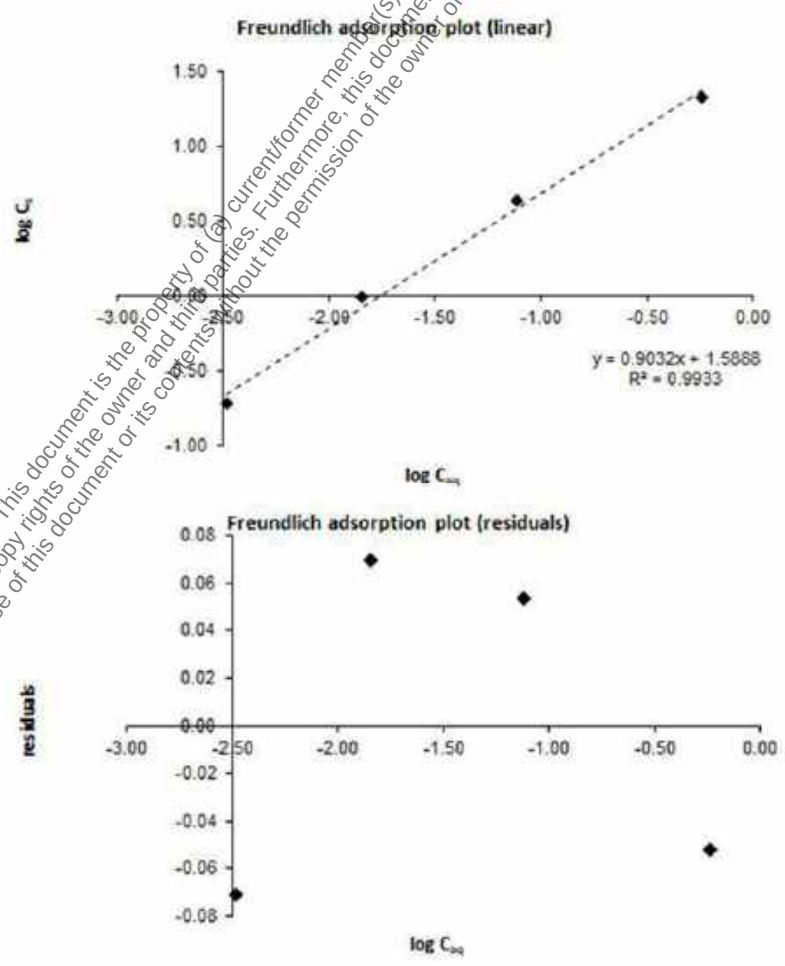
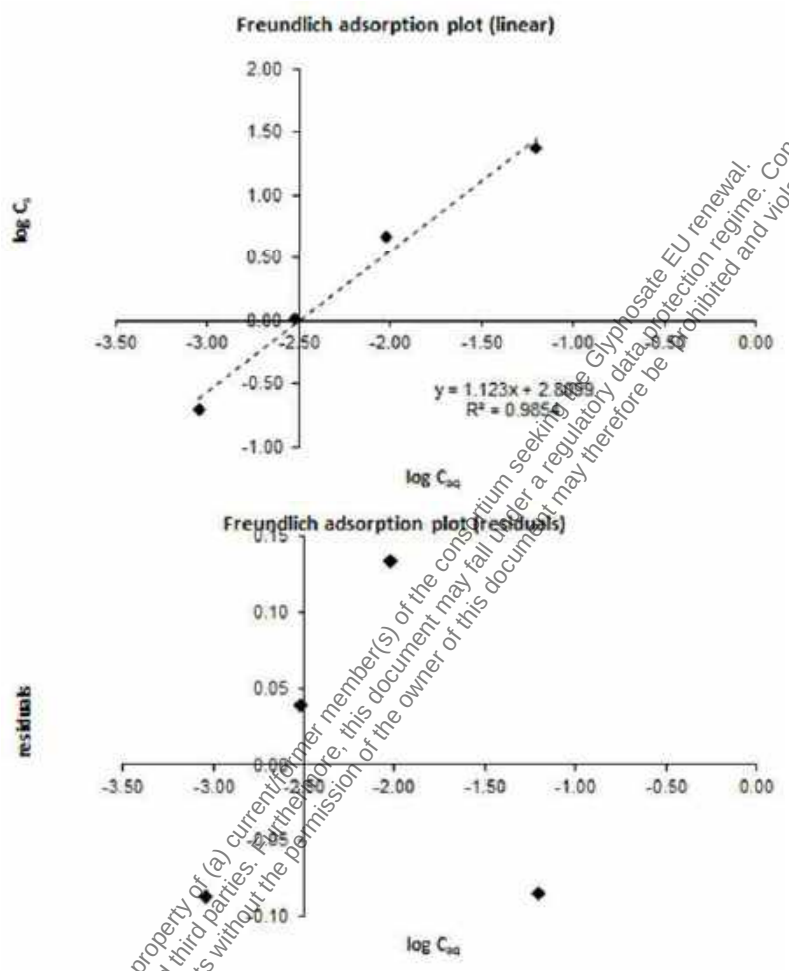


Figure 7.1.3.1.1-35: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Drummer



1. Information on the study

Data point:	CA 7.1.3.1.1/012
Report author	[redacted]
Report year	1986
Report title	HOE 017411, Adsorption/desorption in the soil/water system
Report No	A40783 (B)136/85
Document No	
Guidelines followed in study	No information available
GLP	No information available
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	No information available; From the report title, the compound number HOE 017411 is indicative for the active substance carbendazim. Presumably, the study was erroneously listed in the Monograph (2000).
Short description of results:	No information available
Reasons why the study is not considered relevant/reliable or not considered as key study:	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 7.1.3.1.1/013
Report author	[redacted]
Report year	1978
Report title	Solubility, volatility, adsorption and partition coefficients, leaching and aquatic metabolism of MON 0573 and MON 0101
Report No	MSL-0207
Document No	
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: Adsorption/Desorption in soil Test item: [^{14}C] glyphosate, phosphonomethyl-label (94 % radiochemical purity) and [^{14}C] sodium sesqui salt of glyphosate (95 % chemical purity prior to labelling with [^{14}C]glyphosate) Test soils (soil type): Ray (silt loam), Spinks (sandy loam), Drummer (silty clay loam), Lintonia (sandy loam), Cattail (swamp sediment) pH: 8.1, 4.7, 6.2, 6.5, - (medium not stated) Organic matter: 1.2 %, 2.4 %, 3.4 %, 0.7 %, 1.5 % Soils were sieved to <500 µm or less.

	Experimental conditions: The adsorption phase was carried out at a soil to solution ratio of 1:4 for four hours at 25 °C. Test item was applied at concentrations of 0.1, 1.0, 10 and 20 mg/L in 0.01 N $CaSO_4$. For the desorption step fresh aqueous 0.01 N $CaSO_4$ solution was added to pre-adsorbed soil samples and the resultant samples were re-equilibrated for four hours under continuous agitation. Analytical procedures: Following each adsorption and desorption step soil and supernatant were separated by centrifugation. Radioactivity in supernatants was determined by LSC. Adsorption isotherms were calculated by evaluation of the adsorption data via the indirect method according to the Freundlich equation.
Short description of results:	Glyphosate: $K_{F, OC(ads)}$: 7500 (Ray), 2917 (Spinks), 1823 (Drummer), 3143 (Lintonia), - (Cattail sediment); 1/n: 0.902 (Ray), 0.944 (Spinks), 0.951 (Drummer), 0.782 (Lintonia), 1.010 (Cattail sediment) Sodium sesqui salt of glyphosate: $K_{F, OC(ads)}$: 9583 (Ray), 3333 (Spinks), 2000 (Drummer), 4286 (Lintonia), - (Cattail sediment); 1/n: 1.046 (Ray), 0.979 (Spinks), 0.971 (Drummer), 0.844 (Lintonia), 0.950 (Cattail sediment) Desorption was generally low for both test items and all soils (≤11.5 % of initially adsorbed)
Reasons why the study is not considered relevant/reliable or not considered as key study:	Deviations from OECD Guideline 106 (January 2000): - $CaSO_4$ used instead of $CaCl_2$ - Soil was sieved to mesh size <500 µm - No preliminary tests for adequate equilibration time and soil to solution ratio - Stability of test items under study conditions not reported (neither in $CaSO_4$ solution nor in presence of soil) - No radioactivity material balance establishedNo pre-equilibration of samples
Category study in AIR 5 dossier (L docs)	Category 3b

Relevant articles from literature search

1. Information on the study

Data point:	CA 7.1.3.1.1/014
Report author	Albers, C. *et al.*
Report year	2018
Report title	Soil Domain and Liquid Manure Affect Pesticide Sorption in Macroporous Clay Till
Document No	DOI 10.2134/jeq2018.06.0222 E-ISSN 1537-2537
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - 0.001 M $CaCl_2$ solution used instead of 0.010 M solution, - 10 °C (standard: 20 – 25 °C), - 4 test concentrations (standard: 5), - no investigation of stability insoil by extraction, i.e. no parental mass balances reported - no adequately validated analytical method including LOD and LOQ (required with non-labelled test material)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

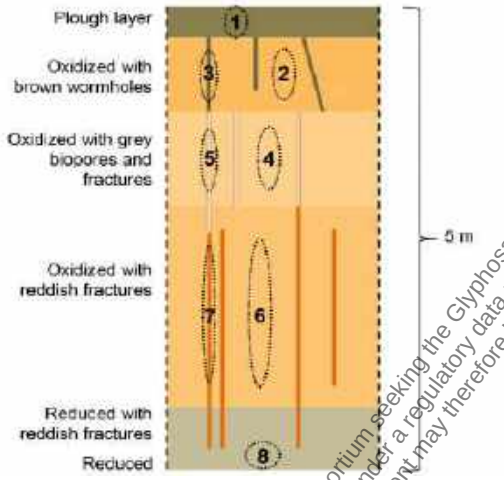
In this study, it was observed that sorption of strongly sorbing pesticide, glyphosate, varied by more than an order of magnitude across soil domains in 3-m-deep clay till profiles with biopores and fractures. Eight soil domains were identified in each of the profiles: five matrix soils and three in the macropores. Glyphosate showed high variation in sorption between fractures and matrix soil from the same depths. The domain-specific sorption of both tebuconazole and glyphosate was, however, overruled by dilute liquid manure. Liquid manure unexpectedly had a greater effect on glyphosate sorption, which was strongly decreased by dissolved organic matter and phosphate in the manure. The variation in sorption across domains, as well as the effects of liquid manure, should be taken into account when assessing leaching risks.

Materials and methods

Soil sampling

Soil was sampled at two locations, Gjorslev (55°20.988′N, 12°23.672′E) and Lund (55°14.698′N, 12°17.418′E) in the Stevns area of southeastern Denmark. At both sites, soil profiles were excavated to a depth of ~5 m. We sampled composite soil samples from eight domains that were clearly separated on the basis of different soil horizons and the presence or absence of biopores and fractures (Figure 7.1.3.1.1-36). The surface of wormholes (Domain 3) was sampled by scraping off the outer 1 to 2 mm of the pore walls. Deeper soil pores surrounding decayed roots were dissected out, and the outer Fe oxides were scraped off with a knife to sample only the 5- to 10-mm wide, inner, greyish part (Domain 5). The surface of even deeper larger fractures with Mn and Fe oxide coatings was sampled by scraping off the outer 1 to 2 mm (Domain 7). At least 240 g soil was sampled from each fracture domain to have sufficient material for sorption experiments and analysis of sediment parameters. The matrix soil samples (bulk soil in the case of the plow layer, Domain 1) were also compositely sampled, comprising ~1 kg from 20 to 50 subsamples. All soil samples were sieved twice through a 2-mm sieve and stored at 2°C. Soil samples from the reduced zone were packed in airtight aluminum tape on location and sieved in a glove box under a reducing N_2/H_2 atmosphere. The fraction <2 mm was stored in anoxic jars at 2°C.

Figure 7.1.3.1.1-36: A schematic representation of the soil profiles in the Gjorslev and Lund sites and their associated soil domains (Domains 1-8). The approximate depth of the lower boundary of each matrix soil domain was (Gjorslev/Lund): Domain 1 (35/35 cm), Domain 2 (105/130 cm), Domain 4 (200/260 cm), and Domain 6 (390/420 cm)



Characterization of the Soil Domains

Soil texture was determined by sieving (0.063-2 mm) and by laser diffraction (<0.063 mm, Mastersizer 3000, Malvern). Water content was determined by drying at 105°C for 24 h. Total carbon and total organic carbon (TOC) were determined on an elemental analyzer (Leco CS-200) on dried (50°C) and crushed samples as they were (total C) or after acid treatment to remove carbonates (TOC). Total inorganic C was calculated as the difference between total C and TOC. The pH was determined in a 1:2.5 soil/liquid slurry with Milli-Q water or 10 mM $CaCl_2$. The $pH_{sorption}$ (i.e., the pH measured at conditions similar to those during the sorption experiments) was also determined with $CaCl_2$, pesticide, and NaN_3 concentrations similar to those used in the sorption experiments. Soil-specific surface area was measured using a Coulter SA 3100 BET analyzer (Coulter Corporation) and calculated using the Brunauer-Emmett-Teller equation. Total Fe and ferrous Fe^{2+} were measured as described by Komadel and Stucki (1988). Iron and manganese oxides were extracted using the citrate-bicarbonate-dithionite (CBD) method and quantified by atomic absorption spectroscopy (PerkinElmer AANALYST 400). Amorphous Fe and Al oxides were extracted using ammonium oxalate solution. Cation exchange capacity was determined by standard method (Chapman, 1965). All analyses of soil parameters were single measurements.

Characterization of Liquid Pig Manure and Soil Extract

Liquid pig manure was sampled from a conventional farm that raised sows and offspring (weaner production) and was stored at 2°C for 4 wk. Topsoil extract was obtained by horizontal rolling of plow layer soil and Milli-Q water (1:1) for 24 h at 22°C. The liquid manure and the topsoil suspension were centrifuged (15 min, 3500 g), and the extracts were stored as frozen subsamples to be used in the subsequent sorption experiments. After thawing, the extracts were sonicated for 30 min before use in sorption experiments. Total organic C in soil and manure extracts and in the aqueous phase of selected sorption experiments was analysed on a TOC analyzer (TOC-Vcph, Shimadzu) after filtration (5 µm polyvinyl difluoride [PVDF], Millipore). Conductivity was determined using a conductivity probe (LE703, Mettler Toledo). The concentrations of major inorganic cations and anions were determined by ion chromatography (Metrohm 819 with a Metrosep A 150/4.0 column). Total Cu, Zn, Al, Ba, Fe, Mn, and S contents were measured on an inductively coupled plasma mass spectrometer (Elan 6100DRC, PerkinElmer) using a multielement scanning method (TotalQuant, PerkinElmer).

Chemicals
(P-methylene-^{14}C)-glyphosate (radiochemical purity = 99 %, specific activity = 122 MBq/mmol) were purchased from Izotop. Glyphosate (purity 97 %) was purchased from Dr. Ehrenstorfer, Germany.

Sorption of Glyphosate
Sorption experiments were performed using a batch-equilibrium method inspired by the Organization for Economic Cooperation and Development (OECD) guideline (OECD, 2000). Eleven-milliliter Pyrex glass vials with 15-mL polypropylene centrifuge vials were used for glyphosate. The final soil/liquid ratio was in all vials 1:10, which in general resulted in between 20 and 95 % sorption of the added pesticide. In each vial, 1 g of soil (wet weight) was mixed with 1 mM $CaCl_2$ solution (8.0 - 9.7 mL) and NaN_3 (20 µL of a 100 g/L solution) was added to repress biodegradation of the pesticides during incubation. One millimolar $CaCl_2$ was used, since it better represented the concentrations in the local soil water than the 10 mM $CaCl_2$ suggested in the OECD guideline. The soil-liquid slurries were then equilibrated at 10°C for 24 h by vertical rotation (7 revolutions/min) before addition of ^{14}C-labeled pesticide and, for the two highest pesticide concentrations, nonradioactive pesticide (both dissolved in 1 mM $CaCl_2$). Initial total concentrations of glyphosate were 30, 120, 1200 (thereof 120 µg/L radioactive glyphosate) and 12,000 µg/L (thereof 120 µg/L radioactive glyphosate). After addition of the pesticides, the vials were rotated at 10°C for another 24 h. The vials were then centrifuged at 1250 g (glass vials) or 3000 g (plastic vials) for 15 min. The pesticide concentration of the aqueous phase was determined by liquid scintillation counting (Tri-Carb 2810 TR, PerkinElmer) of the ^{14}C activity in duplicate 1-mL samples. The ^{14}C activity was counted for 30 min or until 1 % uncertainty (2S, 95 % confidence limit). Sorption to the vials was tested by including reagent blanks without soil, but no such sorption was found. The pesticide concentration in the solid phase (soil) was calculated based on pesticide missing in the aqueous phase.

The sorption experiments were all performed in duplicate. The difference in distribution coefficients between duplicates was <15 %, and in most cases, it was <5 %. All sorption experiments with soil from reduced zones were prepared under a N_2 /H_2 atmosphere in a glove box with solutions that had been flushed with N_2.

Freundlich Sorption Models
Glyphosate sorption was described by an extended Freundlich equation, as suggested by de Jonge *et al.* (2001):

$$C_s = K_{Fex} C_w^{n_{ex}} C_w^{D}$$

where K_{Fex} is the extended Freundlich coefficient, n_{ex} is the extended Freundlich exponent, and D is a parameter that adds extra curvature to the line in a double-logarithmic plot (i.e., increases the concentration sensitivity compared with the simple Freundlich model). The extended Freundlich model was fitted to the experimental data by nonlinear optimization.

Results
Soil Domains
Both soil profiles had a characteristic depth zonation with eight visually different domains based on different layers and the presence or absence of macropores (Figure 7.1.3.1.1-36). At the Gjorslev site, the upper 35 cm was a relatively homogenous dark brown (10YR 3/2) plow layer (Ap horizon, Domain 1) rich in organic matter (Table 7.1.3.1.1-46). The plow layer was followed by an oxidized layer of variable color with a predominantly yellow-brown (10YR 4/4) matrix (Domain 2) perforated by brown (10YR 4/3), vertical wormholes where the soil was enriched in organic matter (Domain 3). Many of the wormholes were present within fractures (geological and desiccation) and extended to a depth of ~ 110 cm. The following layer (105–200 cm) was oxidized with a light brown (10YR 5/3) matrix (Domain 4) and numerous small biopores from decayed plant roots with a diameter of ~ 1 mm. The pores were surrounded by gray (10YR 8/1) pore soil with a diameter of 5 to 10 mm (Domain 5) and a thin, outer layer of Fe oxides. This layer also had gray fractures that extended into the next layer where they changed to reddish. The next layer (200–390 cm) was also oxidized with a light brown (10YR 5/3) matrix (Domain 6) and many parallel fractures (Domain 7). The surface of the larger fractures was coated with Fe and Mn oxides of variable reddish to almost black colors (10YR 4/6). This domain was devoid of visible biopores. The matrix was

reduced at the bottom of the profile (Domain 8), as visible by its gray color (5Y 5/1). The oxidized reddish fracture surfaces extended ~ 50 cm into the reduced zone. Similar horizons and domains were present in the Lund profile, although at slightly different depths (Table 7.1.3.1.1-46). Soil parameters for both profiles are available in Table 7.1.3.1.1-46 and Table 7.1.3.1.1-47.

Table 7.1.3.1.1-46: Main soil parameters from the soil profiles

Domain	Sample depth		TOC†		$pH_{sorption}$‡		Fe_{CBD}§		Mn_{CBD}¶		Surface area	
	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund
	m		%				g kg^{-1}		mg kg^{-1}		m^2 g^{-1}	
1. Plough layer	0.1–0.3	0.2–0.3	0.89	0.67	7.33	7.38	5.6	4.2	286	264	4.1	4.2
2. Matrix	0.6–0.8	0.7–1.2	0.14	0.12	7.30	7.16	9.0	6.4	333	261	16.7	18.5
3. Wormholes	0.4–0.8	0.7–1.2	0.40	0.37	7.88	7.30	7.6	5.9	269	202	12.1	11.3
4. Matrix	1.5–1.6	2.0–2.6	0.05	0.05	8.20	8.26	4.7	3.9	170	153	12.4	13.0
5. Gray macropores	1.1–2.0	2.0–2.6	0.05	0.06	8.19	8.29	2.1	1.1	61	31	11.0	11.6
6. Matrix	2.5–3.5	3.0–3.5	0.05	0.05	8.16	8.31	4.5	2.9	97	97	11.8	12.2
7. Reddish fractures	2.5–3.5	3.0–3.6	0.05	0.06	8.09	8.28	18.8	12.6	778	910	16.0	21.2
8. Reduced zone	4.3–4.5	4.5–4.8	0.18	0.17	8.24	8.53	3.6	2.7	88	44	8.4	11.9

† TOC, total organic C.

‡ $pH_{sorption}$, the pH measured at conditions similar to those during the sorption experiments.

§ Fe_{CBD}, total Fe oxides (extractable with citrate–bicarbonate–dithionite).

¶ Mn_{CBD}, total Mn oxides (extractable with citrate–bicarbonate–dithionite).

Table 7.1.3.1.1-47: Major soil parameters

Domain no.	Description	Sample depth (m) Gjorslev / Lund	$pH_{sorption}$ Gjorslev / Lund	pH_{CaCl2} Gjorslev / Lund	pH_{H2O} Gjorslev / Lund
1	Plough layer	0.1-0.3 / 0.2-0.3	7.33 / 7.38	6.49 / 6.86	7.64 / 7.77
2	Matrix	0.6-0.8 / 0.7-1.2	7.30 / 7.16	6.81 / 6.86	8.09 / 7.92
3	Wormholes	0.4-0.8 / 0.7-1.2	7.88 / 7.30	7.21 / 6.80	8.35 / 7.80
4	Matrix	1.5-1.6 / 2.0-2.6	8.20 / 8.26	7.67 / 7.65	8.67 / 8.63
5	Grey macropores	1.1-2.0 / 2.0-2.6	8.19 / 8.29	7.64 / 7.79	8.77 / 8.77
6	Matrix	2.5-3.5 / 3.0-3.5	8.16 / 8.31	7.62 / 7.47	8.73 / 8.68
7	Reddish fractures	2.5-3.5 / 3.0-3.6	8.09 / 8.28	7.58 / 7.56	8.52 / 8.59
8	Reduced zone	4.3-4.5 / 4.5-4.8	8.24 / 8.53	7.54 / 7.59	N.D. / 8.27

Domain no.	Description	TOC (%) Gjorslev / Lund	TIC (%) Gjorslev / Lund	Surface (m^2/g) Gjorslev / Lund	CEC (cmol/kg) Gjorslev / Lund
1	Plough layer	0.89 / 0.67	0.32 / 0.20	4.1 / 4.2	11.0 / 9.25
2	Matrix	0.14 / 0.12	0.08 / 0.11	16.7 / 18.5	12.5 / 12.2
3	Wormholes	0.40 / 0.37	0.19 / 0.17	12.1 / 11.3	12.0 / 11.9
4	Matrix	0.05 / 0.05	1.95 / 2.41	12.4 / 13.0	8.77 / 7.52
5	Grey macropores	0.05 / 0.06	2.58 / 3.17	11.0 / 11.6	8.80 / 7.75
6	Matrix	0.05 / 0.05	1.88 / 2.98	11.8 / 12.2	8.53 / 7.15
7	Reddish fractures	0.05 / 0.06	1.83 / 2.67	16.0 / 21.2	9.46 / 8.26
8	Reduced zone	0.18 / 0.17	2.21 / 2.85	8.4 / 11.9	7.05 / 4.79

Domain no.	Description	Clay (%) Gjorslev / Lund	Silt (%) Gjorslev / Lund	Fine sand (%) Gjorslev / Lund	Med. sand (%) Gjorslev / Lund	Coarse sand (%) Gjorslev / Lund
1	Plough layer	6.5 / 6.3	41.1 / 39.4	35.9 / 35.7	10.6 / 12.3	6.0 / 6.3
2	Matrix	8.5 / 7.8	46.1 / 43.2	30.0 / 32.3	9.2 / 11.2	6.2 / 5.5
3	Wormholes	8.6 / nd	42.4 / nd	33.2 / nd	9.1 / nd	6.6 / nd
4	Matrix	13.1 / 13.4	42.7 / 43.2	28.8 / 28.5	8.7 / 8.9	6.7 / 6.0
5	Grey macropores	12.9 / 12.5	43.1 / 41.0	27.6/ 25.5	8.8 / 8.8	7.5 / 12.2
6	Matrix	12.9 / 13.6	45.0 / 43.5	27.1 / 26.4	9.0 / 7.4	6.0 / 9.1
7	Reddish fractures	10.6 / 14.1	40.8 / 45.5	24.8 / 26.0	8.8 / 9.9	15.1 / 12.0
8	Reduced zone	10.7 / 14.3	51.3 / 45.5	24.7 / 28.3	8.0 / 7.6	5.3 / 4.3

Sorption Is Domain Specific

Glyphosate sorption followed the Freundlich model with a high concentration dependence ($0.87 < n < 1.32$, Table 7.1.3.1.1-48).

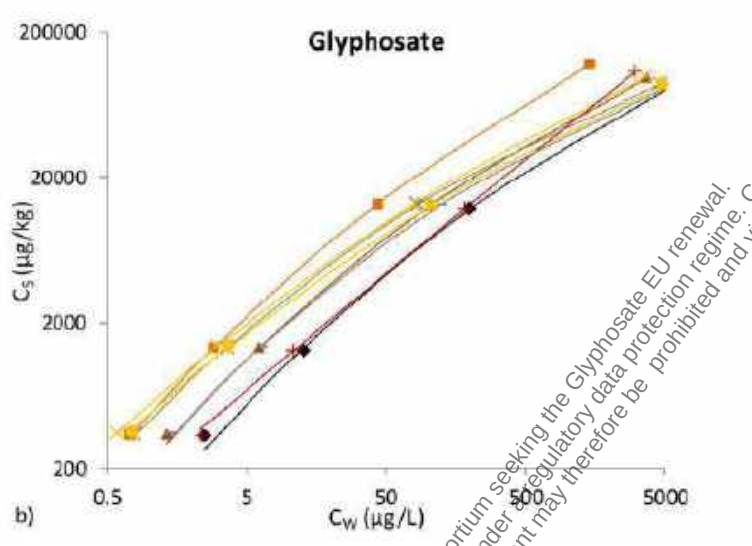
Table 7.1.3.1.1-48: Extended Freundlich parameters (glyphosate) for sorption to eight soil domains in the Gjorslev and Lund profiles

Domain	Glyphosate[1]					
	K_{fex}		$1/n$		D	
	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund
1	130	72.5	1.00	1.06	0.040	0.036
2	443	947	1.04	0.98	0.041	0.043
3	239	496	1.07	0.97	0.045	0.040
4	536	125	0.88	1.12	0.039	0.042
5	3849	33	1.01	1.01	0.053	0.053
6	424	359	0.87	0.91	0.037	0.037
7	124	58	0.92	0.93	0.011	0.000
8	217	281	1.08	1.32	0.049	0.071

[1] K_{fex} the extended Freundlich coefficient; D, a parameter that adds extra curvature to the line in a double-logarithmic plot. K_{fex} equals the predicted distribution coefficient at 1 µg L^{-1} glyphosate.

The extended Freundlich model fitted the sorption data of glyphosate very well, though with a tendency to slightly underestimate sorption at the lowest concentration (Figure 7.1.3.1.1-37). Glyphosate sorption was very concentration dependent (Figure 7.1.3.1.1-37), which is why the extended Freundlich model fitted the sorption data better.

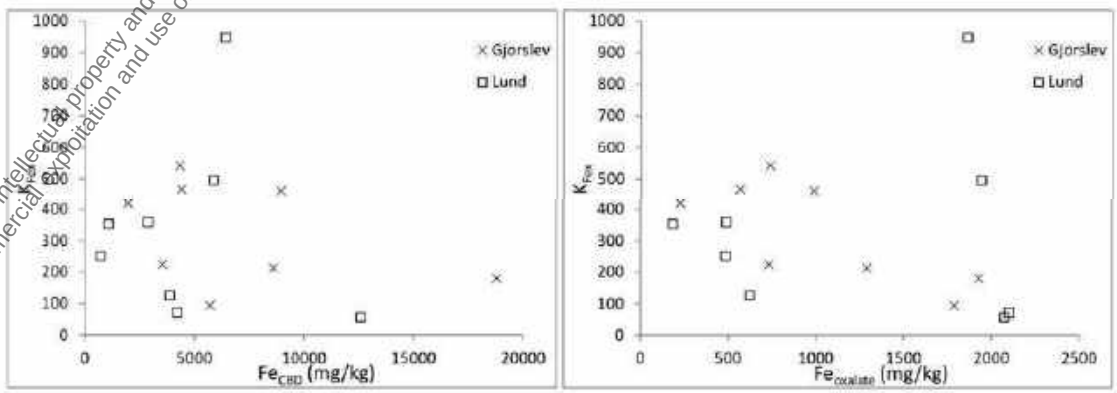
Figure 7.1.3.1.1-37: Sorption isotherms for glyphosate (extended Freundlich model) in the eight soil domains from the Gjorslev profile. Note: Cs is the pesticide concentration in the soil phase, and Cw is the pesticide concentration in the aqueous phase



The concentration dependence can be exemplified by Domain 6 (matrix soil), where the Kd at the lowest glyphosate equilibrium concentrations (0.8–0.9 mg/L) was 377 for Lund and 453 for Gjorslev, whereas at the highest equilibrium concentrations (4.2–4.8 mg/L), the Kd was only 18 for Gjorslev and 22 for Lund. Domain 7 (reddish macropores from same depth as Domain 6) was an exception with low sorption and little concentration dependence, with a Kd of 55 (Lund) to 137 (Gjorslev) at the lowest concentration and 33 to 34 at the highest. Hence, two very different sorption strengths and concentration dependencies were observed from the same soil depth. Also, at the 0.4- to 1.2-m depth, sorption of glyphosate varied in the two domains at both study sites, being twice as high in matrix soil (Domain 2) than in soil from the wormholes (Domain 3). This fits well with the much lower sorption of glyphosate to the plow layer, which shows some similarities with the wormholes.

There was no correlation between Fe oxide content (expressed either as total Fe oxides or amorphous Fe oxides) and glyphosate sorption (expressed as K_{Fex}) (Figure 7.1.3.1.1-38). There was also no correlation when K_{Fex} was plotted against any other measured soil parameter.

Figure 7.1.3.1.1-38: Relationship between total iron oxide concentration (Fe_{CBD}) or amorphous iron oxides ($Fe_{oxalate}$) and sorption of glyphosate (K_{Fex}) in the eight soil domains at the two study sites. K_{Fex} was determined at a µg/L basis and therefore denotes the calculated partitioning coefficient at 1 µg/L



It may be important to consider differences in pesticide sorption between soil domains from the same depth when modeling the risk of pesticide leaching. In clayey tills, most water transport takes place in the macropores; sorption studies, on the other hand, would normally be conducted on bulk soil samples, resembling the matrix samples in the present study. Most sorption studies are furthermore performed only with soil from the plow layer, and leaching in the actual fields may therefore be different from the leaching calculated from such sorption studies. For glyphosate, the leaching would most likely be higher than expected, since sorption to the soil of the upper biopores and especially to the surfaces of the metal oxide coated fractures is lower than in their corresponding matrix domains.

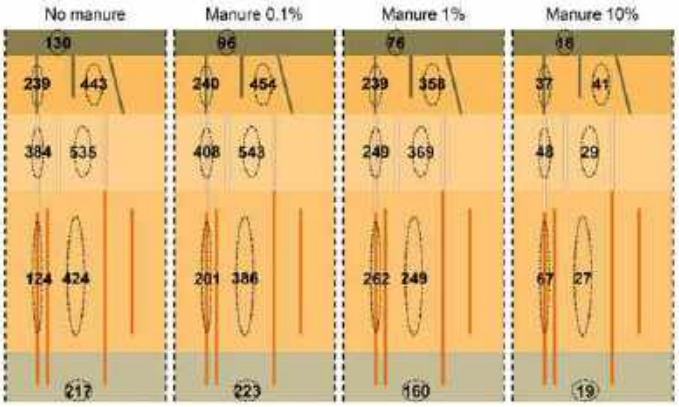
Topsoil Extract and Liquid Manure Extract Reduce Pesticide Sorption

The addition of topsoil extract had an effect on glyphosate sorption, decreasing sorption (K_{Fex}) by 3 to 37 % depending on the domain (Table 7.1.3.1.1-49), and the addition of liquid manure had an even larger effect. Ten percent liquid pig manure changed sorption (K_{Fex}) dramatically, with a decrease of 83 to 95 % in the Gjorslev Domains 1 to 6 and 8, and 76 to 83 % in the corresponding Lund domains (Figure 7.1.3.1.1-39). Manure additionally changed the other extended Freundlich parameters (n and D), as the sorption of glyphosate was less concentration dependent when manure was present.

Table 7.1.3.1.1-49: Sorption parameters for glyphosate with different liquid treatments. Control is without any additions

	Control			Topsoil extract (50%)			Manure (0.1%)			Manure (1%)			Manure (10%)		
Domain	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D
Gjor-1	130	1.06	0.040	86	1.16	0.044	96	1.17	0.046	76	1.09	0.037	16	1.14	0.028
Gjor -2	443	1.04	0.041	338	0.98	0.032	454	0.99	0.036	358	1.07	0.045	41	1.28	0.038
Gjor -3	239	1.07	0.045	193	1.07	0.042	240	1.09	0.048	239	1.08	0.045	37	1.13	0.028
Gjor -4	536	0.88	0.039	354	0.97	0.042	543	0.89	0.039	369	1.03	0.049	29	1.33	0.042
Gjor -5	384	1.01	0.053	295	1.02	0.049	408	0.98	0.049	249	1.14	0.057	28	1.28	0.039
Gjor -6	424	0.87	0.037	283	1.00	0.046	386	0.93	0.042	249	1.08	0.049	27	1.31	0.042
Gjor -7	124	0.92	0.011	142	0.99	0.023	201	0.86	0.012	262	0.98	0.030	67	1.14	0.032
Gjor -8	217	1.08	0.049	169	1.12	0.050	223	1.09	0.051	160	1.17	0.052	19	1.36	0.043
Lund-1	72	1.06	0.036	-	-	-	72	1.01	0.030	35	1.11	0.033	12	1.02	0.019
Lund-2	947	0.98	0.043	-	-	-	1026	0.96	0.044	657	1.00	0.043	47	1.20	0.035
Lund-3	496	0.97	0.040	-	-	-	472	0.96	0.038	262	1.07	0.045	23	1.25	0.036
Lund-4	125	1.12	0.042	-	-	-	244	0.98	0.039	253	1.09	0.050	30	1.27	0.040
Lund-5	353	1.01	0.053	-	-	-	350	1.04	0.055	258	1.09	0.053	18	1.39	0.044
Lund-6	359	0.91	0.037	-	-	-	354	0.97	0.045	241	1.07	0.047	30	1.27	0.040
Lund-7	58	0.93	0.000	-	-	-	116	0.85	0.000	259	0.88	0.016	88	1.12	0.031
Lund-8	251	1.32	0.071	-	-	-	264	1.33	0.073	265	1.33	0.073	14	1.29	0.035

Figure 7.1.3.1.1-39: Effect of liquid pig manure extract (%, v/v) on the sorption (K_{Fex}) of glyphosate in the Gjorslev soil domains. The K_{Fex} equals the predicted distribution coefficient at a glyphosate concentration of 1 µg/L



Why Do Topsoil Extract and Manure Reduce Glyphosate Sorption

Several soil water parameters have been suggested to influence glyphosate sorption. These include pH, phosphate, divalent metal ions like Cu^{2+} and Zn^{2+} and dissolved organic matter.

Change in pH cannot explain the general decrease in sorption when topsoil extract or pig manure was added.

The manure had a high conductivity (21,900 µS/cm, Table 7.1.3.1.1-50). In parallel experiments, it was observed an increase in sorption at increased ionic strengths (data not shown), which has also been reported previously in the literature. The high ionic strength in the manure therefore cannot explain the decreased sorption.

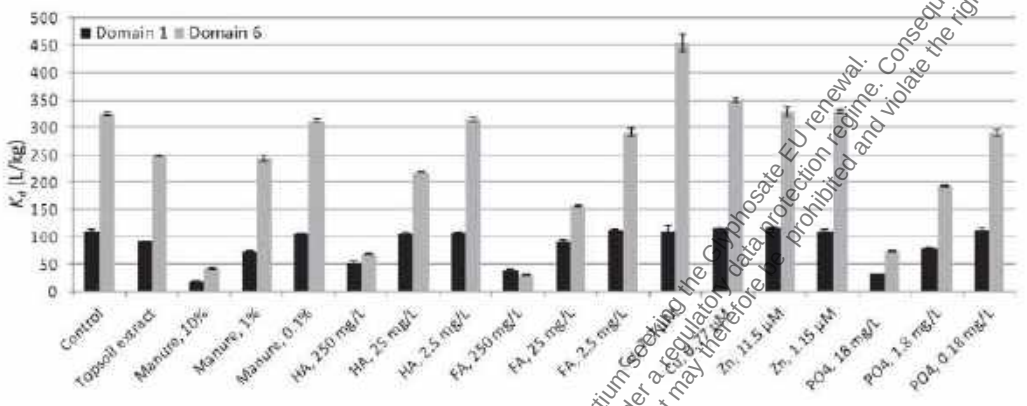
Table 7.1.3.1.1-50: Major analyzed parameters for the liquid manure and topsoil extracts. ND = not determined

Parameter	Unit	Liquid pig manure	Topsoil extract
DOC	mg/L	2648	32
Dry matter	%	1.5	ND
Conductivity	µS/cm	21900	204
PO_4^{3-}	mg/L	182	<0.1
Cu	mg/L	4.9	0.03
Zn	mg/L	7.5	0.12
Fe	mg/L	10.2	22.7
Al	mg/L	0.28	32.0
Na^+	mg/L	623	0.35
K^+	mg/L	2500	0.57
Ca^{2+}	mg/L	<13	3.8
Mg^{2+}	mg/L	<8	0.15
F^-	mg/L	<0.9	1.2
Cl^-	mg/L	1481	4.5
Br^-	mg/L	36.7	0.86
NO_3^-	mg/L	0.64	33.1
SO_4^{2-}	mg/L	10.4	10.3
Ba	mg/L	0.2	0.16
Mn	mg/L	0.4	0.18
S	mg/L	668	11.2
Density		1.02	ND

Both the humic and fulvic acid fractions of soil organic matter decreased glyphosate sorption, when added to soil (Figure 7.1.3.1.1-40).

Divalent metal ions and phosphate would be relevant only with manure addition. Divalent metal ions (Cu^{2+} and Zn^{2+}) at concentrations corresponding to 1 and 10 % pig manure had no effect on sorption in Domain 1 and increased sorption in Domain 6 (Figure 7.1.3.1.1-40). The Cu^{2+} and Zn^{2+} ions are therefore not likely to have caused the manure effect. Phosphate, on the other hand, reduced glyphosate sorption at concentrations corresponding to those in the pig manure (Figure 7.1.3.1.1-40). Both phosphate and dissolved organic matter are therefore likely candidates to explain the manure effect on glyphosate sorption.

Figure 7.1.3.1.1-40: Effect of topsoil extract, liquid pig manure extract, organic matter (humic acids [HA] and fulvic acids [FA]), divalent metals (Cu and Zn), and phosphate on glyphosate sorption (expressed as the distribution coefficient, K_d) in Domains 1 and 6 from the Gjorslev site. Concentrations correspond to the tested manure concentrations. Error bars are minimums and maximums of duplicate samples. Results from experiments with topsoil extract and liquid manure are included for comparison. Controls are without any additions



Conclusion

The study has demonstrated that the sorption of glyphosate varies by an order of magnitude across eight identified soil domains in macroporous clayey till. It was expected that glyphosate would show the strongest sorption in domains with high Fe oxide content. This turned out to be wrong, since there was no correlation between glyphosate sorption and any measured soil parameter, including extractable Fe oxides. The domain-specific sorption of glyphosate was by far overruled by addition of liquid manure that strongly decreased glyphosate sorption due to its content of dissolved organic matter and phosphate. The variation across domains and the effects of solutes like the liquid fraction of manure should be taken into account when using sorption data in assessment of leaching risks. Our results suggest that hydrological modeling should focus more on sorption to fracture surfaces and pay less attention to traditional bulk sorption data when predicting pesticide transport through clay macropores. How much sorption influences leaching will, after all, also depend on general hydrological parameters such as pore size, connectivity, and climatic conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the sorption behaviour of glyphosate to different soil domains (top- and sub-soils) from two agricultural soils in Denmark. The set-up of the experiment was based on the OECD 106 guideline but with significant deviations: The study was conducted with 1 mM $CaCl_2$ solution (standard: 10 mM solution), at 10°C (standard: 20 – 25 °C); at 4 test concentrations (standard: 5), no validation of the analytical methods used, no concentrations in the solid phase were explicitly reported, i.e. no mass balances or parental mass balances were established.

The article is therefore classified as reliable with restrictions i.e. not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/015
Report author	Dollinger, J. *et al.*
Report year	2018
Report title	Contrasting soil property patterns between ditch bed and neighbouring field profiles evidence the need of specific approaches when assessing water and pesticide fate in farmed landscapes
Document No	DOI 10.1016/j.geoderma.2017.09.006 ISSN 0016-7061
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The authors' aim was to evaluate the specificity of ditch material properties to determine whether ditches require an approach that differs from that of field soils when studying water and pesticide fate in farmed landscapes. The authors thus analysed the variations in the pedological, herbicide sorption and flow properties of soil materials along a 2D cross-section of an intermittently flooded ditch in the Roujan catchment of southern France. They found that the upper part of the ditch bed soil profile is composed of 3 horizons that formed after the original creation of the ditch, most likely via the deposition of field-eroded particles and the accumulation of organic matter. These specific horizons have greater porosity, mostly due to their dense root systems, and contain up to 2 times more organic carbon than the neighbouring banks or field soils. Consequently, the hydraulic conductivity is greater, and the sorption of hydrophobic herbicides is up to 2 times greater in ditch bed materials than it is in soils located farther away from the ditch surface. Moreover, significant macroporal flow was evidenced in both profiles but with different contribution to the global flow. The contrasts in the hydrodynamic and sorption properties between both the ditch bed and banks materials likely results in significantly different water and pesticide infiltration patterns in ditches compared to crop fields. Given these differences, they recommend investigating the specific properties of ditch beds when studying and modelling water and pesticide fate in croplands.

Materials and methods

Study site

The studied ditch is located near the outlet of the Roujan catchment (Herault, France). This 91 ha catchment (Figure 7.1.3.1.1-41 and Table 7.1.3.1.1-51) is cultivated mainly by vineyards and a dense network of ditches, 11 km total length, was implemented between the vine fields. Except on the plateau, the soils are directly developed over the Miocene loose sandstone and are organized along a toposequence. The soils depth increase and soil texture evolve consistently with the colluvial accumulations of clay and gravels in the glacis. Nearby the study site the soil is classified as a gleyic cambisol (IUSS Working Group WRB, 2014). A perennial groundwater has developed on the bottom part of the catchment (Figure 7.1.3.1.1-41) and >5 km of ditches (47 % of the total length of ditches) drain this area.

The catchment is subjected to semi-arid Mediterranean climate characterized by scarce high-intensity rainfall events. This specific precipitation pattern results in the periodic flooding of ditches and the rapid fluctuation of the shallow water table in the bottom part of the catchment. The high reactivity of the water table leads to the alternation of downward and upward fluxes in ditch beds during storm events. The studied ditch is chosen near the catchment outlet in order to: i) represent the typical functions of ditch in a perennial groundwater environment (Figure 7.1.3.1.1-41) and ii) be representative of the soil type and ditch characteristics combination that prevail in the 33 ha of the bottom part of the catchment.

Figure 7.1.3.1.1-41: The ditch network over the Roujan catchment in relation with the soils and the perennial groundwater

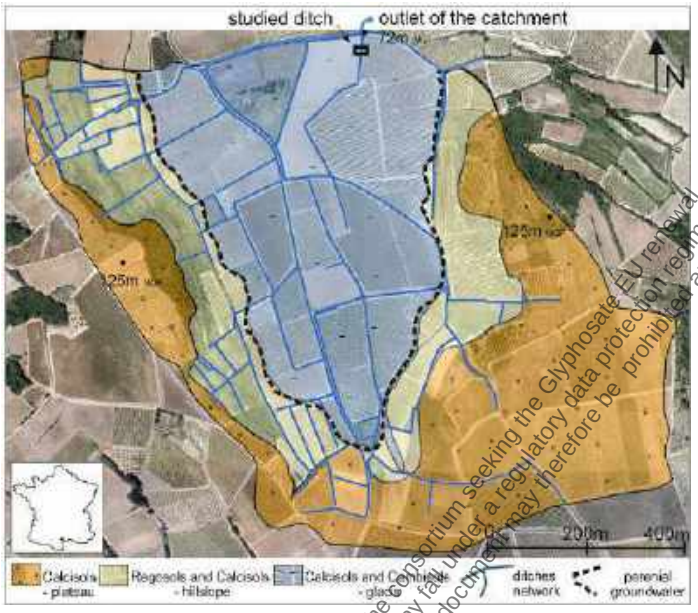


Table 7.1.3.1.1-51: The spatial variability of ditches network over the catchment

Topographic position	Dominant soil types (WRB 2014)	Soil depth	Surface area	Total length of ditch in the network
		m	ha	km
Plateau	Calcisols	0.4–2	36	1.49
Hillslope	Regosols and calcisols	0.2–1.5	22	4.78
Glacis	Calcisols and cambisols	1–4	33	5.48
Catchment			*91*	*11.75*

Experimental design

Characterization of soil properties and core sampling along the cross section

For characterizing and sampling soil heterogeneity of the ditch soil and its vicinity, a 1.50-m-wide, 1.50-m-deep trench was excavated across the ditch in February 2014. The studied ditch is densely vegetated, and roots are present along the entire soil profile to a depth of 1.5 m (Figure 7.1.3.1.1-42A).

A series of morphological parameters, including texture, structure, colour, stone and root abundances, were observed in the field. Soil horizons were determined based on these observations. Bulk densities (ρ_b) were measured by core sampling with 100-cm^3 cylinders, using 6 replicates per horizon. The ρ_b was determined as the ratio between the dry soil mass and the total core sampling volume. Samples of over 500 g were collected from each horizon for further laboratory characterization. Particle size distribution, pH, cation exchange capacity (CEC), organic carbon content (OC), and calcium carbonate ($CaCO_3$) content were measured at the INRA-ARRAS Laboratory (France) (see Table 7.1.3.1.1-52).

Figure 7.1.3.1.1-42: Morphology of the ditch cross-section soil profile. A) Description of the soil profile, B) core sampling scheme. The black lines represent core sampling locations within the soil profile. V and H represent the cores sampling axis direction being, respectively, vertical and horizontal

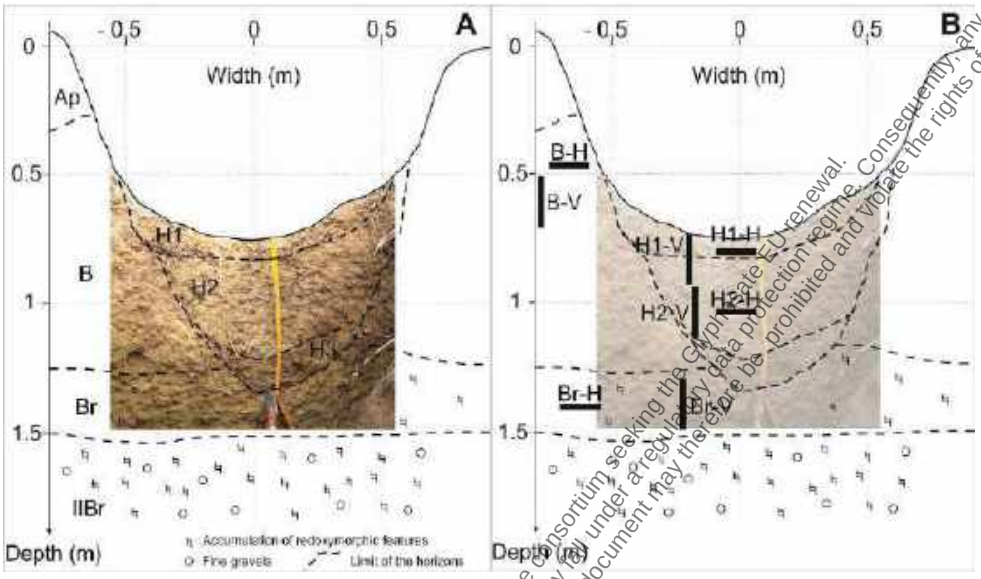


Table 7.1.3.1.1-52: Physico-chemical properties of ditch-bed and banks soils

Horizon	Depth from field topsoil	Structure	Sand	Silt	Clay	OC	CEC	pH	$CaCO_3$	ρ_b
	m		%	%	%	%	cmol kg^{-1}		g kg^{-1}	g cm^{-3}
B	0.4–1.30	Polyhedral subangular blocky – hydromorphic features	7.7	57.2	35.1	0.96	14.2	8.71	247.0	1.36 ± 0.01
H1	0.75–0.82	Stratified	35.9	39.1	25.0	1.58	12.5	8.44	150.0	1.25 ± 0.06
H2	0.82–1.15	Granular	40.3	33.1	26.6	1.56	12.1	8.54	160.0	1.26 ± 0.04
H3	1.15–1.35	Stratified and subangular blocky – hydromorphic features	10.4	56.7	32.9	1.17	14.0	8.59	248.0	1.21 ± 0.05
Br	1.35–1.50	Polyhedral blocky – hydromorphic and redoximorphic features	4.7	58.5	36.8	0.73	14.4	8.63	285.0	1.48 ± 0.03

Four undisturbed soil cores were sampled from each horizon except in the Ap horizon of the bank profile that has no counterpart in the ditch soil profile. These cores were collected by gently pushing stainless-steel cylinders with internal diameters of 15 cm and heights of 20 cm in the soil until the soil surface was approximately 5 cm from the top of the cylinder. The soil around the cylinders was then excavated to facilitate the undisturbed extraction of the monoliths. To characterize the anisotropy of downward vs. lateral water and solute flow, a series of monoliths was sampled vertically and a second series was sampled horizontally (Figure 7.1.3.1.1-42B). Due to the length of the sampled cores, the core sampled in the first horizon below the ditch also included the top of the second horizon; because the third horizon below the ditch was too narrow, it could not be sampled. After extraction, cores were stored at 4°C until undergoing tracer experiments.

Tracer displacement experiments

Tracer displacement experiments were performed on soil cores sampled vertically and horizontally in the ditch bed and bank profiles (Figure 7.1.3.1.1-42B) in order to characterize the water flow patterns of these materials. Because bromide is only present at trace concentrations in the environment and rarely sorbs to soil particles, it was selected as a conservative tracer of water flow for these displacement experiments.

Stainless-steel grids with 6-mm-diameter holes were sealed at the bottom of the columns to prevent soil loss occurring during the infiltration experiments without disturbing the water flux in the columns. Prior to tracer injections, the columns were gradually saturated via capillarity for 48 h to prevent the trapping of gas bubbles in soil pores.

The tracer solutions used for the displacement experiments contained 800 mg/L of bromide (Br^-). At the beginning of the displacement experiments, the saturated columns were manually ponded with a 30-mm water height of the tracer solution. This water height was chosen to mimic the infiltration conditions in the Roujan catchment during intermittent flooding and corresponds to the water level commonly monitored in ditches during flood events with a 1-month return period. The water height was kept constant during the infiltration by adjusting the supply of the tracer solution. A total of 85 mm of solution was supplied to the columns. Among the 16 columns, the pore volumes ranged from 63 to 82 mm. Therefore, the volume of tracer solution supplied during the displacement experiment was always higher than the pore volume of the columns. When the solution supply stopped, the decrease in water head was monitored during the remaining period of ponded water infiltration. Just after all the ponded solution had infiltrated, the columns were ponded again with a constant head of 30 mm, and the columns were flushed with 85 mm of tap water, following the same procedure. During the infiltration and flushing periods, 50 ml fractions of the percolates were collected in glass containers at the outlets of the columns. The sampling frequency varied from 15 s to about 6 min, depending on the columns drainage fluxes. The outlet flowrates were monitored using the timing of sample collection and their precise weights. The inlet flowrates were monitored by weighing the injection tank at 1-s intervals.

The concentrations of bromide in the percolate samples were measured using an ion-specific electrode (Hanna Instruments, HI4002, Lingolsheim). These concentration values were cross-validated with ion chromatography measurements of randomly selected samples. A good fit was found between the ion-specific electrode and the ion chromatograph results (data not shown). The electrical conductivity and pH were also measured in the percolate samples.

Dye tracing of the active macroporosity
Following the displacement experiments, dye tracing was performed on the columns to visualize and quantify the active macroporosity. The dye tracing experiments were also used to visualize the presence or absence of sidewall flow. The displacement experiments were validated when sidewall flow was absent or weak and discontinuous along the sides of the columns. In contrast, when continuous sidewall flow was detected along the sides of the columns, the corresponding displacement experiments were dismissed. A total of 8 columns, containing one sample per horizon and one sample per direction (vertical/lateral) were validated based on dye staining experiments.

The infiltration conditions of dye tracing were identical to those of the displacement experiments, as the fraction of active porosity mobilized for percolation in structured soils likely varies with initial moisture and water head conditions. The columns were thus saturated again via capillarity for 48 h; then, 57 mm of the fluorescent dye sulforhodamine B at a concentration 1 g/L was percolated through the columns with a constant water head of 30 mm. At a concentration of 1 g/L, the sorption sites of sulforhodamine B on soils in contact from all horizons were saturated, which guaranteed homogeneous staining among the columns. After percolation, the columns were sliced into cross-sections approximately 2 cm in height. A marker was placed on the sides of the slices to determine the orientation and superposition of the 7 slices within a given column. The slices were then imaged in a dark chamber with homogeneous LED lighting (3800 K) using a digital camera that was equipped with a 28-mm lens and was positioned 65 cm above the slice. The image resolution was 300 dpi, which corresponds to a pixel size of 71 µm. The illumination and hue saturation of the raw images were corrected using Nikon Capture NX2 software based on the grey and colour scales positioned next to the column slices during imaging. The RGB channels were split, and the colour thresholds were adjusted in each of the channels. The minimum/maximum thresholds applied to all images were 9/255, 118/253 and 4/255 for the R, G and B channels, respectively. The RGB channels were then merged, and the image was binarized. Both bright and dark isolated pixels were removed using the 'Noise' function of the ImageJ software, with a radius of 10 pixels, for white and black pixels, successively. The respective areas of both bright and dark pixels relative to the total area of the column cross-section were then calculated with ImageJ. The dark areas correspond to the stained areas on the cross-sections of the columns.

The volume of macroporosity mobilized during percolation relative to the total porosity (ω) was estimated from the dye coverage area. As dye diffusion in the matrix is limited, due to its short infiltration time, ω was calculated for each column cross-section (i) by multiplying the average dye coverage area per column slice (i.e., top and bottom coverage) by the slice height and dividing it by the total porosity (i.e., the total volume of soil in the slice multiplied by the soil porosity). The average ω per column was also calculated as a geometric mean of the respective ω_i values of the 7 column slices (i).

Inverse modelling of transport properties

Water flow and transport equations

Inverse modelling was performed with the HYDRUS-1D model that solves the Richards and convection-dispersion equations. Four modelling approaches were compared in the first place: single porosity, dual porosity, dual porosity + mobile-immobile (DP + MIM) and dual permeability (see Šimůnek *et al.*, 2003 for a detailed description of these approaches). Only the dual-permeability model provided satisfactory fits of the tracer displacement experiments for most of the columns and is thereby considered in this paper. The column H2-H was the only exception for which the model DP + MIM was better adapted than the dual permeability model. DP + MIM was used to simulate the bromide breakthrough curve of H2-H but is not described in this paper (for the description of the model please refer to Šimůnek *et al.*, 2003). Equations of the dual permeability model are briefly reviewed below.

The dual permeability model assumes that flow and solute transport occur within and between two distinct compartments, namely the macropore compartment, consisting in inter-aggregate or fracture porosities, and the micropore or matrix compartment, consisting in intra-aggregate porosity. The water flow equations in the macroporal and matrix compartments are assumed similar by HYDRUS 1D and given by:

$$\frac{\partial \theta_f(h_f)}{\partial t} = \frac{\partial}{\partial z}\left[K_f(h_f)\left(\frac{\partial h_f}{\partial z}+1\right)\right] - S_f(h_f) - \frac{\Gamma_w}{\omega} \quad (1a)$$

$$\frac{\partial \theta_s(h_s)}{\partial t} = \frac{\partial}{\partial z}\left[K_s(h_s)\left(\frac{\partial h_s}{\partial z}+1\right)\right] - S_s(h_s) - \frac{\Gamma_w}{1-\omega} \quad (1b)$$

where subscript *f* and *s* respectively refers to the fast macroporal compartment and the slow matrix compartment, θ is the water content [L^3/L^3], *h* is the pressure head [L], *K(h)* is the unsaturated hydraulic conductivity function, *S* is a sink or source term [T^{-1}], ω is the ratio of the macroporal volume of fast to the total poral volume of the soil (dimensionless) and Γ_w is the transfer rate between the two compartments [T^{-1}]. The water retention curve $\theta(h)$ and the unsaturated hydraulic function *K(h)* are defined for both compartments using the van Genuchten model. *K(h)* is described as the product of the relative hydraulic conductivity function *Kr* (dimensionless) and the saturated hydraulic conductivity *Ks* [L/T].

The transport equations associated with the dual-permeability formulation for water flow are based on the classical convection-dispersion equation for both the fast macroporal compartment and the slow matrix compartment with an exchange term between the two compartments:

$$\frac{\partial \theta_f c_f}{\partial t} + \rho\frac{\partial s_f}{\partial t} = \frac{\partial}{\partial z}\left(\theta_f D_f \frac{\partial c_f}{\partial z}\right) - \frac{\partial q_f c_f}{\partial z} - \phi_f - \frac{\Gamma_s}{\omega} \quad (2a)$$

$$\frac{\partial \theta_s c_s}{\partial t} + \rho\frac{\partial s_s}{\partial t} = \frac{\partial}{\partial z}\left(\theta_s D_s \frac{\partial c_s}{\partial z}\right) - \frac{\partial q_s c_s}{\partial z} - \phi_s + \frac{\Gamma_s}{1-\omega} \quad (2b)$$

$$\Gamma_s = \omega_{dp}(1-\omega)\theta_s(c_f - c_s) + \Gamma_w c^* \quad (2c)$$

where *c* is the solute concentration [M/L^3], *s* is the sorbed solute concentration [M/M], ρ is the bulk density [M/L^3], *D* is the dispersion coefficient accounting for both molecular diffusion and hydrodynamic dispersion [L^2/T], *q* is the Darcian flux [L/T] ϕ is a sink-source term [$M/(L^3\ T)$], Γs is the mass transfer term for solute between the macroporal and the matrix compartments [$M/(L^3\ T)$] and c^* is equal to c_f. for $\Gamma_w > 0$ c_m for $\Gamma_w < 0$.

Inverse modelling design

The 0.15 m-soil profiles were densely discretized with 101 nodes to facilitate numerical convergence. An initial hydrostatic equilibrium with a zero-pressure at the top of the soil columns was considered. A variable head was imposed at the upper boundary condition. It was fixed at the ponding head value (3 cm) during the injection and rinsing phases and varied between both phases to correspond to the ponding height decreases monitored during the experiments (see Section *Tracer displacement experiments*). The eight following parameters were fitted against the cumulative water outflows heights and bromide concentrations at the outlet of the soil column: θs_s, θs_f, Ks_s, Ks_f, ω, $Disp_s$, $Disp_f$, ω_{dp} with θs and θr respectively the saturated and residual soil water content and *Disp*, the dispersion coefficient [L]. To avoid local minimum, the stability of the fitted parameter set estimated was evaluated using different sets of initial parameters, including the estimated sets themselves. The other hydrodynamic parameters were set according to the textural composition and bulk density of the soils using Rosetta, except θr_f that was set to zero. Note however that since the soil column remained saturated during the whole experiment, the van Genuchten parameters alpha, n and l were not sensitive. The Bromide diffusion coefficient was fixed to 1.67 10^{-9} m^2 s^{-1}. K_{sat} was not adjusted but calculated from the experimental outflow data using Darcy's law. Θs was also not adjusted but calculated from the bulk density data using a pedotransfer function.

Sorption properties of selected herbicides

Two herbicides, diuron and glyphosate, were selected to assess the heterogeneity of sorption properties along the profile of the ditch cross-section. Diuron was extensively used on the Roujan catchment for weed control in vineyards. After it was banned from the list of allowed active molecules in France in 2008, it was replaced by the broad-spectrum herbicide glyphosate. Both herbicides were still measured in the water column of the ditch at the outlet of the catchment in 2016. Glyphosate and diuron exhibit very different physicochemical properties (Table 7.1.3.1.1-53), which may lead to contrasting sorptive patterns along the soil profiles.

The adsorption parameters were assessed according to the procedure described in Dollinger *et al.* (2016), which was adapted from the OECD Guideline 106. Briefly, the soils were air-dried to a target humidity of 10 % then sieved to a size of 2 mm. 10 mL of the ^{14}C-labelled pesticide solution, with concentrations ranging from 5 to 1000 µg/L, were equilibrated with 1 and 2 g of dry soil in glass centrifuge tubes for glyphosate and diuron adsorption experiments, respectively. The tubes were shaken for 24 h, and the radioactivity in the supernatant was measured after centrifugation. Pesticide concentrations in soils were assessed by mass balance between initial and equilibrium concentrations. Both linear (Eq. 3) and Freundlich models (Eq. 4) were fitted to the experimental data.

$$C_s = Kd\ C_w \quad (3)$$

$$C_s = Kf\ C_w^n \quad (4)$$

$$H = \frac{n_{ads}}{n_{des}} \quad (5)$$

where C_s is the amount of sorbed pesticides in the soil at equilibrium (µg kg^{-1}), C_w is the equilibrium concentration in the supernatant (µg/L), *Kd* is the linear sorption coefficient (L/kg), *Kf* (µg$^{(1-n)}$ L^n/kg) and n are the Freundlich coefficients and H is the apparent hysteresis index with n the non-linearity parameter of the Freundlich model and subscripts *ads* and *des* standing for adsorption and desorption isotherms, respectively.

The detailed procedure for the determination of herbicide desorption parameters can be found in Dollinger *et al.* (2016). Briefly, after 24 h of equilibration with a 100 µg/L pesticide solution, the activity in the supernatant was measured and the residual supernatant was removed. An equivalent volume of fresh electrolyte was added, and the tubes were shaken again for 24 h. Five successive desorption steps of 24 h each were then performed. The amount of pesticides sorbed to soils at each step was calculated by mass balance based on radioactivity counting, and experimental data were fitted to Freundlich isotherms (Eq. 5). The hysteresis between adsorption and the corresponding desorption isotherms was represented by the H

parameter (Eq. 3), which was calculated as proposed by Barriuso *et al.* (1994). Sorption is considered to be hysteretic when H <0.7; the lower the value of H is, the more irreversible the sorption is.

Table 7.1.3.1.1-53: Physico-chemical properties of the studied pesticides

Properties		Glyphosate	Diuron
Formula		$C_3H_8NO_5P$	$C_9H_{10}Cl_2N_2O$
Molecular mass	$g\ mol^{-1}$	169.1	233.1
Aqueous solubility at 20 °C	$g\ l^{-1}$	10.5 to 12.0	0.42
Log Kow at pH 7		− 4.1 to − 3.2	2.7
$pKa_1 - pKa_2 - pKa_3$		2.2–5.5–10.2	13.2

From ANSES, 2017, FOOTPRINT, 2015, ChemID, 2017 and chemicalize.org 2017.

Results

Morphology of the ditch bed and bank profiles

Based on field morphological descriptions, two different soil profiles were distinguished along the cross-section: (i) the bank profile, which is composed of 4 horizons, and (ii) the ditch bed profile, which is composed of 5 horizons (Table 7.1.3.1.1-52 and Figure 7.1.3.1.1-42).

The bank soil profile corresponds to the soil pit observed in the vicinity of the ditch by Andrieux *et al.* (1993). According to the World Reference Base, this soil is a tilled gleyic Cambisol (colluvic, clayic). The structure of the first horizon (Ap, which extends from the surface to a depth of 0.4 m) is affected by tillage and deep ploughing operations. The upper cambic horizons B and Br (described in Table 7.1.3.1.1-51) are developed above another deep cambic horizon IIBr (Figure 7.1.3.1.1-42A) that feature both a high clay content and high bulk density values. However, more hydromorphic features and denser root systems are observed closer to the ditch bank surface than they are in the bank soil profile, which is located further away. The ditch bed soil profile corresponds to a succession of 3 ditch-specific horizons (H1, H2, and H3) and the Br and IIBr horizons, which are shared with the bank profile. The H1, H2, and H3 horizons are significantly different from the other horizons. H1 and H2, which are enriched in sand and have platy structures, are different from the bank horizon B, which is siltier and is dominated by a subangular blocky structure. The third horizon, H3, is similar to the bank horizon B in terms of texture but features a stratified structure that differs from that of the bank horizons. These differences indicate that the H1, H2, and H3 horizons were formed by the deposition of field-eroded particles during successive flood events subsequent to the creation of the ditch. The contours of the 3 horizons specific to the ditch bed (H1, H2 and H3; Figure 7.1.3.1.1-42A) thus likely delimit the section of the original ditch. The shape of the horizons is probably due to the regular management of the ditch, including dredging operations. At the location where the profiles were observed, the original ditch only slightly incises the Br horizon that prevailed prior to the creation of the ditch.

The B and Br horizons have very similar physicochemical properties (Table 7.1.3.1.1-52), although horizon B has a slightly greater organic carbon content and a lower bulk density. However, the porosities of horizons B and Br are larger in the vicinity of the ditch surface due to the higher density of the ditch vegetation root system. The upper two ditch bed soil horizons (H1 and H2) contain 1.5 to 2 times more organic carbon than horizon B, which is consistent with the presence of vegetation and higher water contents during the year. Moreover, the bulk densities of the specific ditch bed horizons are significantly lower than those of the other horizons, which is in accordance with their textures, organic matter contents, and dense root channels network. Therefore, the overall porosity of the ditch bed soil profile is higher than that of the bank profile.

In summary, the ditch bed profile and the bank profile have contrasting textural, chemical and structural properties. Moreover, the vertical gradient of the analysed soil properties across the ditch bed horizons is sharper than that across the bank horizons. Both lateral and vertical gradients of soil properties, such as organic matter content or bulk density, are present within the limited spatial area of one square metre between the ditch and the bank. It is therefore expected that flow and sorption properties differ between the ditch and bank soils.

Heterogeneity and anisotropy of water pathways and associated soil pore structure
The results of the displacement experiments and dye staining of the active macroporosity (ω_{dye}) allowed us to compare the hydraulic conductivity and preferential flow patterns of the different horizons and sampling axes in the two soil profiles (Figure 7.1.3.1.1-43, Table 7.1.3.1.1-54) and to relate these flow patterns to the macroporosity patterns (Figure 7.1.3.1.1-44 and Figure 7.1.3.1.1-45). The inverse modelling procedure provides a complementary estimation of the flow mechanisms and soil hydrodynamic properties (Table **7.1.3.1.1-54**). The main interest of the modelling results is the opportunity to estimate the contribution of the fast flow to the global outflow.

The horizontal and vertical saturated hydraulic conductivity values (K_{sat}) at the column scale calculated from the percolation flux data range from very large ($1.7 \cdot 10^{-4}$ m s^{-1} for H2-V) to rather small ($6.9 \cdot 10^{-6}$ m s^{-1} for Br-H) values (Table 7.1.3.1.1-54). Regardless of the horizon, no systematic differences were observed in the measured K_{sat} values between the two sampling axes. With the exception of the second horizon in the ditch bed (H2), the anisotropy of the hydraulic conductivity was small in all samples. Therefore, a mean saturated hydraulic conductivity value was calculated for each horizon, and these values are reported in Table 7.1.3.1.1-54. The mean saturated hydraulic conductivity of the B horizon is slightly smaller than those of the H1 and the H2 horizons, despite important differences in their textures, organic matter contents and structures, as observed in Section *Morphology of the ditch bed and bank profiles*. Horizon H2 is the most conductive horizon due to the large value of its observed vertical K_{sat}, which may be caused by specific local macropore features, such as the snail shells observed in this horizon. The Br horizon is 4 to 15 times less conductive than the other horizons. Accordingly, as generally observed in structured soils, both ditch bed and bank soil profiles exhibit decreasing soil hydraulic conductivity with depth (e.g., Sammartino *et al.*, 2015; Udawatta and Anderson, 2008), but the upper horizons of the ditch bed profile have higher permeability values than those of the bank profile.

The dye tracing experiments reveal information about the active macroporosity patterns (Figure 7.1.3.1.1-44) and, thus, about the heterogeneity of the soil structures between the horizons. In all columns, the dye percolated across the column demonstrating the presence of connected macroporosity along the height of the column. However, the magnitude of this connected macroporosity varied greatly between the horizons. Roots were found to be the main source of flow paths in most horizons, as most stained areas surrounded living or decayed root channels. However, not all living or decayed root channels were stained. Roots were present throughout the entirety of both profiles, but denser networks were located near the ditch surface. Consistently, on average, the active porosity was largest in the cores sampled in the upper horizons of the ditch bed profile (Figure 7.1.3.1.1-42 and Figure 7.1.3.1.1-44, Table 7.1.3.1.1-54). The B horizon exhibits a large anisotropy in ω_{dye}, yielding a very large value of approximately 20 % in the horizontal direction. This anisotropy may be partly explained by the fact that the sampling location of the horizontal column is almost in the ditch sidewalls and is slightly further away for the vertical column (Figure 7.1.3.1.1-42). The H1 and H2 horizons both exhibit a large active porosity, as H2 has the largest ω_{dye} values of all of the horizons. The numerous snail shells, combined with the granular structure present in H2, are likely responsible for its greater active porosity than H1. The active porosity of the Br horizon is significantly smaller than those of the other horizons. Finally, in accordance with the observed variations in saturated hydraulic conductivity, the ditch bed profile exhibits, on average, a larger active porosity than the bank profile. Indeed, although the linear correlation is not statistically significant, K_{sat} generally increases when ω_{dye} increases (Figure 7.1.3.1.1-45).

Figure 7.1.3.1.1-43: Bromide breakthrough curves. The black dashed lines represent the shift between contaminated and clear water injection

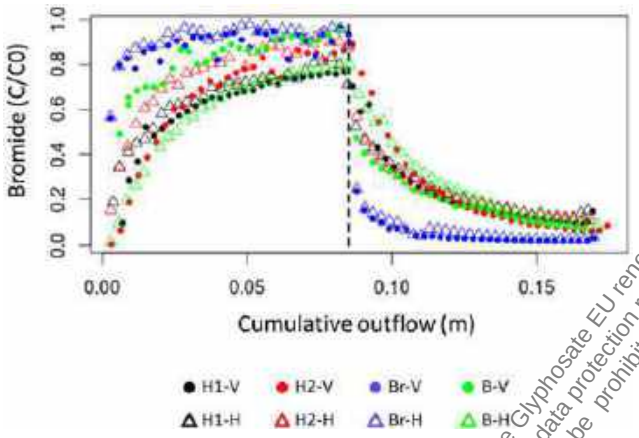


Figure 7.1.3.1.1-44: Imaging of preferential flow patterns within the soil columns. The black areas represent the stained areas at different depths along the soil cores

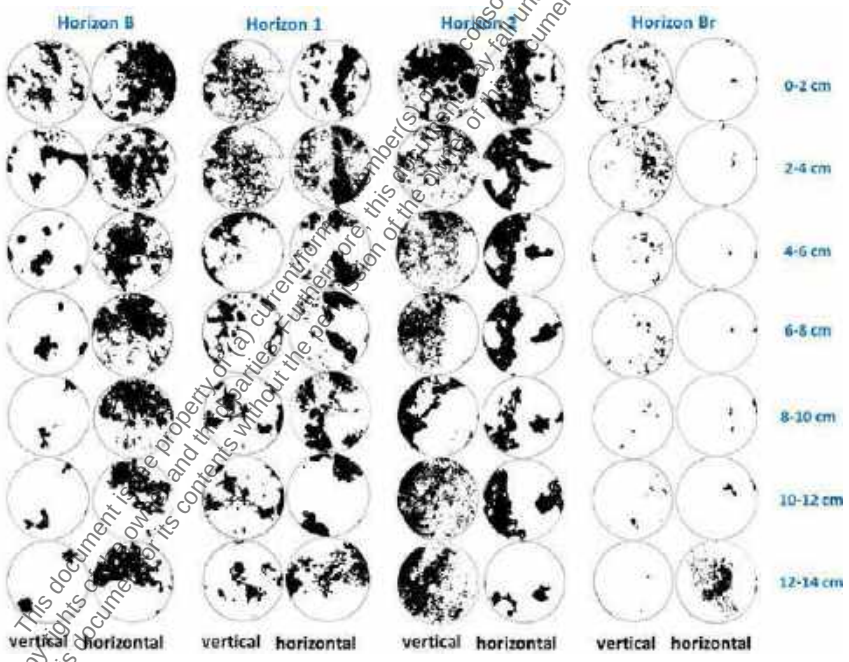


Figure 7.1.3.1.1-45: Evolution of the hydraulic conductivity with the active macroporosity fraction in the set of soil columns

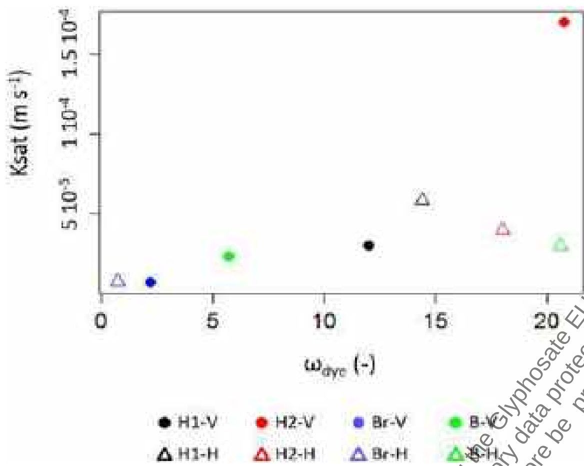


Table 7.1.3.1.1-54: Hydrodynamic properties of the ditch-bed and banks soil horizons

Columns	ᵃK_{sat}	ᶜMean K_{sat}	ᵃω_{dye}	ᵇθr_s	ᵇθs_s	ᵇKs_s	ᵇθs_f	ᵇKs_f	ᵇω	ᵇ$Disp_s$	ᵇ$Disp_f$	ᵇθs_{Ks}	ᵇθs	ᵃRelative contribution of preferential flow
	$m\,s^{-1}$	$m\,s^{-1}$	%	m^3m^{-3}	m^3m^{-3}	$m\,s^{-1}$	m^3m^{-3}	$m\,s^{-1}$	%	m	m	m^3m^{-3}	m^3m^{-3}	%
B-V	$2.34\cdot10^{-5}$	$2.68\,10^{-5}$	5.7 ± 3.5	0.093	0.55	$3.01\cdot10^{-5}$	0.250	$3.07\cdot10^{-4}$	10.0	0.13	0.5	0.51	0.48	53.1
B-H	$3.02\cdot10^{-5}$		20.6 ± 3.5	0.093	0.45	$8.95\cdot10^{-6}$	0.492	$7.76\cdot10^{-5}$	21.0	0.27	24.25	0.46	0.48	69.7
H1-V	$2.99\cdot10^{-5}$	$4.41\,10^{-5}$	12.0 ± 3.6	0.075	0.41	$1.35\cdot10^{-5}$	0.367	$1.13\cdot10^{-4}$	17.0	0.1	0.07	0.40	0.52	63.2
H1-H	$5.82\cdot10^{-5}$		14.4 ± 2.8	0.075	0.45	$2.26\cdot10^{-6}$	0.450	$3.79\cdot10^{-4}$	10.0	0.23	30	0.45	0.52	65.1
H2-V	$1.70\cdot10^{-4}$	$1.05\,10^{-4}$	20.8 ± 5.2	0.077	0.55	$4.91\cdot10^{-5}$	0.400	$5.98\cdot10^{-4}$	22.0	0.1	0.1	0.52	0.52	77.5
H2-H**	$3.99\cdot10^{-5}$		18.0 ± 3.5	0.077	0.49	$1.18\cdot10^{-5}$	0.430	$3.0\cdot10^{-4}$	10.0	0.03	25.84	0.48	0.52	73.3
Br-V	$6.90\cdot10^{-6}$	$7.38\,10^{-6}$	2.2 ± 2.0	0.092	0.40	$1.08\cdot10^{-6}$	0.300	$9.80\cdot10^{-5}$	6.0	0.1	0.47	0.39	0.44	85.3
Br-H	$7.86\cdot10^{-6}$		0.7 ± 1.1	0.092	0.40	$1.08\cdot10^{-6}$	0.300	$9.80\cdot10^{-5}$	7.0	0.1	0.47	0.39	0.44	87.2

ᵃ Values obtained from the experimental data.
ᵇ Values obtained from the inverse modelling of the bromide breakthrough curves.
ᶜ Mean K_{sat} is the mean saturated hydraulic conductivity for a given horizon.
** The values of the hydrodynamic parameters obtained from inverse modelling result from adjustments of a dual permeability model except for the column H2-H for which a dual porosity + mobile-immobile model was used. θr_f always equals 0. For H2-H the saturated water content of immobile compartment θs_{IM} = 0.35, the Ks_s and Ks_f are the K_{sat} values in the slow and fast porous compartments and the K_{sat} of the immobile compartment is null.

Figure 7.1.3.1.1-43 depicts the succession of bromide concentrations measured at the column outlets during displacement experiments. The results are expressed as the ratio of outlet to inlet concentrations. These concentration evolutions could all be satisfactorily simulated using a dual-permeability model except the H2-H for which a dual-porosity model with a mobile-immobile conceptualization of the matrix compartment was needed. For each set of parameters allowing a good reconstitution of the water flow and the bromide leaching pattern (Table 7.1.3.1.1-54), the estimated ω were statistically equivalent to the one obtained with dye tracing (linear correlation: slope = 0.97, intercept = 0, R^2 = 0.92, p-value = $5\cdot10^{-5}$). This highlights the reliability of the simulated parameters, despite the equifinality issue inherent to the large number of fitted parameters.

For all displacement experiments, quantifiable bromide concentrations were measured in the first 50 ml of leachates, which were collected between 15 s and 7 min after injection began. This suggests that preferential flow occurred in all of the columns (e.g., Paradelo *et al.*, 2016), which is consistent with the observation of connected macroporosity in the columns. Additionally, two major shapes of breakthrough curves can be distinguished.

In the columns of horizons B and H, the curve features a gentle increase and decrease in concentration during the injection and rinsing phases, respectively, as well as a maximum concentration that is less than the injected concentration. If it is assumed that macropore flow occurred almost instantaneously at a concentration close to the injected concentration, it follows that, throughout the displacement experiment

in these columns, matrix flow was a significant contributor to outflow, as bromide concentration remained below the injected concentration. Although the volume of the injected bromide solution was chosen to be larger than the overall pore volume of the columns, this volume was likely not sufficient to ensure a renewal of matrix pore water. This hypothesis is confirmed by the modelling results indicating that even if preferential flow contributed up to 77 % to the global outflow for this group of columns, the hydraulic conductivity of the fracture never exceeded 25 time that of the matrix (Table 7.1.3.1.1-54).

The other breakthrough curve shape is observed in the columns of the Br horizon and exhibits a sharp increase and early plateau in bromide concentrations during the injection phase, in which the plateau concentration is close to the injected concentration value. Additionally, a sharp decrease in concentration is observed during the rinsing phase. This pattern is not consistent with the small observed macroporosity of the Br horizon but can be explained by the very poor permeability of the soil matrix. In this case, most of the flow bypasses the soil matrix and flows through a few connected macropores. This hypothesis is confirmed by the modelling results indicating that preferential flow contributed to >85 % to the global outflow for this group of columns and that the hydraulic conductivity of the fracture was >90 time higher than that of the matrix (Table 7.1.3.1.1-54).

In accordance with recent studies relating soil macroporosity and hydraulic conductivity in structured soils, K_{sat} generally rises along with an ω increase (Figure 7.1.3.1.1-45). As ω is related to the root channels density, which decreases with the distance from the ditch surface, the saturated hydraulic conductivity is overall greater in the upper horizons of the ditch bed than in the banks. The contribution of preferential flow to the global outflow, is however greater in the deep bed and bank horizon. This can be explained by the contrasted hydraulic conductivity of the macroporal compartment relative to that of the matrix compartment (Ks_f/Ks_s) and by ω ($R^2 = 0.95$, p-value = $6 \cdot 10^{-4}$).

In sum, it is mainly in their upper horizons that the bank and ditch bed profiles differ in their patterns of water and solute transport. The top horizons of the ditch bed exhibit larger transport properties due to their larger active macroporosities, which are related to their denser rooting patterns. Thus, in contrast with the soil profile from which it originates, the ditch bed profile exhibits larger infiltration and percolation capacities. However, the deeper percolation of water and solutes is limited in both profiles by their common bottom Br horizon, which exhibits low permeability. The differences in the flow patterns may induce significant contrasts in the transfer and retention of herbicides. Indeed, water pathways determine the material surface area that is in contact with the soil solution and its effective contact time during downward seepage. This conditions the herbicide sorption equilibria.

Pesticide sorption heterogeneities among the ditch bed and bank soil profiles

The heterogeneities in herbicide sorption properties among the horizons are presented in Table 7.1.3.1.1-55. The H1 and H2 horizons exhibit the greatest diuron adsorption capacities and lowest desorption capacities, whereas adsorption on B and Br is low and very easily reversible. Therefore, the sorption capacities of the ditch bed profile are larger than those of the bank profile. For glyphosate, the adsorption coefficient of the B horizon is higher than that of the H1 horizon but is lower than that of the H2 horizon, and the desorption hysteresis of the B horizon is smaller and larger than those of the H1 and H2 horizons, respectively. The Br horizon exhibits a lower adsorption coefficient than the B horizon, but they both exhibit a similar desorption hysteresis. Based on the properties of these horizons, it remains unclear whether the ditch bed profile or the bank profile has the greater retention capacity.

Table 7.1.3.1.1-55: Sorption coefficients of the herbicides on ditch soils

Molecule	Horizon	Kf_{ads}	n_{ads}	Kd_{ads}	Kf_{des}	n_{des}	H
		$\mu g^{(1-n)}l^n kg^{-1}$	–	$l\,kg^{-1}$	$\mu g^{(1-n)}l^n kg^{-1}$	–	–
Diuron	B	4.49	0.84	1.86	110.58	0.12	0.98
	H1	14.79	0.83	5.17	114.66	0.32	0.39
	H2	10.57	0.82	3.52	118.74	0.25	0.31
	Br	4.12	0.81	1.49	114.31	0.09	0.97
Glyphosate	B	157.66	0.94	109.90	666.99	0.28	0.29
	H1	77.60	0.93	51.69	675.64	0.14	0.15
	H2	165.24	0.96	129.86	533.29	0.36	0.37
	Br	124.36	0.91	75.38	505.00	0.29	0.32

In summary, the heterogeneities in the sorption coefficients of the two studied pesticide within a given profile are more substantial under the ditch bed than in the banks. Generally, due to the enrichment in organic matter of the ditch bed horizons, the sorption capacities of hydrophobic molecules in the ditch bed profile should be greater than those in the bank profile. Concerning ionisable compounds with low hydrophobicity, a higher sorption capacity of ditch bed profiles is not straightforward. The desorption hysteresis are generally significant in the ditch bed and are weaker or null in the bank soils. This should lower the release of pesticides previously adsorbed in the ditch bed soils as compared to the bank soils.

Conclusion

This study provides the first description of the range of soil properties influencing the magnitude of the water and pesticide exchanges occurring between surface water and groundwater along a ditch cross section profile. These ditch bed soil properties were also for the first time compared with those of the surrounding field soils. The in-situ and laboratory characterization of the physico-chemical properties evidenced distinct soil profiles between both the ditch bed and banks profiles. The ditch bank profile was equivalent to the surrounding field profile. In particular, the ditch bed upper horizons contain up to 2 times more organic carbon than the bank soils. These upper ditch bed horizons being also located closer to the ditch surface than the bank soils, they contain a denser network of plant roots which increases their active macroporosity and in turn their hydraulic conductivity. The deeper horizons share however, great similarities in both profiles, in particular their poor macroporosity, hydraulic conductivity and organic carbon content.

In conclusion, the physicochemical and sorptive properties of specific ditch bed horizons contrast with those of the ditch banks and neighbouring field soils. These differences may thus have different effects on the risk of groundwater contamination by pesticides. On one hand, ditch beds exhibit higher organic matter contents than field soils, possibly limiting the percolation of hydrophobic pesticides due to increased retention in the soil matrix. On the other hand, the upper horizons of ditch beds present larger active macroporosity and transport property values, which favour percolation. The final balance between the two effects, in terms of overall groundwater contamination risk, depends on the local hydrological conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article reports the properties of a soil from a ditch in an agricultural area in the south of France. Mainly, the hydraulic parameters of the different soil layers of the ditch and the surrounding banks are considered and modelled and tracer experiments with bromide are presented.

Sorption experiments with glyphosate were conducted and Freundlich sorption coefficients for the different soil horizons including topsoil and subsoil are reported. However, there was no detailed reporting of data to assess the validity (i.e. mass balances, chemical properties of test substance, solvents used, information about analytical methods and their validation including, LOD, LOQ, temperature, test concentrations, demonstration of stability of the test item).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/016
Report author	Skeff, W. *et al.*
Report year	2018
Report title	Adsorption behaviors of glyphosate, glufosinate, aminomethylphosphonic acid, and 2-aminoethylphosphonic acid on three typical Baltic Sea sediments
Document No	DOI 10.1016/j marchem.2017.11.008 E-ISSN-1872-7581
Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

A batch experiment was conducted to study the adsorption behaviors of glyphosate, glufosinate, aminomethylphosphonic acid (AMPA), and 2-aminoethylphosphonic acid (2-AEP) in marine sediments (mud, silt, and sand) from the Baltic Sea. The experiment took into account the influence of pH, salinity, and temperature on the adsorption behaviors of the studied compounds. In contrast to glufosinate, glyphosate exhibited an adsorption affinity for the three types of sediments. AMPA and 2-AEP showed similar adsorption behaviors on mud and silt, while their adsorption on sand was negligible. The equilibrium adsorption data for glyphosate, AMPA, and 2-AEP on mud and silt fit well with the linear partitioning and Freundlich isotherms, whereas the data for glyphosate on sand could only be fitted with the Freundlich isotherm. The Freundlich distribution coefficients (k_f) were in the range of 6.1-259.5 L/kg for glyphosate, 9.2-39.5 L/kg for AMPA, and 7.7–38.5 L/kg for 2-AEP under the experimental conditions of pH 8.1, temperature = 21°C, and a salt concentration of 8 g/L. The adsorption kinetic was better described by the pseudo-second-order than the pseudo-first-order model, suggesting chemisorption as the adsorption mechanism. The order of adsorption of the compounds on the sediments was: glyphosate >AMPA ≥2-AEP >glufosinate. The adsorption capacity of sediments followed the sequence: mud >silt >sand. Increasing the pH, salinity, or temperature of the solution significantly reduced the adsorption capacity of the compounds. The data obtained in this study provide valuable information on the fate and distribution of the investigated phosphonates in the Baltic Sea.

Materials and methods

Chemicals and reagents

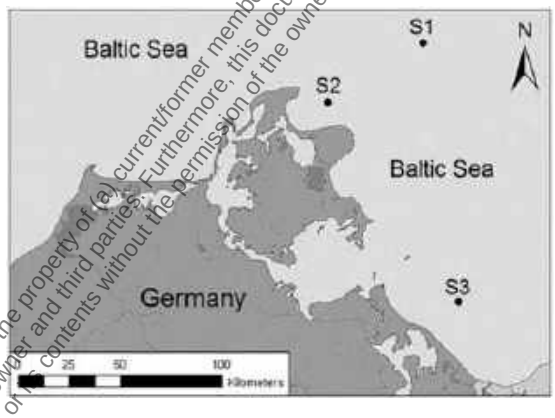
Standards of glyphosate, a glyphosate internal standard (1-2-$^{13}C_2{}^{15}N$ glyphosate), AMPA, an AMPA internal standard (^{13}C ^{15}N AMPA), and glufosinate were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 2-AEP was supplied by Sigma-Aldrich (Taufkirchen, Germany). Stock solutions of these compounds, except the internal standards, were prepared in polypropylene volumetric flasks at a concentration of 100 mg/L by dissolving 5 mg of each compound in 50 mL of LC-MS grade water (VWR International GmbH, Darmstadt, Germany). The stock solutions were stored at 5°C in the dark. A stock solution (66.6 mM) of 9-fluorenylmethyl chloroformate (FMOC-Cl) (purity 99.0 %, Sigma-Aldrich) was prepared by dissolving 1 g in 58 mL of acetonitrile (Walter-CMP GmbH, Kiel, Germany). Borate buffer at pH 9 was prepared by dissolving 1 g of sodium tetraborate decahydrate (Sigma-Aldrich) in 50 mL of

Milli-Q water (Merck kgaA, Darmstadt, Germany). Artificial sea salt, contains all 70 trace elements found in natural seawater in the exact proportions found in nature, was purchased from Tropic Marin®, Germany. Chloroform was supplied by VWR AnalaR Normapure (Germany).

Sediment collection and characterization

Three types of sediment typical of the Baltic Sea were collected from the German Baltic Sea (Figure 7.1.3.1.1-46) which are: S1 from Arkona basin (54° 50′ N, 13° 30′ E), S2 from Tromper Wiek (54° 39′ N, 13° 35′ E), and S3 from Oder Bank (54° 04′ N, 14° 03′ E). The sediments were collected using a multiple corer during research cruise EMB76, in June 2014. Samples of the uppermost sediment were sealed in glass jars, stored at -20°C until dry. No sieving was done but large items such as stones, leaves, grass and animals were removed and the samples were manually homogenized. The bulk sediments were freeze-dried using a Chaist ALPHA 1-4 LD freezer dryer and used as sorbents in this study. The grain sizes of the sediments were determined using a CILAS 1180 particle size analyzer. The TOC content of the sediments was analyzed with an elemental analyzer according to (Leipe *et al.*, 2011). The major and trace elements in the sediments were measured using inductively coupled plasma optical emission spectrometry after acid total digested of the samples. The sediment grain sizes were distributed among the different classes: clay (<2 µm), silt (2-63 µm), and sand (>63 µm). The sediment S1 contained 6.6 % clay, 92.3 % silt, and 1.1 % sand, with a median grain size 20.1 µm. The sediment S2 contained 3.6 % clay, 69.9 % silt, and 26.5 % sand, with a median grain size 41.2 µm. The sediment S3 contained 1.7 % clay, 10.7 % silt, and 87.6 % sand, with a median grain size 156.8 µm. The sediment S1, with organic-rich silt-size sediments, was classified as mud, while the sediment S2 as silt and the sediment S3 as fine sand. The sediment TOC, total phosphorus, and major and trace elements followed the order: mud >silt >fine sand.

Figure 7.1.3.1.1-46: Location of the sampling stations in the German Baltic Sea. The station S1 is in Arkona basin, S2 in Tromper Wiek, and S3 in Oder Bank



Batch sorption experiment

To investigate the possible adsorption of the analytes onto the walls of the centrifuge tubes, the hydrolysis and degradation of the test compounds during the experiment, a control set of tubes was established in which sediment-free artificial seawater samples were spiked with 250 µg of the analytes/L for 48 h. An additional set of tubes containing sediments with unspiked artificial seawater controlled for possible desorption and the contamination of the sediments and media with the target compounds.

To initiate the experiment, artificial seawater was prepared at a salt concentration of 8 g/L. The pH of the solution was 8.1, measured using a conductivity meter (WTW Inolab cond® 720, Germany). Chloroform (0.1 %) was added to the media to inhibit microbial activity. 1 g dry weight of each sediment type was distributed in 15-mL polypropylene centrifuge tubes and mixed with 10 mL of artificial seawater. The tubes were mechanically shaken and incubated for at least 2 days, after which the samples were centrifuged (Megafuge 1.0, Heraeus Instruments) for 3 min at 2500 rpm. Then, 8 mL of each supernatant was transferred to a sediment-free polypropylene centrifuge tube. The samples were then spiked with the target compounds, well shaken at 300 rpm using a mechanical shaker, and 200 µL were then drawn and analyzed

for their initial concentrations of the compounds. Thereafter, the spiked medium was returned to the respective sediment tube, which was then vigorously shaken. This process was conducted in order (i) to avoid any possible adsorption of the compounds onto the sediments at the start of the experiment (T = 0 h) and (ii) to allow the analysis of the phosphonates in same sample matrices during the experimental time, whereas a variety of sample matrices might lead to analytical errors. The experiment was conducted for 48 h at room temperature (21°C), with samples from the aqueous phase taken for analysis at 0, 1, 3, 5, 7, 24, and 48 h. The phosphonates were tested at the following concentrations: 120, 300, 600, 900, and 1200 µg/L. All experiments were performed in duplicate and each sample was measured in triplicate. The target compounds were measured in the aqueous phase. The amounts adsorbed onto the sediments (q_t, µg/g) at time *t* were calculated according to Eq. (1):

$$q_t = (c_0 - c_t)v/m \quad (1)$$

where c_0 is the initial concentration (µg/L), c_t is the concentration at time *t* (µg/L), v is the volume of the solution (L), and m is the dry mass of the sediment (g).

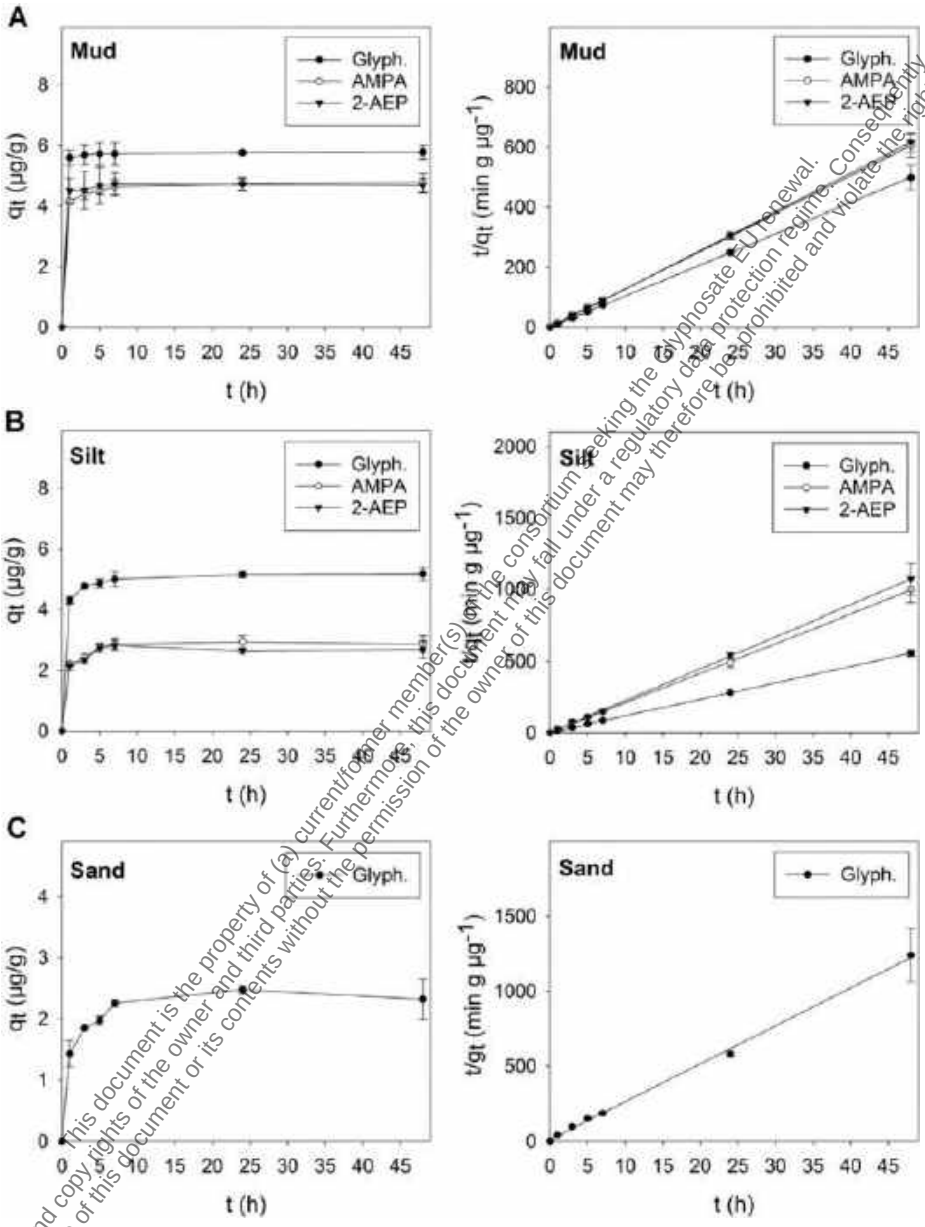
Analysis of organophosphonates
A volume of 200 µL of each supernatant was transferred to 2-mL reaction tubes (Eppendorf, Germany) and diluted to 700 µL using LC-MS grade water. The samples were then treated with 100 µL of the glyphosate and AMPA internal standard solutions, prepared in the same matrix, to obtain a final concentration of 15 µg/L. To derivatize the samples, the pH was adjusted to 9 using 100 µL of 0.07 M borate buffer, after which 100 µL of 33.3 mM FMOC-Cl in acetonitrile was added. The samples were shake-incubated at room temperature for 4 h to allow complete derivatization, filtered through a 45-µm Phenex-RC 15-mm syringe filter (Phenomenex, Germany), and analyzed by LC-MS/MS according to a previously described method (Skeff *et al.*, 2015, 2016). Glyphosate was quantified using the glyphosate internal standard, and AMPA, glufosinate, and 2-AEP using the AMPA internal standard.

Statistical analysis
All adsorption experiments were conducted in duplicate, and the measurements in triplicate. The adsorption study measured the initial and equilibrium concentrations of the target compounds. A *p*-value <0.05 was considered to indicate statistical significance. A one-way ANOVA followed by a Holm-Sidak post-hoc test was carried out using SigmaPlot software (version 13.0, Systat Software Inc.).

Results
Control experiments
A successful adsorption investigation requires the proper controls to rule out both contamination of the aqueous phase or adsorbents with the sorbates and the loss of the sorbates due either to their degradation during the experiment or their adsorption onto the tubes. Controls for both possibilities were therefore established. Data from the first control experiment, in which the compounds were incubated in artificial seawater without sediments, showed a high degree of measurement stability and thus high biological stability of the sorbates during the 48 h and negligible adsorption onto the tubes as well. Furthermore, the stable measurements indicate that the C-P bonds in the organophosphonates are relatively stable and no hydrolysis occurs. Data from the second control experiment, in which the sediments were incubated without sorbates, failed to show the compounds in the aqueous phase and thus confirmed the lack of contamination or desorption. The results of both control experiments demonstrated the validity of the adsorption study.

Kinetic studies and models
The mechanism of glyphosate, glufosinate, AMPA, and 2-AEP absorption onto marine sediments was examined in kinetic studies. The q_t (µg/g) values of glyphosate, AMPA, and 2-AEP between 0 and 48 h are shown in Figure 7.1.3.1.1-47. Whereas glyphosate had an affinity for all three types of sediments, AMPA and 2-AEP adsorbed to mud and silt but not sand. Glufosinate concentrations measured in the aqueous phase remained comparable during the 48 h of the experiment, indicating the lack of significant adsorption ($p > 0.05$) onto the sediments. The presence of a methyl group on the phosphonate of glufosinate might obstruct the formation of surface complexes, thus limiting its adsorption compared to glyphosate.

Figure 7.1.3.1.1-47: Adsorption equilibrium time (left) of glyphosate, AMPA, and 2-AEP on A. mud, B. silt, and C. sand, and the respective pseudo-second order kinetics (right). The figures in the left column are based on a concentration of 600 µg of each compound/L



As shown in Figure 7.1.3.1.1-47, glyphosate, AMPA, and 2-AEP followed similar adsorption kinetics in the mud and silt sediments, and on sand for glyphosate. The adsorption kinetics consisted of two distinct stages: a fast adsorption process in the first hour followed by slow adsorption. The adsorption equilibrium of glyphosate, AMPA, and 2-AEP was reached in 24 h. The amount adsorbed of glyphosate was higher than those of AMPA and 2-AEP for the three types of sediment. The adsorption rate of glyphosate was the highest on mud, followed by silt and sand (96.3 %, 86.2 %, and 38.6 %, respectively). The adsorption rates of AMPA and 2-AEP on mud and silt were similar (~80 % and ~50 %, respectively). The latter observation can be explained that AMPA and 2-AEP own the same functional groups (i.e. each contains phosphonate and amino group), resulting in their similar interactions with sediments.

Lagergren pseudo-first-order and pseudo-second-order models were employed as kinetic models to investigate the rate-controlling steps involved in the adsorption of glyphosate, AMPA, and 2-AEP onto sediments. The linearized Lagergren pseudo-first-order [Eq. (2)] and pseudo-second-order [Eq. (3)] equations are as follows:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (2)$$

$$t/q_t = 1/k_2 q_e^2 + t/q_e \quad (3)$$

where q_e and q_t are the amount of phosphonates (µg/g) adsorbed onto the marine sediments at equilibrium and time t (min), respectively, and k_1 (min^{-1}) and k_2 (g/µg min) are the equilibrium rate constants of the pseudo-first-order and pseudo-second-order models, respectively. The best-fit model was selected based on the values of the linear regression correlation coefficient (R^2). The pseudo-second-order kinetic model efficiently predicted the kinetic behavior of the three compounds on sediments, based on the high R^2 values (0.9982-0.9999), whereas a poor fit of the data was obtained with the pseudo-first-order kinetic model ($R^2 < 0.85$). The rate constant k_2, the q_e values, and the corresponding linear regression correlation coefficient (R^2) were calculated from the linear plots of t/q_t vs. t (Figure 7.1.3.1.1-47) and are shown in Table 7.1.3.1.1-56. The good fit obtained with the pseudo-second-order model suggested chemisorption as the rate-limiting step, presumably between the functional groups of the compounds (i.e., the phosphonate, carboxylate, and amino groups) and the sediment surfaces through the sharing or exchange of electrons. As can be seen from Table 7.1.3.1.1-56, the calculated adsorption capacity values ($q_{e\,cal}$) from the second order model are well comparable to the experimental adsorption capacity values ($q_{e\,exp}$). Thus, the adsorption kinetics of the three phosphonates on the sediments is more precisely described by the mechanism of surface site-sorbates reaction of pseudo-second-order model. The adsorption capacity of the three compounds followed the sequence glyphosate >AMPA ≥2-AEP.

Table 7.1.3.1.1-56: Pseudo-second order kinetic parameters for the adsorption of glyphosate, aminomethylphosphonic acid (AMPA), and 2-aminoethylphosphonic acid (2-AEP) onto Baltic Sea mud, silt, and fine-sand sediments under the experimental condition of 600 µg/L initial concentrations, 8 g salt/L, pH = 8.1, temperature = 21 °C

Sediment	Pseudo-second-order				
	Equation	R^2	$q_{e,\,exp}$ (µg/g)	$q_{e,\,cal}$ (µg/g)	K_2 (g/µg min)
Mud					
Glyphosate	Y = 0.1730X + 0.4567	0.9999	5.7804	5.7789	0.0655
AMPA	Y = 0.2093X + 2.4457	0.9999	4.7778	4.7633	0.0179
2-AEP	Y = 0.2146X + 1.4743	0.9998	4.6772	4.6705	0.0312
Silt					
Glyphosate	Y = 0.1879X + 2.3688	0.9999	4.6998	5.1685	0.0157
AMPA	Y = 0.3371X + 3.6885	0.9997	2.9070	2.9318	0.0308
2-AEP	Y = 0.5931X + 6.7319	0,9997	2.6925	1.6432	0.0522
Sand					
Glyphosate	Y = 0.4218X + 10.449	0.9982	2.3708	2.8043	0.0170
AMPA	NA	NA	NA	NA	NA
2-AEP	NA	NA	NA	NA	NA

NA: not applicable.

Adsorption isotherms

Linear partitioning and Freundlich models are common adsorption isotherms that were applied in this study to describe the adsorption equilibrium of glyphosate, AMPA, and 2-AEP on marine sediments. Linear partitioning is described by Eq. (4) and the linear formula of the Freundlich isotherm is shown in Eq. (5):

$$q_e = k_d c_e \quad (4)$$

$$\log q_e = \log k_f + 1/n \log c_e \quad (5)$$

where c_e is the concentration (µg/L) in the aqueous phase at equilibrium, and k_d (L/g) the distribution coefficient for the sediment/solution ratio (1/10). The k_d values (Table 7.1.3.1.1-57) were obtained from the slope of the linear plots of q_e (µg/g) vs. c_e (µg/L) (Figure 7.1.3.1.1-48). k_f (µg/g) is the Freundlich constant (i.e. sorption capacity), and 1/n an empirical parameter related to the intensity of adsorption. The values for k_f and 1/n (Table 7.1.3.1.1-57) were determined from the intercept and slope of the plots log q_e vs. log c_e (Figure 7.1.3.1.1-48). As shown in Figure 7.1.3.1.1-48, both isotherms described the equilibrium adsorption of the three phosphonates on mud and silt. The Freundlich model had a slightly better fit than the linear partitioning model based on the higher R^2 values (0.96 and 0.99), which suggests that the adsorption takes place on heterogeneous surfaces. It is important to point out that the concentrations of the compounds tested in this study of marine sediments were lower than those typically used in soil adsorption studies, as they were considered representative of conditions in the marine ecosystem. Thus, fitting of the data to both models might be a result of the narrow concentration range (120-1200 µg/L) tested in this study.

The k_d values obtained from linear partitioning were in the range of 55.2 to -259.5 L/kg for glyphosate, 10.0-39.5 L/kg for AMPA, and 7.7-38.5 L/kg for 2-AEP. Data on glyphosate adsorption in the sandy sediment could only be fitted with the Freundlich model (R^2 = 0.99), not with linear partitioning (R^2 = 0.607), which suggested adsorption saturation by the sand as the glyphosate concentration increased. The AMPA and 2-AEP concentrations measured in the aqueous phase were relatively stable, indicative of their difficult adsorption onto sand.

In the Freundlich isotherm, higher k_f values represent a larger adsorption capacity. The calculated k_f values for glyphosate, AMPA, and 2-AEP were in the range 129.8-397.7 L/kg, 25.3-73.5 L/kg, and 19.9-70.1 L/kg, respectively. The k_d and k_f values obtained in this study clearly demonstrated the higher adsorption capacity of glyphosate than of the other studied compounds. The parameter 1/n represents the linearity of the relationship between C_{aq} and $C_{sediment}$, with a lower 1/n value indicating a less homogeneous distribution of the adsorption site energy on the sediments. For all of the tested compounds, the 1/n values were <1: 0.527-0.917 for glyphosate, 0.784-0.899 for AMPA, and 0.779-0.882 for 2-AEP. The higher 1/n value of glyphosate implied that the variability of the sediment adsorption sites had a smaller effect on its adsorption than was the case for AMPA or 2-AEP. The 1/n values for the three compounds decreased according to the sequence mud >silt >sand, reflecting the increasingly difficult (i.e., concentration-dependent) adsorption process.

The influence of sediment organic carbon content on the adsorption of glyphosate, AMPA, and 2-AEP was determined by examining their correlations. The sediment organic carbon normalized distribution coefficient (k_{oc}) was calculated from the Freundlich isotherm using Eq. (6). The results are provided in Table 7.1.3.1.1-57:

$$K_{oc} = (k_f \times 100)/TOC\% \quad (6)$$

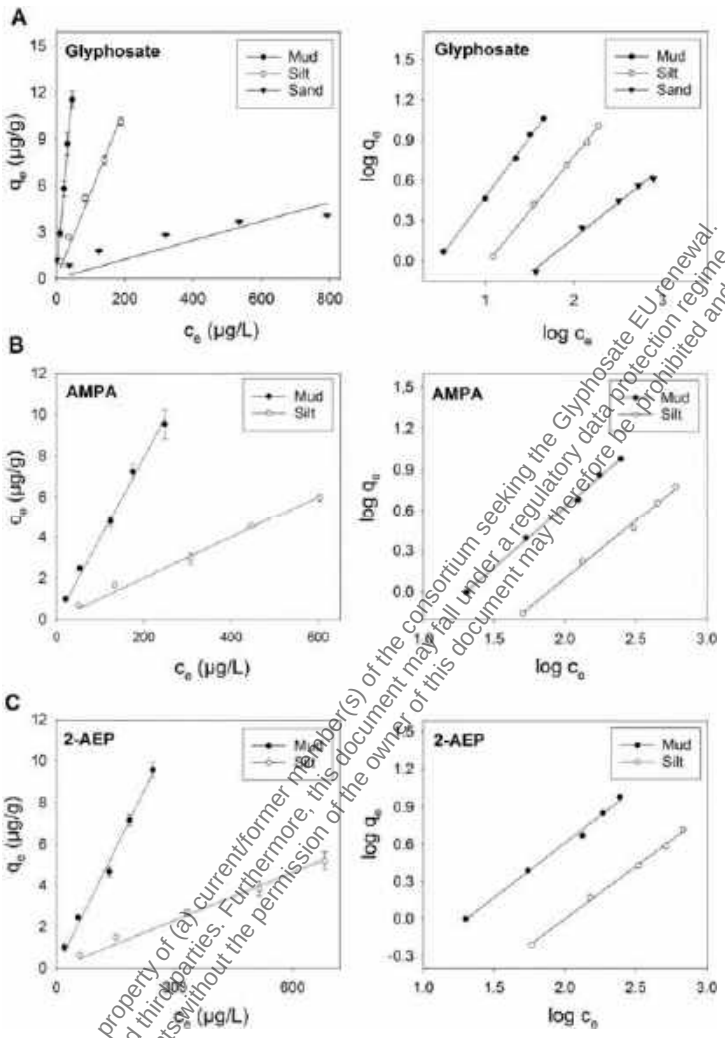
The k_{oc} values of glyphosate were in the range of 5706-86,540 L/kg, but were higher in the sandy sediment, which had the lowest TOC content (0.15 %). This result demonstrated that sediment organic carbon content is not a determining factor in glyphosate adsorption. For AMPA and 2-AEP, the k_{oc} values decreased with the decreasing TOC content, which suggested that the adsorption of both compounds was more sensitive to the organic carbon content of the sediments than glyphosate. The soil mineral composition, which includes aluminium and iron oxides, is a major factor governing glyphosate and AMPA adsorption. In this study, a positive correlation was also determined between the aluminium and iron contents of the sediments and the adsorption of glyphosate, AMPA, and 2-AEP.

Table 7.1.3.1.1-57: Parameters obtained from the linear partitioning and Freundlich adsorption isotherms of glyphosate, AMPA, and 2-AEP on mud, silt, and sandy sand sediments under the experimental condition of 8 g salt/L, pH =8.1, temperature = 21 °C

Sediment	Linear partitioning model		Freundlich model			
	K_d (L/kg)	R^2	K_f (L/kg)	K_{oc} (L/kg)	1/n	R^2
Mud						
Glyphosate	259.5	0.994	397.7	7152.9	0.917	0.999
AMPA	39.5	0.992	73.5	1321.9	0.889	0.998
2-AEP	38.5	0.992	70.1	1259.0	0.882	0.998
Silt						
Glyphosate	55.2	0.990	141.5	5706.1	0.849	0.999
AMPA	9.2	0.988	25.3	1019.4	0.784	0.996
2-AEP	7.7	0.987	19.9	802.4	0.779	0.998
Sand						
Glyphosate	6.1	0.607	129.8	86,540.0	0.527	0.992
AMPA	NA	NA	NA	NA	NA	NA
2-AEP	NA	NA	NA	NA	NA	NA

NA: not applicable.

Figure 7.1.3.1.1-48: Linear partitioning isotherm (left) and Freundlich isotherm (right) of A. glyphosate, B. AMPA, and C. 2-AEP on marine mud, silt, and sandy sediments



Effect of environmental factors

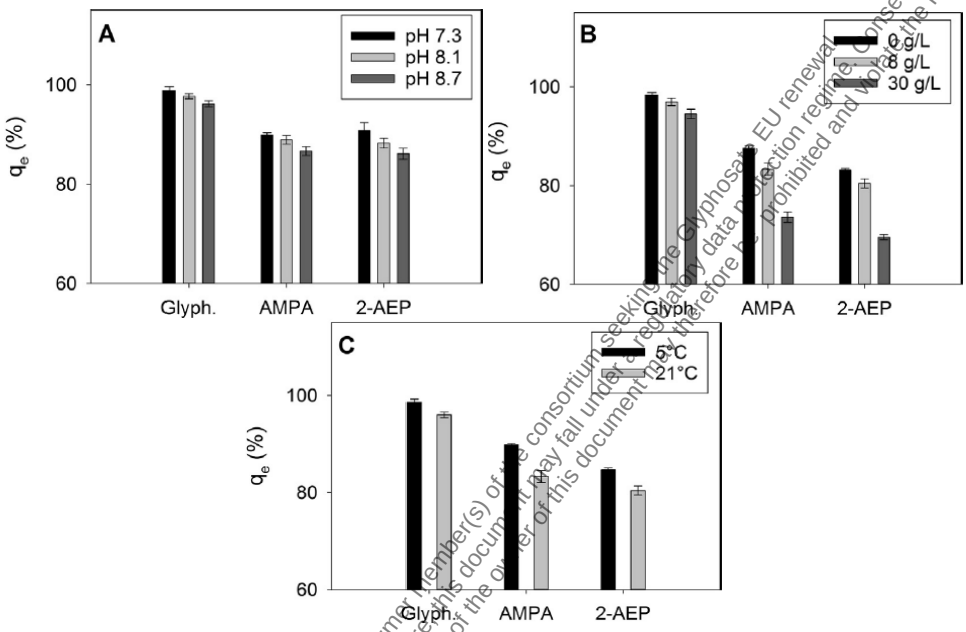
The impact of environmental factors, including the pH, salinity, and temperature of the medium, on the adsorption behaviors of glyphosate, AMPA, and 2-AEP was investigated in the mud sediment. The choice of this sediment is due to its greater adsorption capacity for the compounds than silt and sandy sediments. The initial concentrations of the three compounds in the salinity and temperature tests was 300 µg/L, and in the pH test 120 µg/L.

Effect of the medium pH

To elucidate the effect of the pH of the medium on phosphonate adsorption onto the mud sediment, artificial seawater (a salt concentration of 8 g/L, 0.1 % $CHCl_3$) was adjusted to three different pH values (7.3, 8.1, 8.7) reflecting the variability of the pH of Baltic Sea water. The pH was adjusted using concentrated HCl and NaOH. The mud sediment samples were incubated for 48 h in the corresponding medium and then spiked with the test compounds. The experiment was then conducted as described for the standard experiment at 21 °C. As seen in Figure 7.1.3.1.1-49A, adsorption of the three compounds increased significantly ($p < 0.05$) as the pH decreased from 8.7 to 7.3. The results suggested the similar effects of a change in seawater pH on 2-AEP and AMPA. As the pH of the medium increases, the positive surface charge of the sediment decreases and may become negative. Thus, in this study, the decreased adsorption may have been due to the reduced coordination between the phosphonate group of the compounds and the

surface of the sediments. In addition, a higher pH may enhance the release of native organic matter from the sediment into solution, thereby reducing the sediment adsorption capacity of the target compounds.

Figure 7.1.3.1.1-49: The influence of A. pH, B. salinity, and C. temperature on the adsorption of glyphosate, AMPA, and 2-AEP onto mud sediment. The data are based on duplicate experiments, each consisting of triplicate measurements. The initial concentrations of the compounds were 120 µg/L in the pH experiment and 300 µg/L in the salinity and temperature experiments



Effect of solution salinity

Salinity (ionic strength) may have an important influence on the adsorption behavior of amphoteric compounds, including glyphosate, AMPA, and 2-AEP, in seawater-sediment systems. To investigate its effect, media containing three different salt concentrations (0, 8, 30 g/L) were prepared. The salt-free medium (0 g/L) consisted of LC-MS grade water, presumably free of salt. The experiment was run at pH 8.1 and 21°C. The results revealed the negative correlation between the adsorption of the compounds and the salinity of the medium (Figure 7.1.3.1.1-49B). The adsorption capacity increased significantly ($p < 0.05$) as the salt concentration decreased from 30 g/L to 0 g/L, an effect attributable to ion exchange. At pH 8.1, glyphosate and AMPA carry negative charges related to the phosphonate (both molecules) and carboxylate (glyphosate) groups and positive charges related to the amino group (both compounds). Most sediment surfaces carry a net negative charge but at pH 8.1 positive charges in sediment organic matter might be exposed. Therefore, changing the ionic composition of the medium may influence the adsorption process, by promoting competition for ion-exchangeable sites. Alternatively, complexes between the phosphonates and cations such as Ca^{2+} and Mg^{2+}, present in the medium, may form that have a lower adsorption affinity for the sediments than do free compounds, such that their adsorption decreases with increasing salinity. According to the results, the various salt concentrations had similar effects on the adsorption behaviors of 2-AEP and AMPA, perhaps because they have the same functional groups.

Effect of temperature

To examine the influence of temperature on the adsorption behaviors of glyphosate, AMPA, and 2-AEP, two different temperatures (5°C and 21°C) were tested. As shown in Figure 7.1.3.1.1-49C, the amount of adsorbed compounds increased significantly ($p < 0.05$) as the temperature decreased, indicating that adsorption was an exothermic process. The amount of adsorbed glyphosate, AMPA, and 2-AEP increased differentially as the temperature decreased; at rates of 1.5 %, 6.5 %, and 4.3 %, respectively. This may have been due to the different effects of temperature on the water solubility of the compounds. In general, the

solubility of chemical substances improves as the temperature rises, such that the amounts entering the solid phase will be lower when equilibrium is reached. Moreover, an increase in the temperature of the medium could increase the solubility of organic matter in sediments, thus increasing the competition with phosphonates for sediment adsorption.

Environmental implications

Mud sediments can act as a sink for glyphosate as well as for AMPA and 2-AEP, based on the high adsorption affinities of these compounds (>96 % and >78 %, respectively). In silt, the three compounds were distributed between the water and adsorption to the sediment, with a higher tendency of the latter. This result clearly supports the need for bioavailability and toxicity studies of benthic as well as pelagic organisms. Sandy sediments had a weak adsorption capacity for glyphosate, and a negligible adsorption capacity for glufosinate, AMPA, and 2-AEP. Therefore, these compounds can be easily moved from Baltic Sea regions characterized by sandy sediments to those with mud or silt sediments. The pH, salinity, and temperature data demonstrated that the variability of these parameters significantly influences the adsorption behaviors of glyphosate, AMPA, and 2-AEP. A decrease in either the seawater pH or the temperature enhanced the adsorption of these compounds onto marine sediments. Thus, their mobility is a more important factor in warmer than in colder marine systems. Furthermore, the warming effect induced by climate change may influence the fate of phosphonates in the marine environment. The negative correlation between salinity and adsorption suggested the greater mobility of these compounds in marine than in freshwater systems. In the Baltic Sea, salinity varies greatly from south to north, and from east to west, increasing from 2 to 4 in the northern area up to 20-30 in the southwestern area of the sea. Thus, the distribution of glyphosate, AMPA, and 2-AEP in Baltic Sea water and sediments is most likely spatially dependent. These results provide basic information about the fate of these phosphonates in the Baltic Sea and highlight the importance of monitoring these compounds in marine water and sediments, especially in semi-closed seas such as the Baltic Sea, where contaminants may cause acute effects.

Conclusion

In this work, the adsorption of glyphosate, glufosinate, AMPA, and 2-AEP onto mud, silt, and sandy sediments of the Baltic Sea was investigated. Glufosinate had no adsorption affinity for any of the sediments tested. Data on the adsorption kinetics of the other compounds could be well fitted with a second-order rate model. The adsorption rate followed the order glyphosate >AMPA ≥2-AEP >glufosinate. Linear partitioning and Freundlich isotherms described the adsorption of glyphosate, AMPA, and 2-AEP on mud and silt. However, only glyphosate showed important adsorption onto the sandy sediment and its behavior could be well modeled with the Freundlich isotherm. The adsorption capacity of the sediments decreased in the order mud >silt >sand. Inverse correlations between the pH, salinity, and temperature of the medium and the adsorption of glyphosate, AMPA, and 2-AEP were determined. This study showed that a small difference in the chemical structure of amphoteric substances such as glyphosate and glufosinate can lead to large differences in their adsorption behaviors.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the sorption of glyphosate and AMPA to sediments of the Baltic Sea. Sediments are out of scope of EU data requirements for adsorption data. There was no detailed reporting of data to assess the validity (i.e. mass balances, test items not sufficiently described, information about LOD, LOQ).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/017
Report author	Gómez Ortiz, A.M. *et al.*
Report year	2017
Report title	Sorption and desorption of glyphosate in mollisols and ultisols soils of Argentina
Document No	DOI 10.1002/etc.3851 E-ISSN 1552-8618
Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

In Argentina, glyphosate use has increased exponentially in recent years as a result of the widespread adorption of no-till management combined with genetically modified glyphosate-resistant crops. This massive use of glyphosate has created concern about its potential environmental impact. Sorption–desorption of glyphosate was studied in 3 Argentinean soils with contrasting characteristics. Glyphosate sorption isotherms were modeled using the Freundlich equation to estimate the sorption coefficient (K_f). Glyphosate sorption was high, and the K_f varied from 115.6 to 1612 $mg^{1-1/n}L^{1/n}/kg$. Cerro Azul soil had the highest glyphosate sorption capacity as a result of a combination of factors such as higher clay content, cation exchange capacity, total iron, and aluminum oxides, and lower available phosphorus and pH. Desorption isotherms were also modeled using the Freundlich equation. In general, desorption was very low (<12 %). The low values of hysteresis coefficient confirm that glyphosate strongly sorbs to the soils and that it is almost an irreversible process. Anguil soil had a significantly higher desorption coefficient (K_{fd}) than the other soils, associated with its lower clay content and higher pH and phosphorus. Glyphosate high sorption and low desorption to the studied soils may prevent groundwater contamination. However, it may also affect its bioavailability, increasing its persistence and favoring its accumulation in the environment. The results of the present study contribute to the knowledge and characterization of glyphosate retention in different soils.

Materials and Methods

Soils

Soil samples were taken from agricultural fields of Cerro, Tandil, and Anguil, The studied soils are located in areas of high agronomic land use and have different edaphoclimatic conditions. Four composite soil samples from the top 15 cm of topsoil were collected from each field. Samples were homogenized, air-dried, and sieved to a particle size of 2 mm. A subsample of each replicate was used for physicochemical analysis of the soils (see Table 7.1.3.1.1-58). Particle size distribution was measured using the pipette method; organic carbon content was measured according to the Walkley-Black method; CEC was determined by displacement with 1M ammonium acetate at pH 7; soil pH was measured by electrode in a soil:water ratio of 1:2.5; available phosphorus (P-Bray) was determined according to Bray and Kurtz; total iron (Fe) was determined by atomic absorption spectrophotometry; and exchangeable aluminum (Al) was measured according to the Al method.

Table 7.1.3.1.1-58: Main characteristics of the sampled locations and soil physicochemical properties

	Soil		
	Anguil	Cerro Azul	Tandil
Altitude (masl)	157	280	256
Annual average temperature (°C)	15.3	20.5	13.7
Mean annual precipitation (mm)	760	1844	993
Latitude	36°35′54″S	27°39′42″S	37°36′0.1″S
Longitude	63°58′31″W	55°26′25″W	59°04′29″W
Soil type	Mollisol	Ultisol	Mollisol
Main textural class	Loam	Clay	Loam
pH	6.3 A	4.9 C	5.4 B
Clay (%)	14.7 C	78.5 A	23.0 B
Silt (%)	45.6 A	15.4 C	40.9 B
Sand (%)	39.6 A	6.1 C	36.0 B
Organic carbon (%)	1.3 C	2.4 B	3.4 A
P-Bray (mg/kg)	29.6 A	7.6 C	17.1 B
CEC (meq/100 g)	17.4 C	20.6 B	25.2 A
Ca^{2+} (meq/100 g)	8.1 B	5.6 B	14.7 A
Mg^{2+} (meq/100 g)	2.9 B	3.2 B	5.1 A
K^{+} (meq/100 g)	3.2 A	1.2 A	2.8 A
Na^{+} (meq/100 g)	0.3 A	0.2 A	0.5 A
Al^{3+} (meq/100 g)[b]	0.15 B	0.69 A	0.11 B
Total Fe (%)[b]	1.08 B	8.40 A	0.81 B

[a]Different letters indicate differences among soils ($p < 0.05$).
[b]From Gianelli et al. [8].
CEC = cation exchange capacity; P-Bray = available phosphorus.

Chemicals
Stock solutions for the standard curves and the isotherm study solutions were prepared using analytical pure glyphosate (99.9 %). For analytical procedures HPLC - grade methanol and HPLC - grade acetonitrile were purchased commercially. Nanopure water was obtained by purifying demineralized water.

Sorption isotherms
The sorption isotherms were performed according to the batch equilibrium method. First, 2 g of soil was shaken with 40 mL of a 0.01M $CaCl_2$ solution. After 24 h, glyphosate was spiked at different initial concentrations (C_0): 0, 0.5, 1, 5, 10, and 20 mg/L. The suspensions were shaken for another 24 h at constant temperature (20°C). Afterward, tubes were centrifuged, and an aliquot (3 mL) of the aqueous solution was analyzed for glyphosate concentration. Each initial concentration was tested by duplicate for each soil sample. These laboratory duplicates were averaged, finally obtaining data of 4 replicate isotherms per soil.

Desorption isotherms
The desorption isotherms were performed using the spiked soil with the C_0: 5 mg/L solution from the sorption isotherm studies. This concentration is equivalent to the commonly used dose in the field per year (6 L/ha/yr) considering 5 cm depth of soil. After the sorption study, the aqueous phase was carefully discarded to avoid any soil loss during manipulation. The volume of the solution that was removed was replaced with 0.01M $CaCl_2$, and the soil was re-suspended and shaken at a constant temperature for another 24 h. Then, samples were centrifuged and glyphosate was measured in the aqueous solution to quantify the glyphosate that desorbed from the soil matrix. This procedure was repeated at 48 and 72 h by removing the aqueous solution and adding again $CaCl_2$. The amount of adsorbed glyphosate at each desorption step was calculated as the difference between the initially adsorbed concentration and the desorbed amount.

Glyphosate analysis
To quantify the remaining glyphosate in the aqueous solution, an aliquot of 3 mL was transferred to a 15-mL polyethylene flask, and 0.5 mL of borate buffer solution (0.04 mM $Na_2B_4O_7$ 10 H_2O, pH 9) and

0.5 mL of acetonitrile were added. Samples were shaken vigorously, then derivatized with 0.5 mL of 9-fluorenylmethylchloroformate dissolved in acetonitrile (6 g/L) and incubated overnight at room temperature. As a cleanup step, CH_2Cl_2 was added to the samples to remove any organic impurities and minimize matrix effects. The aqueous fraction was separated from the organic solvent by centrifuging. The supernatant was collected and filtered and then analyzed by liquid chromatography coupled to a tandem mass spectrometer (MS/MS).

Chromatographic analysis was carried out using a Waters ACQUITY1 ultra-performance liquid chromatography system. Target molecules were detected by a triple quadrupole MS/MS Quattro Premier XE (Waters). The equipment was operated with an electrospray ionization source in positive mode. To take into account the matrix effect of each soil, standard curves were prepared using a background solution of each soil obtained after shaking with $CaCl_2$ 0.01 M. After separating the solid phase from the aqueous phase, the solution was used to prepare each point of the standard curves by adding the corresponding glyphosate concentration. A sample without any glyphosate was also analyzed to check the concentration of presorbed glyphosate. In all cases, the background solution had non-detectable levels of glyphosate. The limit of detection was 0.1 µg/L, and the limit of quantification was 0.5 µg/L.

Sorption modeling
Following the experimental design proposed by the Organisation for Economic Co-operation and Development Guidelines for the Testing of Chemicals, Test No. 106, the measured glyphosate in the aqueous solution was used to estimate the remaining glyphosate sorbed to the soil (Cs).

$$C_s = M_s/M_{soil} = (C_0 - C_w)V_0/M_{soil} \quad (1)$$

where C_s is the concentration of glyphosate adsorbed to the soil at equilibrium (mg/kg), M_s is the mass of glyphosate sorbed to the soil at sorption equilibrium (mg), M_{soil} is the dry mass of the soil sample (kg), C_0 is the initial tested concentration of glyphosate in contact with the soil sample (mg/L), C_w is the analytically measured mass concentration of glyphosate in the aqueous phase at sorption equilibrium (mg/L), and V_0 is the initial volume of the aqueous phase in contact with the soil sample (mL).

The Freundlich equation was used to describe sorption and desorption isotherms

$$C_s = K_f C_w^{1/n} \quad (2)$$

where K_f ($mg^{1-1/n} L^{1/n}/kg$) is the Freundlich sorption coefficient and 1/n is the Freundlich exponent (K_f and 1/n will hereafter refer to sorption and K_{fd} and $1/n_d$ to desorption). The K_f coefficient indicates the affinity of the substance to the soil matrix, and 1/n indicates the degree of linearity between the amounts adsorbed and the concentration in the solution.

The hysteresis coefficient (H) for the sorption/desorption isotherms was calculated according to the equation

$$H = (1/n_d)/(1/n) \quad (3)$$

where 1/n and $1/n_d$ are the Freundlich slopes obtained for the sorption and desorption isotherms, respectively.

Statistical analysis
For the isotherm sorption and desorption studies, each soil sample was analyzed in duplicate. Laboratory duplicate samples were averaged, and the isotherm curves were then modeled using the NLIN procedure of SAS software. Statistical analyses of the soil properties and of the estimated sorption and desorption parameters were performed using a completely randomized design with 4 replicates per soil. Analysis of variance was performed using the PROC GLM procedure to evaluate differences in the Freundlich parameters at a significance level of 5 %.

Results and Discussion

Soil characteristics

Tandil and Anguil soils correspond to a loam texture, while Cerro Azul is classified as clay. Cerro Azul soil had a significantly higher clay content, followed by Tandil and then Anguil ($p < 0.05$). On the other hand, the organic carbon content and CEC were significantly higher in Tandil, followed by Cerro Azul and Anguil soil ($p < 0.05$). Anguil soil had significantly higher pH and P-Bray values than Tandil and Cerro Azul ($p < 0.05$). Regarding the exchangeable cations, significant differences were observed only for Ca^{2+} and Mg^{2+}, following the order Tandil>Cerro Azul>Anguil ($p < 0.05$). The highest Al^{3+} and Fe contents were found in Cerro Azul soil, denoting its Ultisol origin.

Sorption isotherms

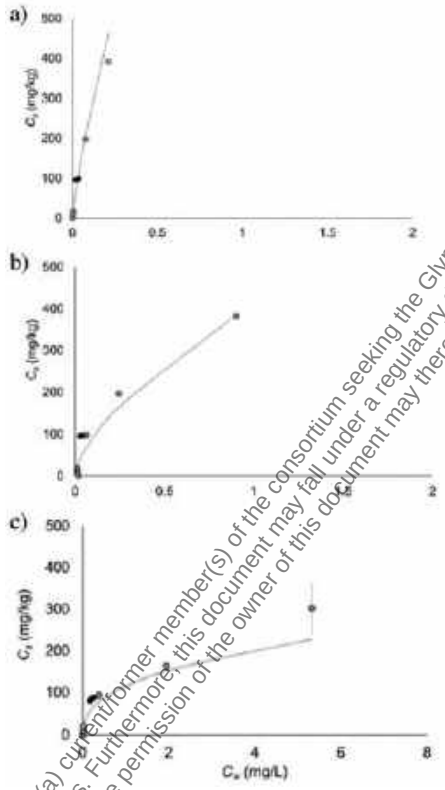
Glyphosate sorption and desorption isotherms are shown in Figure 7.1.3.1.1-50. The K_f values for glyphosate were very high and ranged from 115.6 to 1612 (Table 7.1.3.1.1-59), being generally higher than those usually reported in the literature. Glyphosate K_f was significantly higher in Cerro Azul compared with Tandil and Anguil soil ($p<0.05$) (Table 7.1.3.1.1-59). The values of 1/n ranged from 0.4 to 0.8 (Table **7.1.3.1.1-59**). Isotherms exhibited an L-type (1/n<1) curve according to the classification of Giles *et al.* This indicates that sorption is not constant as the concentration of the herbicide increases and that the sorption sites become saturated with increasing glyphosate concentration. In the case of Tandil and Anguil soils, glyphosate was almost completely sorbed to the soil at low initial concentrations; and as the concentration increased, sorption became less efficient (Figure 7.1.3.1.1-50). Isotherms of this type occur when the adsorbent has a high initial affinity for the herbicide until the sorption sites become saturated. In contrast, the Cerro Azul isotherm exhibits an almost linear relationship between the amount of sorbed glyphosate and its concentration at equilibrium in the solution (Figure 7.1.3.1.1-50), with 1/n values closer to 1 (Table 7.1.3.1.1-59). Therefore, it can be assumed that the number of sorption sites remains almost constant even at high concentrations. The reason glyphosate sorption was significantly higher in Cerro Azul soil can be explained by the soil's textural composition. At the soil's pH, the negatively charged glyphosate molecule can be complexed with cations released from the clays via a cation exchange reaction with solution protons. On the other hand, Fe and Al oxides also play an important role in glyphosate sorption because the phosphonate group of glyphosate establishes coordination links with the interchangeable surfaces of Fe^{3+} and Al^{3+} cations. In this sense, the lower soil pH of Cerro Azul could also be favoring sorption via Fe and Al oxides because as the pH decreases, these oxides become more protonated, increasing the affinity toward the negatively charged glyphosate molecule. Therefore, aside from cation exchange reactions, glyphosate may strongly bond through ligand exchange with the metal ions (Fe or Al) at the surface of the clay minerals. This mechanism has been proposed for other organic weak acids, and hence it can be applied to glyphosate.

Table 7.1.3.1.1-59: Glyphosate Freundlich sorption and desorption parameters for Anguil, Cerro Azul, and Tandil soils[a]

	Sorption			Desorption[b]							
							Percentage[c]				
Soil	K_f ($mg^{1-1/n}L^{1/n}/kg$)	1/n	r^2	K_{fd} ($mg^{1-1/n}L^{1/n}/kg$)	$1/n_d$	r^2	1°	2°	3°	Total[d]	H^e
Cerro Azul	1612.0 (859.8) C	0.8 (0.5) A	0.97–0.99	101.2 (2.9) C	0.01 (0.0) C	0.99–0.99	0.7 (0.1)	0.6 (0.0)	0.5 (0.1)	1.6 (0.0)	0.01 (0.0) B
Tandil	412.6 (50.0) B	0.5 (0.07) AB	0.98–0.99	105.4 (1.7) B	0.02 (0.0) B	0.99–0.99	0.8 (0.1)	0.6 (0.0)	0.3 (0.0)	1.9 (0.5)	0.04 (0.0) B
Anguil	115.6 (12.0) B	0.4 (0.2) B	0.90–0.99	117.5 (0.6) A	0.20 (0.0) A	0.99–0.99	4.5 (0.3)	3.6 (0.1)	3.3 (0.3)	32.3 (4.1)	0.4 (0.2) A

[a]Mean values of 4 replicates, standard deviation in parentheses. Different letters indicate significant differences among soils ($p < 0.05$).
[b]Desorption from initial glyphosate aqueous concentration $C_0 = 5$ mg/L.
[c]Percentage of desorbed glyphosate in the 1°, 2°, and 3° desorption cycle.
[d]Total desorbed glyphosate after 3 successive desorption cycles.
[e]Hysteresis coefficient ($H = 1/n_d/1/n$).

Figure 7.1.3.1.1-50: Adsorption (gray dots) and desorption (black dots) isotherms for (a) Cerro Azul, (b) Tandil, and (c) Anguil soils. Error bars represent standard deviations; Black dotted line represents the Freundlich model fit. Note different x axis scale for Anguil soil. C_s = concentration of glyphosate adsorbed to the soil at equilibrium; C_w = analytically measured mass concentration of glyphosate in the aqueous phase at sorption equilibrium



Desorption isotherms

The K_{fd} values of the studied soils ranged from 101.2 to 117.5 $mg^{1-1/n}kg^{-1}L^{1/n}$ (Table 7.1.3.1.1-59). Anguil soil had the highest K_{fd}, while Cerro Azul had a significantly lower desorption coefficient than the rest ($p < 0.05$). The total desorbed glyphosate at the end of the desorption study was 1.6 and 1.9 % for Cerro Azul and Tandil, respectively, whereas in Anguil soil desorption reached 12 % (Table 7.1.3.1.1-59). The values of $1/n_d$ ranged from 0.01 to 0.2 (Table 7.1.3.1.1-59). The irreversibility of glyphosate sorption was confirmed by the lower values of $1/n_d$ with respect to 1/n. The more pronounced curvature of the desorption isotherms suggests that more energy is required to desorb the molecules than that needed for the sorption process. In consequence, hysteresis coefficients were low, ranging from 0.01 to 0.4 (Table 7.1.3.1.1-59). When comparing the 3 soils, desorption and hysteresis coefficients were significantly higher in Anguil. This can be explained by the lower clay content and lower CEC, as well as the significantly higher pH and available phosphorus, which affect glyphosate sorption mechanisms in an inverse way, as explained before. Nevertheless, desorption hysteresis can be considered significant in all the studied soils because the hysteresis coefficient was <0.7, indicating that glyphosate sorption is nearly an irreversible process.

The fact that glyphosate binds strongly to the studied soils and that desorption was very low has a major implication for glyphosate bioavailability. Glyphosate's biological degradation is strongly limited in soils that have high glyphosate affinity and low desorption.

The results obtained in the present study indicate that sorption of glyphosate increases in soils with high contents of Al^{3+}, Fe, and clays as well as low pH and phosphorus content. This situation favors greater glyphosate retention and, therefore, lower desorption, which would reduce the likelihood of leaching and

therefore the potential risk of groundwater contamination. However, glyphosate bioavailability can also be reduced, increasing its persistence and therefore contributing to its accumulation in the environment. These results contribute to the knowledge about glyphosate retention in soils and allow the identification of behavior patterns of this extensively applied herbicide in different edaphic scenarios. This is of major importance for the development of decision-making tools and criteria to reduce the potential negative impacts on soil and groundwater resources.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes an adsorption/desorption experiment with glyphosate on three different agricultural soils from Argentina which have not been tested for their applicability to EU conditions due to the supportive character of the overall information in the article (insufficient information to assess validity, i.e. no mass balance, previous exposure to other chemicals not documented).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/018
Report author	Munira, S., Farenhorst, A.
Report year	2017
Report title	Sorption and desorption of glyphosate, MCPA and tetracycline and their mixtures in soil as influenced by phosphate
Document No	DOI 10.1080/03601234.2017.1361773 E-ISSN 1532-4109
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) - Temperature: 5 °C, - 0.01 M KCl
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Phosphate fertilizers and herbicides such as glyphosate and MCPA are commonly applied to agricultural land, and antibiotics such as tetracycline have been detected in soils following the application of livestock manures and biosolids to agricultural land. Utilizing a range of batch equilibrium experiments, this research examined the competitive sorption interactions of these chemicals in soil. Soil samples (0–15 cm) collected from long–term experimental plots contained Olsen P concentrations in the typical (13 to 20 mg/kg) and elevated (81 to 99 mg/kg) range of build–up phosphate in agricultural soils. The elevated Olsen P concentrations in field soils significantly reduced glyphosate sorption up to 50 %, but had no significant impact on MCPA and tetracycline sorption. Fresh phosphate additions in the laboratory, introduced to soil prior to, or at the same time with the other chemical applications, had a greater impact on reducing glyphosate sorption (up to 45 %) than on reducing tetracycline (up to 13 %) and MCPA (up to 8 %) sorption. The impact of fresh phosphate additions on the desorption of these three chemicals was also statistically significant, but numerically very small namely <1 % for glyphosate and tetracycline and 3 % for MCPA. The presence of MCPA significantly reduced sorption and increased desorption of glyphosate,

but only when MCPA was present at concentrations much greater than environmentally relevant and there was no phosphate added to the MCPA solution. Tetracycline addition had no significant effect on glyphosate sorption and desorption in soil. For the four chemicals studied, we conclude that when mixtures of phosphate, herbicides and antibiotics are present in soil, the greatest influence of their competitive interactions is phosphate decreasing glyphosate sorption and the presence of phosphate in solution lessens the potential impact of MCPA on glyphosate sorption. The presence of chemical mixtures in soil solution has an overall greater impact on the sorption than desorption of individual organic chemicals in soil.

Materials and Methods

Chemicals

Analytical grade glyphosate (99.9 %), MCPA (99 %), tetracycline (98 %), potassium dihydrogen phosphate (KH_2PO_4), (99 %) and potassium chloride (100 %) were obtained commercially. Radioactive [phosphonomethyl–^{14}C] glyphosate (99 %; specific activity 50 mCi/mmol), [2–methyl–4–chlorophenoxyacetic acid ^{14}C] MCPA (98 %; specific activity 55 mCi/mmol) and [7–^{3}H (N)] tetracycline (98 % radiochemical purity; specific activity 20 Ci/mmol) were obtained commercially.

Table 7.1.3.1.1-60: Selected soil physical and chemical properties as mean with standard error

Organic Carbon [a] (%)	pH[b]	Fe_2O_3[c] (mg kg^{-1})	Al_2O_3[c] (mg kg^{-1})	Ca[d] (mg kg^{-1})	Clay[e] %	Silt[e] %	Sand[e] %
2.81 ± 0.04	4.7± 0.02	237±7.93	6.41±0.64	1152±2.35	20	20	60

[a]Soil organic carbon content was determined using combustion technique with a high temperature induction furnace.[39]
[b]Soil pH was determined using a 10 ml 0.01M $CaCl_2$ solution and 2 g soil solution ratio.[40]
[c]Extractable Fe and Al were extracted with diethylenetriaminepentaacetic acid (*DTPA*),[41] and 0.01M $CaCl_2$,[42] respectively, and extracts were analyzed by ICP.
[d]Extractable Ca was also measured by ICP using ammonium acetate as an extractant.[43]
[e]data adapted from Grant et al.[36]

Soil characteristics and experimental design

Soil samples (0–15 cm) were collected in spring 2013 from experimental plots that were arranged in a randomized complete block design with four replications and were located at the University of Manitoba Carman Field Research Station, Manitoba, Canada. All plots were under a flax and durum wheat rotation and received urea fertilizers at an annual rate of 50 and 90 kg N/ha, respectively. For this study, samples were collected from the replicated plots that had also received eight years (2002–2009) of annual mono ammonium phosphate (MAP) applications at rates of 80 kg P/ha, as well as from control plots that did not receive MAP application during these years. The rotation was continued from 2010 to 2013 but after 2010 no phosphate was applied. In each plot, composite soil samples were collected using a Dutch auger with ten samples per plot and the auger was cleaned in between plots. The soil is classified as an Orthic Black Chernozem based on the Canadian System of Soil Classification, which is approximately equivalent to the Udic Boroll subgroup in the U.S. Soil Taxonomy. Key soil properties are listed Table 7.1.3.1.1-60.

Impact of phosphate in solution on herbicides and antibiotic sorption and desorption

Batch equilibrium procedures using 50–mL centrifuge Teflon tubes (duplicates) followed the OECD guideline 106 with air-dried soil (2 g) and a soil/solution ratio of 1:5 with 0.01 M KCl as the background electrolyte Soil slurries were rotated in the dark at 5°C from 0 to 24 h (pre–incubation), from 24 to 48 h (sorption) and from 48 to 72 h (desorption) with phosphate added at 0 h, 24 h and/or 48 h, or never added, depending on the treatment (Table 7.1.3.1.1-61). Radiolabelled chemical solutions contained 1 mg/L analytical–grade glyphosate, MCPA or tetracycline, with 6.67 x 10^5 Bq/L ^{14}C–labelled glyphosate, 3.83 × 10^5 Bq/L ^{14}C–labelled MCPA or 4.17 x 10^5 Bq/L ^{3}H–labelled tetracycline, respectively. The concentration 1 mg/L represented environmentally–relevant concentrations of herbicides and antibiotics detected in agricultural soils or animal manure. At 48 h, tubes were centrifuged at 10,000 rev/min for 10 min and subsamples (1 mL) of the supernatant (duplicates) were added to scintillation vials (7 mL) containing 5 mL 30 % Scintisafe scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method). The sorption distribution constant, Kd (L/kg), of glyphosate, MCPA or tetracycline was quantified by Cs/Ce, where Cs is the concentration of the organic chemical in soil at equilibrium (mg/kg) and Ce is the concentration of the organic chemical in the equilibrium solution (mg/L). The concentration of the organic chemical in soil was calculated by the difference between the radioactivity in the initial solution and the equilibrium

solution. The soil organic carbon coefficient, Koc (L/kg) of glyphosate, MCPA or tetracycline was calculated by dividing the Kd value by 0.0281 which was the fraction of soil organic carbon in soil.

Table 7.1.3.1.1-61: Addition of phosphate during pre–incubation, sorption and desorption steps

Code	Pre-incubation from 0 h to 24 h	Sorption from 24 h to 48 h	Desorption from 48 to 72 h
n,n,n	No P added	No P added	No P added
n,n,P	No P added	No P added	P added at 48 h
P,n,n	P added at 0 h	No P added	No P added
P,n,P	P added at 0 h	No P added	P added at 48 h
n,P,P	No P added	P added at 24 h	P added at 48 h

n = no phosphate added during pre-incubation, sorption and/or desorption step; P = phosphate added at time 0 h at the start of the pre-incubation step or at time 24 h at the start of the sorption step; or at time 48 h at the start of the desorption step.

Impacts of MCPA and tetracycline in solution on glyphosate sorption and desorption in the presence and absence of fresh phosphate

Experiments followed similar protocols as described for *n,n,n*; *n,n,P*; and *P,n,P* in Table 7.1.3.1.1-61 above and also added to soil (at 0 h) were MCPA, tetracycline (Tetra), or their mixtures (M/T). MCPA, Tetra, and M/T were added at concentrations of 1 or 11 mg/L. The glyphosate solution was always added at 24 h and contained 1 mg/L analytical–grade glyphosate with 6.67×10^5 Bq/L ^{14}C–labelled glyphosate.

Table 7.1.3.1.1-62: Effect of phosphate fertilizer on MCPA and tetracycline sorption and desorption in soil. See Table 7.1.3.1.1-61 for an explanation of the treatment labels

Treatment	Kd (L kg^{-1})		Desorption (%)	
	MCPA	Tetracycline	MCPA	Tetracycline
n,n,n	5.35 A	134.49 A	27.45 B	0.51 B
n,n,P	5.28 A	129.02 A	29.63 A	0.73 A
P,n,n	5.00 B	117.50 B	29.04 A	0.69 A
P,n,P	5.00 B	122.55 B	30.18 A	0.71 A
n,P,P	4.99 B	117.55 B	29.91 A	0.74 A

Effect of the pre–sorbed phosphate on the sorption of glyphosate, MCPA and tetracycline

This batch equilibrium experiment only used the soil samples obtained from the plots that had not received phosphate fertilizer applications.

Effect of the pre–sorbed MCPA on glyphosate sorption

Experiments followed similar protocols as described for the pre–sorbed phosphate above. The glyphosate solution contained 1 mg/L analytical–grade glyphosate with 6.67×10^5 Bq/L ^{14}C–labelled glyphosate.

Statistical analysis

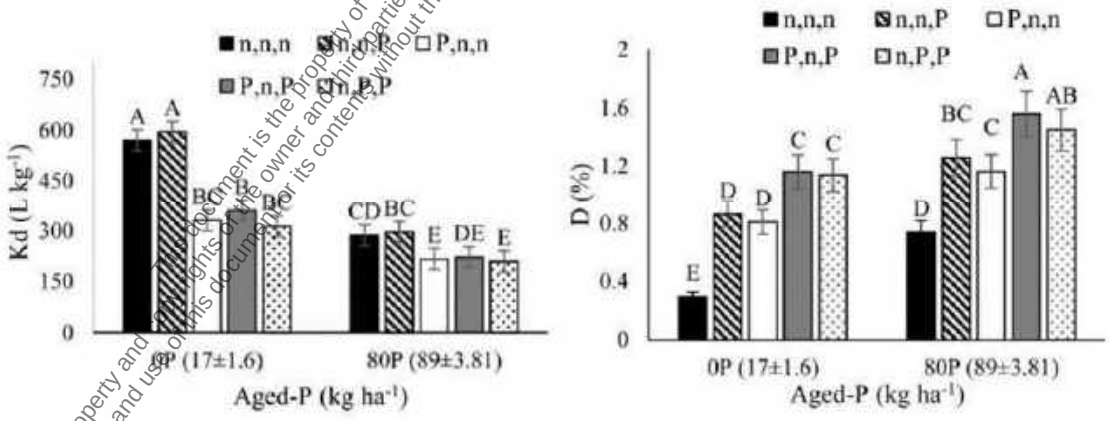
Statistical analyses were carried out using SAS software version 9.4 for Windows. Prior to each analysis, data sets were checked for outliers, normality of residuals and homogeneity of variances. Residuals were normally distributed and variances were homogeneous. For the Kd values, data were analyzed by using normal distribution and for the % desorption by beta distribution. Two–way ANOVA in PROC GLIMMIX was used to quantify the effect of field aged–P (0P, 80P) and fresh–P addition (0, 11 mg/L) on Kd values and % desorption of MCPA, tetracycline, and glyphosate in soil. One–way ANOVA in PROC GLIMMIX

was utilized to determine the effect of retained phosphate in soil on glyphosate, MCPA and tetracycline sorption, and of retained MCPA in soil on glyphosate sorption. Both in the presence and absence of fresh phosphate, two–way ANOVAs in PROC GLIMMIX were carried out to quantify the effect of field aged-P (0P, 80P) and of the concentrations (0, 1, 11 mg/L) of MCPA, tetracycline, or MCPA–tetracycline mixtures on glyphosate Kd values. For fresh phosphate added at 48 h only, or at both 0 h and 48 h, and in the absence of fresh phosphate, two way ANOVAs in PROC GLIMMIX were carried out to quantify the effect of field aged–P (0P, 80P) and of the concentration (0, 1, 11 mg/L) of MCPA, tetracycline, or MCPA–tetracycline mixtures on the percent of glyphosate desorbed. For all ANOVAs, the separation of treatment means was performed using the Tukey's test ($p < 0.05$).

Results

Kd values on average ranged from 209 to 596 L/kg for glyphosate (Figure 7.1.3.1.1-51), from 118 to 135 L/kg for tetracycline, and from 4.99 to 5.37 L/kg for MCPA (Table 7.1.3.1.1-62). Koc values ranged from 6105 to 25,496 L/kg for glyphosate, from 3,928 to 4,901 L/kg for tetracycline, and from 156 to 209 L/kg for MCPA. These results are within the ranges observed in previous studies of the sorption of glyphosate, tetracycline and MCPA in soils. Glyphosate (<2 %) (Figure 7.1.3.1.1-51) and tetracycline (<1 %) desorption was always small but MCPA desorption ranged from 26 to 31 % (Table 7.1.3.1.1-62). Phosphate significantly reduced glyphosate sorption in soil (Figure 7.1.3.1.1-51). Without laboratory-added phosphate, glyphosate Kd values were 50 % smaller in soil containing 81 to 99 mg/kg Olsen P than in soil containing 13 to 20 mg/kg Olsen P. Regardless of whether MCPA, tetracycline or MCPA/tetracycline mixture were added to soils in the laboratory, field aged–P always significantly reduced glyphosate Kd values. When phosphate was added to soil solution at either 0 h or 24 h, it had the same significant effect on reducing glyphosate sorption with glyphosate Kd values being reduced by 37–45 % in field soils containing 13 to 20 mg P/kg, and by 23–27 % in field soils containing 81 to 99 mg P/kg (Figure 7.1.3.1.1-51).

Figure 7.1.3.1.1-51: Effect of phosphate fertilizer on glyphosate sorption and desorption in soil. Potassium dihydrogen phosphate was added prior or during glyphosate addition for the sorption study and prior, during and/or post stage of glyphosate addition for the desorption study (see Table 7.1.3.1.1-61 for labels and details)



In the presorbed phosphate experiment, the soil retained 9.8, 18.5 and 32.4 mg P/kg for the additions of 11, 22, 44 mg P/L respectively, and glyphosate sorption was significantly reduced by 41 % (11 mg P/L), 52 % (22 mg P/L) and 65 % (44 mg P/L) (Figure 7.1.3.1.1-52).The amount of field aged–P in soil had no significant impact on MCPA and tetracycline sorption in soil. However, fresh phosphate added to soil solution significantly reduced tetracycline Kd values by 8–13 % and MCPA Kd values by 7–8 % (Table **7.1.3.1.1-62**). The competitive effect of phosphate on MCPA and tetracycline sorption was not dependent on when the phosphate was added in the laboratory (either 0 h or 24 h) (Table 7.1.3.1.1-62). In the presorbed phosphate experiment, phosphate significantly reduced MCPA sorption by 10 % and tetracycline sorption

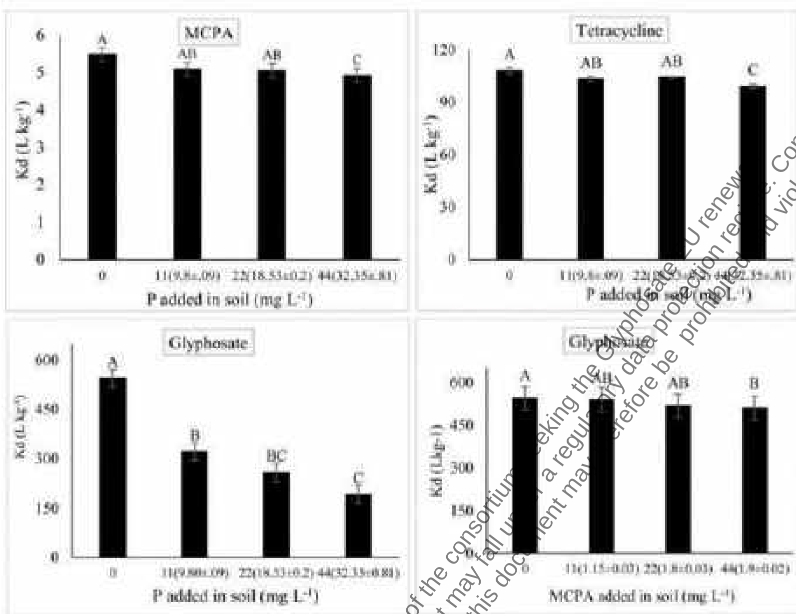
by 8 % for the addition of 44 mg P/L (Table 7.1.3.1.1-64, or Figure 7.1.3.1.1-52). However, there was no impact on MCPA or tetracycline sorption when phosphate additions were 11 or 22 mg P/L. Glyphosate desorption was significantly greater in field soils containing 81 to 99 mg/kg Olsen P (0.74 %) than in soils containing 13 to 20 mg/kg Olsen P (0.29 %) (Figure 7.1.3.1.1-51). Regardless of whether MCPA, tetracycline or MCPA/tetracycline mixture were added to soils in the laboratory, field aged–P always significantly increased glyphosate desorption. Fresh phosphate additions at 0 h, 24 h or/and 48 h to soil solutions in the laboratory also significantly increased glyphosate desorption by 0.52–0.84 % in soils containing 13 to 20 mg/kg Olsen P and by 0.52–0.82 % in field soils containing 81 to 99 mg/kg Olsen P (Figure 7.1.3.1.1-51). The amount of field aged–P in soil had no significant impact on MCPA and tetracycline desorption in soil, but the addition of fresh phosphate to soil solutions in the laboratory significantly increased desorption of MCPA by 2–3 % and tetracycline by 0.18–0.23 % (Table **7.1.3.1.1-62**).

Table 7.1.3.1.1-63: Effect of MCPA (0, 1, 11 mg/L), tetracycline (0, 1, 11 mg/L) and MCPA/tetracycline mixtures (0, 1, 11 mg/L) on sorption and desorption of glyphosate in soil in the presence and absence of phosphate

		No P		P at 0 h		P at 0 h and 48 h	
Chemicals	Concentration (mg L^{-1})	Kd ($L kg^{-1}$)	D (%)	Kd ($L kg^{-1}$)	D (%)	Kd ($L kg^{-1}$)	D (%)
MCPA	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	409.73 A	0.53 A	424.99 A	1.11 A	271.09 A	1.42 A
	11	370.88 B	0.60 B	382.32 B	1.16 A	278.44 A	1.43 A
Tetracycline	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	415.64 A	0.54 A	426.02 A	1.04 A	283.50 A	1.36 A
	11	415.94 A	0.55 A	426.02 A	1.08 A	271.72 A	1.45 A
MCPA-tetracycline mixtures	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	426.02 A	0.53 A	444.58 A	1.12 A	283.50 A	1.39 A
	11	318.05 B	0.66 B	386.72 B	1.15 A	290.51 A	1.44 A

The competitive effect of phosphate on MCPA, tetracycline and glyphosate desorption was not dependent when phosphate was added to soil solution (either at 0 h, 24 h or 48 h). The number of times that phosphate was added had no significant effect on MCPA and tetracycline desorption (Table 7.1.3.1.1-62). However, glyphosate desorption was greater when phosphate was added twice (*P,n,P*, or *n,P,P*) rather than once (*P,n,n* or *n,n,P*) but glyphosate desorption remained <2 % in all cases (Figure 7.1.3.1.1-51). MCPA and MCPA/tetracycline mixtures added at 11 mg/L significantly reduced glyphosate Kd values and increased glyphosate desorption, but only when no phosphate was added to the soil solution (Figure 7.1.3.1.1-53, Table 7.1.3.1.1-63). MCPA and MCPA/tetracycline mixtures added at 1 mg/L had no significant effect on glyphosate sorption and desorption (Table 7.1.3.1.1-63). Tetracycline had no significant effect on glyphosate Kd values and desorption, regardless of whether it was added to soil at 1 or 11 mg/L, and whether or not phosphate was added to soil solution (Table 7.1.3.1.1-63). Thus, the effect of MCPA/tetracycline mixtures on glyphosate sorption and desorption was due to MCPA.MCPA addition significantly reduced glyphosate Kd values by 14 % (Figure 7.1.3.1.1-53) and glyphosate desorption by 0.1 % (Figure 7.1.3.1.1-53). In the pre–sorbed MCPA experiment, the addition of 11, 22, 44 mg MCPA/L the soil retained 1.2, 1.8 and 1.9 mg MCPA/kg, respectively. The pre–sorbed MCPA significantly reduced glyphosate sorption by 6 % for the addition of MCPA at 44 mg/L, but there was no impact on glyphosate sorption when additions were at 11 or 22 mg/L (Table 7.1.3.1.1-64, Table 7.1.3.1.1-62 S, or Figure 7.1.3.1.1-52).

Figure 7.1.3.1.1-52: Effect of pre–sorbed phosphate concentrations on MCPA, tetracycline and glyphosate sorption, and of pre–sorbed MCPA concentrations on glyphosate sorption in soil. Numbers on x–axis in parenthesis refer to mean (± standard error) of measured pre–sorbed phosphate and MCPA



Discussion

The addition of phosphate at either 0 h or 24 h yielded the same impact on glyphosate sorption (Figure 7.1.3.1.1-51), in agreement with the findings of Gimsing *et al.* (2004) who also reported that the timing of phosphate additions had no significant effect. Glyphosate and phosphate have shown to compete for the same sorption sites in soil. Application of phosphate with glyphosate in solution reduced glyphosate sorption because phosphate is preferentially sorbed over glyphosate by available sorption sites. Glyphosate Kd values were significantly smaller in soils containing elevated Olsen P concentrations than in soils containing typical Olsen P concentrations. This elevated Olsen P concentrations resulted from eight years of annual phosphate application from 2002 to 2009, with soils being sampled for this study in 2013. These results indicate that phosphate persists in agricultural soils and occupies sorption sites that otherwise would be available sorption sites for glyphosate. In–addition, in the pre–sorbed phosphate experiment, glyphosate sorption was also reduced with increasing phosphate application to soil thus indicating that phosphate from recently fertilizer applications will also occupy sorption sites otherwise available for glyphosate sorption. Given the moderately acidic conditions (soil pH 5), the sorption sites that phosphate occupies are positively charged Fe/Al–oxides. When phosphate ($H_2PO_4^{-1}$) is retained by Fe/Al–oxides, the Fe/Al–oxides will yield a net negative charge, leading to an electrostatic repulsion between the Fe/Al–oxides and glyphosate (H_2G^-) in soil. However, a portion of glyphosate molecules that were sorbed by available positively charged Fe/Al-oxides. The addition of phosphate after this sorption increased glyphosate desorption (Figure 7.1.3.1.1-51) possibly because phosphate is able to displace glyphosate bound to Fe/Al–oxides as the bonding forces between phosphate and Fe/Al–oxides are stronger than the bonding forces between glyphosate and Fe/Al–oxides. Under the experimental conditions with the soil slurries being at a pH 5, the molecules of MCPA (pKa D 3.73) are predominantly negatively–charged.

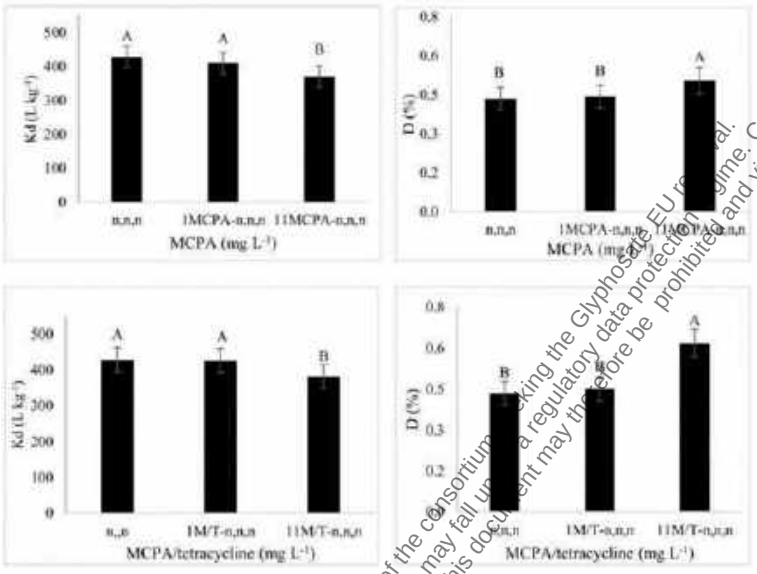
Table 7.1.3.1.1-64: Effect of pre–sorbed phosphate (0, 11, 22, 44 mg/L) on glyphosate, MCPA and tetracycline sorption and pre–sorbed MCPA on glyphosate sorption (L/kg) in soil

Concentration (mg L^{-1})	Glyphosate	MCPA	Tetracycline	Glyphosate
0	544.60 A	5.48 A	108.22 A	544.6 A
11	321.78 B	5.09 AB	103.39 AB	540.8 AB
22	258.49 BC	5.05 AB	104.35 AB	518.25 AB
44	192.96 C	4.93 C	99.32 C	510.25 B

MCPA and tetracycline sorption was only significantly reduced at the highest rate because more Fe/Al-oxides were net negatively charged and repelling MCPA and tetracycline molecules. The effect of phosphate on reducing sorption was less for MCPA and tetracycline than for glyphosate. Under moderately acidic conditions, Fe/Al–oxides are the dominant sorption sites for glyphosate and phosphate because both have a phosphonic acid group. However, MCPA (i.e., carboxyl and phenyl groups) and tetracycline (i.e. tricarbonylamide carbonyl, amine and hydroxyl groups) have other functional groups and sorption sites for MCPA and tetracycline can include under moderately acidic conditions humic substances and clay minerals in addition to Fe/Al–oxides in soils. MCPA had no longer a significant effect on glyphosate sorption when phosphate was added to the soil solution. The molecular size of phosphate (0.25 nm) is smaller than glyphosate (0.43 nm) and MCPA (0.77 nm). Therefore, it is possible that phosphate is preferentially sorbed over glyphosate and MCPA. Thus, when both phosphate and MCPA were added to the soil solution, phosphate occupied the sorption sites that may otherwise be available to MCPA and suppressed the effect of MCPA on glyphosate sorption. In the pre–sorbed experiment, in the absence of phosphate additions, MCPA reduced glyphosate sorption because pre–sorbed MCPA occupied some sorption sites which may otherwise be accessible to glyphosate.

MCPA was weakly retained with Koc values ranging from 156 to 209 L/kg while glyphosate and tetracycline were strongly retained with Koc values ranging from 6,105 to 25,496 and 3,928 to 4,901 L/kg, respectively. It has been reported that organic molecules are considered relatively mobile when Koc value ranges from 150 to 500 L/kg. Thus, given these Koc values, MCPA is relatively mobile in soil because it is only weakly retained, unlike glyphosate and tetracycline. Glyphosate is very strongly retained in soil and is less likely to be mobile in matrix flow than MCPA, regardless of the amounts of phosphate or MCPA that can compete with glyphosate for sorption sites in soil. In contrast, the presence of recent phosphate applications to agricultural soils may increase the mobility of MCPA to deeper depths but only when applied at relatively large phosphate fertilizer rates.

Figure 7.1.3.1.1-53: Effect of MCPA and MCPA/tetracycline mixtures on glyphosate sorption and desorption in soil. Potassium dihydrogen phosphate with MCPA or MCPA/tetracycline were added prior glyphosate for the sorption study and prior, or post stage of glyphosate addition for the desorption study: (see Table 7.1.3.1.1-61 for labels and details)



Conclusion

Field–aged phosphate had no significant effect on MCPA and tetracycline sorption and desorption but significantly reduced glyphosate sorption up to 50 % and increased glyphosate desorption by 0.45 %. Pre-sorbed phosphate had a greater impact on reducing glyphosate sorption than on reducing MCPA and tetracycline sorption. The addition of fresh phosphate in the laboratory also significantly decreased glyphosate sorption (up to 45 %) and increased glyphosate desorption (up to 0.87 %) and the impact on reducing MCPA and tetracycline sorption (<13 %) and increasing MCPA and tetracycline desorption (<3 %) was significant but smaller than the impact on glyphosate. Glyphosate and tetracycline were strongly retained in soil with Kd values >100 L/kg and desorption less than 2 %. In contrast, MCPA was weakly retained in soil with Kd values <6 L/kg and desorption was above 25 %. Hence, even in soils with a large phosphate build–up, glyphosate will be less mobile in matrix flow than MCPA. MCPA but not tetracycline additions significantly decreased glyphosate sorption, but only when MCPA was present at concentrations ten times greater than typically detected in agricultural soils and there was no phosphate added to the herbicide solutions.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes an OECD 106 experiment with glyphosate on a Canadian soil considering the influence of phosphate additions. The article shows some deviations from the validity criteria for EU guidelines (temperature, usage of 0.01 M KCl instead of 0.01 M $CaCl_2$, no mass balance and no demonstration of test item stability).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/019
Report author	Munira, S. *et al.*
Report year	2017
Report title	Phosphate and glyphosate sorption in soils following long-term phosphate applications
Document No	DOI 10.1016/j.geoderma.2017.10.030 ISSN 0016-7061
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Phosphate and glyphosate molecules compete for sorption sites in soil. The objective of this study was to quantify the impact of Olsen P concentrations in two contrasting soils on phosphate and glyphosate sorption. Soils were a sandy clay loam soil rich in iron oxides (SCL-Fe_2O_3) and a clay loam soil rich in calcium carbonates (CL-$CaCO_3$). The phosphate Freundlich sorption coefficient (K_f) ranged from 3 to 68 $L^{1/n}$ $mg^{1-1/n}$ kg^{-1} in the SCL-Fe_2O_3 and from 21 to 76 $L^{1/n}$ $mg^{1-1/n}$ kg^{-1} in the CL-$CaCO_3$. Glyphosate sorption coefficient (Kd) ranged from 293 to 1173 L/kg in the SCL-Fe_2O_3 but only 99 to 141 L/kg in the CL-$CaCO_3$. Glyphosate K_d and phosphate K_f values decreased significantly with increasing Olsen P concentrations in both soils. Glyphosate K_d values were further significantly reduced when phosphate was added to the slurry solutions, but phosphate K_f values were not impacted by the presence of glyphosate in solutions. We conclude that annual phosphate fertilizer applications leave phosphate concentrations in Prairie soils to the extent that soils have a lesser capacity to retain glyphosate and phosphate that are subsequently applied, but glyphosate residues will not influence phosphate sorption.

Methods

Chemicals

Chemicals used were analytical grade glyphosate (99.9 % purity) from Sigma-Aldrich Co., St. Louis, MO; [phosphonomethyl-^{14}C]glyphosate (99 % radiochemical purity; specific activity 50 mCi/mmol) from American Radiolabeled Chemicals Inc., St. Louis, MO; Roundup Ultra2® (49 % active ingredient and 51 % other ingredients, CAS No. 70901-12-1) from Monsanto Chemical Company; and analytical grade potassium dihydrogen phosphate (KH_2PO_4) (99 % chemical purity), potassium chloride (100 % chemical purity) and calcium chloride, dehydrate (> 95 % chemical purity) from Fisher Scientific, Fair Lawn, NJ.

Soil characteristics and experimental design

This study utilized soil samples (0–15 cm) obtained from long-term experimental plots under a durum wheat and flax rotation near Carman (49° 29.7′ N, 98° 2.4′ W) and near Forrest (50° 1.2′ N, 99° 53.3′ W) Manitoba, Canada. Soil profiles at both sites were classified based on the Canadian System of Soil Classification as Orthic Black Chernozems, which is equivalent to the Udic Boroll subgroup in the U.S. Soil Taxonomy. The experimental design at each site was a randomized complete block design with four mono ammonium phosphate fertilizer treatments and four replicates plots. Treatments were a control (no phosphate applications), and plots receiving annual applications of mono ammonium phosphate fertilizers at 20, 40, and 80 kg P/ha or 20P, 40P, and 80P, respectively, from 2002 to 2009. For all plots that received mono ammonium phosphate, 20 kg P/ha was placed near the seed to enhance fertilizer use efficiency, a common practice in Canadian Prairie agriculture. For the 40 and 80 kg P/ha treatments, to avoid seedling toxicity, the additional mono ammonium phosphate was broadcast and then

incorporated. From 2010 to 2013, the rotation was continued but no phosphate was applied. Application of urea fertilizer differed by year. Generally, durum wheat received 90 kg N/ha and flax 50 kg N/ha. From each plot, composite samples were collected in spring, 2013 using a Dutch auger with ten (Carman) to eight (Forrest) samples per plot and cleaning the auger between plots. Soil samples were air-dried and sieved (< 2 mm) prior to soil property analyses and sorption experiments. The Carman soil has a sandy clay loam texture and is relatively high in iron oxides (SCL-Fe_2O_3), whereas the Forrest soil has a clay loam texture and is relatively high in calcium carbonates (CL- $CaCO_3$) (Table 7.1.3.1.1-65). Available phosphate was extracted using the Olsen (0.5 M $NaHCO_3$, pH 8.5) phosphorus test. 2 g of air-dried soil and 40 mL of 0.5 N $NaHCO_3$ solution was mixed in a 50 mL Erlenmeyer flask. Flasks (duplicates) were shaken horizontally (200 excursions/min). Equilibrium solutions were filtered through Whatman No. 2 filter paper and phosphate concentrations were determined colorimetrically.

Table 7.1.3.1.1-65: Selected soil physical and chemical properties as mean with standard error

Soil	Organic Carbon[a] (%)	pH[b]	Fe_2O_3[c] (mg kg^{-1})	Al_2O_3[c] (mg kg^{-1})	Ca[d] (mg kg^{-1})	Clay[e] %	Silt[e] %	Sand[e] %
SCL-Fe_2O_3	2.81 ± 0.04	4.7 ± 0.02	237 ± 7.93	6.41 ± 0.04	3252 ± 38	20	20	60
CL-$CaCO_3$	3.2 ± 0.07	7.3 ± 0.02	12.52 ± 0.22	1.07 ± 0.47	4704 ± 158	30	39	31

[a] Soil organic carbon content was determined using combustion technique with a high temperature induction furnace (Nelson and Sommers, 1996).
[b] Soil pH was determined using a 10 mL 0.01 M $CaCl_2$ solution and 2 g soil solution ratio (Jones, 2001).
[c] Extractable Fe and Al were extracted with diethylenetriaminepentaacetic acid (*DTPA*) (Whitney, 2011) and 0.01 M $CaCl_2$ (Barnhisel and Bertsch, 1982) respectively, and extracts were analyzed by *ICP*.
[d] Extractable Ca was also measured by *ICP* using ammonium acetate as an extractant (Warncke and Brown, 2011).
[e] Data adopted (Grant et al., 2013).

Phosphate sorption

Phosphate sorption was determined by batch equilibrium using either 0.01 M $CaCl_2$ or 0.01 M KCl as the background electrolyte. Batch equilibrium procedures followed standard protocols using a soil/solution ratio of 1:10 and an equilibrium time of 24 h. Two experiments were conducted utilizing soil samples: (1) from all plots at each site to quantify the effect of Olsen P concentrations on phosphate sorption in soil and (2) from control and 80P plots at each site to quantify the effect of Roundup Ultra2 additions to soil slurries on phosphate sorption in soil.

Effect of field-aged phosphate concentrations on sorption of phosphate

In the first experiment, potassium dihydrogen phosphate solutions (20 mL) at concentrations of 5, 10, 25, 50, 100, 150, 250 or 500 mg P/L were added to air-dried soil (2 g) in 50-mL centrifuge tubes (duplicates) and shaken horizontally (120 excursions/min) at room temperature (23 ± 2 °C) for 24 h. Equilibrium solution was centrifuged (6100 G for 10 min) and filtered (0.45 µm). Phosphate concentration was determined colorimetrically by the molybdate blue method. Linearized Freundlich isotherm has been specified as: The phosphate sorption coefficient, K_f ($L^{1/n}$ $mg^{1-1/n}$ kg^{-1}), was calculated using the linearized form of Freundlich equation: log q= log K_f + 1/n log C. Where q represents phosphate sorption in soil at equilibrium (mg/kg), C represents phosphate concentration of equilibrium solution (mg/L), and 1/n represents the Freundlich slope. In addition, the Freundlich P sorption isotherm was used to determine the equilibrium P concentration (EPCo) at log q = 0, which is the concentration at which neither sorption nor desorption occurs and hence can be used to define whether a soil is likely to act as a sink (sorption) or source (desorption) of P. EPCo levels above 0.025 mg/L suggest an increased risk of eutrophication because of P transport in soluble form.

Effect of glyphosate formulation on sorption of phosphate

In the second experiment, stock solutions of 150 mg P/L were prepared with and without 100 mg/L Roundup Ultra2 in the solution. The 100 mg/L Roundup Ultra 2 was equivalent to 378 mg glyphosate/kg soil. The 150 mg P/L solution was used because previous studies have proposed that this parameter (P150) is the most optimum single point in the isotherm reflective of the phosphate sorption capacity in soils. Batch equilibrium procedures were carried out as described above. The phosphate sorption coefficient, Kd (L/kg), was calculated by q/C, where q represents phosphate sorption by soil at equilibrium (mg/kg) and C represents phosphate concentration of equilibrium solution (mg/L).

Glyphosate sorption

Glyphosate sorption was determined by batch equilibrium with the initial glyphosate solution containing 1 mg/L analytical-grade glyphosate and 6.67 × 104 Bq/L ^{14}C–labelled glyphosate. Two experiments were conducted utilizing soil samples: (1) from all plots to quantify at each site the effect of Olsen P concentrations on glyphosate sorption, and (2) from control and 80P plots to quantify at each site the effect of fresh phosphate additions to soil slurries on glyphosate sorption in soil.

Impact of field-aged phosphate concentrations on sorption of glyphosate

Batch equilibrium procedures followed the OECD guideline 106 using a soil/solution ratio of 1:5, an equilibrium time of 24 h and 0.01 M $CaCl_2$ or 0.01 M KCl as background electrolyte. Glyphosate solutions (10 mL) were added to air-dried soil (2 g) in 50-mL centrifuge Teflon tubes (duplicates) and slurries were rotated in the dark at 5 °C for 24 h. Equilibrium solution was centrifuged (6100 G for 10 min) and subsamples (1 mL) of supernatant were added in duplicated 7-mL scintillation vials containing 5 mL of 30 % Scintisafe scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method) (LS 6500 Beckman Instruments, Fullerton, CA). The glyphosate sorption distribution constant, Kd (L/kg), was calculated by Cs/Ce, where Cs represents glyphosate sorption by soil at equilibrium (mg/kg) and Ce represents glyphosate concentration of equilibrium solution (mg/L). The difference between the added radioactivity and radioactivity in the supernatant was assumed to be the proportion of glyphosate having been sorbed.

Impact of fresh phosphate addition on sorption of glyphosate

Experiments followed similar batch equilibrium sorption protocols as described above. In this experiment, potassium dihydrogen phosphate was added to the initial glyphosate solution at rates equivalent to 11, 22 and 44 mg P/kg soil, or an estimated 20, 40 and 80 P kg/ha, respectively, when assuming the fertilizer being present in the top 15-cm layer of a soil with a bulk density of 1200 kg/m^3.

Statistical analysis

Statistical analyses were carried out using SAS software version 9.3 for Windows (SAS Institute Inc. 2002-2010). Prior to each analysis, data sets were checked for outliers, normality of residuals and homogeneity of variances. Residuals were normally distributed and variances were homogeneous. The paired *t*-test ($P < 0.05$) was used to test for the effect of background electrolyte solution (0.01 M $CaCl_2$ versus 0.01 M KCl) on glyphosate K_d or phosphate K_f and EPCo. For both background electrolyte solutions and at each site, simple linear regression analyses ($P < 0.05$) were carried out to estimate glyphosate K_d and phosphate K_f values using Olsen P concentration as the independent variable. In each of the glyphosate K_d and phosphate K_f figures, the slopes of regression lines developed for SCL-Fe_2O_3 and CL- $CaCO_3$ were compared by including dummy variables in PROC REG to test whether the responses of sorption to increasing Olsen P concentrations was influenced by soil type. Simple linear regression analysis was also carried out to estimate glyphosate K_d values by using the added fresh phosphate concentration as an independent variable. The slopes of the regression lines developed for the 0P (control) and 80P plots in both soils were compared by including dummy variables in PROC REG to test whether the responses of sorption to increasing potassium dihydrogen phosphate concentration was influenced by Olsen P concentrations (0P, 80P). Simple linear regression analyses were carried out to determine the relationship between glyphosate K_d and phosphate K_f values by using K_f as an independent variable. Simple linear regression analyses ($P < 0.05$) were also carried out to estimate EPCo values by using Olsen P as an independent variable for CL-$CaCO_3$ soil. Graphical plot fitting of EPCo as a function of Olsen P showed that data did not fit well with simple linear regression for the SCL-Fe_2O_3 soil.

Results

Effect of background electrolyte solutions on sorption of phosphate and glyphosate

The types of ions in solution had a significant effect on phosphate and glyphosate sorption, except for glyphosate sorption in the CL-$CaCO_3$ soil (Table 7.1.3.1.1-66). Phosphate K_f values in both soils were significantly greater in experiments with 0.01 M $CaCl_2$ than experiments with 0.01 M KCl (Table **7.1.3.1.1-66**).

Table 7.1.3.1.1-66: Statistical parameters (Paired t-tests) on the effect of background electrolyte solution (0.01 M $CaCl_2$ versus 0.01 M KCl) on glyphosate (L/kg) and phosphate sorption coefficient ($L^{1/n}$ mg $^{1-1/n}$ kg^{-1}) in soils

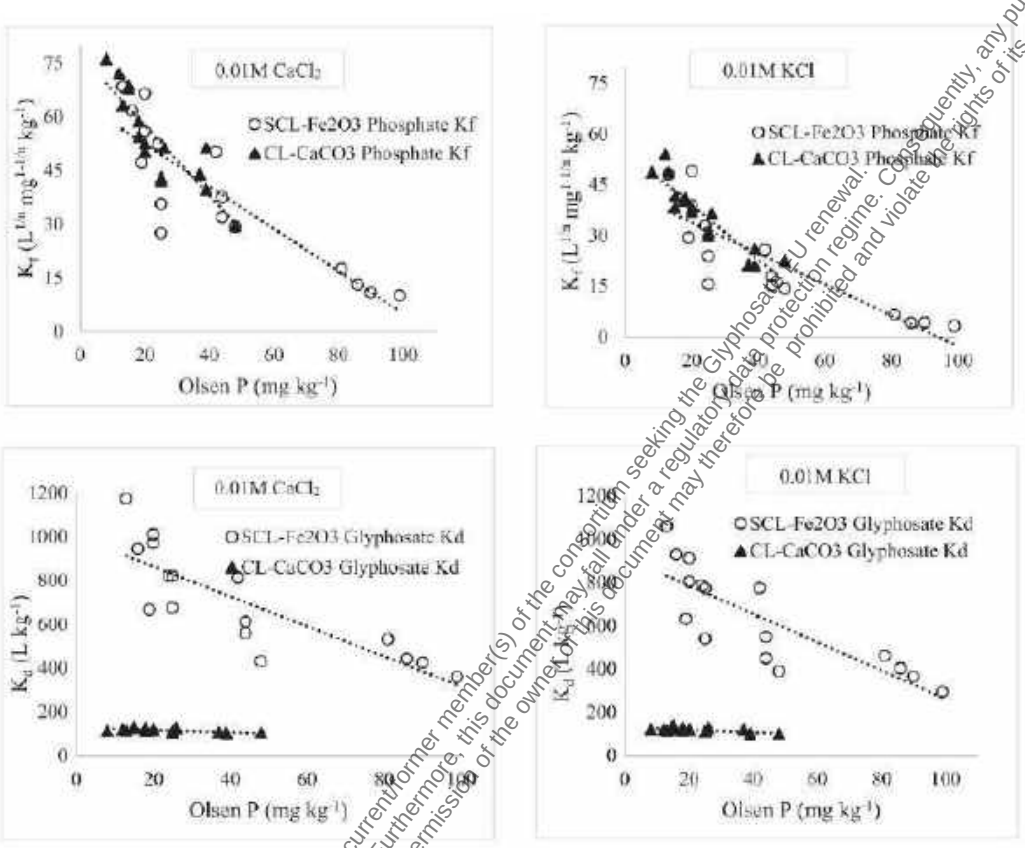
Sorption Parameter	Soil	Mean		DF	t Value	Pr > |t|
		0.01 M $CaCl_2$	0.01 M KCl			
Phosphate sorption coefficient, K_f	SCL-Fe_2O_3	38.47	23.11	15	11.14	< 0.0001
	CL-$CaCO_3$	54.08	36.04	15	10.94	< 0.0001
Glyphosate sorption coefficient, K_d	SCL-Fe_2O_3	703	632	15	5.89	< 0.0001
	CL-$CaCO_3$	116	117	15	− 1.55	0.1430
Phosphate equilibrium concentration, EPCo	SCL-Fe_2O_3	0.007	0.04	15	− 2.29	0.0366
	CL-$CaCO_3$	0.006	0.015	15	− 4.72	< 0.0003

Phosphate K_f values were on average 54 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in CL-$CaCO_3$ and 38 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} SCL-Fe_2O_3 with $CaCl_2$ but on average 36 L1/n mg $^{1-1/n}$ kg^{-1} in CL-$CaCO_3$ and 23 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} SCL-Fe_2O_3 with KCl. Thus, when 0.01 M $CaCl_2$ was used with the SCL- Fe_2O_3 and CL-$CaCO_3$ soils but also when KCl was used with the CL-$CaCO_3$ soil, phosphate likely formed stable complexes with a portion of Ca^{2+} in soil solution and precipitated. In batch equilibrium experiments with 0.01 M $CaCl_2$, precipitation with Ca^{2+} occurs more readily for phosphate than glyphosate. For glyphosate sorption, K_d values were on average 116 L/kg in CL-$CaCO_3$ and 703 L/kg SCL- Fe_2O_3 with $CaCl_2$, and on average 117 L/kg in CL-$CaCO_3$ and 632 L/kg SCL-Fe_2O_3 with KCl. In calcareous soils, Ca^{2+} in forms a bridge between negatively charged soil colloids and glyphosate molecules in soil and, because of the already high free calcium content in the CL-$CaCO_3$ soil, the addition of Ca with 0.01 M $CaCl_2$ solution had no impact on glyphosate sorption. For the SCL-Fe_2O_3 soil, glyphosate sorption was greater with 0.01 M $CaCl_2$ than 0.01 M KCl, suggesting that glyphosate was able to form complexes with Ca^{2+} in solution for enhanced sorption.

Effect of field-aged phosphate concentrations on sorption of phosphate

Despite being exposed to similar long-term phosphate fertilizer treatments, Olsen P ranged from 13 to 99 mg/kg in the acidic SCL-Fe_2O_3 soil but only from 8 to 48 mg/kg in the calcareous CL-$CaCO_3$ soil. Olsen P concentrations by treatment were on average 17 (control), 24 (20P), 44 (40P) and 89 (80P) mg/kg in the SCL- Fe_2O_3 soil and 13 (control), 18 (20P), 24 (40P) and 41 (80P) mg/kg in the CL-$CaCO_3$ soil. The Olsen P test was originally developed for calcareous soils and can overestimate plant available P in acidic soils, such as the SCL-Fe_2O_3. Olsen P measures the $NaHCO_3$ extractable phosphate in soil, but calcareous soil may also contain slow release inorganic phosphate (apatite minerals) extracted by 1 M HCl. Olsen P concentrations ranged from 8 to 99 mg/kg in this research which is within the typical range of 8 to 114 mg/kg that has been reported for soils in North America. Hence, the findings from this research on the sorption pattern of phosphate and glyphosate in soil would be applicable to a wider range of soils in North America. Phosphate K_f values significantly decreased with the increasing concentrations of Olsen P in soil (Figure 7.1.3.1.1-54).

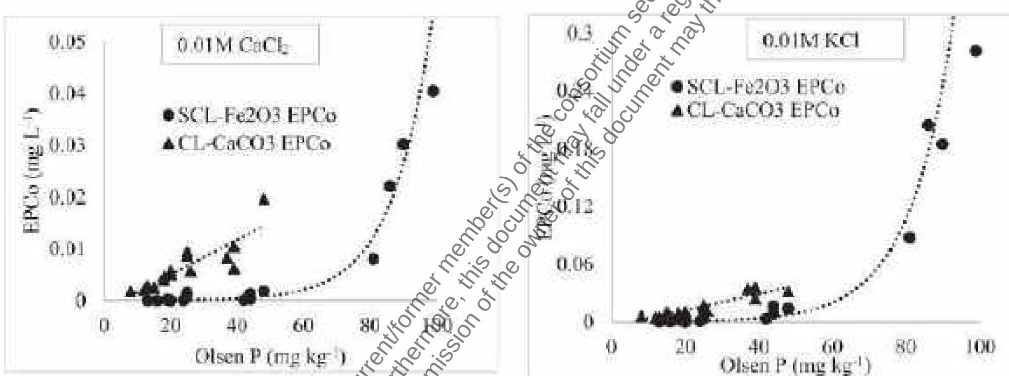
Figure 7.1.3.1.1-54: Effect of Olsen P concentrations in soil on glyphosate and phosphate sorption in SCL-Fe_2O_3 and CL-$CaCO_3$ soils, as determined by batch equilibrium experiments using 0.01 M $CaCl_2$ or 0.01 M KCl as background electrolyte solutions. All regression equations are significant at $P < 0.05$



The SCL- Fe_2O_3 and CL-$CaCO_3$ soils showed relatively similar phosphate sorption (Figure 7.1.3.1.1-54). Phosphate K_f values ranged from 3.2 to 68 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in the SCL- Fe_2O_3 soil with 1/n values between 0.37 and 0.92, and from 21 to 76 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in the CL-$CaCO_3$ soil with 1/n values between 0.68 and 0.92. These values are within the range of other studies (Bertrand *et al.*, 2003; Jalali, 2007; Shafqat and Pierzynski, 2014). A maximum reduction of phosphate K_f value was observed in SCL- Fe_2O_3 soil. The phosphate K_f value in SCL- Fe_2O_3 was reduced by 95 % in soil containing 99 mg/kg Olsen P relative to soil containing 13 mg/kg Olsen P. Thus, P accumulation in soil reduced the capacity of soil to hold Wang *et al.* (2015) also reported that sorption of P decreased with the in- creasing concentrations of Olsen P because long-term application of P fertilizer leads to the accumulation of P in soil. In their study, they showed that long-term (5 to 15 years) application of phosphate significantly reduced phosphate sorption by 56 % in soil containing 53 mg/kg Olsen P relative to soil containing 15 mg/kg Olsen P. Olsen P concentrations significantly predicted phosphate K_f (Figure 7.1.3.1.1-54) in both SCL- Fe_2O_3 and CL-$CaCO_3$. The effect of Olsen P concentrations on reducing phosphate sorption was more pronounced for SCL- Fe_2O_3 than CL-$CaCO_3$. For the phosphate K_f, the regression slopes were significantly different between the soils in case of 0.01 M KCl but not with 0.01 M $CaCl_2$ because the presence of Ca in solution led to the possibility of precipitation of phosphate-Ca^{2+} complexes in both soils. Generally, in calcareous soil, Ca forms precipitation with the added phosphate in soil solution. For 0.01 M KCl, the CL- $CaCO_3$ showed a significantly steeper slope than SCL- Fe_2O_3 (Figure 7.1.3.1.1-54) because, with increasing Olsen P concentrations, more sorption sites remained available in SCL- Fe_2O_3. CL-$CaCO_3$ soil has less sorption sites available for the added phosphate than SCL- Fe_2O_3 soil because calcareous soils contain slow-release phosphate (e.g., octacalcium phosphate and apatite) which occupy sorption sites that otherwise would be available for the added phosphate.

EPCo significantly increased with increasing concentrations of Olsen P in both SCL- Fe_2O_3 and CL-$CaCO_3$ (Figure 7.1.3.1.1-55). EPCo values ranged from 0 to 0.281 mg/L, depending on the background electrolyte solution and soil (Figure 7.1.3.1.1-55). EPCo values in both soils were significantly greater in the experiments with 0.01 M KCl than experiments with 0.01 M $CaCl_2$ (Table 7.1.3.1.1-66) because of the formation of Ca^{2+}-phosphate complexes in both soils with 0.01 M $CaCl_2$. All EPCo levels were below the threshold value of 0.025 mg/L except in the 80P plots. The average calculated EPCo values for the four replicated 80P plots was 0.031 mg/L for CL-$CaCO_3$ and 0.190 mg/L for SCL- Fe_2O_3 with 0.01 M KCl, and 0.025 mg/L for SCL- Fe_2O_3 with 0.01 M $CaCl_2$. Although this suggest that prairie soils have a low risk for soluble P transport, a recent review reported that a significant portion of phosphate in Prairie soils can be transported as dissolved P during snow melt runoff. Phosphate can be transported from the agricultural soil when phosphate fertilizer is applied in excess of crop requirements and also from plant residues during snow melt.

Figure 7.1.3.1.1-55: Effect of Olsen P concentrations in soil on the phosphate equilibrium concentration, (EPCo) in SCL-Fe_2O_3 and CL-$CaCO_3$ soils determined by batch equilibrium experiments using 0.01 M $CaCl_2$ or 0.01 M KCl as background electrolyte solutions. Olsen P All regression equations are significant at P < 0.05



Effect of glyphosate formulation on sorption of phosphate

Commercially available glyphosate formulation had no impact on phosphate sorption in soil because there were no significant differences in phosphate sorption between treatments with and without Roundup Ultra2 additions to soil slurries. Gimsing and Borggaard (2001) also found that, when glyphosate was added following phosphate additions to goethite, glyphosate did not displace the sorbed phosphate. In a recent article that was published in the magazine "No-Till Farmer", a statement was made that "20-25 percent of the dissolved reactive phosphorus in runoff is caused by glyphosate [use]" because of the assumption that glyphosate residues in soil decreases phosphate retention in soil. However, in our batch-equilibrium study that utilized very high rates of Roundup Ultra2, there was no significant difference in phosphate sorption between treatments with and without Roundup Ultra2 additions to soil slurries. Thus, given our findings, the recent concerns stated in Barrera (2016) are unlikely to be applicable to the Prairie soils that were included in our studies.

Effect of field-aged phosphate on sorption of glyphosate

Glyphosate K_d values significantly decreased with the increasing concentrations of Olsen P in both SCL- Fe_2O_3 and CL-$CaCO_3$ (Figure 7.1.3.1.1-54). Glyphosate K_d values ranged from 293 to 1173 L/kg in the acidic SCL- Fe_2O_3 soil and from only 99 to 141 L/kg in the calcareous CL- $CaCO_3$ soil (Figure 7.1.3.1.1-54), and these values are within the range of other studies (Farenhorst *et al.*, 2008; Kumari *et al.*, 2016; Sørensen *et al.*, 2006). Long-term application of phosphate fertilizer in soil reduced glyphosate sorption because pre-sorbed phosphate occupied the sorption sites that would otherwise be available to glyphosate. A maximum reduction in glyphosate K_d value was observed in SCL- Fe_2O_3 soil. The K_d value was reduced by 75 % in soil containing 99 mg/kg Olsen P relative to soil containing 13 mg/kg Olsen P in SCL- Fe_2O_3. Thus, results indicate that glyphosate and phosphate compete for the same sorption sites in

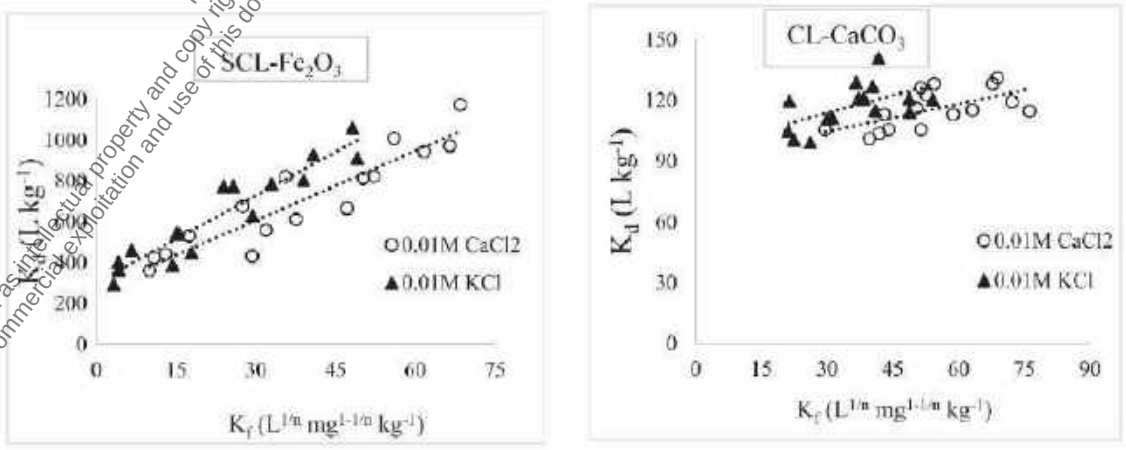
soil. Similar observations have been made by de Jonge *et al.* (2001) who reported that long-term (60 to 100 years) application of phosphate significantly reduced glyphosate sorption by 50 % in soil containing 59 mg/kg Olsen P relative to soil containing 6 mg/kg Olsen P.

Olsen P concentrations significantly predicted glyphosate K_d (Figure 7.1.3.1.1-54) in both SCL- Fe_2O_3 and CL-$CaCO_3$. With both 0.01 M $CaCl_2$ and 0.01 M KCl, the slopes of the regressions predicting glyphosate K_d were significantly different between soils with the SCL- Fe_2O_3 showing steeper slopes than CL-$CaCO_3$ (Figure 7.1.3.1.1-54). Regardless of the solution used, the sorption of glyphosate was greater in SCL- Fe_2O_3 than CL-$CaCO_3$ be- cause of the importance of Fe_2O_3 in providing sorption sites for the negatively charged glyphosate in acidic soils. Research findings indicate that the presence of iron-oxide and soil pH had a stronger influence on glyphosate than phosphate sorption. The SCL- Fe_2O_3 soil contained 94 % more Fe-oxides and 83 % more Al-oxides than the CL-$CaCO_3$ soil (Table 7.1.3.1.1-65), and glyphosate sorption was greater in SCL-Fe_2O_3 soil because glyphosate sorption has been shown to be positively correlated with Fe/Al-oxides. In addition, glyphosate sorption was greater in SCL- Fe_2O_3 (pH 4.7 to 5) than CL-$CaCO_3$ (pH 7.3 to 7.5) soil because glyphosate sorption is negatively correlated with soil pH. This is because with increasing soil pH, an increasing portion of the glyphosate molecules become negatively charged with glyphosate molecules existing as HG^{2-} (~ 100 %) (net negative charge of glyphosate is 2^-) at pH 7.3–7.5, and soil colloid deprotonation increases with soil colloids having a net negative charge in Prairie soils when soil pH > 6. Hence, regardless of the background electrolyte solutions, the sorption of glyphosate was always relatively low in the CL-$CaCO_3$ soil (Figure 7.1.3.1.1-54). Thus, the effect of Olsen P concentrations on reducing glyphosate sorption was more pronounced for SCL-Fe_2O_3 than CL-$CaCO_3$. For example, with 0.01 M KCl, glyphosate K_d was reduced by 39 % when the phosphate concentration increased from 17 mg/kg (control) to 44 mg/kg (40P plots) in SCL-Fe_2O_3 but by only 11 % when the phosphate concentration increased from 13 mg/kg (control) to 41 mg/kg (80P plots) in CL-$CaCO_3$.

Association between glyphosate K_d and phosphate K_f in relation to field-aged phosphate

Phosphate K_f and glyphosate K_d values were positively correlated (Figure 7.1.3.1.1-56). Thus, agreeing with previous studies suggesting phosphate and glyphosate have similar sorption pattern in soil. However, regardless of the background electrolyte solution, phosphate K_f and glyphosate K_d were more strongly correlated in SCL-Fe_2O_3 than CL-$CaCO_3$. Hence, glyphosate and phosphate may compete more strongly for sorption sites in acidic soils with high Fe/Al-oxides content than in calcareous soils. In both soils and under both electrolyte background solutions, phosphate sorption was more strongly reduced by Olsen P concentrations than glyphosate sorption was reduced by Olsen P concentrations. Thus, long-term application of phosphate fertilizer has an overall greater impact on reducing phosphate sorption than glyphosate sorption.

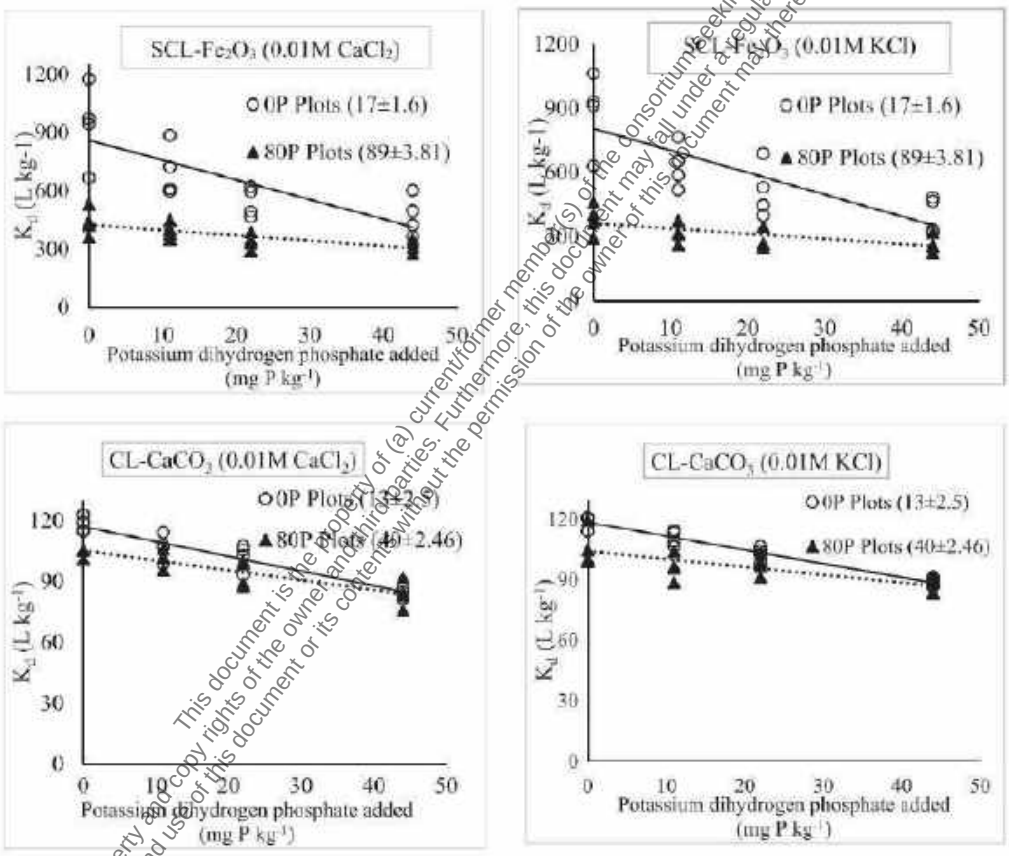
Figure 7.1.3.1.1-56: Association between glyphosate K_d and Phosphate K_f in SCL-Fe_2O_3 and CL-$CaCO_3$ soils with sorption being determined by batch equilibrium experiments using 0.01 M $CaCl_2$ or 0.01 M KCl as background electrolyte solutions. All regression equations are significant at P < 0.05



Effect of fresh phosphate addition on the sorption of glyphosate

Regardless of the background electrolyte solution and soil, the potassium dihydrogen phosphate additions to soil slurries significantly decreased glyphosate K_d values (Figure 7.1.3.1.1-57). Addition of fresh phosphate significantly reduced glyphosate sorption because the chemicals competed for the same sorption sites as they have similar phosphonate functional groups. Gimsing and Borggaard (2002) studied the competitive sorption effect of fresh phosphate on glyphosate in soil and concluded that phosphate is preferentially sorbed over glyphosate. In addition to this, sorption of phosphate lowers the zero point charge of sorption sites such as Fe/Al-oxides, potentially increases the net negative charge on the oxide surfaces and thereby increasing the electrostatic repulsion between glyphosate and soil oxides.

Figure 7.1.3.1.1-57: Effect of potassium dihydrogen phosphate concentrations on glyphosate sorption in SCL-Fe_2O_3 and CL-$CaCO_3$ soils with low (0P) or high (80P) Olsen P concentrations. Potassium dihydrogen phosphate was added to glyphosate in soil slurries during batch equilibrium experiments using 0.01 M $CaCl_2$ and 0.01 M KCl. All regression equations are significant at $P < 0.05$. The values in parentheses in each legend represent mean values of Olsen P and standard error



Fresh phosphate significantly predicted glyphosate K_d (Figure 7.1.3.1.1-57) in both SCL- Fe_2O_3 and CL-$CaCO_3$. The regression slope was significantly steeper for 0P plots (control) than 80P plots in both soils and regardless of the background electrolyte solution (Figure 7.1.3.1.1-57). Thus, the effect of potassium dihydrogen phosphate addition in reducing glyphosate K_d values was less in soils that had greater Olsen P concentrations because less sorption sites were available for the added phosphate to compete with glyphosate molecules. This impact of phosphate already in soil was larger in SCL- Fe_2O_3 than CL-$CaCO_3$ because in CL-$CaCO_3$ soil at pH 7.3–7.5, glyphosate molecule existed as HG^{2-} (~ 100 %) leading to less sorption, both in the presence and absence of fresh phosphate. Thus, the competitive effect of phosphate on

glyphosate is stronger in soils that are acidic and contain substantial amount of Fe-oxides than in calcareous soils.

Conclusion
The sorption of phosphate and glyphosate was reduced due to the long-term addition of phosphate fertilizer in two Prairie soils. The impact of Olsen P on reducing glyphosate sorption was more pronounced in the acidic (iron-oxide rich) sandy clay loam than the calcareous (calcium carbonate rich) clay loam soil, both with or without the addition of potassium dihydrogen phosphate. Regardless of the background electrolyte and soil type, phosphate sorption was more strongly reduced by the Olsen P concentrations than glyphosate sorption. The reduction of glyphosate sorption due to the application of potassium dihydrogen phosphate was greater in soils containing low Olsen P concentrations. The equilibrium phosphate concentration was above the threshold level for eutrophication only in soils that had exceptionally high phosphate concentrations i.e., the soils had received annual applications of mono ammonium phosphate at rates of 80 kg/ha for eight years. Commercially formulated glyphosate had no influence on phosphate sorption suggesting that glyphosate residues in soils have no impact on phosphate sorption or mobility.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a sorption experiment with phosphate and glyphosate to Canadian agricultural soils. Some validation criteria of the underlying OECD 106 study protocol were not met, or insufficient information is reported (i.e. no material balance, stability of test item not demonstrated, no pre-equilibration of samples).

The article is therefore classified as reliable with restrictions and was. not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/020
Report author	Zhelezova, A. *et al.*
Report year	2017
Report title	Effect of Biochar Amendment and Ageing on Adsorption and Degradation of Two Herbicides
Document No	DOI 10.1007/s11270-017-3392-7 ISSN 0049-6979
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

The article was found relevant for multiple data points. The summary is provided under CA 7.1.2.1.1/010.

1. Information on the study

Data point:	CA 7.1.3.1.1/021
Report author	Cassigneul, A. *et al.*
Report year	2016
Report title	Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study
Document No	DOI 10.1016/j.scitotenv.2015.12.052 E-ISSN: 1879-1026
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

The article was found relevant for multiple data points. The summary is provided under CA 7.1.2.1.1/011.

1. Information on the study

Data point:	CA 7.1.3.1.1/022
Report author	Munira, S. *et al.*
Report year	2016
Report title	Phosphate fertilizer impacts on glyphosate sorption by soil
Document No	Chemosphere 153 (2016) 471-477
Guidelines followed in study	DOI 10.1016/j.chemosphere.2016.03.028 E-ISSN 1879-1298
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

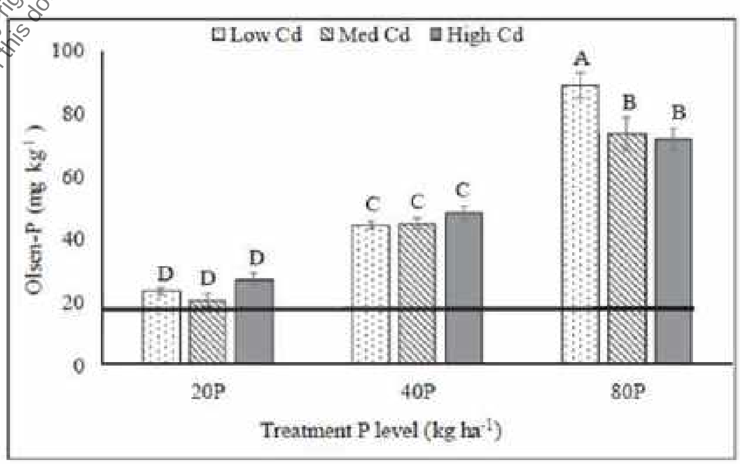
This research examined the impact of field-aged phosphate and cadmium (Cd) concentrations, and fresh phosphate co-applications, on glyphosate sorption by soil. Soil samples were collected in 2013 from research plots that had received, from 2002 to 2009, annual applications of mono ammonium phosphate (MAP) at 20, 40 and 80 kg P/ha and from products containing 0.4, 70 or 210 mg Cd/kg as an impurity. A series of batch equilibrium experiments were carried out to quantify the glyphosate sorption distribution constant, Kd. Extractable Cd concentrations in soil had no significant effect on glyphosate sorption. Glyphosate Kd values significantly decreased with increasing Olsen-P concentrations in soil, regardless of the pH conditions studied. Experiments repeated with a commercially available glyphosate formulation showed statistically similar results as the experiments performed with analytical-grade glyphosate. Co-applications of MAP with glyphosate also reduced the available sorption sites to retain glyphosate, but less so when soils already contain large amounts of phosphate. Glyphosate Kd values in soils ranged from 173 to 939 L/kg under very strong to strongly acidic condition but the Kd was always <100 L/kg under moderately acidic to slightly alkaline conditions. The highest Olsen-P concentrations in soil reduced Kd values by 25-44 % relative to control soils suggesting that, under moderately acidic to slightly alkaline conditions, glyphosate may become mobile by water in soils with high phosphate levels. Otherwise, glyphosate residues in agricultural soils are more likely to be transported off-site by wind and water-eroded sediments than by leaching or runoff.

Materials and Methods

Experimental design and soil characteristics

Soil samples (0 -15 cm) with a sandy clay loam texture were collected in the spring 2013 from research plots situated under a durum wheat and flax rotation near Carman, Manitoba, Canada. The soil is classified as an Orthic Black Chernozem. The experimental plot was a randomized complete block design with 10 treatments and 4 replicates per treatment. In each of the forty plots, the composite soil sample consisted of ten samples collected in the plot using a Dutch augur. Treatments were a control (neither phosphate nor Cd applications), and plots receiving from 2002 to 2009 annual applications of mono ammonium phosphate (MAP) fertilizers that originated from three different phosphate rock sources containing 0.4, 70 or 210 mg Cd/kg, or low, medium and high Cd, respectively (Grant *et al.*, 2013). MAP from these three sources was applied to plots at 20, 40 and 80 kg P/ha, or 20P, 40P and 80P, respectively. For all plots that received MAP, 20 kg P/ha was placed near the seed to enhance fertilizer use efficiency, a common practice in Canadian Prairie agriculture. For the 40 and 80 kg P/ha treatments, to avoid seedling toxicity, the additional MAP was broadcasted and then incorporated in soil. From 2010 to 2013, the rotation was continued but no phosphate or Cd was applied. Nitrogen fertilizer varied by year to optimize yields. The typical rate of N applied was 90 kg N/ha in durum wheat and 50 kg N/ha in flax. Soil samples were air-dried and sieved (<2 mm) prior to soil properties analysis and sorption studies. Soil was digested with nitric acid and total Cd was determined by inductively coupled plasma (ICP). Extractable Cd was extracted with diethylene triamine pentaacetic acid (DTPA) ICP. Various factors have been shown to influence the efficiency of micronutrient extraction by DTPA, including extraction temperature and shaking time. Available phosphate was extracted using Olsen ($NaHCO_3$) phosphorus test. Soil physical and chemical properties that are known to influence glyphosate and phosphate sorption by soil, but did not significant vary across the plots by treatment, were also determined. Soil organic carbon content was determined using combustion technique with a high temperature induction furnace. Extractable Fe_2O_3 and Al_2O_3 were extracted with DTPA and 0.01 M $CaCl_2$, respectively, and extracts were analyzed by ICP. Extractable Ca was also measured by ICP using ammonium acetate as an extractant. Results were soil organic carbon content: 2.80 % (mean) ±0.04 (standard error) (n = 16, number of plots analyzed); extractable Fe_2O_3: 246 ± 5 mg/kg (n = 40), extractable Al_2O_3: 6.4 ± 0.65 mg/kg (n = 16); and extractable Ca: 2252 ± 40.57 mg/kg (n = 16). Given that the study focused on Cd and P applications as treatments, the concentrations of extractable and total Cd, as well as Olsen-P in all plots were determined. We did not expect to see treatment differences for the other parameters that were measured (i.e., extractable Fe_2O_3, Al_2O_3, and Ca). Fe_2O_3 was also measured in all plots as previous studies have demonstrated that there is a strong positive association between Fe_2O_3 concentrations and phosphate or glyphosate sorption in soils. Since our results indicated no treatment differences induced by Cd and P applications on Fe_2O_3 concentrations extractable Al_2O_3, and Ca were quantified for 16 plots only (i.e., Control, 20P, 40P and 80P plots).

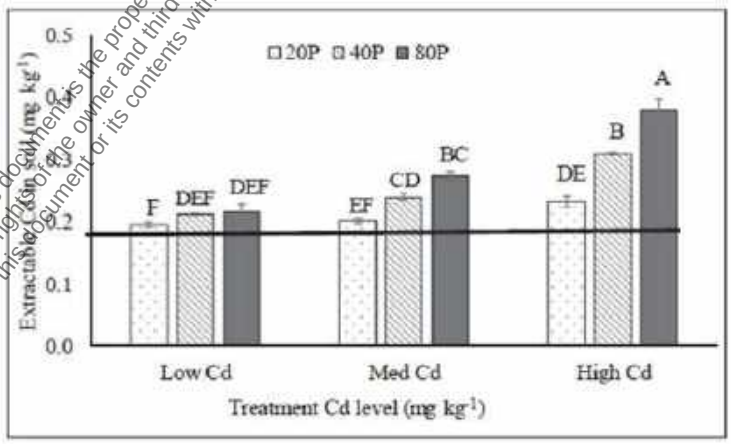
Figure 7.1.3.1.1-58: Effect of phosphate fertilizers with different Cd levels on Olsen P concentrations in soil. The solid line indicates the concentration of Olsen P in control plots



Sorption studies

Chemicals used in the sorption studies were: analytical grade ammonium phosphate monobasic (98 % chemical purity) and glyphosate (99.9 % purity), ^{14}C-labelled glyphosate [phosphonomethyl-^{14}C] (99 % radiochemical purity; specific activity 50 µCi), and Roundup Ultra 2 (49 % active). Active ingredient was potassium salt of N-(phosphonomethyl) glycine. Glyphosate sorption was determined by batch equilibrium with the initial solution containing 1 mg/L glyphosate and 6.67 x 10^4 Bq/L ^{14}C-labelled glyphosate. Batch equilibrium procedures followed the OECD guideline 106 using a soil/solution ratio of 1:5 and an equilibrium time of 24 h (OECD, 2000). Initial solution was added to soil in centrifuge Teflon tubes (duplicates) and slurries were rotated in the dark for 24 h. A constant 5 ^{0}C temperature was utilized to minimize risks for biodegradation. Equilibrium solution was centrifuged and subsamples of supernatant were added in duplicated scintillation vials containing Scintisafe scintillation cocktail. Vials were lightly shaken and stored in the dark for 24 h to disperse the chemiluminescence before the radioactivity was measured. Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method). The glyphosate sorption distribution constant, Kd (L/kg) was calculated by Cs/Ce, whereby Cs = glyphosate sorption by soil at equilibrium (mg/kg), and Ce = glyphosate concentration of equilibrium solution (mg/L). The effects of field-aged phosphate and Cd concentrations on glyphosate sorption were examined at pH conditions ranging from 3.6 to 7.3. This first experiment utilized soils from all forty plots and the range in pH was induced using different types of ions in the initial solution (0.01M HCl, 0.01M $CaCl_2$, 0.01M KCl, 0.01M KOH or dH_2O). For the control and high Cd 80P plots, the experiments were repeated but then using the Tier 2 parallel method with tubes being sampled at 1, 2, 4, 6, 8 and 24 h. The two subsequent experiments utilized soils from the plots labelled as low Cd and with 20P, 40P or 80P levels. In one experiment, for slurry pH conditions ranging from 3.6 to 7.3, batch equilibriums procedures were repeated but using Roundup Ultra 2 in 0.01 M HCl, 0.01 M $CaCl_2$, 0.01 M KCl, 0.01 M KOH or dH_2O to verify experimental results for a formulated product. In the other experiment, for slurry pH conditions range from 4.7 to 5.4, the effect of fresh phosphate additions on glyphosate sorption by soil was examined by adding analytical grade MAP to analytical glyphosate in 0.01 M $CaCl_2$, 0.01 M KCl and dH_2O solutions. The amounts of MAP added was equivalent to 11, 22 and 44 mg P/kg, or an estimated 20, 40 and 80 P kg/ha, respectively, assuming the fertilizer being present in the top 15 cm layer of a soil with a bulk density of 1200 kg/m^3.

Figure 7.1.3.1.1-59: Effect of phosphate fertilizers with different Cd levels on DTPA-extractable Cd in soil. The solid line indicates the concentration of extractable Cd in control plots



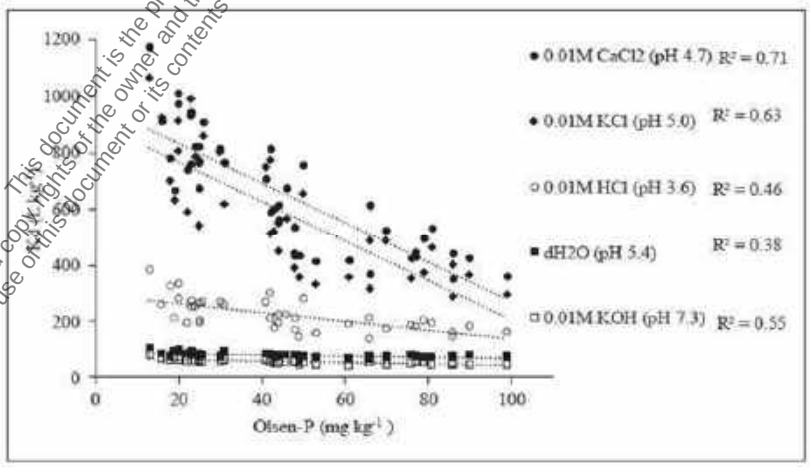
Statistical analyses were completed using SAS software version 9.3 for Windows. Two-way analysis of variance (ANOVA) and multiple means comparison (Tukey's) tests were conducted to determine the effect of phosphate fertilizer (20P, 40P, 80P) and Cd (low, medium, high) treatment on Olsen-P concentrations, extractable Cd concentrations and total Cd concentrations in soil. For each pH (ionic solution), multiple linear regression analyses were carried out to predict glyphosate Kd values by using Olsen-P and extractable Cd concentrations as independent variables. Repeated measure analysis was used to determine the effect of

shaking time (0.5, 1, 2, 4, 6, 8 and 24 h) by using phosphate levels and time as independent variables. Two way ANOVA and multiple means comparison (Tukey's) tests were utilized to quantify the effects of field-aged (20P, 40P, 80P) and fresh phosphate additions (11, 22 and 44 mg P/kg) on glyphosate Kd values. One way ANOVA and multiple means comparison (Tukey's) tests were applied to quantify the impact of using Roundup Ultra 2 versus analytical-grade glyphosate on Kd values in soils.

Results and Discussion

Glyphosate Kd values ranged from 43 to 1173 L/kg which is in agreement with glyphosate Kd values reported in agricultural soils. There were no significant differences in glyphosate sorption by soil when using either Roundup Ultra 2 or analytical-grade glyphosate, suggesting that other ingredients in the commercial formulation had no impact on the sorption behaviour of the active ingredient glyphosate in soil. The additions of MAP fertilizers from 2002 to 2009 had a significant effect on phosphate concentrations in 2013 (Figure 7.1.3.1.1-58). Olsen-P concentrations ranged from 13 to 99 mg/kg across plots and significantly decreased from 80P > 40 P > 20P plots. Total Cd concentrations in soil ranged from 0.42 to 0.98 mg/kg across plots but there were no significant treatment effects. Thus, the amount of Cd in the MAP fertilizers applied had no significant effect on the total Cd concentrations in 2013. DTPA-extractable Cd concentration ranged from 0.19 to 0.41 mg/kg, within the typical range of 0.1 - 0.5 mg/kg reported for soils (International Cadmium Association, 2015). There was a significant interaction, between the rate of phosphate fertilizer applied and the amount of Cd that the phosphate fertilizer contained, on extractable Cd concentrations in soil (Figure 7.1.3.1.1-59). For the 80P plots, extractable Cd concentrations significantly decreased in the order of high Cd > med Cd > low Cd. For the 40P plots, extractable Cd concentrations significantly decreased in the order of high Cd > (med Cd = low Cd). In 20P plots, only the high and low Cd treatments had significantly different extractable Cd concentrations. Despite these significant differences, extractable Cd concentrations in soil had no significant influence on glyphosate Kd values. The Cd concentrations in our field plots are those typically encountered in agricultural soils, but we recognize that in a batch equilibrium experiment, Zhou *et al.* (2004) demonstrated that the co-application of exceptionally large quantities of Cd to glyphosate solutions (i.e., 562 mg Cd/kg soil) can increase glyphosate sorption by approximately 1.6 times fold, relative to control soil. Increased Olsen-P concentrations in soil was a significant factor ($P < 0.0001$) in the regression analysis to explain reduced glyphosate Kd values in soil.

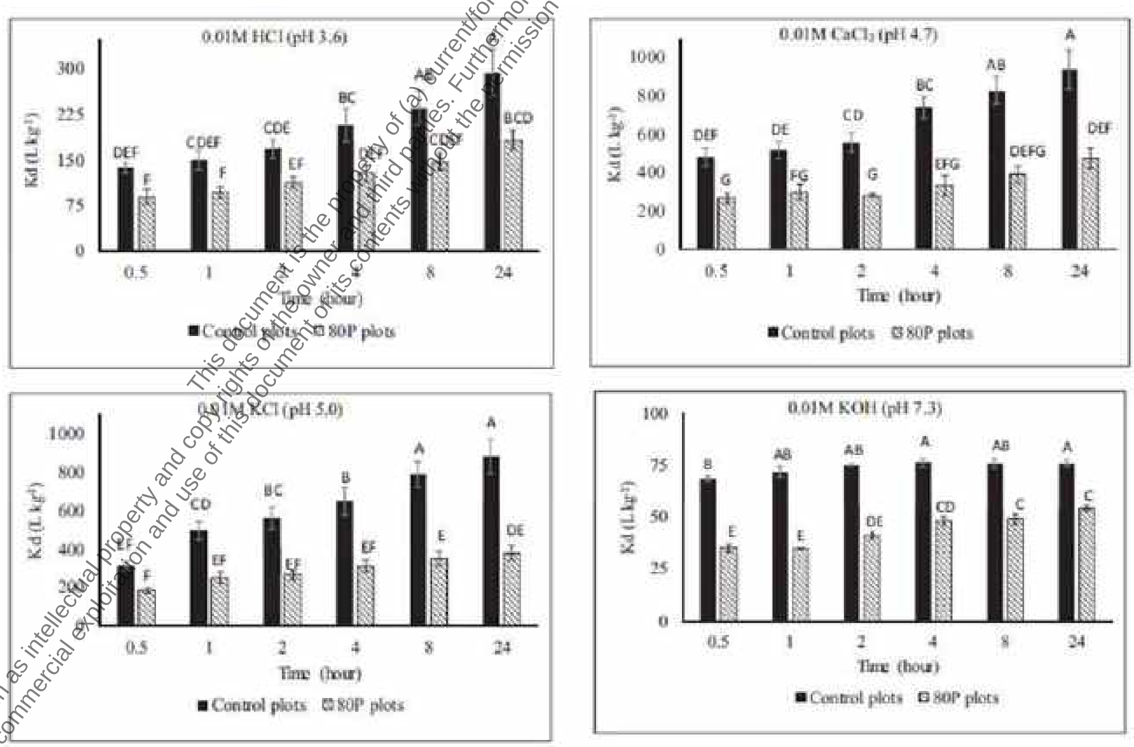
Figure 7.1.3.1.1-60: Relation between Olsen-P concentrations in soil and the glyphosate sorption distribution constant, Kd, with soil slurries being under different pH conditions. All regression equations are significant at P < 0.0001



Regardless of the ionic solution used in the batch equilibrium experiments, increased Olsen P concentrations significantly decreased glyphosate sorption by soil (Figure 7.1.3.1.1-60). A maximum reduction in glyphosate sorption occurred at a pH of 5 (0.01 M KCl solution) when the Olsen-P concentrations was on average 89 mg/kg Olsen P and the glyphosate Kd value was reduced by 57 %, relative

to the control plots that contained on average 18.75 mg/kg Olsen-P (Figure 7.1.3.1.1-60). Our results are in agreement with the findings of de Jonge *et al.* (2001) who also reported that field-aged phosphate in soil reduces glyphosate sorption by soil. The iron oxides content of the Orthic Black Chernozem used is within the range of that observed in other Prairie soils in Canada suggesting the competitive effect of phosphate on glyphosate sorption could be applicable to a wider range of soils in the Prairie region of Canada particularly with low pH and high Fe content. At pH 5.4, in both 80P and control, time had no significant effect on glyphosate Kd values and sorption was always significantly smaller in 80P than control plots. For all other pH conditions, glyphosate sorption approached equilibrium at approximately 8 h because there were no significant differences in glyphosate Kd values between 8 and 24 h (Figure 7.1.3.1.1-61). For these pH conditions, glyphosate sorption was almost always significantly smaller in 80P than control plots, regardless of the time, except for 0.5, 1 and 2 h under pH 3.6 and 0.5 h under pH 5.0 (Figure 7.1.3.1.1-61). In general, longer shaking hours resulted in greater numerically differences in glyphosate Kd values between control and 80P plots. Regardless of the ionic solution used (Figure 7.1.3.1.1-62), there was a significant interaction ($P < 0.01$) between field-aged and fresh phosphate on glyphosate sorption. In general, regardless of the amount of aged phosphate in soil, the addition of fresh MAP to the ionic solutions numerically reduced glyphosate Kd values, suggesting that phosphate and glyphosate compete for the same sorption sites in soil and that phosphate is preferentially sorbed when added with glyphosate to soil. Additions of 11 mg P/kg to the 0.01 M $CaCl_2$ solutions had no significant effect on glyphosate Kd values, except in the 20 P plots containing relatively small Olsen-P concentrations (Figure 7.1.3.1.1-62). The addition of 22 or 44 mg P/kg to the 0.01 M $CaCl_2$ solutions always significantly reduced glyphosate Kd values, except the addition of 22 mg P/kg to 80 P plots (Figure 7.1.3.1.1-62). For the largest co-application (44 mg P/kg), glyphosate Kd values were reduced on average by 52 % in 20P plots, but by only 37 % in the 80P plots. Additions of 11, 22 or 44 mg P/kg to 0.01 M KCl solutions always significantly reduced glyphosate Kd values except for 80 P plots for which only the addition of 44 mg P/kg resulted in a significant reduction in glyphosate Kd values (Figure 7.1.3.1.1-62).

Figure 7.1.3.1.1-61: Time dependent sorption study of glyphosate Kd values in control and 80P plots

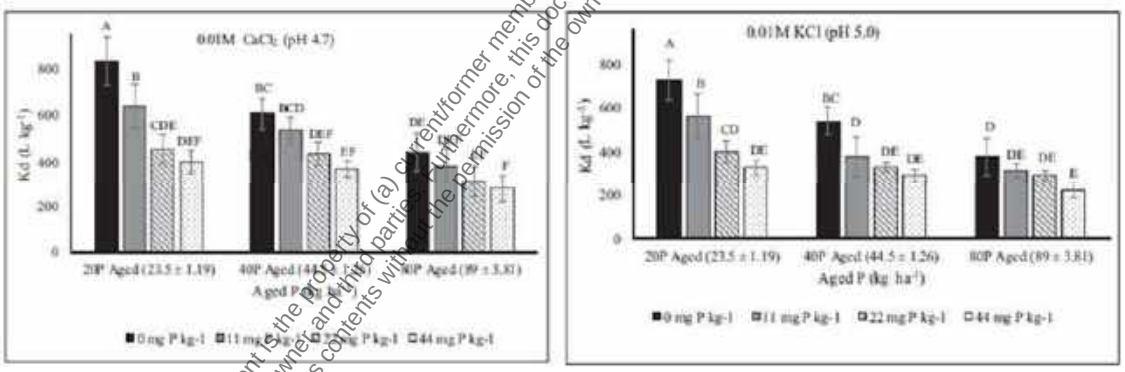


For the 44 mg P/kg co-application, glyphosate Kd values were reduced on average by 54 % in 20P plots, but by 42 % in the 80P plots. Thus, the largest impact of fresh MAP applications on reducing sorption sites for glyphosate occurred in soils with smaller field-aged phosphate concentrations because more sorption sites were available for competition in the plots that had low field-aged phosphate concentrations. In general, glyphosate Kd values were largest at pH 4.7 (0.01 M $CaCl_2$) when glyphosate molecules mainly exist as H_2G^- (~85 %) and HG^{2-} (~15 %), and at pH 5.0 (0.01M KCl) when glyphosate molecules mainly exist as H_2G^- (~75 %) and HG^{2-} (~25 %). The soil used in this study had already a relatively large Ca^{2+} content (2252 ± 40.57 mg/kg), and using 0.01 M KCl, would allow K^+ to replace Ca^{2+} on the exchange site of organic-clay complexes which may interact with glyphosate forming stable complexes. Glyphosate Kd values were greater at pH 3.6 (0.01 M HCl), than pH 5.4 (dH_2O) (Figure 7.1.3.1.1-60). At pH 3.6, a greater amount of soil colloids is net positively-charged, promoting the sorption of glyphosate molecules that mainly exist as H_2G^- (~95 %) and H_3G (~5 %). Sorption was less at pH 5.4 than at pH 3.6 because the amount of negatively- charged soil colloids increases with soil pH, and glyphosate molecules mainly exist as $H2G^-$ (~60 %) and $HG2^-$ (~40 %) at pH 5.4. The lowest sorption was observed at pH 7.3 (0.01 M KOH), as the negatively charged soil colloids increased and glyphosate molecules existed as $HG2^-$ (~100 %).

Conclusion

Analytical-grade glyphosate showed similar results as a commercially-available glyphosate formulation. Long-term additions of phosphate fertilizers to soils will reduce the capacity of the soil to bind glyphosate under a wide range of pH conditions, but the impurities of Cd in these fertilizers have no impact on glyphosate sorption.

Figure 7.1.3.1.1-62: Effect of co-applying mono ammonium phosphate with glyphosate in solution, for batch equilibrium experiments using 0.01 M $CaCl_2$ (pH 4.7) and 0.01 M KCl (pH 5.0)



Fresh applications of phosphate fertilizers to most soils will significantly reduce the availability of sorption sites for glyphosate. However, this reduction in sorption site availability will be small in soils that have exceptionally high phosphate levels and do not have many sorption sites available for phosphate or glyphosate. Cd concentrations typically found in agricultural fields are not high enough to influence the binding capacity of glyphosate in soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a sorption experiment with glyphosate on a Canadian agricultural soil considering different treatments with phosphate fertilizer. Some information on soil and study design are not reported (i.e. soil characteristics, mass balances, amount of soil, no information on chromatographic methods used, stability of test item not demonstrated), so no final validity check is possible.

The article is therefore classified as reliable with restrictions. Therfore, data were not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/023
Report author	Sidoli, P. *et al.*
Report year	2016
Report title	Glyphosate and AMPA adsorption in soils: laboratory experiments and pedotransfer rules
Document No	DOI 10.1007/s11356-015-5796-5 E-ISSN 1614-7499
Guidelines followed in study	OECD Guideline 106 (2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) Lack of information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Adsorption of the herbicide glyphosate and its main metabolite AMPA (aminomethylphosphonic acid) was investigated on 17 different agricultural soils. Batch equilibration adsorption data are shown by Freundlich adsorption isotherms. Glyphosate adsorption is clearly affected by equilibration concentrations, but the nonlinear AMPA adsorption isotherms indicate saturation of the adsorption sites with increasing equilibrium concentrations. pH_{CaCl2} (i.e. experimental pH) is the major parameter governing glyphosate and AMPA adsorption in soils. However, considering pH_{CaCl2} values, available phosphate amount, and amorphous iron and aluminium oxide contents by using a nonlinear multiple regression equation, obtains the most accurate and powerful pedotransfer rule for predicting the adsorption constants for these two molecules. As amorphous iron and aluminium oxide contents in soil are not systematically determined, we also propose a pedotransfer rule with two variables—pH_{CaCl2} values and available phosphate amount—that remains acceptable for both molecules. Moreover, the use of the commonly measured pH_{water} or pH_{KCl} values gives less accurate results compared to pH_{CaCl2} measurements. To our knowledge, this study is the first AMPA adsorption characterization for a significant number of temperate climate soils.

Materials and Methods
Soil properties
Seventeen surface top soils were sampled in different agricultural plots with variable land uses and fertilization practices under intensive agriculture. The sample site is located in a quaternary fluvio-glacial corridor near Lyon in southeastern France. They are loamy to sandy-loamy soils, characterized by a

decarbonation state on surface and large amounts of amorphous iron and aluminium oxides issued from the weathering of primary minerals (Table 7.1.3.1.1-67). Fresh soil samples were air-dried, sieved to 2 mm, and stored in the dark at 4 °C, before measuring their physicochemical properties. Crystallized oxy-hydroxides (Fe_{DCB} and Al_{DCB}) were extracted by the Mehra-Jackson method (1960), and amorphous oxy-hydroxides (Fe_{ox} and Al_{ox}) by the Tamm method (1992) (Table 7.1.3.1.1-67). The experimental 1:5 soil pH_{CaCl2}, hereafter referred to as 'pH_{CaCl2}', was measured in batch supernatants with a pH microelectrode (Inlab Flex-Micro). These soils showed wide ranges of pH_{CaCl2} (5.1 to 7) and clay content (8.9 to 15.3 %) and, except soil 11, contained less than 2 % organic carbon (Table 7.1.3.1.1-67).

Chemical reagents and analysis
Glyphosate adsorption was studied with its ^{14}C-radiolabeled form (phosphonomethyl-^{14}C)-glyphosate (4.36 MBq/mg, radiochemical purity 96.32 %) purchased from Izotop (Hungary). Unlabeled solid glyphosate and AMPA products (purity ≥98 %) were purchased from Dr Ehrenstorfer (CIL Cluzeau, Sainte-Foy la Grande, France). Stock solutions were prepared in MilliQ water (storage at 4 °C for 1 month).

Table 7.1.3.1.1-67: Physicochemical properties of studied soils. Crystallized oxy-hydroxides (Fe_{DCB} and Al_{DCB}) and amorphous oxy-hydroxides (Fe_{OX} and Al_{OX}) were extracted by the Mehra-Jackson method (1960) and the Tamm method, respectively

Soil	pH_{CaCl2}	pH_{water}	pH_{KCl}	Organic C (g kg^{-1})	Olsen P (g kg^{-1})	Al_{DCB} (g kg^{-1})	Fe_{DCB} (g kg^{-1})	Al_{ox} (g kg^{-1})	Fe_{ox} (g kg^{-1})	Clay %	Silt %	Sand %	CEC (meq 100 g^{-1})
1	6.8	7.7	6.8	14.1	0.10	2.1	9.8	1.7	3.1	12.3	36.4	49.1	7.6
2	5.1	6.1	4.9	14.0	0.05	11.1	2.7	2.2	2.7	15	37.3	45.5	8.3
3	5.8	6.5	5.7	13.0	0.10	2.4	10.9	2.0	2.9	15.1	39.1	43.6	8.2
4	5.9	6.9	6.0	13.6	0.07	2.6	11.2	1.9	2.8	14.9	38.1	45	8.1
5	5.9	7.5	6.6	16.0	0.09	2.5	12.8	2.0	2.7	15.3	41.5	40.8	8.2
6	5.8	7.0	6.1	14.7	0.07	2.4	11.5	1.9	2.7	15	40.3	42.5	8.3
7	5.9	6.9	6.1	13.4	0.08	2.2	11.5	1.8	2.6	15	39	44.2	8.4
8	6.1	7.2	6.3	8.3	0.11	1.8	8.8	1.5	2.6	11.4	34.3	52.8	6.0
9	6.2	7.3	6.5	14.8	0.06	2.5	13.5	2.1	3.2	15.4	36.3	45.7	8.2
10	5.5	6.7	5.8	17.3	0.06	2.5	11.5	2.0	2.9	14.8	35.6	47.3	7.7
11	7.0	8.0	7.2	23.1	0.20	1.5	8.6	1.2	3.0	11.8	42.3	42.8	9.6
12	6.1	7.0	6.1	16.1	0.08	2.1	11.8	1.9	3.0	13.9	34.1	49.5	8.2
13	5.1	6.3	5.0	12.9	0.04	10.6	2.1	1.8	2.8	14.6	40	43.8	6.6
14	6.2	7.3	6.5	9.2	0.08	1.6	6.8	1.3	2.2	8.9	31.5	58.1	5.0
15	6.3	7.4	6.3	9.1	0.12	1.6	6.9	1.4	2.1	8.9	29.5	60.4	5.6
16	5.4	6.5	5.6	7.2	0.11	1.8	7.7	1.5	2.5	9.7	30.6	58.3	4.4
17	5.9	6.7	5.9	15.0	0.14	2.0	10.4	1.7	2.7	12.7	32.4	52.7	7.6
Mean	5.9	7.0	6.1	13.6	0.09	3.1	9.3	1.8	2.7	13.2	36.4	48.4	7.4
Standard deviation	0.5	0.5	0.5	3.7	0.04	2.8	3.2	0.3	0.3	2.2	3.7	5.9	1.4
Min	5.1	6.1	4.9	7.2	0.04	1.5	2.1	1.2	2.1	8.9	29.5	40.8	4.4
Max	7.0	8.0	7.2	23.1	0.20	11.1	13.5	2.2	3.2	15.4	42.3	60.4	9.6

The glyphosate concentration was obtained by measuring ^{14}C-glyphosate activity, which was counted with a liquid scintillation analyzer (Packard Tricarb® 2300TR). After adding a scintillator (Aquasafe 300 Plus, Zinsser Analytic), the radioactivity was measured in 2 mL of supernatant. The minimal measured ^{14}C-glyphosate radioactivity is 30 dpm/mL which corresponds to 0.09 µg/L.
AMPA analysis was done on an Acquity ultra-performance liquid chromatography system (UPLCTM, Waters) interfaced to a triple quadrupole mass spectrometer (Quattro Premier XE, Waters). Due to its low molecular weight, a derivatization step with FMOC-chloride in the presence of a borate buffer is required prior to analysis. Extraction is done online with an SPE cartouche (Oasis HLB 25 µm 2.1×20 mm) before separation in an Acquity UPLC HSS column (T3 1.8 µm×2.1 mm×100 mm). The quantification limit is 0.05 µg/L.

Isotherm adsorption coefficients (K_f)

Sorption experiments were run according to a normalized method (OECD guideline 106, 2000) with a 1/5 soil-weight/solution-volume ratio in 15-mL centrifuge plastic tube. Equilibrium - tested with a 1 mg/L solution - was obtained after 24 h. After 12 h of pre-equilibration with a $CaCl_2$ solution (0.01 M), the equilibrated soil suspensions were spiked with a pesticide solution and agitated during 24 h (darkness, 20 °C). After centrifugation (3000 rpm, 30 min, 20 °C), the supernatants were filtrated with 0.2 µm cellulose acetate and analyzed for pesticide concentrations. Blanks (each soil without spiking) did not reveal any presence of either molecule in the soils before the experiments. No adsorption was measured on tubes and filters used for batch experiments. The adsorption isotherm was obtained by the relationship between adsorbed concentration per weight (Cs, mg/kg) compared to the equilibrium concentration per volume of solution (Ce, mg/L) according to the Freundlich equation. Six solute concentrations were tested, 0.05, 0.2, 0.5, 1.0, 3.0 and 5.0 mg/L, for both glyphosate and AMPA. For glyphosate, which was studied with its ^{14}C radiolabeled form, the initial radioactivity was 6000 dpm/mL in tubes. The experiments were run as triplicates. The Freundlich parameters Kf and $1/n_f$ were estimated by using a nonlinear fitting programme (XLStat, Excel 5.0).

Parametric linear and nonlinear regression for pedotransfer rule determination
The relationship between the K_f parameter and soil properties was studied for each pesticide (XLStat, Excel 5.0) by multiple linear and nonlinear regression analyses.

Results and discussion
Freundlich adsorption isotherms
The Freundlich isotherm equation adjusts accurately the experimental data (R^2>0.99). High experimental glyphosate K_f values, $K_{f\text{-}exp}$, were obtained, ranging between 32 and 540 mg/kg(L/mg)$^{-nf}$ (Table **7.1.3.1.1-68**) in agreement with previous studies. In the case of AMPA, $K_{f\text{-}exp}$ values between 33 and 392 mg/kg (L/mg)$^{-nf}$ (Table 7.1.3.1.1-68) are in the same high adsorption range as glyphosate.

Table 7.1.3.1.1-68: Experimental Freundlich isotherm coefficients $K_{f\text{-}exp}$ (mg/kg (L mg $^{-1}$)$^{-nf}$) and $1/n_{f\text{-}exp}$ (-) for glyphosate and AMPA, and K_f recalculated for averaged $1/n_{f\text{-}exp}$ glyphosate ($1/n_{f\text{-}avg}$ = 0.93) and AMPA ($1/n_{f\text{-}avg}$ = 0.78)

Soils	Glyphosate				AMPA				
	$K_{f\text{-}exp}$	$1/n_{f\text{-}exp}$	R^2	K_f (for $1/n_{f\text{-}avg}$)	R^2	$K_{f\text{-}exp}$	$1/n_{f\text{-}exp}$	R^2	K_f (for $1/n_{f\text{-}avg}$)	R^2
1	34	0.86	1.00	36	1.00	63	0.74	1.00	67	1.00
2	540	0.97	0.99	475	1.00	392	0.80	1.00	365	1.00
3	143	0.98	1.00	143	1.00	140	0.72	1.00	162	1.00
4	92	1.09	1.00	73	0.99	212	0.79	1.00	206	1.00
5	135	0.92	0.99	139	1.00	226	0.81	1.00	208	1.00
6	132	0.98	0.99	140	0.99	191	0.80	1.00	183	1.00
7	100	0.90	1.00	106	0.99	143	0.75	1.00	154	1.00
8	99	0.94	1.00	97	1.00	119	0.81	1.00	111	1.00
9	103	0.91	1.00	110	1.00	158	0.81	0.99	147	0.99
10	235	0.94	1.00	230	1.00	242	0.77	1.00	253	1.00
11	32	0.83	1.00	34	1.00	33	0.78	1.00	33	1.00
12	91	0.94	1.00	89	1.00	134	0.82	1.00	122	1.00
13	285	0.99	0.99	243	0.99	284	0.79	1.00	210	1.00
14	42	0.98	1.00	40	1.00	91	0.80	1.00	87	1.00
15	41	0.88	1.00	43	1.00	56	0.73	1.00	61	1.00
16	233	0.94	1.00	229	1.00	198	0.78	1.00	198	1.00
17	111	0.97	1.00	103	1.00	107	0.82	1.00	99	1.00
Min	32	0.83		34		33	0.72		33	
Max	540	1.09		475		392	0.82		365	
Mean	144	0.93		137		164	0.78		157	
Standard deviation	121	0.06		106		88	0.03		79	

Table 7.1.3.1.1-69: Glyphosate and AMPA K_f coefficients calculated by multiple nonlinear regression from Eq. (2): $Kf = C\, e^{\sum_{i=1}^{n} a_i X_i}$

Soil variables X_n	Constant	pH measurements			Olsen P (g kg^{-1})	Al_{ox} (g kg^{-1})	Fe_{ox} (g kg^{-1})	R^2
		1:5 soil pH_{CaCl2}	pH_{KCl}	pH_{water}				
Regression coefficient values	C	a_1	a'_1	a''_1	a_2	a_3	a_4	
4 variables (pH, Olsen P, Al_{ox}, Fe_{ox})								
Glyphosate	**5.1×10^{5}**	**−1.7**			**−1.1**	**−0.4**	**1.6**	**0.94**
	3.6×10^{5}		−1.25		−5.6	−0.1	0.1	0.65
	1.3×10^{6}			−1.30	−7.3	−0.4	0.3	0.61
AMPA	**1.8×10^{4}**	**−0.9**			**−4.4**	**0.6**	**−0.1**	**0.92**
	3.6×10^{3}		−0.50		−5.4	1.3	−0.7	0.81
	3.8×10^{3}			−0.40	−5.4	1.2	−0.8	0.78
2 variables (pH, Olsen P)								
Glyphosate	1.9×10^{6}	−1.6			−2.6			0.88
	3.5×10^{5}		−1.24		−5.3			0.65
	6.7×10^{5}			−1.17	−6.0			0.60
AMPA	6.1×10^{4}	−0.9			−7.7			0.88
	2.1×10^{4}		−0.7		−9.6			0.73
	3.2×10^{4}			−0.7	−9.8			0.70
1 variable (pH)								
Glyphosate	1.33×10^{6}	−1.59						0.82
	2.23×10^{5}		−1.24					0.61
	2.74×10^{5}			−1.12				0.51
AMPA	4.67×10^{4}	−0.97						0.69
	1.18×10^{4}		−0.63					0.37
	1.18×10^{4}			−0.63				0.37

For both molecules, the pedotransfer rule including four variables and the simplified rule including two or one variable(s) are calculated for pH_{CaCl2}, pH_{KCl} or pH_{water}. Value parameters corresponding to the pedotransfer rule including the four pH_{CaCl2}, Olsen P, Al_{ox} and Fe_{ox} variables are indicated in bold

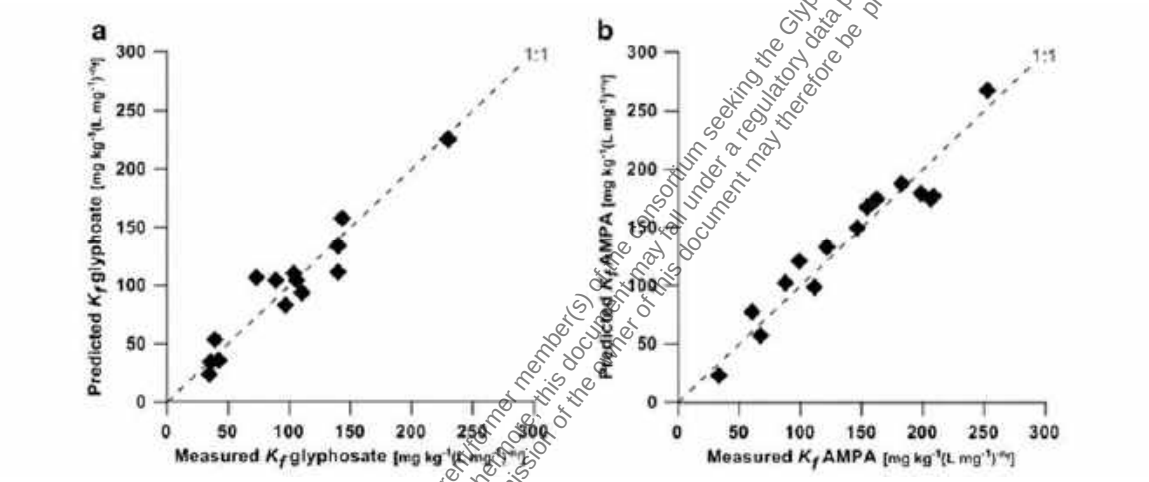
These values are consistent with those obtained by Baez *et al.* (2015) for molisols and alfisols. Experimental values of the $1/n_{f\text{-}exp}$ coefficients vary between 0.83 and 1.09 for glyphosate, and between 0.72 and 0.82 for AMPA. As the $1/n_{f\text{-}exp}$ values are different, the glyphosate and AMPA $K_{f\text{-}exp}$ datasets cannot be compared directly. Indeed, even if the $K_{f\text{-}exp}$ value is similar, the isotherm can be very different because of the $1/n_{f\text{-}exp}$ value. For each molecule, the $1/n_{f\text{-}exp}$ values had a low standard deviation, allowing to calculate an average $1/n_{f\text{-}exp}$ value, i.e. $1/n_{f\text{-}avg}$, of 0.93 (±0.06) and 0.78 (±0.03) for glyphosate and AMPA, respectively (Table **7.1.3.1.1-68**). New K_f Freundlich coefficients were recalculated for each soil by using this averaged $1/n_{f\text{-}avg}$ value (Table 7.1.3.1.1-68). For both molecules, this second fit to the Freundlich equation is very precise ($R^2 \geq 0.99$) and allows the comparison between soils. For AMPA, the $1/n_{f\text{-}avg}$ value of less than one (i.e. 0.78) indicates that adsorption is strongly limited by the availability of sorption sites. However, the high glyphosate $1/n_{favg}$ value of 0.93 means that adsorption is less governed by the availability of adsorption sites than AMPA. Therefore, despite the similar atomic composition of glyphosate and AMPA, they probably do not sorb in the same way onto the studied soils.

Pedotransfer rule for glyphosate and AMPA adsorption prediction

Regression analysis was restricted to soils with a higher experimental pH_{CaCl2} than both glyphosate pKa_3 and AMPA pKa_2, i.e. pH_{CaCl2}>5.4. This limitation allowed defining adsorption rule when the same ionic form of either glyphosate or AMPA dominates in solution. Thus, soils 2 and 13 (pH_{CaCl2} values=5.1) were excluded from the data analysis. First, a linear multiple regression was tested to relate K_f to every combination of measured soil properties, but the adjustment accuracy was very weak (R^2<0.75). In our study, nonlinear consideration sharply improves the fit of both glyphosate and AMPA K_f (R^2>0.92). Of the ten variables studied, nonlinear regression analysis appears optimized when considering the four variables: pH_{CaCl2} value, available phosphate, and amorphous aluminium and iron oxide amount (Table 7.1.3.1.1-68) and Figure 7.1.3.1.1-63), for both glyphosate and AMPA K_f adsorption coefficients. As in earlier studies, the highest correlation was found between glyphosate K_f and pH - in our study pH_{CaCl2} - (Table 7.1.3.1.1-70).

For the pH_{CaCl2} here studied (between 5.4 and 7.0), deprotonation of the phosphonic group results in the dominant glyphosate net-2^- (2^-) and dominant AMPA net-1^- charged (1^-) forms (Figure 7.1.3.1.1-64). For soils with high pH values, high repulsion forces with negative charges act on the amorphous oxide surfaces and sorption is reduced. In the regression analysis, both glyphosate and AMPA K_f values positively correlate with amorphous iron and aluminium oxides (Table 7.1.3.1.1-70), with higher correlations calculated for amorphous aluminium oxides that probably are more reactive in the studied soils. A negative correlation between available phosphate and glyphosate K_f values is observed (Table 7.1.3.1.1-70) where both molecules compete for the same adsorption sites on oxide surfaces, thus reducing glyphosate adsorption in the presence of phosphate (Gimsing *et al.* 2004b).

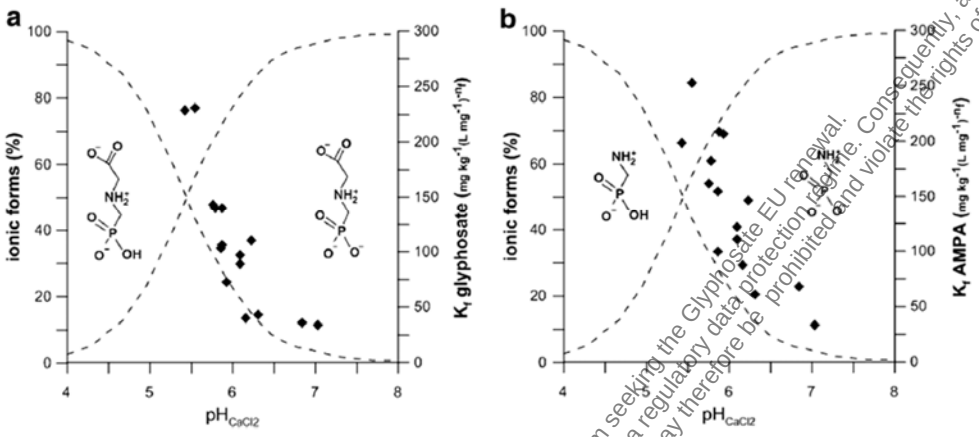
Figure 7.1.3.1.1-63: Comparison between measured and predicted Kf coefficients with a nonlinear pedotransfer rule for a glyphosate (with $l/n_{f\text{-}avg}$ = 0.93) and b AMPA (with $l/n_{f\text{-}avg}$ = 0.78). The *dotted lines* represent a 1 to 1 straight line. Glyphosate and AMPA pedotransfer rules include pH $_{CaCl2}$, Olsen P, Al_X and Fe_{OX} variables



A negative correlation between AMPA K_f values and available phosphate suggests a similar competition for adsorption on oxide surface sites by reducing AMPA sorption when phosphate is present. The more strongly negative correlation between phosphate and K_f values for AMPA than for glyphosate (Table **7.1.3.1.1-70**) indicates a higher competitive adsorption between phosphate and AMPA than glyphosate.

To evaluate the sensitivity of the variables affecting the pedotransfer rule, the number of variables was initially reduced to two dominant parameters, i.e. pH_{CaCl2} and available phosphate amount. The resulting equation explains 88 % of the variations in the K_f of glyphosate and AMPA (see R^2, Table 7.1.3.1.1-68). Considering only variable pH_{CaCl2} - the most significant of all four variables – decreases the accuracy adjustment for AMPA (R^2 0.69, Table 7.1.3.1.1-68), whereas that for glyphosate is only slightly modified (R^2 0.87, Table 7.1.3.1.1-68). Thus, it seems possible to arrive at an acceptable estimate of glyphosate adsorption with an equation with just one variable, i.e. pH_{CaCl2}, whereas for AMPA, the two variables pH_{CaCl2} and available phosphate amount are needed.

Figure 7.1.3.1.1-64: Distribution of Freundlich coefficients K_f as a function of pH_{CaCl2} and dominance of dissociated glyphosate forms (a) and dissociated AMPA forms (b) in solution. Bjerrum diagram taken from (Sheals *et al.* 2002) for glyphosate (a) and same diagram suggested as hypothesis for AMPA (b). Glyphosate pKa_3 = 5.46 (Tomlin 1997), AMPA pKa_3 = 5.4 (Chen *et al.* 2009)



Constraints in applying pedotransfer adsorption equations

To verify the accuracy of the proposed sorption multiple regression, as Paradelo *et al.* 2015 showed that this might be dependent upon the study site, we collected published data concerning pedotransfer rules for testing them in our model. To our knowledge, AMPA adsorption instead of glyphosate sorption is rarely described in the literature. Nevertheless, none of the published work describes all four variables - pH_{CaCl2}, available phosphate, and amorphous iron and aluminium contents - for several soils. We thus carried out an in-depth study on the effect of the pH-measuring method on predicting the glyphosate K_f (Table **7.1.3.1.1-68**). The parameters for the adsorption equations with four and two variables, or even one variable, were recalculated for pH_{water} or pH_{KCl} values, as these are more commonly measured parameters than experimental pH_{CaCl2}. We then did the same work for AMPA equations as a comparison. The choice of pH clearly affected the accuracy of an equation with four variables, as R^2 varied from 0.94 with pH_{CaCl2} to ≤0.65 with pH_{water} and pH_{KCl} for glyphosate, and from 0.92 to ≤0.81 for AMPA. This decrease in the adjustment accuracy was obviously also noted for regressions with two variables - R^2 going from 0.88 to ≤0.65 for glyphosate and from 0.88 to ≤0.73 for AMPA—and one variable (R^2 going from 0.88 to ≤0.61 for glyphosate and from 0.69 to ≤0.37 for AMPA). Glyphosate K_f coefficients are much more affected by the pH measurement method than those of AMPA, but the pH variable in exponential glyphosate equations is systematically associated with higher correlation coefficients than in the AMPA ones (Table **7.1.3.1.1-70**). These results clearly show that the type of pH measurement plays a crucial role for the prediction of glyphosate and AMPA adsorption coefficients. Since no simple relationship can be established between experimental pH_{CaCl2} and pH_{water} (R^2 0.76) or pH_{KCl} (R^2 0.80), a model validation for glyphosate cannot be based on available published data.

Table 7.1.3.1.1-70: Correlation matrix of Kf glyphosate ($1/n_{f\text{-}avg}$ = 0.93) and K_f AMPA ($1/n_{f\text{-}avg}$ = 0.78) with soil parameters by multiple nonlinear regression

Correlation matrix of K_f glyphosate ($1/n_{f\text{-}avg}$=0.93) and K_f AMPA ($1/n_{f\text{-}avg}$ =0.78) with soil parameters by multiple nonlinear regression

Variables	pH_{CaCl2}	Olsen P (%)	Al_{ox} (%)	Fe_{ox} (%)
$K_{f\ glyphosate}$	−0.83	−0.36	0.43	0.05
$K_{f\ AMPA}$	−0.82	−0.67	0.68	0.10

Conclusions

High adsorption coefficients calculated for glyphosate and AMPA molecules depend upon the experimental pH_{CaCl2} value (1:5 soil/solution), the available phosphate content, and the amorphous aluminium- and iron oxide contents. These four key soil parameters combined in an exponential regression equation provide a precise description of a pedotransfer rule for K_f prediction. To our knowledge, our AMPA dataset contains the first published data for adsorption on a significant number of natural soils. Because of low $1/n_f$ values, prediction of the AMPA K_f is strongly related to the soil-solution concentration, contrary to glyphosate. Changes in pH strongly affect adsorption by modifying the ionic state of glyphosate and AMPA and the available amorphous oxide surface sorption sites. Phosphate competes with both molecules for adsorption, but more strongly with AMPA. Considering only the two variables, pH_{CaCl2} and available phosphate content, leads to a satisfactory prediction of the adsorption constants. For both molecules, pH_{CaCl2} is the most reliable explanatory variable. However, pH_{CaCl2} is only rarely measured during batch experiments, even though most of such experiments use $CaCl_2$ as the solute. Replacing pH_{CaCl2} by pH_{KCl} or by pH_{water} - that are more frequently measured in soils - as the variable in the pedotransfer rule does not allow adjusting K_f with sufficient precision. Since simple relation does not exist between pH_{KCl} and pH_{CaCl2} - or between pH_{water} and pH_{CaCl2} - a complementary soil characterization with pH_{CaCl2} value therefore appears necessary for the application of the pedotransfer rule. However, such a measurement is easier and faster than the implementation of sorption experiments. The acquisition of supplementary data on various soils will lead to a better validation of the pedotransfer rules for glyphosate and AMPA. Finally, the strong adsorption observed in the studied soils, which are rather depleted in organic carbon, takes place on mineral fractions. Hence, the—little studied—geological materials present in the unsaturated zone might also strongly adsorb glyphosate and AMPA. The adsorption of these molecules on such materials should thus be studied as well.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes batch adsorption experiments with glyphosate and AMPA on 17 soils from France. The OECD 106 guideline was considered. However, not all parameters were reported to check the validity of the study (i.e. no material and mass balances established, stability of test item not reported, chromatographic method for analysis of glyphosate not reported).

The article is therefore classified as reliable with restrictions and was not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/024
Report author	Dollinger, J. *et al.*
Report year	2015
Report title	Glyphosate sorption to soils and sediments predicted by pedotransfer functions
Document No	DOI 10.1007/s10311-015-0515-5 ISSN 1610-3653
Guidelines followed in study	None
Deviations from current test guideline	Not relevant, modelling study with no experimental data determined
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Glyphosate is the most applied herbicide for weed control in agriculture worldwide. Excessive application of glyphosate induces water pollution. The transfer of glyphosate to freshwater and groundwater is largely controlled by glyphosate sorption to soils and sediments. Sorption coefficients are therefore the most sensitive parameters in models used for risk assessment. However, the variations in glyphosate sorption among soils and sediments are poorly understood. Here we review glyphosate sorption parameters and their variation with selected soils and sediment. We use this knowledge to build pedotransfer functions that allow predicting sorption parameters, Kd, Kf and n, for a wide range of soils and sediments. We gathered glyphosate sorption parameters, 101 Kf, n and equivalent Kd, and associated soil properties. These data were then used to perform stepwise multiple regression analyses to build the pedotransfer functions. The linear (Kd) and Freundlich (Kf, n) pedotransfer functions were bench marked against experimental data. We found the following major points: (1). Under current environmental conditions, sorption is best predicted by the Kd pedotransfer function. (2) The pedotransfer function is Kd = 7.20*CEC – 1.31 *Clay + 24.82 (Kd in L/kg, CEC in cmol/kg and clay in %). (3) Cation exchange capacity (CEC) and clay content are the main drivers of Kd variability across soils and sediments. Freundlich parameters are additionally influenced by pH and organic carbon. This suggests that the formation of complexes between glyphosate phosphonate groups and soil–exchanged polyvalent cations dominates sorption across the range of analyzed soils.

Materials and Methods

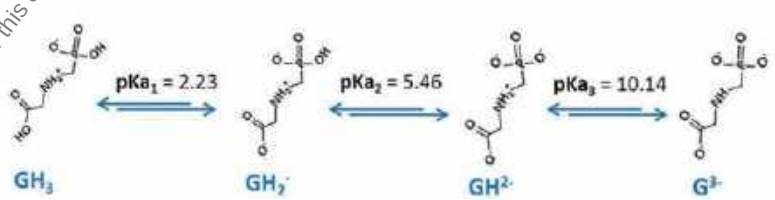
Physical and chemical properties of glyphosate

Glyphosate [N–(Phosphonomethyl)glycine] is a weak acid with strong hydrophilicity and very high water solubility (Table 7.1.3.1.1-71). Speciation of this zwitterionic molecule varies with the pH of the surrounding environment (Figure 7.1.3.1.1-65). The main species within the soil pH range are GH_2^- and GH^{2-}, corresponding to net negative charges of one and two, respectively (Figure 7.1.3.1.1-65).

Table 7.1.3.1.1-71: Physicochemical properties of glyphosate

Properties		References
Formula	$C_3H_8NO_5P$	ANSES (2015), FOOTPRINT (2015)
Molecular mass (g mol^{-1})	169.1	ANSES (2015), FOOTPRINT (2015)
Aqueous solubility at 20 °C (g L^{-1})	10.5 to 12.0	ANSES (2015), FOOTPRINT (2015)
Log Kow at pH 7	−4.1 to −3.2	ANSES (2015), FOOTPRINT (2015)
pKa_1–pKa_2–pKa_3	2.2–5.5–10.2	ANSES (2015), FOOTPRINT (2015)
Vapor pressure at 25 °C (mPa)	1.31×10^{-2}	ANSES (2015), FOOTPRINT (2015)
Henry's law constant at 25 °C (Pa m^3 mol^{-1})	2.10×10^{-7}	ANSES (2015), FOOTPRINT (2015)

Figure 7.1.3.1.1-65: Speciation of glyphosate through the entire soil pH range from Albers *et al.* (2009), Borggaard (2011) and Maqueda *et al.* (1998)



Data mining

We extensively reviewed the literature to assemble a database of observed glyphosate sorption coefficients to both soils and sediments and the associated substrate properties (Table 7.1.3.1.1-72). We found 23 studies reporting sorption parameters for one or more soils or sediments. The soils or sediments for which glyphosate sorption measurements were carried out originated from four continents (Europe, Asia and North and South America) and exhibited highly varied texture and properties. The experimental conditions

varied greatly. For example, the initial concentrations in the liquid phase ranged from 0.01 to more than 1000 mg/L. Only coefficients of sorption to unmodified soils or sediments were included in the database. Measured coefficients of sorption to organic soils were included in the database, but only those measured for sorption to mineral soils, i.e., with an organic matter content lower than 20 % (IUSS 2014) were used for the statistical analyses. Several studies have reported that sorption coefficients depend strongly on the background electrolyte. Therefore, only sorption coefficients obtained with classical background electrolyte, either Milli–Q water or $CaCl_2$, were included in the database. Among the 101 sorption parameters registered in the database (Table 7.1.3.1.1-72), 69 were measured with $CaCl_2$, as the background electrolyte. Statistical analyses were only performed for sorption parameters measured with $CaCl_2$ (designated as "sample A").

Sorption isotherms
For sample A, approximately two–thirds of the sorption models were nonlinear Freundlich (Table **7.1.3.1.1-73**). To establish a pedotransfer function for Kd, we approximated equivalent Kd values by linearizing the Freundlich models over the actual range of the initial aqueous concentrations of the batch experiment used for model fitting (Table 7.1.3.1.1-73). The relative difference between Kf and its equivalent, Kd (Kd_{eq}), was approximately 30 % on average.

Statistical analyses
Pedotransfer functions aim to predict the sorption parameters Kd, Kf and n from selected substrate properties. Some of the properties, especially CEC, iron– and aluminum oxides or phosphorus content, were not available for all soils or sediments (Table 7.1.3.1.1-72). This lack of data induced a subsampling of sample A for the establishment of pedotransfer functions for the Kd and Kf parameters. This sample is designated as "sample B". The sample used for the establishment of the pedotransfer function for the n parameter excluded sorption studies that investigated only one concentration and, thereby, did not consider the possibility that n differs from 1. This sample is designated as “sample C”.

The statistical analyses were performed using the R statistical computing software. Correlation analyses were performed using the default "1 m" function of the R software. The three pedotransfer functions for the estimation of linear and nonlinear sorption models were established by forward and backward stepwise multiple regression analyses of the substrate properties and the Kd_{eq}, Kf and n parameters. The stepwise multiple regression analyses were performed using the default "step" function of the R software.

The validity of the Kd pedotransfer function is strongly supported by the fact that sorption processes do not depend on the pesticide concentration. However, in sample A, the n values ranged from 0.48 to 1.05, with a mean value of 0.83, indicating saturation of the sorption sites at high glyphosate concentrations. A complementary multiple regression between n, the substrate properties and the experimental conditions (C_{max} and *R)* was performed. The resulting equation (see Eq. 4) indicates the linearity range under various conditions.

Finally, we evaluated the accuracy of the predicted equilibrium partitioning of glyphosate between the soil and water by using the sorption parameters provided by the Kd or Kf/n pedotransfer functions. The evaluation was performed for 11 initial concentrations (0.01, 0.04, 0. 10, 0.40, 1, 4, 10, 40, 100, 400 and 1000 mg/L) in the liquid phase by comparing the predicted soil–to–water glyphosate concentration ratios, as obtained by the pedotransfer–estimated sorption parameters, to those obtained by the batch–fitted sorption parameters. The aqueous and soil concentrations were calculated for all sample B soils and sediments using the numerical solver as described previously.

Table 7.1.3.1.1-72: Glyphosate sorption parameters and associated soil or sediment properties' database

Substrate type and origin	Texture Sand-silt-clay (%)	pH	CEC cmol kg^{-1}	Organic carbon %	Clay %	Phosphorus mg kg^{-1}	Fe_{ox} and Al_{ox} g kg^{-1}	Kf L kg^{-1} n^{-1}	n	Aqueous concentration min–max mg L^{-1}	Solid/liquid ratio g mL^{-1}	Aqueous phase	References
Soil (Italy)	63.4:22.6:14.0	8.11[a]	na	0.70	14.00	na	na	43.01	0.79	0.20–120.00	1/5	$CaCl_2$	Accinelli et al. (2005)
Soil (USA)	93.5:2.7:3.8	7.20 [a]	na	0.94	3.80	na	na	62.16	0.88	0.20–120.00	1/5	$CaCl_2$	Accinelli et al. (2005)
Soil (France)	Sandy loam	5.10 [a]	na	0.82	10.50	1240.00	298.73	34.50	0.97	0.73–60.13	1/5	$CaCl_2$	Al-Rajab et al. (2008)
Soil (France)	Silt clay loam	6.30 [a]	na	1.45	30.60	3240.00	48.57	33.60	1.00	0.73–60.13	1/5	$CaCl_2$	Al-Rajab et al. (2008)
Soil (France)	Clay loam	7.90 [a]	na	1.91	34.90	2740.00	35.67	16.60	1.00	0.73–60.13	1/5	$CaCl_2$	Al-Rajab et al. (2008)
Soil (Finland)	Sandy loam	6.10[b]	na	0.47	17.00	1.60	na	166.00	0.97	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	5.80[b]	na	2.88	46.00	3.00	na	55.00	0.92	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	5.60[b]	na	0.54	58.00	1.10	na	249.00	0.91	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	5.80[b]	na	2.57	13.00	27.40	na	44.00	0.90	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	5.20[b]	na	0.72	4.00	17.10	na	55.00	1.00	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	6.00[b]	na	7.06	41.00	4.10	9.66	97.00	1.03	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	6.00[b]	na	2.96	47.00	1.50	11.22	41.00	1.02	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	6.40[b]	na	5.93	4.00	5.30	9.26	97.00	0.95	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	5.90[b]	na	1.77	4.00	0.90	6.70	51.00	0.86	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	8.10[b]	na	2.67	41.00	88.70	na	58.00	0.93	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	7.90[b]	na	2.50	30.00	22.40	na	113.00	0.87	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	7.10[b]	na	2.35	21.00	8.80	na	93.00	0.98	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	6.80[b]	na	0.75	8.00	2.80	na	90.00	0.98	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Clay	6.60[b]	na	7.05	41.00	5.80	na	179.00	1.00	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Sandy loam	6.30[b]	na	5.93	4.00	6.40	na	121.00	0.96	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Silty loam	5.40[b]	na	7.90	5.00	10.00	4.48	159.00	0.98	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Silty loam	5.60[b]	na	4.50	4.00	3.60	4.29	802.00	0.93	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Silty loam	5.40[b]	na	1.30	8.00	3.50	4.57	59.00	0.76	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Muddy clay	6.90[b]	na	12.60	57.00	52.00	na	41.00	0.91	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Soil (Finland)	Organic soil	5.20[b]	na	26.00	79.00	5.30	17.09	54.00	1.11	2.00–10.00	1/5	H_2O	Autio et al. (2004)
Sediment (France)	35.9:39.1:25.0	8.44[c]	12.50	1.58	25.00	19.00	2.74	1[illegible].00	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	46.3:33.1:26.6	8.54[c]	12.10	1.56	26.60	6.00	2.62	302.00	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	4.7:58.5:36.8	8.63[c]	14.40	0.73	36.80	5.00	3.27	1[illegible].38	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	7.7:57.2:35.1	8.71[c]	14.20	0.96	35.10	5.00	3.[illegible]	109.90	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	9.7:54.6:35.7	7.30[c]	23.20	0.54	35.70	11.00	3[illegible]	302.63	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	25.5:42.0:32.5	7.93[c]	26.10	3.88	32.50	93.00	3[illegible]	262.41	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	77.9:13.9:8.2	6.03[c]	6.34	0.47	8.20	142.00	1[illegible]	39.07	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Sediment (France)	7.8:62.8:29.8	6.89[c]	17.60	3.67	29.60	144.00	3[illegible]	318.82	1.00	0.005–1.00	1/10	$CaCl_2$	Bailly et al. (2015)
Soil (Sweden)	7.3:46.2:46.5	7.20[c]	28.40	4.00	46.50	na	na	118.00	0.95	0.01–0.10	1/10	$CaCl_2$	Bergström et al. (2011)
Soil (Sweden)	3.3:40.6:56.1	7.40[c]	33.60	0.00	56.10	na	na	165.00	1.03	0.01–0.10	1/10	$CaCl_2$	Bergström et al. (2011)

Table 7.1.3.1.1-72: continued

Substrate type and origin	Texture	pH	CEC	Organic carbon	Clay	Phosphorus	Fe_{ox} and Al_{ox}	Kf	n	Aqueous concentration min–max	Solid/liquid ratio	Aqueous phase	References
	Sand-silt-clay (%)		cmol kg^{-1}	%	%	mg kg^{-1}	g kg^{-1}	L kg^{-1} n^{-1}		mg L^{-1}	g mL^{-1}		
Soil (Sweden)	87.8/4.5/7.7	7.40[a]	4.70	2.00	7.70	na	na	40.00	0.92	0.01-0.10	1/10	$CaCl_2$	Bergström et al. (2011)
Soil (Sweden)	95.4/4.6/0.0	6.40[a]	1.80	1.00	0.00	na	na	28.70	0.82	0.01-0.10	1/10	$CaCl_2$	Bergström et al. (2011)
Soil (Spain)	82.8/11.0/7.0	7.90[a]	5.20[a]	1.00	7.00	na	36.70	93.00	0.73	0.10-5.00	2/5	H_2O	[illegible] et al. (2007)
Soil (Spain)	59.0/13.0/10.0	7.30[a]	4.60[a]	0.00	10.00	na	126.50	154.00	0.74	0.10-5.00	2/5	H_2O	[illegible] et al. (2007)
Soil (Malaysia)	Sandy loam	6.70	7.10[a]	1.30	10.00	na	na	83.30	0.85	0.10-5.00	1/5	$CaCl_2$	[illegible] et al. (1997)
Soil (Malaysia)	Muck	4.70	24.10[a]	30.50	22.50	na	na	417.00	0.71	0.10-5.00	1/5	$CaCl_2$	[illegible] et al. (1997)
Soil (France)	10.0/44.0/46.0	7.60[a]		3.90	46.00	na	4.40	33.90	1.00	0.10-5.00	1/5	[illegible]	Dousset et al. (2007)
Soil (France)	53.0/25.0/22.0	6.60[a]		1.50	22.00	na	5.10	51.30	1.00	0.10-5.00	1/5	[illegible]	Dousset et al. (2007)
Soil (Denmark)	Sandy	7.60	12.60	1.70	15.00	na	na	30.90	0.78	0.03-67.00	1/20	[illegible]	de Jonge and Wollesen de Jonge (1999)
Soil (Denmark)	Sandy	6.10	12.60	1.70	15.50	na	na	28.50	0.73	0.03-67.00	1/20	$CaCl_2$	de Jonge and Wollesen de Jonge (1999)
Soil (Denmark)	Sandy	6.30	12.60	1.70	15.50	na	na	28.40	0.75	0.03-67.00	1/20	$CaCl_2$	de Jonge and Wollesen de Jonge (1999)
Soil (Denmark)	Sandy	6.50	12.60	1.70	15.50	na	na	48.40	0.77	0.03-67.00	1/20	$CaCl_2$	de Jonge and Wollesen de Jonge (1999)
Soil (Chile)	Clay loam	4.79[a]		6.60	18.00	na	na	12.13	1.00	[illegible]	1/10	H_2O	Kogan et al. (2003)
Soil (Finland)	Clay	6.02[a]		7.10	41.00	4.20	9.56	98.60	1.00	[illegible]	1/5	H_2O	Laitinen et al. (2006)
Sediment (Germany)	Sandy	7.7[a]		0.34	0.00	0.00	0.61	1.30	[illegible]	[illegible]	1/2	$CaCl_2$	Litz et al. (2011)
Soil (France)	Rendzina over limestone	8.4[a]	6.40	1.86	8.80	3500.00	2.51	17.60	[illegible]	0.20-10.00	1/5	H_2O	Mamy and Barriuso (2005, 2007)
Soil (France)	Rendzina over limestone	8.2[a]	7.10	1.86	9.30	2720.00	2.58	34.80	[illegible]	0.20-10.00	1/5	$CaCl_2$	Mamy and Barriuso (2005, 2007)
Soil (France)	Clay loam	8.36[a]	17.80	2.00	37.60	2490.00	15.20	70.10	0.88	0.20-10.00	1/5	H_2O	Mamy and Barriuso (2005, 2007)
Soil (France)	Clay loam	8.2[a]	20.60	1.89	37.70	2360.00	14.96	71.60	0.80	0.20-10.00	1/5	$CaCl_2$	Mamy and Barriuso (2005, 2007)
Soil (France)	Clay loam	6.3[a]	16.40	1.01	27.40	1400.00	8.58	60.00	0.83	0.20-10.00	1/5	H_2O	Mamy and Barriuso (2005, 2007)
Soil (France)	Clay loam	7.6[a]	15.90	0.96	23.50	1310.00	7.81	236.00	0.77	0.20-10.00	1/5	$CaCl_2$	Mamy and Barriuso (2005, 2007)
Soil (Greece)	13.4/64.1/22.6	8.0[a]	28.30[a]	0.00	22.60	na	7.80	13.79	0.77	50.00–300.00	1/25	$CaCl_2$	Piccolo et al. (1994)
Soil (UK)	46.2/36.8/17.0	5.8[a]	18.30[a]	5.73	17.00	na	0.10	40.64	0.77	50.00–300.00	1/25	$CaCl_2$	Piccolo et al. (1994)
Soil (Germany)	81.5/12.6/6.0	4.6[a]	10.70[a]	0.23	6.00	na	2.40	51.14	0.58	50.00–300.00	1/25	$CaCl_2$	Piccolo et al. (1994)
Soil (France)	17.9/2.4/16.0	8.3[a]	11.40[a]	0.45	16.00	na	25.00	152.85	0.44	50.00–300.00	1/25	$CaCl_2$	Piccolo et al. (1994)
Soil (Brazil)	46.0/7.0/47.0	5.2[a]	14.18	3.20	47.00	80.00	222.80	162.90	0.98	0.42-6.72	1/5	$CaCl_2$	Prata et al. (2003)
Soil (Brazil)	38.0/60.0/56.0	5.0[a]	12.32	2.50	56.00	60.00	277.10	215.70	0.99	0.42-6.72	1/5	$CaCl_2$	Prata et al. (2003)

Substrate type and origin	Texture	pH	CEC	Organic carbon	Clay	Phosphorus	Fe_{ox} and Al_{ox}	Kf	n	Aqueous concentration min–max	Solid/liquid ratio	Aqueous phase	References
	Sand-silt-clay (%)		cmol kg^{-1}	%	%	mg kg^{-1}	g kg^{-1}	L kg^{-1} n^{-1}		mg L^{-1}	g mL^{-1}		
Soil (Denmark)	Sandy	5.3[a]	na		1.64	na	2.21	403.50	1.00	0.30	1/5	$CaCl_2$	Strange-Hansen et al. (2004)
Soil (China)	–	7.20[a]	23.80		34.20	na	209.50	31.19	0.82	21.00–469.00	1/25	H_2O	Wang et al. (2006)
wetland Sediment (Canada)	Sandy	6.70[a]	14.30	1.20	9.10	na	na	172.90	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Wetland sediment (Canada)	Sandy	6.40[a]	16.60	3.80	11.30	na	na	152.60	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Wetland sediment (Canada)	Sandy	6.90[a]	9.30	0.80	4.90	na	na	251.90	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Wetland sediment (Canada)	Sandy	7.20[a]	10.20	8.70	5.60	na	na	193.10	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Wetland sediment (Canada)	Sandy	7.30[a]	9.90	9.60	6.70	na	na	124.90	1.00	1.00	1/10	$CaCl_2$	Xu et al. (2009)
Soil (Denmark)	Sandy	5.3[b]	na	1.32	4.20	50.55	3.21	107.00	0.62	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.4[b]	na	1.06	4.20	63.49	2.47	80.60	0.62	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.6[b]	na	1.28	4.20	87.39	2.98	83.50	0.66	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.8[b]	na	1.06	4.20	72.15	2.55	79.40	0.68	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.2[b]	na	1.29	4.20	18.32	2.73	121.20	0.69	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.3[b]	na	1.33	4.20	27.70	3.36	141.20	0.63	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.5[b]	na	1.30	4.20	44.58	3.43	118.10	0.67	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.3[b]	na	1.40	4.20	44.50	3.24	111.50	0.62	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.5[b]	na	1.26	4.20	12.07	3.23	126.80	0.56	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.7[b]	na	1.26	4.20	18.56	2.95	120.00	0.58	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.6[b]	na	1.21	4.20	36.31	2.76	92.00	0.59	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	4.9[b]	na	1.36	4.20	32.08	3.27	116.20	0.61	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.2[b]	na	1.33	4.20	9.08	3.46	154.00	0.62	0.04–65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.5[b]	na	1.14	4.20	13.07	3.44	138.80	0.65	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.4[b]	na	1.29	4.20	24.28	3.65	136.60	0.63	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy	5.5[b]	na	1.20	4.20	25.57	3.32	119.80	0.63	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.2[b]	na	1.28	10.80	6.19	6.45	214.20	0.56	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.2[b]	na	1.26	10.80	13.26	5.26	165.10	0.60	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.3[b]	na	1.25	10.80	24.52	5.35	132.60	0.67	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.4[b]	na	1.23	10.80	58.74	5.71	106.40	0.71	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.3[b]	na	1.21	10.80	8.75	5.28	171.30	0.58	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.3[b]	na	1.40	10.80	15.56	5.04	144.00	0.59	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)
Soil (Denmark)	Sandy loam	6.3[b]	na	1.44	10.80	24.79	4.94	151.30	0.65	0.04-65.00	1/18	$CaCl_2$	de Jonge et al. (2001)

Table 7.1.3.1.1-72: continued

Substrate type and origin	Texture	pH	CEC	Organic carbon	Clay	Phosphorus	Fe_{ox} and Al_{ox}	Kf	n	Aqueous concentration min–max	Solid/liquid ratio	Aqueous phase	References
	Sand-silt-clay (%)		cmol kg^{-1}	%	%	mg kg^{-1}	g kg^{-1}	L kg^{-1} n^{-1}		mg L^{-1}	g mL^{-1}		
Soil (Brazil)	81.7:2.0:16.3	4.00[a]	2.00	0.83	16.30	28.77	212.00	31.32	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	81.7:2.0:16.3	5.00[a]	2.00	0.83	16.30	28.77	212.00	17.89	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	81.7:2.0:16.3	6.00[a]	2.00	0.83	16.30	28.77	212.00	10.59	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	81.7:2.0:16.3	7.00[a]	2.00	0.83	16.30	28.77	212.00	15.52	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	27.7:42.0:30.3	4.00[a]	4.00	0.38	30.30	3.12	289.40	0.87	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	27.7:42.0:30.3	5.00[a]	4.00	0.38	30.30	3.12	289.40	0.83	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	27.7:42.0:30.3	6.00[a]	4.00	0.38	30.30	3.12	289.40	1.37	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	27.7:42.0:30.3	7.00[a]	4.00	0.38	30.30	3.12	289.40	0.00	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	19.7:26.0:54.3	4.00[a]	11.00	2.56	54.30	16.93	236.70	4.45	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	19.7:26.0:54.3	5.00[a]	11.00	2.56	54.30	16.93	236.70	3.44	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	19.7:26.0:54.3	6.00[a]	11.00	2.56	54.30	16.93	236.70	2.51	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)
Soil (Brazil)	19.7:26.0:54.3	7.00[a]	11.00	2.56	54.30	16.93	236.70	2.01	1.00	3405.00	2/5	$CaCl_2$	da Cruz et al. (2007)

Note that sorption parameters included in the database were measured on unmodified soil or sediment and with background electrolyte being either water (H_2O) or calcium chloride ($CaCl_2$). Some parameters were not included in the database because of unit incoherencies (e.g., Jacobsen et al. 2008)

ns not available

[a] pH_{H2O}

[b] pH_{CaCl2}

[c] CEC meq/100 g

Table 7.1.3.1.1-73: Statistical characteristics of the database subsamples

	Sample A					Sample B					Sample C				
	Nobs.	Mean	Median	Min	Max	Nobs.	Mean	Median	Min	Max	Nobs.	Mean	Median	Min	Max
Kf (L kg^{-1} s^{-1})	69	108.16	111.50	0.83	403.50	36	96.35	81.00	0.83	297.02	51	118.89	118.10	1.89	297.02
n	69	0.83	0.88	0.48	1.05	36	0.74	0.80	0.75	1.05	51	0.77	0.75	0.48	1.05
Kd_{ini} (L kg^{-1})	69	73.96	38.89	0.06	403.50	36	47.54	20.05	0.83	318.82	51	72.61	44.78	0.06	318.82
pH	69	6.10	6.30	3.60	8.71	36	6.38	6.85	4.00	8.71	51	6.20	6.30	3.60	8.71
OC (%)	69	1.79	1.29	0.00	9.60	36	2.01	1.69	0.00	9.60	51	1.80	1.29	0.00	6.44
CEC (cmol kg^{-1})	36	13.25	12.55	1.80	33.60	36	16.25	12.55	1.80	33.60	19	15.21	12.60	1.80	33.60
Clay (%)	69	18.32	10.80	0.00	56.10	36	23.36	24.25	0.00	56.10	51	16.11	10.80	0.00	56.10
$P_{MEHLICH}$ (mg kg^{-1})	52	297.88	25.18	0.00	3240.00	36	321.32	16.93	3.12	2760.00	40	382.36	29.89	0.00	3240.00
Fe_{ox}–Al_{ox} (g kg^{-1})	49	80.87	5.04	0.61	298.73	36	157.32	212.00	2.58	289.40	36	28.00	3.44	0.61	298.73

Sample A all data with $CaCl_2$ as the background electrolyte. *Sample B* data used for the calibration of Kf and Kf pedotransfer functions. *Sample C* data used for the calibration of the n pedotransfer function

OC organic carbon, *CEC* cation exchange capacity, Fe_{ox}–Al_{ox} iron and aluminum oxides, *Nobs.* number of observations

Results and Discussion

Database and sample characteristics

The soils and sediments used in the glyphosate sorption measurements displayed great variability in their origins and properties. This variability was preserved in the subsampling of the database for pedotransfer function calibration, as seen in Table 7.1.3.1.1-73. Indeed, the three subsamples of the database displayed similar distributions of properties and parameters values. The 0.01–1000 mg/L concentration range (Table 7.1.3.1.1-74) was also preserved by the subsampling of the database. This range covers all possible environmental glyphosate concentrations from concentrations found during spraying to those found in runoff and groundwater. It is interesting to note that the data presented in Table 7.1.3.1.1-75 exhibited highly significant correlations between some basic soil properties: The CEC was correlated with organic carbon or iron– and aluminum oxides, and the clay content was correlated with iron– and aluminum oxides. In contrast, there was no correlation between clay and CEC, suggesting a large influence of the within–sample variation in clay mineralogy (Table 7.1.3.1.1-75).

Table 7.1.3.1.1-74: Initial aqueous concentration used for the linear approximation if Freundlich isotherms

	Initial aqueous concentrations (mg L^{-1})	Frequency of use in experimental design (%)
Class 1	0.01–0.02–0.04–0.06–0.08	56
Class 2	0.10–0.20–0.40–0.60–0.80	59
Class 3	1.00–2.00–4.00–6.00–8.00	55
Class 4	10.00–20.00–40.00–60.00–80.00	32
Class 5	100.00–200.00–400.00–600.00–800.00–1000.00	12

Table 7.1.3.1.1-75: The Pearson correlation coefficients matrix among soil properties

Parameters	CEC (cmol kg^{-1})	OC (%)	Clay (%)	Phosphorus (mg kg^{-1})	Fe_{ox} and Al_{ox} (g kg^{-1})
pH	0.407*	NS	0.250*	0.339*	NS
	(36)	(69)	(69)	(52)	(49)
CEC	–	0.666***	NS	NS	−0.691**
		(36)	(36)	(25)	(19)
OC	–	–	NS	NS	NS
			(69)	(52)	(49)
Clay	–	–	–	NS	0.631***
				(52)	(49)
Phosphorus	–	–	–	–	NS
					(49)

The number in brackets corresponds to the number of observations for the given correlation. "***", "**" and "*" represent correlation significance levels of 0.001, 0.01 and 0.05, respectively

NS correlation is not significant, *OC* organic carbon, *CEC* cation exchange capacity, Fe_{ox}–Al_{ox} iron- and aluminum oxides

Table 7.1.3.1.1-76: The Pearson correlation coefficients matrix between sorption parameters and soil properties or experimental conditions

Parameters	pH	CEC (cmol kg^{-1})	OC (%)	Clay (%)	Phosphorus (mg kg^{-1})	Fe_{ox} and Al_{ox} (g kg^{-1})	C_{max} (mg L^{-1})	log(R)
Kd_{ox}	NS	0.659***	0.380**	NS	NS	NS	−0.364**	NS
	(69)	(36)	(69)	(69)	(52)	(49)	(69)	(69)
Kf	NS	0.669***	0.255*	NS	NS	−0.527***	−0.551***	−0.432***
	(69)	(36)	(69)	(69)	(52)	(49)	(69)	(69)
n	0.531***	NS	0.351*	0.760***	0.361*	0.560***	−0.666***	0.489***
	(51)	(19)	(51)	(51)	(40)	(36)	(51)	(51)

The number in brackets corresponds to the number of observations for the given correlation. "***", "**", and "*", represent correlation significance levels of 0.001, 0.01 and 0.05, respectively

NS correlation is not significant, *OC* organic carbon, *CEC* cation exchange capacity, Fe_{ox}–Al_{ox} iron- and aluminum oxides, C_{max} maximal initial concentration (mg L^{-1}), *log(R)* log-transformed solid–liquid ratio (g mL^{-1})

Table 7.1.3.1.1-77: Pedotransfer function for the estimation of linear (Kd) and Freundlich (Kf–n) sorption isotherms

Pedotransfer function	Sample	Soil parameters	Equation	R^2	RMSEP
Kd	B	CEC, Clay	Kd = 24.821 + 7.199 * CEC − 1.307 * Clay	0.48***	7.59 (8.7 %)[a]
Kf	B	CEC, Clay, OC	Kf = 50.904 + 9.246 * CEC − 1.985*Clay − 11.811 * OC	0.52***	16.36 (16.9 %)[b]
n	C	Clay, pH	*n* = 0.505 + 0.007 * Clay + 0.024 * pH	0.62***	[illegible] (10.8 %)[c]

OC organic carbon (%), *CEC* cation exchange capacity (cmol kg^{-1}), clay (%)

*** Correlations are significant at the level 0.001

[a] RMSEP expressed as a percentage of the mean Kd value (87.02 L kg^{-1})

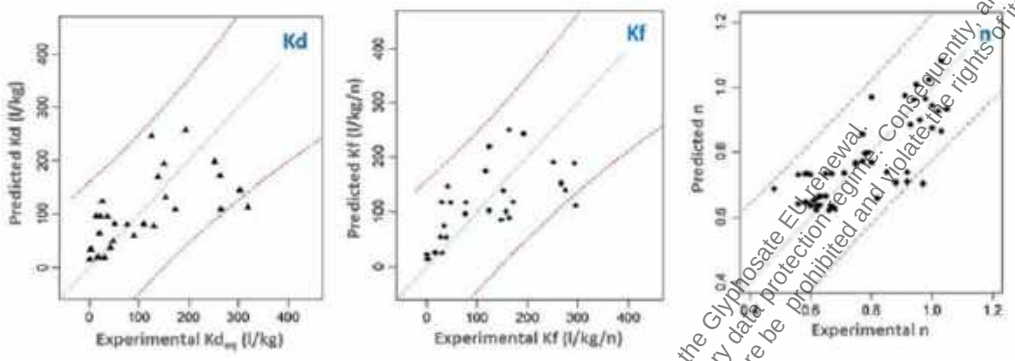
[b] RMSEP expressed as a percentage of the mean Kf value (96.32 L kg^{-1} n^{-1})

[c] RMSEP expressed as a percentage of the mean n value (0.77)

Glyphosate sorption: mechanisms and prediction

The Pearson correlation coefficients (Table 7.1.3.1.1-76) showed that the Kd_{eq} and Kf values are primarily correlated with CEC and, secondarily, with organic carbon content and Fe_{ox}–Al_{ox} content. They also show that n exhibits significant correlation with all of the selected soil properties, with the exception of CEC. The multiple regression analysis (Table 7.1.3.1.1-77) provided pedotransfer functions that accurately fit the observed Kd_{eq}, Kf and n values. The functions account for 48–62 % of the variation in the sorption parameters. Visual inspection of the disparity between the measured and predicted Kd_{eq}, Kf and n sorption parameters did not reveal systematic departures from the regression, except for one outlier corresponding to high Kd_{eq} and Kf values measured on a sediment containing a particularly high organic carbon content (Figure 7.1.3.1.1-66). The multiple regression analyses high–lighted the points that CEC is the main predictor of Kd_{eq} and Kf variation and that clay is a useful predictor. Furthermore, we found that organic carbon was a predictor for Kf only. The analyses also revealed that clay and pH are significant predictors of *n*. These results suggest that the formation of complexes between the glyphosate phosphonate groups and the soil exchanged polyvalent cations is the dominating sorption mechanism across the entire range of analyzed soils. This is indicated by the primary role of CEC in controlling Kd_{eq} and Kf variability. Given the high correlation between CEC and Fe_{ox}–Al_{ox} in our sample, it is likely that the influence of the latter property was masked by that of the former. Additionally, we found that clay content explained only approximately 5 % of the Kd_{eq} and Kf variability (Table 7.1.3.1.1-76, Table 7.1.3.1.1-77). Significant correlations were found between organic carbon and Kd_{eq} or Kf (Table 7.1.3.1.1-76), although organic carbon only slightly increased the R^2 value obtained in the multiple regression analyses of Kf. Organic carbon appeared to be strongly correlated with CEC, indicating the significant contribution of organic matter to CEC; this correlation may explain the correlation of organic carbon with the sorption parameters. There is a general consensus that a rise in pH negatively affects the sorption of glyphosate. However, the multiple regression analyses did not detect any influence of pH on Kd_{eq}, and Kf variability.

Figure 7.1.3.1.1-66: Multiple regression analysis of the sorption coefficients (Kd, Kf, *n)* and soil properties. The sorption coefficients predicted from the pedotransfer functions presented in Table 7 were plotted against the sorption coefficients (Kf, *n*) fitted from the experimental data and for Kd_{eq}, against the linearized sorption coefficients



Here, pH and clay explained most of the n parameter variability (Table 7.1.3.1.1-76 and Table 7.1.3.1.1-77). The positive correlation of n with pH may be related to the increased negative charges for both glyphosate (Figure 7.1.3.1.1-65) and the soil, favoring the formation of complexes with soil–exchanged polyvalent cations. Despite the increasing electrostatic repulsion, a rise in pH appears to reduce the potential saturation of sorption sites for high initial concentrations by favoring cation bridging between glyphosate and the soil. The variability of the sorption parameters that is not predicted by the multiple regressions may be largely attributed to the varying experimental conditions among the studies measuring glyphosate sorption to soils and sediments (Table 7.1.3.1.1-72). If different parameters are considered to be possible predictors in the multiple regressions, they enable a fit to a regression function (Eq. 4) with a better performance (R^2 = 0.69) than that of the regression using only basic soil properties as predictors for *n.*

$$n = 0.920 - 0.028 \times \log(C_{max}\ (mg/L)) + 0.064 \times \log(R(g\ m/L)) + 0.005 \times clay\ (\%) \qquad (4)$$

A small R implies a limited amount of sorption sites.

Correlations between Kf, Kd_{eq} and the solid–to–liquid ratio or the maximal initial concentration (Table **7.1.3.1.1-76**) are further evidence of the influence of the experimental conditions on the sorption. However, unlike the case of the *n* parameter, inclusion of the experimental conditions (C_{max}, *R*) in the multiple regression analyses did not increase the predictive performance of the regression for Kd_{eq} and Kf. It must be noted that the pedotransfer functions could be improved with additional experimental sorption studies designed to closely mimic the environmental conditions and with pH and CEC analyzed with the standardized methods [pH_{H2O}, Metson CEC (cmol/kg)].

Use of pedotransfer functions for risk assessment
The linear sorption coefficient Kd can be predicted by a pedotransfer function requiring the knowledge of only two properties, the clay content and CEC. The prediction performance is good with an RMSEP of less than 10 % of the mean glyphosate Kd_{eq} (Table 7.1.3.1.1-77). However, Figure 7.1.3.1.1-67a shows that the errors in the predicted soil–to–water concentration ratios vary largely according to the initial concentration of water. The errors are moderate for initial liquid–phase concentrations below 10 mg/L, indicating that the Kd pedotransfer function predicts sorption relatively accurately for concentrations below this threshold. The 10 mg/L may correspond to the threshold above which the concentration independence of the sorption process can no longer be assumed. This assumption can be checked by examining the variation in n given by Eq. 4. Figure 7.1.3.1.1-68 presents the departure from linearity assumed to occur when *n* is below 0.9 across a range of clay content values and initial glyphosate concentrations.

Figure 7.1.3.1.1-67: Distributions of the prediction errors for linear sorption isotherms and nonlinear sorption isotherms. Δ represents the difference (%) at a given initial concentration between the predicted and the measured ratio of concentrations between soil and water. a Ratios predicted by the Kd pedotransfer function (linear isotherm estimation) and b ratios predicted by the Kf and *n* pedotransfer function (nonlinear isotherm estimation)

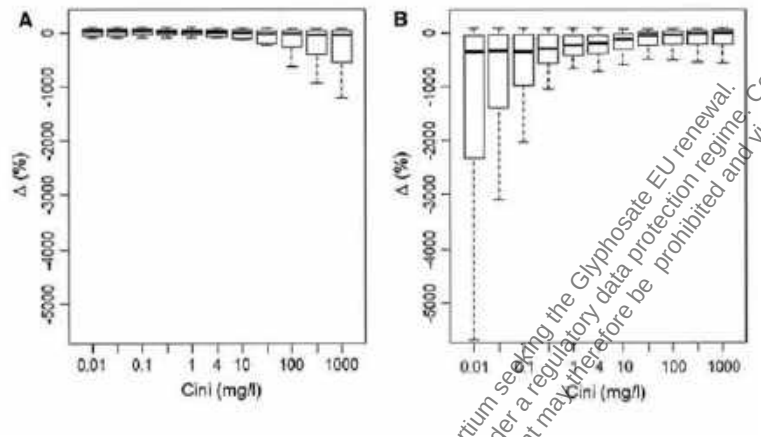
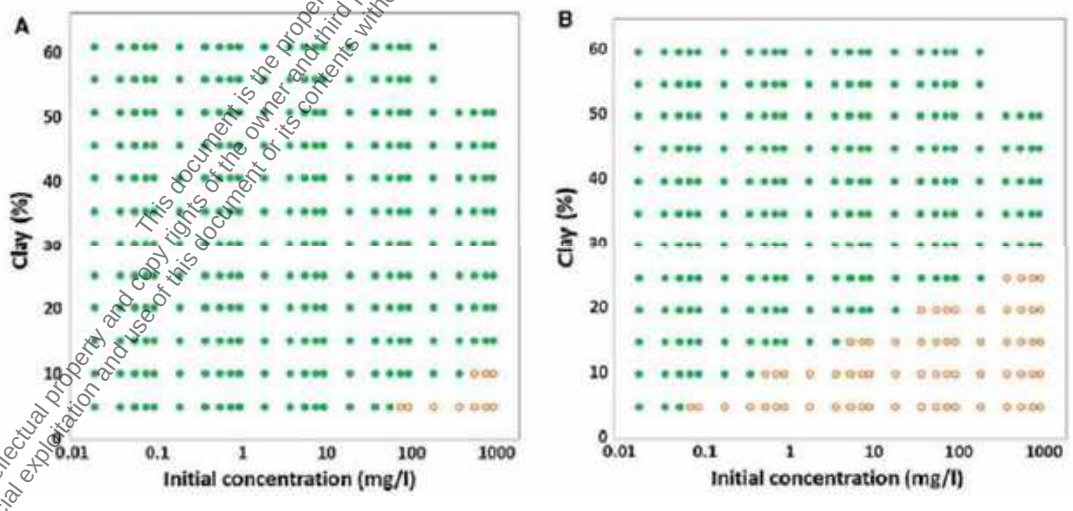


Figure 7.1.3.1.1-68: Linearity range of sorption isotherms in relation to the clay content and initial glyphosate concentrations in the liquid phase. Plain green dots represent 0.9 <n <1.05, and empty orange dots represent n values lower than 0.9 (bottom right), A n values were calculated from Eq. 4 with a solid–to–liquid ratio of 1:1 (g/ml). B n values were calculated from Eq. 4 with a solid–to–liquid ratio of 1:20 (g/ml). Note that for a solid–to–liquid ratio of 1:1, the saturation of sorption sites occurs at initial concentration higher than 100 mg/L for clay content varying between 0 and 10 %, whereas for the 1:20 ratio, the saturation for the same clay content starts at initial concentrations of approximately 0.1 mg/L



The Freundlich isotherms can be satisfactorily predicted by two pedotransfer functions requiring the knowledge of four properties, namely the organic carbon and clay contents, CEC and pH (Table **7.1.3.1.1-77**). As seen in Figure 7.1.3.1.1-68, the prediction errors of the soil–to–water concentration ratio exceed 1000 % for concentrations between 0.01 and 0.40 mg/L and 500 % for concentrations up to 10 mg/L

(Figure 7.1.3.1.1-67b). Thus, the sorption estimated by the combination of *n* and Kf pedotransfer functions is significantly underestimated for initial concentrations below 10 mg/L. This may be due to the multiplication of properties used to estimate the sorption parameters and the accumulation of inherent bias of the two pedotransfer functions. However, it must also be noted that for concentrations higher than 10 mg/L, the predictions using the estimated Freundlich model parameters show slightly smaller errors than those using the estimated linear isotherms. The application of the Kf pedotransfer function is therefore only advisable for estimating sorption for very high liquid–phase concentrations, a condition that is relatively rarely found in the current environmental conditions.

Conclusion
Sorption to soils and sediments controls the fate of glyphosate in the environment and thus the potential risk of freshwater and groundwater contamination. Glyphosate sorption appeared to be controlled mainly by cation exchange capacity, clay and organic carbon content and pH. This suggests that the mechanism driving glyphosate sorption over the range of soil and sediment investigated is the complex formation between the phosphonate group of glyphosate and the soil–exchanged polyvalent cations. Robust pedotransfer function for the estimation of glyphosate Kd was built from multiple regression analysis of the literature data. This Kd pedotransfer function enables prediction of glyphosate sorption for a wide range of soils and sediments with a limited number of properties and with reasonable accuracy for most environmental conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article estimates pedotransfer functions for the adsorption of glyphosate to soil based on review of existing published data. However, no new experimental data is presented neither existing data is evaluated regarding their quality in conduct according to OECD 106 or the EU Evaluators Checklist.

The article is therefore classified as reliable with restrictions, i.e. not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/025
Report author	Kanissery, R.G. *et al.*
Report year	2015
Report title	Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate
Document No	DOI 10.2134/jeq2014.08.0331 E-ISSN 1537-2537
Guidelines followed in study	USEPA guidelines for adsorption studies (USEPA, 2008)
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

The article was found relevant for multiple data points. The summary is provided under CA 7.1.2.1.1/013.

1. Information on the study

Data point:	CA 7.1.3.1.1/026
Report author	Tévez, H., dos Santos, A.M.
Report year	2015
Report title	pH dependence of Glyphosate adsorption on soil horizons
Document No	DOI 10.18268/BSGM2015v67n3a13 ISSN 1405-3322
Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Pesticides bring many problems to the environment and to human health. The first rationale for their use is increased food production. Glyphosate N-(phosphonomethyl)glycine (PMG) is a non-selective, post emergent, and broad spectrum herbicide, very well known for its extensive application in agriculture worldwide. PMG adsorption experiments were carried out in three horizons of a Typic Haplustoll soil from the Province of Santiago del Estero, Argentina. Adsorption isotherms were fitted using Freundlich and Langmuir models. The affinity constants (K_F and K_L), the adsorption intensity (l/n) and the maximum surface coverage (Γ_{max}) were obtained. The results show the dependence of the parameters K_L and Γ_{max} with pH and also with the different horizons and particle size.

Materials and Methods

Chemicals

All chemicals utilized were of analytical reagent grade and were used without further purification. All solutions and soil dispersions were prepared using Milli-Q water. All PMG solution concentrations ranged from 0.05 to 10 mM prepared daily.

Study area

Climate is semiarid mesothermal, with an average annual temperature of 19.6 °C and rainfall of between 600 and 750 mm per year concentrated in the spring-summer period. Samples were taken up to 130 cm of depth from three very well differenced horizons classified as Ap (0 - 18 cm), AB (18 - 50 cm) and BC (105 - 130 cm).

Characterizations

The fresh soil samples were air-dried and ground. pH was measured in 0.01 M $CaCl_2$ solution. Organic matter (OM) content and soils chemical analysis were determined by the dichromate oxidation method. The available phosphorus (P) is the inorganic P, that is extractable at pH 8.5 and was determined following the experimental procedure described in Olsen *et al.,* 1954 and Page *et al.,* 1982. The total surface area (Sw) was measured by H_20 adsorption (Torres-Sanchez and Falasca, 1997). The total iron oxides (Fe_{tot}) and amorphous iron oxides (Fe_{amorph}) were established by dithionite (Holmgren, 1967) and oxalate method (McKeague, 1967), respectively. Soils samples were mixed with Lithium Metaborate/Lithium Tetraborate ($LiBO_2$ / $Li_2B_4O_7$) and fused in a furnace. The molten melt was completely dissolved in acidic media of 5 % nitric acid. This solution was analyzed for major and selected trace elements by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). The sample composition is reported as oxide percentage. The mineralogical composition and quantitative analysis of the soils were determined by X-ray Diffraction (XRD) and using the Rietveld method (Rietveld, 1969). Point of zero net proton charge (PZNPC) or point of zero salt effect (PZSE) is the pH where the net adsorption of protons and hydroxyl ions on the surfaces is independent of electrolyte concentration. Titration curves, when surface charge is plotted against pH, frequently showed a common intersect ion point that match with PZNPC.

Table 7.1.3.1.1-78: Characteristics of agriculture soils profile from Santiago del Estero/Argentina

Horizon	pH ($CaCl_2$ 1:2.5)	OM ($g C.Kg^{-1}$)	P ($\mu g.g^{-1}$)	Sw ($m^2.g^{-1}$)	Fe_{amorph} ($mg.g^{-1}$)	Fe_{tot} ($mg.g^{-1}$)	PZNPC (pH)
Ap	5.90	23.30	43.34	188	0.239	1.66	7.1
AB	5.75	17.10	6.67	259	0.158	1.91	7.9
BC	6.02	12.10	1.19	242	0.095	0.99	8.4

Adsorption experiment

The adsorption of herbicide by the soils was studied using batch experiments. Solutions of different concentration of glyphosate was added to soil samples dispersions. Dispersions were kept in constant agitation overnight at constant pH, ionic strength and room temperature to reach equilibrium. The sample was filtered and adsorbed glyphosate was calculated from the difference between the total added ligand and the supernatant concentration (Ce). PMG was evaluated by ion chromatography. Two plastic anion columns were coupled in series to serve both as pre-column and analytical chromatographic column. The typical experimental error is lower than 5 % for all results.

Table 7.1.3.1.1-79: Chemical Analysis of agriculture soils profile from Santiago del Estero, Argentina

Horizon	Chemical Analysis (%)						
	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O
Ap	64.6	12.25	3.51	1.49	1.14	1.70	2.43
AB	63.0	13.65	4.10	1.34	1.40	1.50	2.53
BC	63.0	14.05	4.42	1.44	1.62	1.56	2.66

pH effect

The pH dependence of the glyphosate uptake by soil horizons was investigated using batch isotherm experiments in a pH range from 2 to 8 with a soil concentration of 9.1 g/L and different initial concentrations of PMG at a constant ionic strength of 0.1 M of KNO_3. The pH was measured using a Metrohm 644 pH-meter with a combined glass microelectrode. Adsorption experiments were conducted in triplicate following the procedure described above. There were no significant differences within each replicate ($p < 0.01$). The expressed values represent the average of the obtained results.

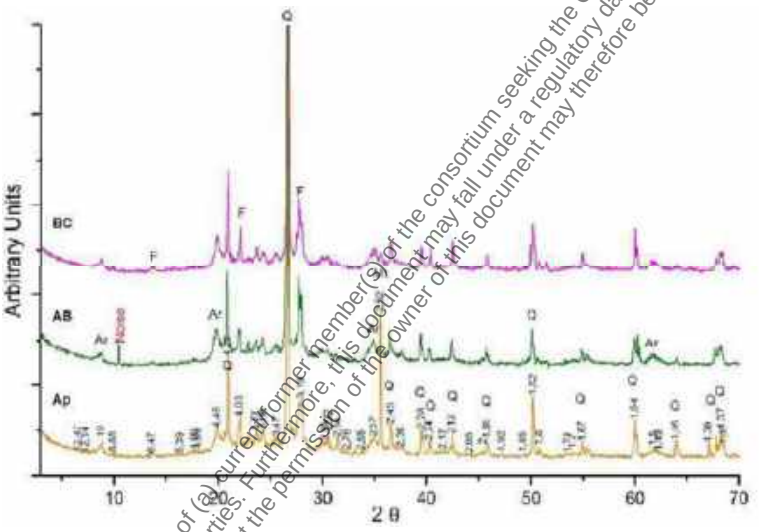
Isotherms Modeling

The relationship between the ligand uptake and the sorbate equilibrium concentration as constant temperature is known as the adsorption isotherm. The adsorbent capacity of a certain material is related to the material balance adsorption: the sorbate that disappears from solution must be in the adsorbent. Freundlich and Langmuir models were chosen and applied for describing the equilibrium data.

Table 7.1.3.1.1-80: Mineralogical Composition of agriculture soils profile from Santiago del Estero, Argentina. Values in parenthesis represent estimated standard deviations

	Mineralogical Composition (%)				
Horizon	Quartz	Sanidine Feldspar	Andesine Feldspar	Illite	Magnetite
Ap	45.2 (0.4)	9.6 (0.9)	24.7 (0.8)	18.6 (1.4)	1.3 (0.2)
AB	39.8 (0.5)	9.6 (0.8)	23.5 (0.7)	25.9 (1.5)	1.2 (0.3)
BC	46.2 (0.4)	9.3 (0.9)	19.9 (0.9)	24.7 (1.3)	1.2 (0.3)

Figure 7.1.3.1.1-69: XRD or the three soil horizons. Q: Quartz, Ar: Clay, F: Feldspar, Mt: Magnetite

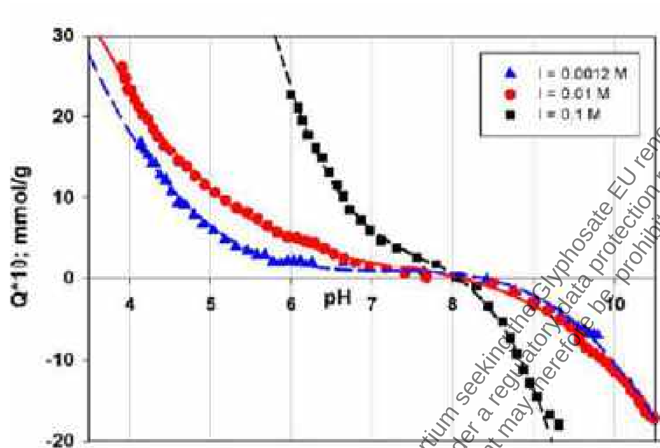


Results and Discussion

Soil characteristics, chemical analysis, mineralogical composition and quantitative analysis are presented in Table 7.1.3.1.1-78, Table 7.1.3.1.1-79 and Table 7.1.3.1.1-80 respectively. XRD of the three soil horizons are shown in Figure 7.1.3.1.1-69. The experimental curves of PZNPC recorded for the BC horizon are illustrated in Figure 7.1.3.1.1-70. Similar behavior was found for all the horizons that showed PZNPC values in the range of 7.1 - 8.1 (Table 7.1.3.1.1-78) following the sequence: Ap<AB<BC. PZNPC value can be explained by the absence of clay minerals with a negative permanent charge, while the presence of 2: 1 clays shift the PZNPC to lower pH values (Table 7.1.3.1.1-80). The higher PZNPC value for the horizons corresponds to horizon BC that contains similar amount of quartz, lower amount of feldspars (andesine) and high amount of illite. PZNPC increase with andesine feldspar content and OM decrease. The determination coefficients of a linear fit were $R^2_{andesine} = 0.9971$ and $R^2_{OM} = 0.9189$. The analysis of the three parameters variations in a 3D plot presented a determination coefficient of $R^2 = 1.0000$ and a constant variance test of $p < 0.0001$. The PMG adsorption isotherms of soils dispersions equilibrated at different pH values are shown in Figure 7.1.3.1.1-71. The Freundlich model parameters values (K_Γ , and l/n) were calculated and are given in Table 7.1.3.1.1-81. The 1/n values vary between 0.1 and 1, which indicates that this model could be used for interpreting the data. The correlation between experimental and calculated curves had a p-level between 0.137 and 0.0035; the determination coefficients were between 0.7578 and 0.9953 for different pHs and horizons.

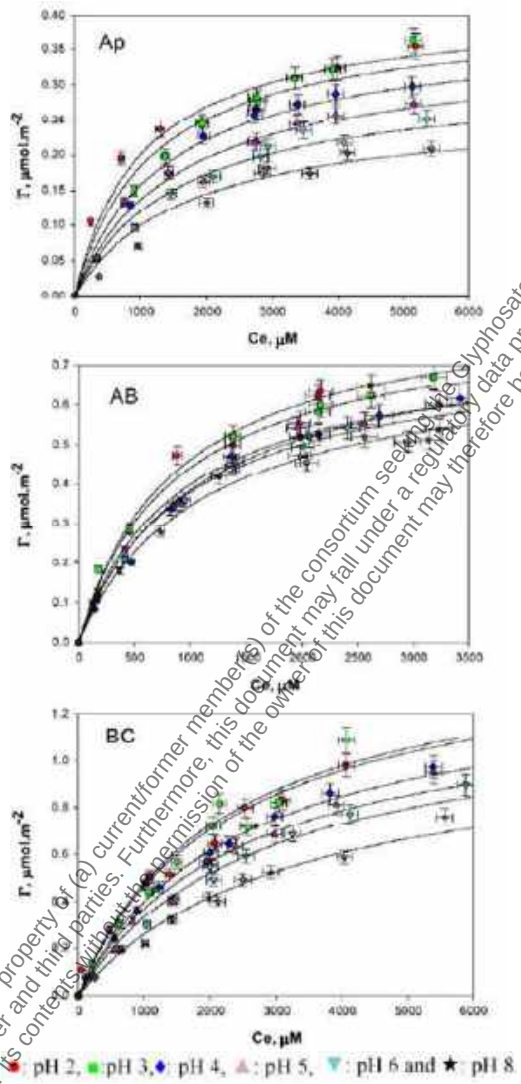
The Langmuir model was also applied to make an interpretation of PMG adsorption isotherms on soil dispersions equilibrated at different pH values. This is shown in Figure 7.1.3.1.1-71, where solid lines are calculated using this model and Γ_{max} and K_L are given.

Figure 7.1.3.1.1-70: Potentiometric titration curves or the dispersions or the BC horizon at three ionic strengths (I = ½$\Sigma_i C_i Z_i$)



The isotherm model parameters were obtained by a non-linear optimization using the Solver-Excel tool. The parameters values were obtained from the plot of the inverse of the surface coverage as a function of the inverse of the equilibrium concentration. Results of the adsorption and surface coverage calculations were normalized with Sw data and the various horizons were contrasted. The correlation between experimental and calculated curves had a p-level between 0.050 and 0.001; the determination coefficients (R^2) obtained were between 0.9300 and 0.9999; and were higher than those obtained using the Freundlich model. Thus, the Langmuir model would better represent the adsorption process of PMG on the Santiago del Estero Province soil.

Figure 7.1.3.1.1-71: Adsorption isotherm or PMG on horizon Ap, AB and BC. Solid lines are calculated using Langmuir model with constants and maximum surface coverage



The dependence of the surface coverage with PMG concentration in the various horizons at constant pH = 5 is shown in Figure 7.1.3.1.1-72. Horizon Γ_{max} sequence is Ap<AB<BC. This behavior is similar to those found for PZNPC. The dependence of the surface coverage with pH in the various horizons is also shown in Figure 7.1.3.1.1-71. The adsorption capacity increases from pH 8 to 2. This pH effect was normally observed during the adsorption of anionic species. Consequently, PMG interaction with the surface occurs throughout the anionic chemical groups (carboxylate or phosphonate) and not through the amine gr up (pKa = 10.14) that is positively charged at the studied pH range (Figure 7.1.3.1.1-73). The surface coverage decrease, $\Delta\Gamma_{max}$ for horizon Ap is around 41 % for this pHs range (Table 7.1.3.1.1-82). This difference is lower for horizons BC, 27 %, and AB, 12 %. The highest adsorption capacity is obtained by horizon BC followed by horizon AB, and the lowest for horizon Ap. A similar sequence was obtained for PZNPC (Table 7.1.3.1.1-78), indicating that the horizon with higher positive surface charge presents higher PMG surface coverage. The ratio of the Γ_{max} of the horizons ($R_{H1/H2}$) was calculated where H1 and H2 denote two different horizons, Γ_{maxH1} and Γ_{maxH2} indicate the maximum coverage of H1 and H2 horizons, respectively. This ratio between the horizons BC and AB was $R_{BC/AB}$ = 46 %, between horizons BC and Ap was $R_{BC/AP}$ = 72 % and between horizon AB and Ap was $R_{AB/Ap}$ = 50 %. These percentages are opposed to the

phosphate content that follows the order Ap > AB > BC. The highest adsorption constants correspond to horizon AB (Table 7.1.3.1.1-82). The changes in the adsorption affinity between horizon BC and AB reach ΔK_L = 46 % while horizon BC decreases 73 % in respect to horizon Ap.

Figure 7.1.3.1.1-72: Adsorption isotherm of PMG on horizon AP, AB and BC at pH 5

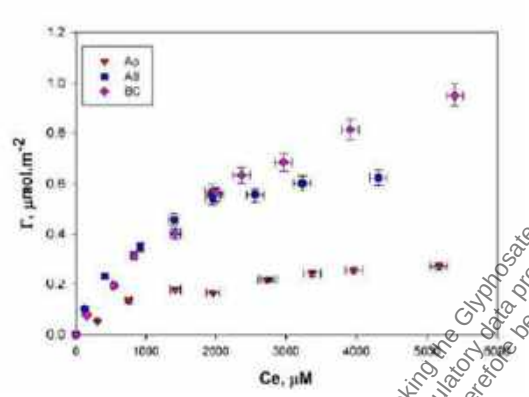
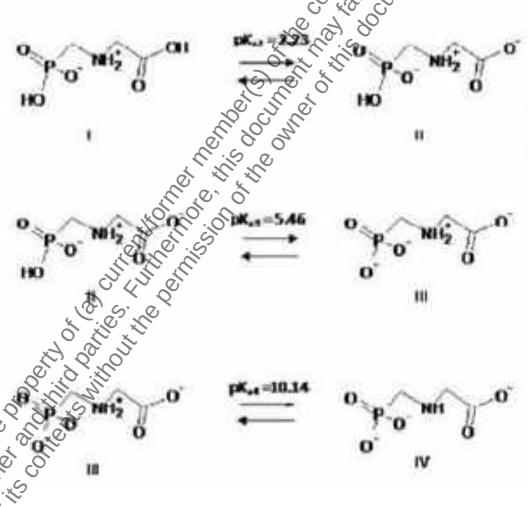


Figure 7.1.3.1.1-73: PMG acid-base equilibrium



The greater slope of the adsorption curves in the AB horizon indicate that PMG binds more strongly to the active sites of this horizon. Thus, the active site of PMG adsorption on the AB horizon could be the surface iron atoms and the higher adsorption in this horizon is directly related to higher iron content.

The adsorption on horizon BC does not reach maximum coverage in experimental conditions. The adsorption isotherms with a low initial slope describe an adsorption process with characteristic adsorption constants of low energy interaction (Figure 7.1.3.1.1-71). The constant and the equilibrium reactions of acid-base dissociation of glyphosate (Barja and dos-Santos-Afonso, 1998) are shown in Figure 7.1.3.1.1-73, where I, II and III are the main species presents in the studied pH range.

Table 7.1.3.1.1-81: Freundlich parameters (in $\mu mol^{1-1/n}\ m^{-2}$) for glyphosate adsorption on Santiago del Estero Province soils

Horizon		Ap			AB			BC	
pH	K_f*10^3	1/n	R^2	K_f*10^3	1/n	R^2	K_f*10^3	1/n	R^2
2	7.3	0.46	0.9449	19.7	0.44	0.9743	5.5	0.64	0.9842
3	6.7	0.47	0.9953	18.4	0.45	0.9894	4.9	0.65	0.9759
4	5.9	0.47	0.9646	16.2	0.46	0.9654	4.7	0.63	0.9852
5	5.6	0.46	0.9729	16.2	0.45	0.9652	4.6	0.63	0.9892
6	5.0	0.48	0.7578	15.1	0.46	0.9862	4.3	0.62	0.9807
8	3.8	0.47	0.9453	14.3	0.45	0.9749	3.0	0.64	0.9892

Conclusions

The major factor in PMG adsorption on soil samples is given by the pH, which could be due to the influence of this parameter on the PMG molecule and on the surface charge of the soil particles. PMG adsorption increase with acidity, and this increase correspond to the adsorption of a ligand with a negative net charge. Sorption of glyphosate in soils is similar to the adsorption of the organic molecule on the soil components such as clay minerals, iron oxides and OM. For these soils with a low organic matter contents and/or similar amounts of clay in the various horizons, the adsorption would be determined by the content of phosphorous, iron oxide and the specific surface. Regarding the relative adsorption capacity of the soil, the adsorption process has a different behavior profile, where the deeper horizon (BC) has a higher capacity retention for this herbicide.

Table 7.1.3.1.1-82: Langmuir parameters for PMG adsorption on soils or Santiago del Estero Province, Argentina

Horizon		Ap			AB			BC	
pH	Γ_{max} $\mu mol.m^{-2}$	K_L $L.mmol^{-1}$	R^2	Γ_{max} $\mu mol.m^{-2}$	K_L $L.mmol^{-1}$	R^2	Γ_{max} $\mu mol.m^{-2}$	K_L $L.mmol^{-1}$	R^2
2	0.41	0.91	0.9572	0.81	0.93	0.9936	1.50	0.40	0.9331
3	0.40	0.87	0.9923	0.82	0.93	0.9833	1.49	0.42	0.9664
4	0.38	0.73	0.9873	0.78	0.92	0.9925	1.36	0.41	0.9955
5	0.34	0.67	0.9889	0.76	0.92	0.9932	1.27	0.40	0.9854
6	0.31	0.60	0.9633	0.74	0.91	0.9974	1.23	0.39	0.9858
8	0.24	0.41	0.9424	0.72	0.90	0.9926	1.10	0.28	0.9830

The lower adsorption in the AB and Ap horizons could be influenced by the higher content of phosphorus. However, the strength of the interaction, as given by the Langmuir Model Constant K_L is larger on horizon A B and would be linked to the illite and iron oxide content that have a better distribution in AB. It should be noted that the Langmuir adsorption model is the best fit to the adsorption experimental results in these soils, although the Freundlich model has a good fit for some pHs. Given the adsorption extent found in this study, it is expected that pesticides will be retained in these soils. This strong interaction could prevent the pesticides movement into the ground water. On the other hand, this retention rate could result in the release of the herbicide on the environment due to displacement by runoff.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the adsorption of non-labelled glyphosate to topsoil and subsoil of an agricultural soil from Argentina. The pH-dependency was investigated in addition. However, there was no detailed reporting of data to assess the validity (i.e. mass balances, detailed chemical properties of test substance, solvents used, information about analytical methods and their validation including, LOD, LOQ, temperature, test concentrations).

The article is therefore classified as reliable with restrictions, i.e. no use of data in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/027
Report author	Jodeh S. *et al.*
Report year	2014
Report title	Fate and Mobility of Glyphosate Leachate in Palestinian Soil Using Soil Column
Document No	ISSN 2028-2508
Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

In recent years, pesticides were used heavily in Palestine, which led to the contamination of soil and water and causing many diseases. Many studies focused on the impact of pollutants such as pesticides and oil on soil, humans, animals, plants and the environment in general. Using column study the amount of glyphosate in soil decreases with increasing depth of soil, where it is for 0–30 cm (11 ppm) >30–60 cm (6 ppm) >60-100 cm (2 ppm) due to organic content and metal oxides founded in soil that can form stable complexes with glyphosate. When we increased the concentration of glyphosate, the amount of glyphosate (contaminant) in leachate where found to be 25 x (15.96 ppm) >15 x (3.91) >5 x (3 ppm) column. The behavior of glyphosate leachate fits the first order reaction and the isotherm is in according with the Freundlich adsorption equation with R^2 value 0.98, *k* value 6.4 and n value 1.07 which indicates good adsorption to soil.

Materials and Methods
Chemicals
Glyphosate (purity 98.5 %) was purchased commercially. Other chemicals like carbon disulfide, copper nitrate and chloroform were available at the university department of chemistry. All chemicals and solvents used in the experiment were of high performance liquid chromatography and high purity.

Acid digestion of soil
To find the metals in soil and $HClO_4$ (70 %) and HF (40 %) were added then heated to incipient (near dryness). HF were added again and heated to dryness then $HClO_4$ and distilled water were added and heated to incipient. The remaining residue was dissolved in HCl and water. Volume was made up to the 100 mL

volume and stored in polyethylene bottle. Fe and Cu in the supernatant were determined by AAS. The physicochemical soil properties (Table 7.1.3.1.1-83) were determined using standard methods.

Sampling site and Collection

The soil was sampled in three layers; 0–30 cm, 30–60 cm and 60–100 cm from agricultural locations in Nablus, Mount Gerizim before herbicide treatment of the fields. The soil samples were mixed well separately. The soil used for chemical analysis was air dried, sieved to 2 mm stored in the dark at room temperature and protected from humidity. Basic physicochemical properties of soil were conducted on soil before any treatment with glyphosate**.**

Table 7.1.3.1.1-83: Physico–chemical characteristics of the soil column

Soil texture • Sand [%]	35%
• Silt [%]	57.5%
• Clay [%] •	7.5%
Moisture %	3.3%
Moisture correction factor (mcf)	1.033
pH	7.63
Organic Carbon %	2.11%
Organic Matter %	3.63%
Conductivity(µs)	530
N%	0.1934%
Ca CO_3 (mg)	0.795
Cu (mg/kg)	44
Fe (mg/kg)	1982.27
Available Phosphorous (P) (mg/kg)	62.41

Leachate extraction columns

Leachate extraction columns consist of four columns of polyvinyl chloride (PVC) pipe. A metal mesh screen was placed at the bottom end of each column and a plastic bottle was placed under each column to collect water. Soil column was washed with distilled water to remove air bubbles from soil and to ensure that the pH of leachate water from each column is neutral.

Glyphosate application to soil–column experiment

Glyphosate contains the monoisopropylamine salt of glyphosate (N–(phosphonomethyl)–glycine) (360 g/L) was applied to each column with concentrations; 5 X, 15 X and 25 X, where X equals amount of glyphosate applied to soil yearly (nearly 2 L/dunom), numbers (5, 15, 25) are the years of applying glyphosate to soil. Blank soil samples were used as controls without glyphosate addition. The concentrations of glyphosate added to soil columns are listed in Table 7.1.3.1.1-84.

Leachate

Leachate was collected from each column in plastic bottle at the end of every period. Leachate volumes were determined gravimetrically. Leachate water was centrifuged to remove solid particles and then the supernatant was filtered before analysis. Glyphosate extracted by the method described below and derivatized using the method shown below then measured by Spectrometer at 435 nm.

Procedure for Solid–Phase Extraction (SPE) of glyphosate from water samples
A cation exchange resin was used for the pre concentration and cleanup of glyphosate. A slurry of the Amberlite IR–120, Na–ion exchange resin (cationic) was made in 10 mL distilled water and packed into a narrow glass column, plugged with glass wool at the bottom. The resin was rinsed with distilled water and then with 1 M HCl at a flow rate of 2 mL/min several times before sample application. The pH of water sample spiked with glyphosate was adjusted to 2 and amine group of glyphosate was converted into its protonated form. The protonated sample (25 mL) was passed through the column at a flow rate of 0.5 mL/min in order to have maximum exchange of protonated sample. After the loading step, the sorbent was washed with 25 mL of 2 M NaCl solution (used as eluent) at the same flow rate. The eluted solution was evaporated to about 10 mL at 70ºC then evaluated by the proposed method.

Table 7.1.3.1.1-84: Main characteristics of soil after application of glyphosate at different depths

Column	Depth (cm)	PH	C %	O.M %	N %	Available P mg/kg	CaCO3 %	Fe mg/kg	Cu mg/kg
Blank	0-30	7.45	1.56	2.69	0.145	7.91	0.211	1941	43
	3-60	7.78	1.53	2.64	0.082	5.3	0.245	1997	38
	60-100	7.7	1.36	2.33	0.024	5.27	0.292	2008	52
5x	0-30	7.55	2.08	3.58	0.321	66.62	0.147	1853	30
	30-60	7.86	2.05	3.53	0.270	48.49	0.161	1953	35
	60-100	7.72	2.03	3.49	0.250	45.71	0.199	2000	64
15x	0-30	7.68	2.08	3.58	0.373	93.53	0.194	1909	35
	30-60	7.75	2.02	3.48	0.356	66.1	0.197	2053	44
	60-100	7.88	1.99	3.42	0.305	53.6	0.208	2103	52
25x	0-30	7.49	2.21	3.80	0.423	95.04	0.178	1909	24
	30-60	7.56	2.05	3.53	0.378	88.31	0.206	1985	29
	60-100	7.66	2.01	3.46	0.309	74.13	0.200	2032	34

Derivatization procedure of glyphosate
Glyphosate was derivatized using carbon disulfide to convert the amine group into dithiocarbamic acid. The dithiocarbamate group was used as chelating group for reaction with transition metal ion Cu (II). The resultant yellow colored complex was measured at 435 nm using UV–Spectrophotometer. Carbon disulfide (1 % CS_2) solution was prepared and an aliquot of glyphosate were added to a series of 100 mL separating funnels followed by the addition of CS_2 solution. Then the mixture was shaken for 3 minutes for the formation of dithiocarbamic acid. An ammonical solution of Cu(II) (1000 mg/L) was added to the mixture, shaken again vigorously to form complex with dithiocarbamic acid and then kept for separation of two phases. The yellow colored chloroform layer containing the complex was separated in a 10 mL flask and diluted with ethanol. The absorbance of the complex was measured at 435 nm.

Soil columns after glyphosate application
At the end of the experiment, soil columns were cut into three parts. Three samples were taken from each part, air dried and stored in an air tight polythene bottle to analyze their parameters in soil lab at An Najah National University. Glyphosate were extracted from the three parts of soil columns, derivatized and measured spectrophotometrically.

Batch sorption experiment
Sorption kinetics was analyzed by altering the contact time at a constant concentration of 20 and 30 ppm per vessel for determination of an appropriate equilibrium time at room temperature for the sorption isotherm experiments. They were shaken for 1, 2, 4, 6, 8, 24, 48 and 72 hours, respectively. Samples were equilibrated and processed.

Adsorption isotherm experiment
Soil samples were air–dried, sieved, stored in the dark at room temperature (23°C), and protected from humidity. Sorption experiments were carried out using the standard batch equilibration method. A series of

five selected glyphosate concentrations were carried out to determine the adsorption isotherms of glyphosate on soil. The adsorption measuring steps were as follows:

- 200 mL of a PTFE vessels containing 25 g air dried weight soil.
- 100 mL aqueous solutions containing 0–50 mg/L glyphosate were equilibrated for 24 h at room temperature on a reciprocating shaker at low speed 120 excursions per minute.
- The supernatant equilibrium concentration is obtained after centrifuging at 3000 rpm (round per minute) for 20 minutes.
- Blank without glyphosate was also equilibrated. The equilibrium concentrations of each soil were measured spectrophotometrically after derivatization.

Consequently, the differences between the initial and equilibrium concentrations were assumed to be due to sorption onto soil. Sorption isotherms were obtained by plotting the amount of glyphosate sorbed per weight of soil at equilibrium (Qe, µg/g) versus the amount of glyphosate per volume of solution at equilibrium (Ce, µg/mL). The sorption data were described using the Freundlich equation:

$$Qe = K_f . C_e^{nf} \qquad \text{eq. 1}$$

where Qe is the concentration of glyphosate sorbed onto the solid phase (µg/g), Ce is the concentration of glyphosate in solution at equilibrium (µg m/L), and Kf (in $\mu g^{1-nf} mL^{nf} g^{-1}$) and nf are empirical constants which are related to the adsorption phenomenon and calculated by regression analysis. Kf can be considered as a characterisation of the intensity of sorption, modulated by the deviation from the unity of the nf exponent.

Glyphosate extraction from soil samples
Homogenized soil sample (10 g) was extracted for 60 min with 25 mL of 2 M NH_4OH solution. The extraction was repeated three times. The pH of eluted sample was re–adjusted to pH 5.4 and was evaluated by the proposed method. Each recovery was performed in triplicates.

Results and Discussion
Batch sorption experiments
The sorption kinetics of the soil were studied to determine an appropriate shaking time for the sorption isotherm experiments. Readings were recorded until 72 hours, no changes in concentrations were observed after 24 hours for all samples, and therefore 24 hours were chosen as equilibrium time for the sorption isotherm experiment due to the quick degradation of glyphosate. The equilibrium adsorption data over the range of concentrations studied here were used to fit Freundlich adsorption equation (eq. 1). The values of n within the range of 2–10 represent good adsorption. Higher values of *k* indicate high adsorption capacity. The isotherm equilibrium results for the examined soil are shown in Figure 7.1.3.1.1-74. Freundlich isotherm constants (*k* & n) for glyphosate, the correlation coefficient "R" were obtained from Figure 7.1.3.1.1-74 and listed in Table 7.1.3.1.1-85. Glyphosate sorption at 25°C in the studied soils was evidenced to be a kinetics process, with a reasonable equilibration time of 24 hours. Literature usually reports Freundlich adsorption constants for glyphosate adsorption by soils which are consistent with that founded in our study. It is indicated from Table 7.1.3.1.1-85 and Figure 7.1.3.1.1-74 that "n" of glyphosate adsorption is higher than 1. The adsorption isotherms for the soil is of S–type, which indicates the easiness of the adsorption, mainly at higher concentrations.

Figure 7.1.3.1.1-74: Adsorption isotherm of glyphosate for Palestinian soil

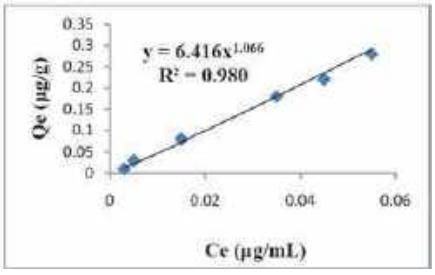


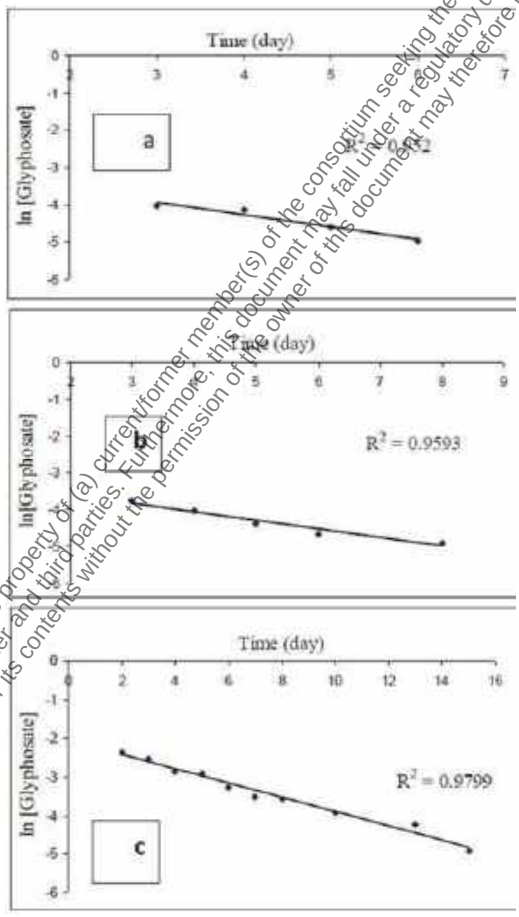
Table 7.1.3.1.1-85: Freundlich isotherm constants for glyphosate

Coefficient	K	1/n	n	R^2
Glyphosate	6.41	0.93	1.07	0.98

Glyphosate in leachate

It is indicated that the amount of glyphosate detected in leachate decreases with increasing time. It takes time for 25 x >15 x >5 x until the inability to detect glyphosate in leachate for concentrations less than 1 ppm. Doubling the concentration of glyphosate increases the amount glyphosate (contaminant) in leachate. The above resulting curves shows that the best fit of the glyphosate degradation data was obtained using a first–order reaction as shown in Figure 7.1.3.1.1-75. DT_{50} values of glyphosate was 2, 3 and 3.75 days for 5 x, 15 x & 25 x column respectively. This indicate relatively rapid degradation.

Figure 7.1.3.1.1-75 Plot of time vs. Ln concentration for 5 X (a), for 10 X and (c) for 25 X times glyphosate

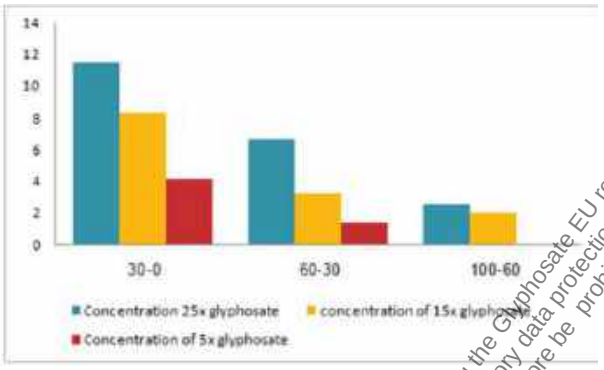


Glyphosate in column soil

The results indicated that the glyphosate mobility in the soil columns increased with application rate. With more glyphosate applied, more glyphosate in the soil columns was capable of moving out of the columns. Amount of glyphosate detected in soil columns was increased in the order: 25 x >15 x >5 x. The amount of glyphosate was decreased with depth increasing due to decreasing organic content. It means that the adsorption tendency decreases as the depth increases. No glyphosate detected in 60–100 cm depth as shown in Figure 7.1.3.1.1-76. This due to low concentration of glyphosate less than 1 ppm that couldn't be measured by the method used here. Lowest concentration was used also most of glyphosate adsorbed on

the upper layer of soil (0–30 cm). This study indicates that glyphosate can be extensively mobile in soil environment if it is applied on soils unable to retain the molecule long enough for its microbial degradation. This may also lead to herbicide leaching to lower soil layers where a limited biological activity occurs.

Figure 7.1.3.1.1-76: Concentration (mg/L) of glyphosate in soil column at different depths



The effect of organic matter

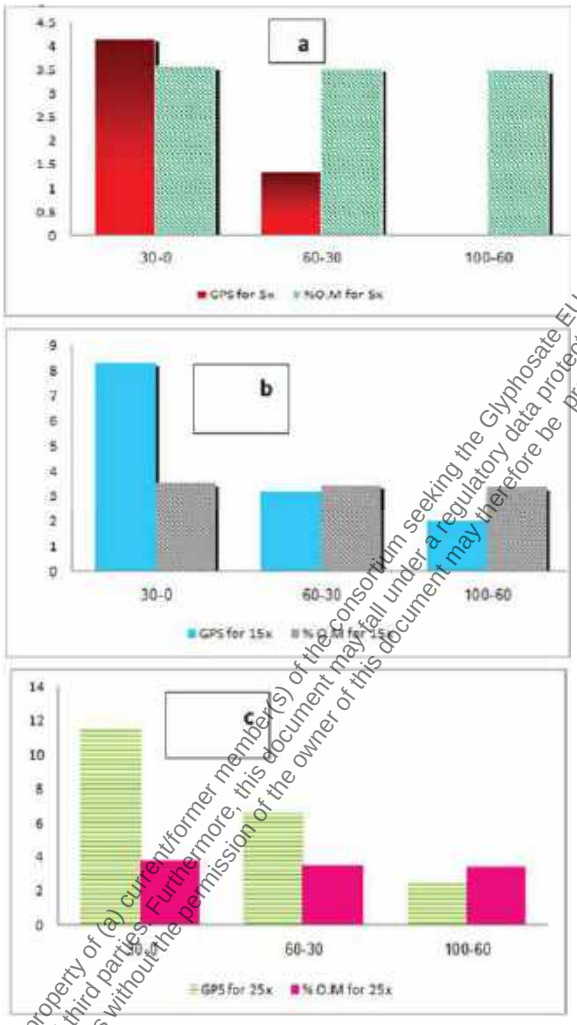
Soil organic matter consists of a variety of components. These include, in varying proportions and many intermediate stages:

- Raw plant residues and microorganisms (1 to 10 %).
- "Active" organic traction (10 to 40 %).
- Resistant or stable organic matter (40 to 60 %) also referred to as humus.

Table 7.1.3.1.1-84 shows that organic matter content of the soil at different depths ranges between 2–3.8 % which is considered as a moderate organic matter soil. Organic matter content of the soil at different depths for each column nearly the same as shown in Figure 7.1.3.1.1-77. It is indicated that organic matter only may not affect the adsorption of glyphosate at different depths and it could affect sorption in two ways:

- Reducing glyphosate sorption by blocking sorption sites.
- Increasing glyphosate sorption because poorly ordered aluminium and iron oxides with high sorption capacity are favored at higher soil organic matter content.

Figure 7.1.3.1.1-77: Organic matter content in 5 X (a), 15 X (b) and 25 X (c) column and concentrations of glyphosate at certain depths



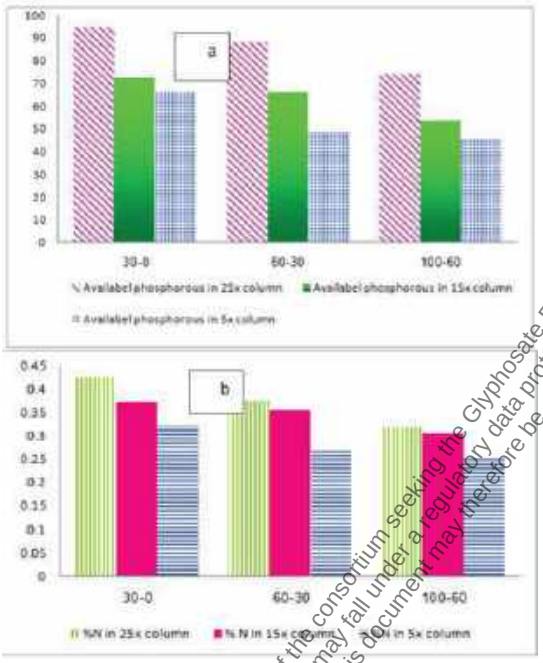
The effect of soil metals

The high sorption values for glyphosate can be in part due to the pH values of soils and to the presence of iron oxides, copper and other metals that can form stable complexes with glyphosate. Glyphosate coordinates strongly to Cu, and Cu–glyphosate complexes formed seem to have higher ability to be adsorbed on the soil than free glyphosate. Copper acts as a bridge between the soil and glyphosate. At these pH values glyphosate is a di–anion and both the carboxylate and the phosphonate functional groups in its molecule are deprotonated, being able to compete for the surface adsorption sites on the metal oxides.

Available phosphorous after glyphosate application

Figure 7.1.3.1.1-78 shows that the amount of phosphorous in soil columns after application of glyphosate increased this indicates degradation of glyphosate to its components where phosphorous is one of the degradation products. Glyphosate could be source of phosphorous, nitrogen and carbon in soil as it is shown in Figure 7.1.3.1.1-78 and Table 7.1.3.1.1-83. The nitrogen content of soil has been increased after glyphosate application to soil columns due to biodegradation of glyphosate.

Figure 7.1.3.1.1-78: Phosphorous content (a) and nitrogen content (b) in soil columns after application of glyphosate



Conclusion

Adsorption is an important process in determining the fate of glyphosate in soil. The texture for soil used has been found to be silty clay and the total organic matter (TOM) close to 4 %. Batch equilibrium technique was used to evaluate the extent of glyphosate adsorption on soil as adsorbent. Isotherm is in accord with the Freundlich adsorption equation with R^2 value 0.98; the parameters of this isotherm have been calculated. The adsorption isotherm was fit the S–type isotherm according to Giles. The values of "n" in Freundlich equitation was more than one indicating good adsorption for glyphosate with the soil used. Freundlich constant "*k*" indicates the tendency of glyphosate in this study to be adsorbed on soil particles. *k* increases with increasing the soil minerals and decreases with increasing the depth of soil where the main binding mechanism for glyphosate is the covalent bond between the herbicide and the metals from soil oxides, and so the adsorption decreasing due to decreasing the organic matter content as depth increases. Many factors affect the adsorption of glyphosate as phosphorous content, pH, and temperature. The high sorption values for glyphosate can be in part due to the presence of metal oxides that can form stable complexes with glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes a column leaching andadsorption tests with non-labelled glyphosate with a Palestinian agricultural soil.

Due to analytical method insensitivity, the lowest rate examined in the column leaching experiment was 5 times the yearly application rate. In addition, some essential information necessary for assessment of validity of both experiments is not reported (i.e. mass balances, equilibration solution not specified).

The article is classified as not reliable for the column leaching experiment and as reliable with restrictions for the adsorption experiment, i.e. it was not used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.1/028
Report author	Rampoldi, E., *et al.*
Report year	2014
Report title	Carbon-14-Glyphosate Behavior in Relationship to Pedoclimatic Conditions and Crop Sequence
Document No	DOI 10.2134/jeq2013.09.0362 E-ISSN 1537-2537
Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

The article was found potentially relevant for multiple data points. The summary is provided under CA 7.1.2.1.1/014.

1. Information on the study

Data point:	CA 7.1.3.1.1/028
Report author	Bergström, L. *et al.*
Report year	2011
Report title	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil
Document No	DOI 10.2134/jeq2010.0179 E-ISSN 1537-2537
Guidelines followed in study	OECD Guideline 106 Guideline
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

The article was found potentially relevant for multiple data points. The summary is provided under CA 7.1.2.1.1/017.

Assessment of pH dependency of adsorption parameters of glyphosate

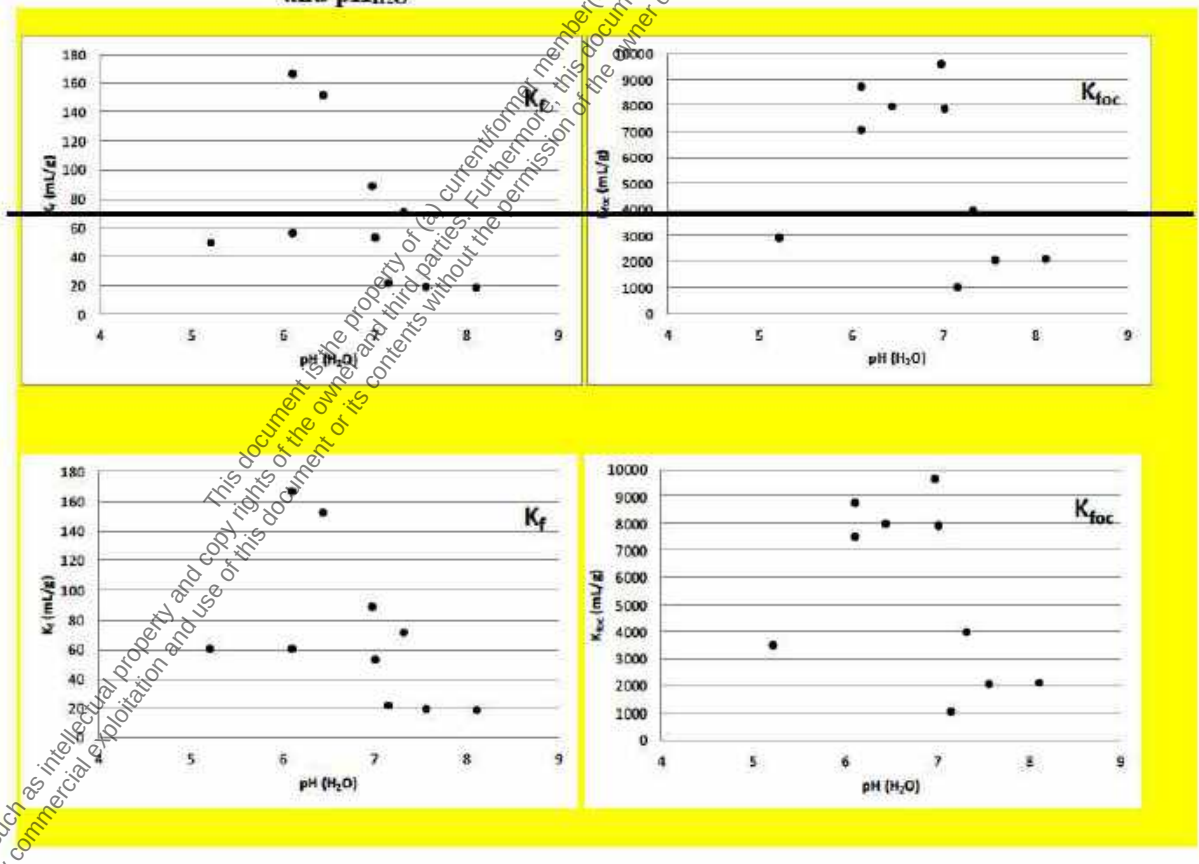
Following evaluation of data from existing and new adsorption studies, this resulted in 10 sets of Freundlich adsorption data derived from 10 soils from the new study by [redacted] (2020, CA 7.1.3.1.1/001, CA 7.1.3.1.1/002 and CA 7.1.3.1.1/030).

The pH dependency of the adsorption parameters $K_{F(ads)}$ and $K_{F, OC(ads)}$ was assessed using the German Input Decision Tool 3.3 (Holdt, G. *et al.* (2012). Recommendations for simulation calculation to predict environmental concentrations for active substances of plant protection products and their metabolites in groundwater (PECgw) in the National Authorisation procedure in Germany) using pH values measured in H_2O. For glyphosate, there is ~~no~~ a significant correlation between the pH-value and the adsorption coefficient $K_{F(ads)}$ but not between pH-value and $K_{F, OC(ads)}$. ~~Therefore, it is concluded that the adsorption behaviour of glyphosate is not pH dependent.~~

Table 7.1.3.1.1-86: Glyphosate: Correlation parameters for $K_{F(ads)}$ and $K_{F, OC(ads)}$ values and pH_{H2O} values

Compound	Parameter	Kendall tau (stringency of the correlation)	p (level of significance)
Glyphosate	$K_{F(ads)}$	~~-0.494~~ -0.539	~~0.059~~ 0.039
	$K_{F, OC(ads)}$	~~-0.315~~ -0.315	~~0.243~~ 0.243

Figure 7.1.3.1.1-79: Glyphosate: Correlation between $K_{F(ads)}$ values and pH_{H2O} as well as $K_{F, OC(ads)}$ and pH_{H2O}



CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

Table 7.1.3.1.2-87: Adsorption/desorption studies on AMPA

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.3.1.2/001	[redacted] 2020	Batch adsorption	AMPA	-	Report not yet available
CA 7.1.3.1.2/002	[redacted], 2003	Batch adsorption	AMPA	Invalid	
CA 7.1.3.1.2/003	[redacted], 2002	Batch adsorption	AMPA	Valid	
CA 7.1.3.1.2/004	[redacted] 1996	Batch adsorption	AMPA	Supportive	
CA 7.1.3.1.2/005	[redacted] 1993	Batch adsorption	AMPA	Invalid	
CA 7.1.3.1.2/006	[redacted] 1993	Batch adsorption	AMPA	Valid	

Table 7.1.3.1.2-88: Adsorption/desorption – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.3.1.2/007	Skeff *et al.*, 2018	Batch adsorption	Glyphosate, AMPA	Reliable with restrictions	Summary under CA 7.1.3.1.1/015
CA 7.1.3.1.2/008	Sidoli *et al.*, 2016	Batch adsorption	Glyphosate, AMPA	Reliable with restrictions	Summary under CA 7.1.3.1.1/022

Table 7.1.3.1.2-89: Summary of soil adsorption parameters for AMPA

Study	Soil Type	OC (%)	pH ($CaCl_2$)	pH (H_2O)	K_D (mL/g)	$K_{D, OC}$ (mL/g)	K_F (mL/g)	$K_{F, OC}$ (mL/g)	1/n
[redacted] 1993, CA 7.1.3.1.2/006	SLI Soil #4, sand	1.34	-	7.4 [1]	-	-	15.7	1160	0.752
	SLI Soil #5, clay loam	0.93	-	7.6 [1]	-	-	53.9	5650	0.791
[redacted], 2002, CA 7.1.3.1.2/003	Lufa 2.1, sand	0.9	5.2	5.8 [2]	-	-	16.746	1861	0.665
	Lufa 2.2, loamy sand	2.3	5.6	6.2 [2]	-	-	189.714	8248	0.5506
Geometric mean (if not pH dependent) (n = 4)							**40.5**	**3167**	-
Arithmetic mean (if not pH dependent) (n = 4)							-	-	**0.690**
pH dependence								No	

[1] Measured in 1:1 soil:water solution
[2] Converted during assessment of pH dependency by Input Decision tool v3.3 of Federal Environment Agency (UBA)

1. Information on the study

Data point:	CA 7.1.3.1.2/001
Report author	[redacted]
Report year	2020
Report title	Adsorption/Desorption of ^{14}C-AMPA in Six Soils
Report No	S19-23618
Document No	
Guidelines followed in study	
Deviations from current test guideline	OECD Guideline 106 (January 2000) -
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

At the time of submission, the study had not yet been completed. The final study report will be submitted in August 2020 after the study is expected to be completed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
At the time of submission, the study had not yet been completed. The final study report will be submitted in August 2020 after the study is expected to be completed..

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.2/002
Report author	[redacted]
Report year	2003
Report title	Aminomethylphosphonic acid: adsorption-desorption
Report No	IF-02/00005220
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - No preliminary tests for determination of equilibration time performed - Incomplete parental mass balance (<90 %) - Use of indirect method for evaluation though formation of NER was >10 %
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The adsorption/desorption behaviour of [^{14}C]aminomethylphosphonic acid (AMPA) was studied in three soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 2 °C using the indirect method.

Soil	Origin	Texture (USDA)	pH [1]	OC [2] [%]
Schwalbach	Germany	Silt loam	5.13	1.59
Hofheim	Germany	Silt loam	5.10	1.24
Bergen-Enkheim	Germany	Silty clay	7.43	2.25

[1] pH values were derived from aqueous 0.01 M $CaCl_2$ suspensions
[2] OC: Organic carbon

For the definitive phase, the adsorption and desorption steps of the study were carried out at a soil to solution ratio of 1:5 for 24 hours using pre-equilibrated air-dried soils. Nominal concentrations of [^{14}C]AMPA were 0.05, 0.3, 1, 2.5, and 5 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$. The whole desorption procedure was repeated on the solid phase with a further quantity of 0.01 mol/L $CaCl_2$ without test item.

Material balances were 96.65 to 100.45 % of applied radioactivity (% AR) for soil Schwalbach, 96.72 to 99.22 % AR for soil Hofheim and 96.55 to 99.89 % AR for soil Bergen-Enkheim.

Within the parental mass balance test 65.28, 69.12 and 27.13 % AR could be extracted after the desorption steps for soils Schwalbach, Hofheim and Bergen Enkheim, respectively. Considering residues in aqueous adsorption and desorption supernatants non-extractable residues amounted to approx. 29.5, 22.4 and 50.3 % AR for soils Schwalbach, Hofheim and Bergen Enkheim. In aqueous supernatants and soil extracts AMPA amounted to at least 95 % AR.

At the end of the adsorption phase 94.94 to 97.85 %, 94.13 to 97.02 % and 86.40 to 92.82 % AR were adsorbed to soils Schwalbach, Hofheim, and Bergen-Enkheim, respectively.

The adsorption coefficients $K_{F(ads)}$ calculated based on the Freundlich isotherms of the four test soils ranged from 33.9 to 137.4 mL/g and the normalized adsorption coefficients $K_{F, OC(ads)}$ (normalized to organic carbon content) ranged from 1507 to 8642 mL/g. The Freundlich exponents 1/n were in the range of 0.907390 to 0.982426.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[14C]AMPA	
Code	CFQ12959
Specific activity	55 mCi/mmol
Radiochemical purity	98.6 % by HPLC, 98.3 % by TLC
Chemical purity	Not provided

2. Test Soils

The soils were collected fresh from the field before study start (upper horizon of 0 to 20 cm), sieved to a particle size of ≤2 mm and stored at ambient conditions in the laboratory. The soils were air-dried before application. The locations of soil collection were of no agricultural use and no plant protection products were used for several years. The characteristics of test soils is summarised in the table below.

Table 7.1.3.1.2-90: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	Schwalbach	Hofheim	Bergen-Enkheim
Geographic Location			
City	North-east of Schwalbach/Limes	North of Hofheim and south of Kelkheim	South-east of Bergen and north-east of Enkheim
State	Not provided	Not provided	Not provided
Country	Germany	Germany	Germany
Textural Class (USDA)	Silt loam	Silt loam	Silty clay
Sand (50 µm – 2 mm)	10.9	29.9	16.7
Silt (2 µm – 50 µm)	68.2	52.3	41.4
Clay (< 2 µm)	20.9	17.8	41.9
pH			
- in $CaCl_2$	5.13	5.10	7.43
- in water	6.09	6.06	8.30
Organic Carbon	1.59	1.24	2.25
Organic Matter [1]	2.74	2.14	3.88
Cation Exchange Capacity (meq/100 g)	14.6	13.5	28.9
Water Holding Capacity			
maximum (%)	48.5	43.0	49.4
Bulk Density (disturbed) (g/cm³)	1.00	1.12	1.06

[1] Calculated as: OM [%] = OC [%] × 1.724
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Plastic centrifuge tubes (750 mL) were used as test systems. The experiments were performed in duplicate.

In preliminary tests, the optimal soil-to-solution ratio and the stability of the test item in 0.01 M $CaCl_2$ solution were determined. The stability of AMPA (parental mass balance) was investigated in the course

of the definitive test following the desorption phase (no extraction performed following the adsorption phase).

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:5 (10 g soil (dry weight equivalents)/50 mL solution). APMA was applied at nominal concentrations of 0.05, 0.3, 1, 2.5 and 5 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 24 hours in the dark at 20 ± 2 °C under continuous agitation. For the desorption phase of the study, the volume of the aqueous solution removed after the adsorption step was replaced by an equal volume of 0.01 mol/L $CaCl_2$ without test item. The mixture was agitated 24 h and centrifuged as in the adsorption step. The whole desorption procedure was repeated on the solid phase with a further quantity of 0.01 mol/L $CaCl_2$ without test item.

2. Analytical Procedures

Following each adsorption or desorption step, the aqueous supernatant was separated from the soil by centrifugation and the amount of Test item in the supernatants was analysed by liquid scintillation counting (LSC).

Following the desorption steps, the remaining adsorbed test item based on the highest test concentration used was extracted two times from soil using 1 M NH_3 at ambient temperature. The ratio of extraction solvent and soil was approximately 1:1 (volume:soil dry weight). Specimen agitation was performed for 1 hour. After shaking, the extraction solvent was removed from the slurry by centrifugation. The residual radioactivity in soils was determined by combustion/LSC. Soil extracts were analysed by LSC and TLC-radiodetection to determine the stability of the test item and to establish the parental mass balance.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Material balances were 96.65 to 100.45 % AR for soil Schwalbach, 96.72 to 99.22 % AR for soil Hofheim and 96.55 to 99.89 % AR for soil Bergen-Enkheim.

B. STABILITY OF TEST ITEM

AMPA was sufficiently stable in aqueous 0.01 mol/L $CaCl_2$ solution. Furthermore, recovery of AMPA in aqueous 0.01 mol/L $CaCl_2$ solution, which was agitated with soil following separation by centrifugation and application to the clear supernatants, ranged from 89.8 to 98.1 % AR. Within the parental mass balance test 65.28, 69.12 and 27.13 % AR could be extracted after the desorption steps for soils Schwalbach, Hofheim and Bergen Enkheim, respectively. Considering residues in aqueous adsorption and desorption supernatants non-extractable residues amounted to approx. 29.5, 22.4 and 50.3 % AR for soils Schwalbach, Hofheim and Bergen Enkheim. In aqueous supernatants and soil extracts AMPA amounted to at least 95 % AR.

C. FINDINGS

At the end of the adsorption phase 94.94 to 97.85 %, 94.13 to 97.02 % and 86.40 to 92.82 % AR were adsorbed to soils Schwalbach, Hofheim, and Bergen-Enkheim, respectively (Table 7.1.3.1.2-91). The adsorption coefficients $K_{F(ads)}$ of test item calculated based on the Freundlich isotherms of the four test soils ranged from 33.9 to 137.4 mL/g (mean: 86.4 mL/g) and the normalized adsorption coefficients $K_{F,\ OC(ads)}$ (normalized to organic carbon content) ranged from 1507 to 8642 mL/g (mean: 5746 mL/g). The Freundlich exponents 1/n were in the range of 0.907390 to 0.982426 (mean: 0.937734 (Table 7.1.3.1.2-92).

At the end of the desorption phase, 1.41 to 2.27 %, 1.61 to 5.22 % and 6.07 to 10.58 % of the initially adsorbed amount was found desorbed from soils Schwalbach, Hofheim and Bergen-Enkheim, respectively.

Table 7.1.3.1.2-91: [^{14}C]AMPA: Percentage of adsorbed/desorbed in soils (mean values)

Soil	Test Concentration (nominal) [mg/L]									
	Adsorption [1]					Desorption [2]				
	0.05	0.3	1.0	2.5	5.0	0.05	0.3	1.0	2.5	5.0
Schwalbach	*97.03*	*95.44*	*97.41*	*96.12*	*97.00*	*1.44*	*2.22*	*1.72*	*1.58*	*2.21*
Hofheim	*96.69*	*95.46*	*95.61*	*96.48*	*94.39*	*1.70*	*3.30*	*3.57*	*4.36*	*2.92*
Bergen-Enkheim	*92.66*	*86.96*	*90.83*	*88.85*	*87.49*	*6.15*	*11.10*	*8.43*	*9.70*	*10.08*

[1] End of adsorption phase, values expressed as percentage of applied radioactivity
[2] Sum of steps one and two of desorption phase, values expressed as percentage of applied radioactivity
Values calculated in the course of writing this summary are given in *italics*

Table 7.1.3.1.2-92: [^{14}C]AMPA: Adsorption parameters in soil at 20 °C

Soil	Adsorption			
	K_F [mL/g]	1/n	R^2	$K_{F, OC}$ [mL/g]
Schwalbach	137.4	0.982426	0.978367	8642
Hofheim	87.9	0.923385	0.989020	7089
Bergen-Enkheim	33.9	0.907390	0.989228	1507

III. CONCLUSIONS

The individual results of the adsorption coefficients on basis of soil organic carbon ($K_{F, OC(ads)}$), assessed with the aid of the Freundlich adsorption isotherm were: Schwalbach test system 8642 mL/g with 1/n of 0.982426; Hofheim test system 7089 mL/g with 1/n of 0.923385; Bergen-Enkheim test system 1507 mL/g with 1/n of 0.907390.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The test was performed using the indirect method for determination of adsorption following the decrease of the test item in aqueous supernatant. This is allowed for the definitive phase following the current EU OECD 106 Evaluators Checklist in case the stability of the test item had been demonstrated in terms of the parental mass balance (PMB). The parental mass balances (PMB) were below 90 % AR to result in NER >10 % for all soils.

The results of the study are thus considered as invalid. Further evaluation of results according to the Evaluators Checklist is shown for informative reasons only.

Assessment and conclusion by RMS:

Results of the parental mass balance are not reported in detail, hence no f-factor can be specified and the check for system error cannot be performed. Therefore, the results of the study are considered as not reliable and an evaluation following the EFSA OECD 106 Evaluators Checklist is considered not necessary. However, for the sake of completeness results of the evaluation according to EFSA OECD 106 Evaluators Checklist are provided below.

Table 7.1.3.1.2-93: Results of evaluation according to EFSA OECD 106 Evaluators Checklist for AMPA

	Units	Bergen-Enkheim	Schwalbach	Hofheim
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw mL	1:5	1:5	1:5
Parental mass balance (at highest conc.)	%	<90 [1]	<90 [1]	<90 [1]
Adsorbed percentage	%	86.4-92.8	94.9-97.9	94.1-97.0
K_D x (soil:solution ratio)		6.4-13.0	18.8-45.6	16.0-32.6
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	33.929 (26.699-43.116)	137.371 (84.902-222.264)	87.913 (64.660-119.528)
$^{ads}1/n$ (95 % confidence interval)	-	0.907 (0.830-0.985)	0.982 (0.863-1.102)	0.923 (0.844-1.003)
$^{ads}R^2$	-	0.989	0.978	0.989
$^{ads}K_{F,OC}$	L/kg OC	1508	8640	7090
K_{FE} / K_F	-	- [2]	- [2]	- [2]

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] Extraction performed was not exhaustive (NER >10 %) resulting in a PMB <90 %.
[2] The check for systemic errors (expressed as K_{FE} / K_F) could not be performed due to missing results of parental mass balance test providing the f-factor necessary for the calculations.

Figure 7.1.3.1.2-1: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Bergen-Enkheim

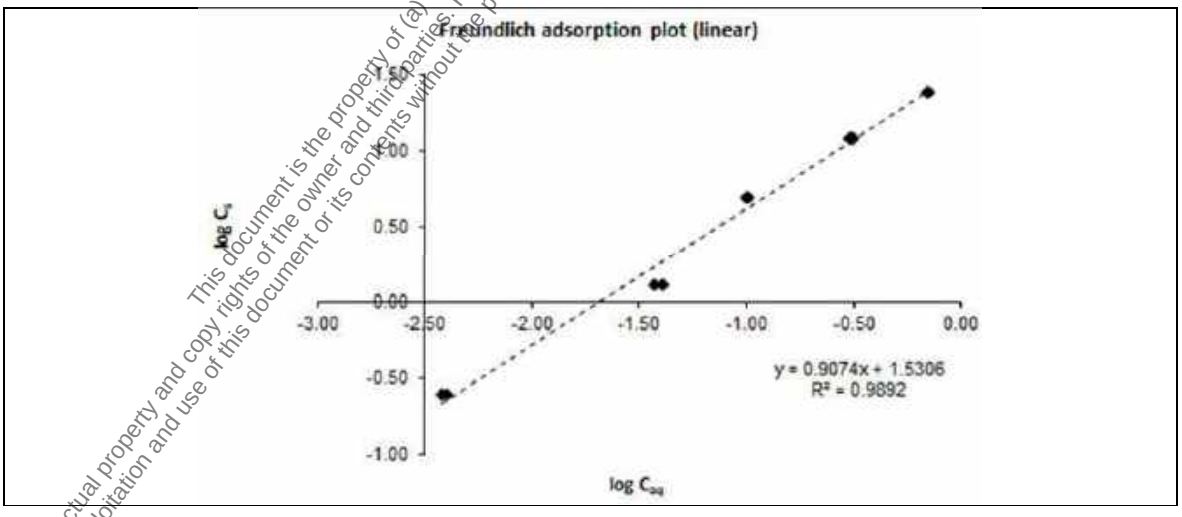


Figure 7.1.3.1.2-1: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Bergen-Enkheim

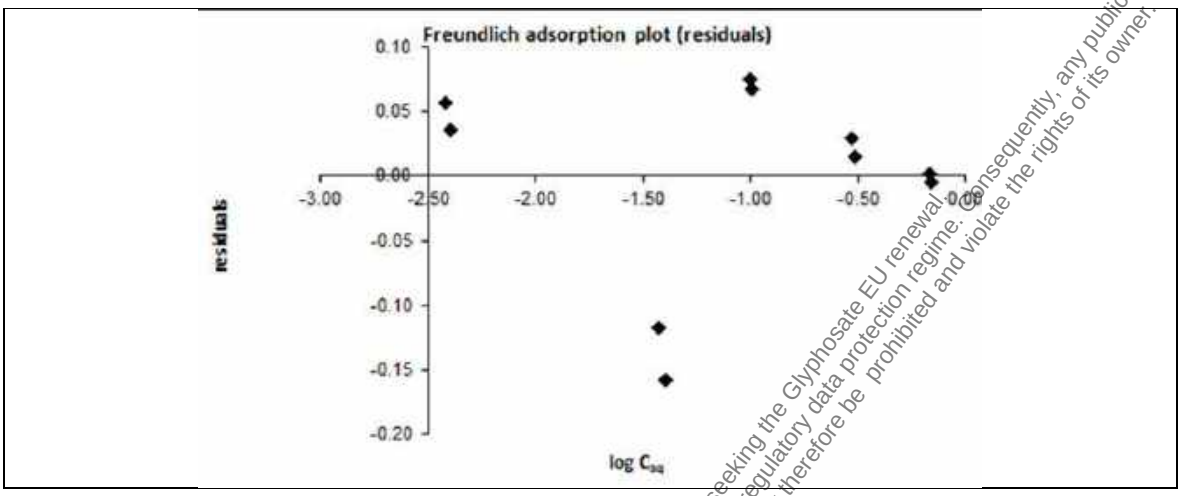


Figure 7.1.3.1.2-2: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Schwalbach

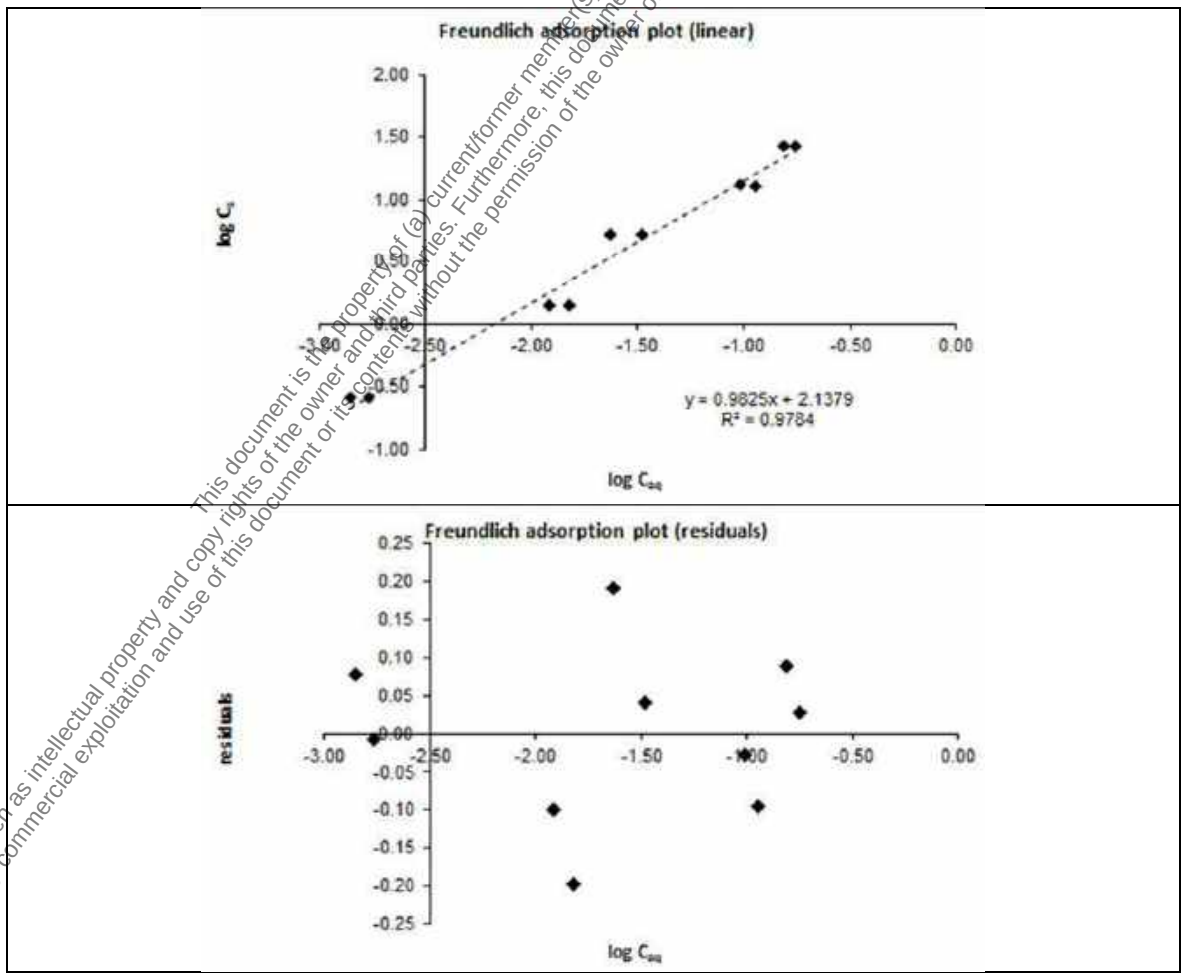
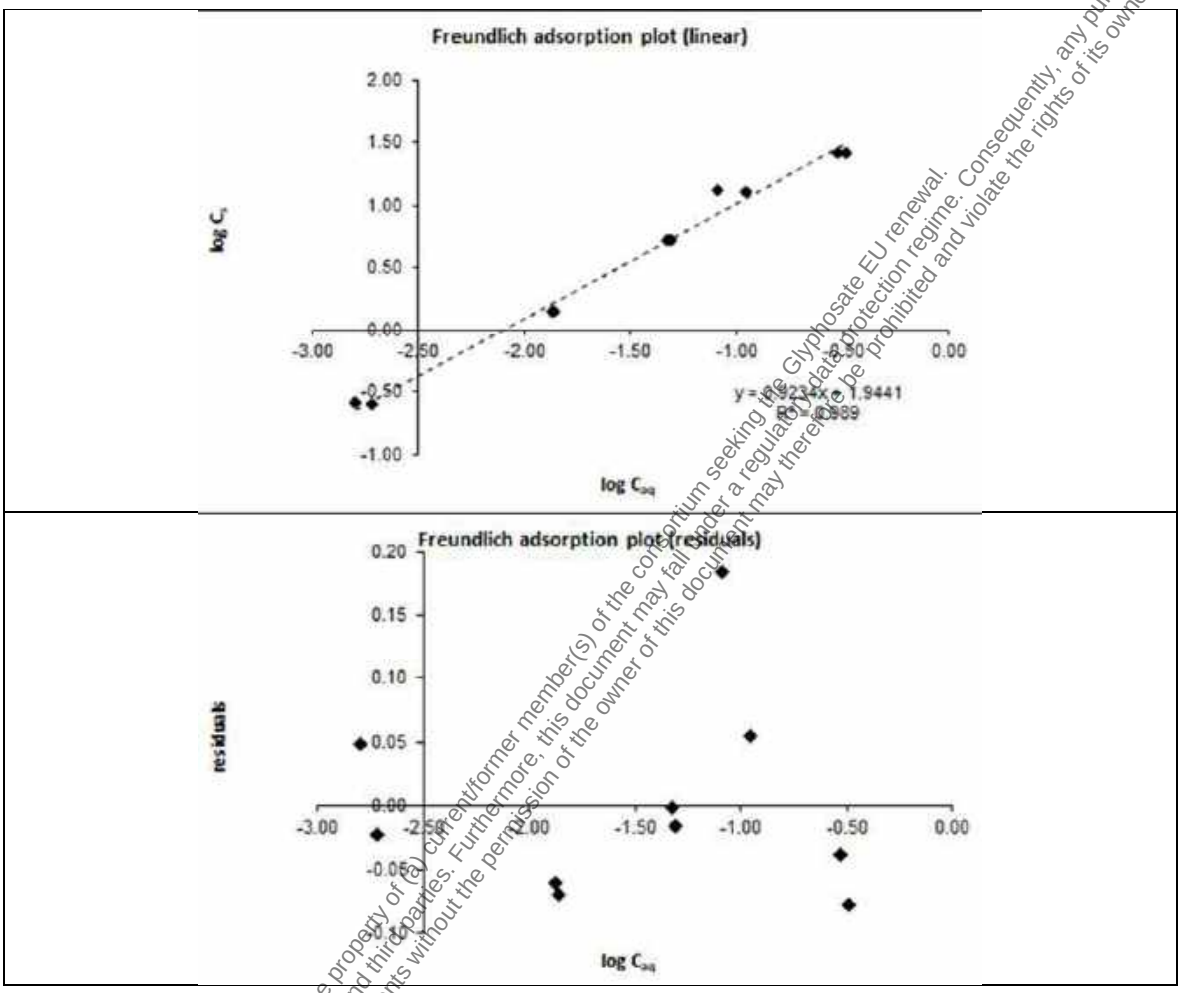


Figure 7.1.3.1.2-3: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Hofheim



1. Information on the study

Data point:	CA 7.1.3.1.2/003
Report author	[redacted]
Report year	2002
Report title	Adsorption/desorption behaviour of AMPA on soil according OECD Guideline 106 (adopted January 2000)
Report No	PR02/007
Document No	
Guidelines followed in study	OECD Guideline 106
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Soil 3A showed high K_{FE}/K_F value of 1.6 indicating systemic errors according to EFSA Evaluators Checklist
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The adsorption / desorption behaviour of [^{15}N]aminomethylphosphonic acid (AMPA) was studied in three soils in batch equilibrium experiments in the laboratory at 22 ± 2 °C using the indirect method.

Soil	Origin	Texture (USDA)	pH [1]	OC [%]
Lufa 2.1	Germany	Sand	5.2	0.9
Lufa 2.2	Germany	Loamy sand	5.6 ± 0.4	2.3 ± 0.2
Lufa 3A	Germany	Sandy Silty Loam	7.1 ± 0.0	2.6 ± 0.7

[1] pH values were derived from aqueous 0.01 M $CaCl_2$ suspensions

For the definitive phase, the adsorption step was carried out for 48 hours at a soil to solution ratio of 1:50 for soils Lufa 3A and Lufa 2.2 and 1:25 for soil Lufa 2.1 using pre-equilibrated samples of air-dried soils. Nominal concentrations of AMPA were 10.0, 3.0, 1.0, 0.30 and 0.10 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$.

The desorption step was conducted using each soil and each concentration of AMPA with a single desorption cycle for 48 hours.

Following the desorption step, between 4.1 and 49.4 % of the initially adsorbed amounts of AMPA were desorbed.

Recovery of AMPA was 95.8 % for soil Lufa 2.1, 92.3 % for Lufa 2.2 and 91.1 % for Lufa 3A after 48 h of equilibration within the preliminary parental mass balance test.

The adsorption coefficients $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 16.746 to 189.714 mL/g (arithmetic mean: 78.52 mL/g). The Freundlich exponents 1/n were in the range of 0.5506 to 0.6710 (arithmetic mean: 0.629). The corresponding, calculated $K_{F, OC(ads)}$ values varied between 1119 and 8248 mL/g (arithmetic mean: 3743 mL/g).

The desorption constants $K_{F(des)}$ of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 21.38 to 49.48 mL/g (arithmetic mean: 37.55 mL/g). The Freundlich exponents 1/n were

in the range of 0.9729 to 0.9894 (arithmetic mean: 0.9790). The corresponding, calculated $K_{F, OC(des)}$ values varied between 1607 and 2376 mL/g (arithmetic mean: 2045 mL/g).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[15N]Aminomethylphosphonic acid (stable labelled)	
Lot No.	UCL01/95
Specific activity	Not provided
Purity	98.8 %

2. Test Soils

The standard soils were air-dried at ambient temperature before application. The characterisation of test soils used is summarised in the table below.

Table 7.1.3.1.2-94: Physico-chemical properties of test soils

Parameter	Results		
Soil Designation	Lufa 2.1	Lufa 2.2	Lufa 3A
Geographic Location			
City	Not provided	Not provided	Not provided
State	Not provided	Not provided	Not provided
Country	Germany	Germany	Germany
Textural Class (USDA)	Sand	Loamy sand	Sandy Silty Loam
Sand (50 µm – 2 mm)	87.2	75.3 ± 2.0	47.3 ± 2.3
Silt (2 µm – 50 µm)	9.0	16.6 ± 1.4	35.9 ± 2.2
Clay (< 2 µm)	3.8	8.1 ± 1.2	16.9 ± 0.1
pH - in $CaCl_2$	5.2	5.6 ± 0.4	7.1 ± 0.0
Organic Carbon (%)	0.9	2.3 ± 0.2	2.6 ± 0.7
Organic Matter (%) [1]	1.5	4.0	4.5
Cation Exchange Capacity (mval/100 g)	6	11 ± 2	19 ± 5
Water Holding Capacity			
maximum (g H_2O ad 100 g soil DW)	30	50 ± 5	50 ± 7
Bulk Density (disturbed) (g/cm³)	1.42	1.15 ± 0.038	1.1 ± 0.12

[1] Calculated using the conversion factor as follows: % organic matter = % organic carbon × 1.72
DW: Dry weight, USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

The test system for batch equilibrium experiments consisted of 250 mL glass bottles with polymer screw caps.

In preliminary tests, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times (for soil Lufa 2.2 only) and the stability of the test item in 0.01 M $CaCl_2$ were determined.

All experiments were performed in duplicates.

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:50 (2 g soil (dry weight equivalents)/ 100 mL solution) for soils Lufa 3A and Lufa 2.2 and 1:25 (4 g soil (dry weight equivalents)/ 100 mL solution)for soil Lufa 2.1. Test item was applied at nominal test concentrations of 10.0, 3.0, 1.0, 0.30 and 0.10 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 48 hours at 22 ± 2 °C under continuous agitation.

The desorption phase was performed by supplying pre-adsorbed soil samples with fresh aqueous 0.01 M $CaCl_2$ solution for each test concentration. The resultant samples were re-equilibrated for 48 hours at 22 ± 2 °C under continuous agitation.

2. Analytical Procedures

The aqueous supernatant after each adsorption and desorption step was separated by centrifugation and the AMPA residues in the supernatant were analysed by gas chromatography-mass spectrometry (GC-MS). The chromatographic method was a validated method from a water/sediment study. Applicability of the method on determination of AMPA in 0.01 M $CaCl_2$ supernatants was demonstrated within the current study. The limit of quantitation (LOQ) was 0.029 µg/mL.

In the preliminary mass balance test, the soils were extracted for 15 minutes at ambient temperature in an ultrasonic bath using aqueous NaOH solution (4 NaOH pellets dissolved in 10 mL water) after the adsorption step. The soil extracts were analysed by GC-MS following centrifugation.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. STABILITY OF TEST ITEM

Mean mass balances after 48 h of equilibration during the parental mass balance test were 95.8, 92.3, and 91.1 % for soil Lufa 2.1, Lufa 2.2, and Lufa 3A, respectively.

B. FINDINGS

At the end of the adsorption phase 25.4-70.0 %, 69.0-96.8 %, and 22.9-63.9 % of the applied test material were adsorbed to soils Lufa 2.1, Lufa 2.2, and Lufa 3A, respectively (see Table 7.1.3.1.2-95).

The adsorption constants $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 16.746 to 189.714 mL/g (arithmetic mean: 78.52 mL/g). The Freundlich exponents 1/n were in the range of 0.5506 to 0.6710 (arithmetic mean: 0.629) indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 1119 and 8248 mL/g (arithmetic mean: 3743 mL/g; see Table 7.1.3.1.2-95).

At the end of the desorption phase, 24.33-49.04 %, 4.1-18.7 % and 19.55-48.05 % of the initially adsorbed amount was desorbed in soils LUFA 2.1, LUFA 2.2 and LUFA 3A, respectively.

The desorption coefficients $K_{F(des)}$ of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 21.38 to 49.48 mL/g. The Freundlich exponents 1/n were in the range of 0.9729 to 0.9894. The corresponding, calculated $K_{F, OC(des)}$ values varied between 1607 and 2376 mL/g.

Table 7.1.3.1.2-95: [^{15}N]AMPA: Percentage of adsorbed and desorbed in soils (mean values)

Soil	Test Concentration [mg/L]									
	Adsorption [1]					Desorption [2]				
	10.0	3.0	1.0	0.30	0.10	10.0	3.0	1.0	0.30	0.10
Lufa 2.1	25.4	34.8	47.1	52.0	70.0	49.04	45.08	37.13	31.92	24.33
Lufa 2.2	69.0	82.4	90.6	96.8	n.d.	18.7	13.6	8.25	4.1	n.d.
Lufa 3A	22.9	31.0	39.9	50.7	63.9	48.05	42.85	39.25	31.1	19.55

[1] End of adsorption phase, mean values expressed as percentage of applied radioactivity
[2] End of desorption phase, mean values expressed as percentage of applied radioactivity
n.d.: not detected

Table 7.1.3.1.2-96: [^{15}N]AMPA: Freundlich adsorption/desorption parameters in soil at 22 °C

Soil	Adsorption				Desorption			
	K_F [mL/g]	1/n	R^2	$K_{F, OC}$ [mL/g]	K_F [mL/g]	1/n	R^2	$K_{F, OC}$ [mL/g]
Lufa 2.1	16.746	0.6650	0.9953	1861	21.38	0.9747	0.9999	2376
Lufa 2.2	189.714	0.5506	0.9983	8248	49.48	0.9894	1.0000	2151
Lufa 3A	29.087	0.6710	0.9995	1119	41.78	0.9729	0.9995	1607

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the three test soils ranged from 16.746 to 189.714 mL/g. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 1119 and 8248 mL/g.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is considered valid for soils Lufa 2.1 and 2.2. Although relatively low 1/n values were obtained the results are considered acceptable since all relevant quality checks confirmed the reliability of the results, and the study was performed using a validated analytical method.
Results for soil Lufa 3A are considered as supportive due to a high K_{FE}/K_F of 1.6 indicating potential systemic errors resulting from loss of test item. Therefore, Freundlich coefficients $K_{F(ads)}$ of soil Lufa 3A should be excluded from risk assessment. However, in general it could be possible to derive single concentration K_D values from the parental mass balance test for soil Lufa 3A.

Assessment and conclusion by RMS:

All relevant quality checks as part of confirming the acceptability of the study and of the reported endpoints were performed. These checks confirmed that the parental mass balance of 91.1-95.8 %, and % adsorption of 22.8-97.1 % were all acceptable for all soils (see Table 7.1.3.1.2-97). Systematic errors estimated via K_{FE}/K_F were shown to be low (i.e. ≤1.2) for soils 2.1 and 2.2. For soil 3A systemic errors were shown to be high with K_{FE}/K_F of 1.6. The validity of the analytical method was confirmed over the entire range of concentrations measured (LOQ at least two orders of magnitude lower than lowest test concentration). In general, the use of the indirect method was appropriate based on a K_D x soil/solution ratio >0.3 in all soils. The graphical fits of the Freundlich equation are presented below based on the standard linear regression form using log-log transformed data alongside the associated residual plots (see Figure 7.1.3.1.2-4 and

Figure 7.1.3.1.2-6). The R^2 of the standard linear regressions ranged from 0.994 to 0.999 and the visual fit of the standard regression were acceptable.

Table 7.1.3.1.2-97: AMPA: Evaluation of result according to EU OECD 106 Evaluators Checklist

	Units	3A	2.1	2.2
Adsorption method	-	indirect	indirect	indirect
Soil:solution ratio	g dw mL	1:50	1:25	1:50
Parental mass balance (at highest conc.)	%	91.1	95.8 [1]	92.3
Adsorbed percentage	%	22.7-65.4	25.1-70.8	69.0-97.1
K_D x (soil:solution ratio)		0.3-1.9	0.3-2.4	2.4-36.2
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	29.086 (28.191-30.010)	16.744 (15.380-18.229)	189.555 (175.875-204.299)
$^{ads}1/n$ (95 % confidence interval)	-	0.671 (0.655-0.687)	0.664 (0.623-0.706)	0.550 (0.522-0.578)
$^{ads}R^2$	-	0.999	0.994	0.997
$^{ads}K_{F,OC}$	L/kg OC	1119	1861	8242
K_{FE} / K_F	-	1.6	1.2	1.1

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] Parental mass balance established at a soil:solution ratio of 1:50.

Figure 7.1.3.1.2-4: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 3A

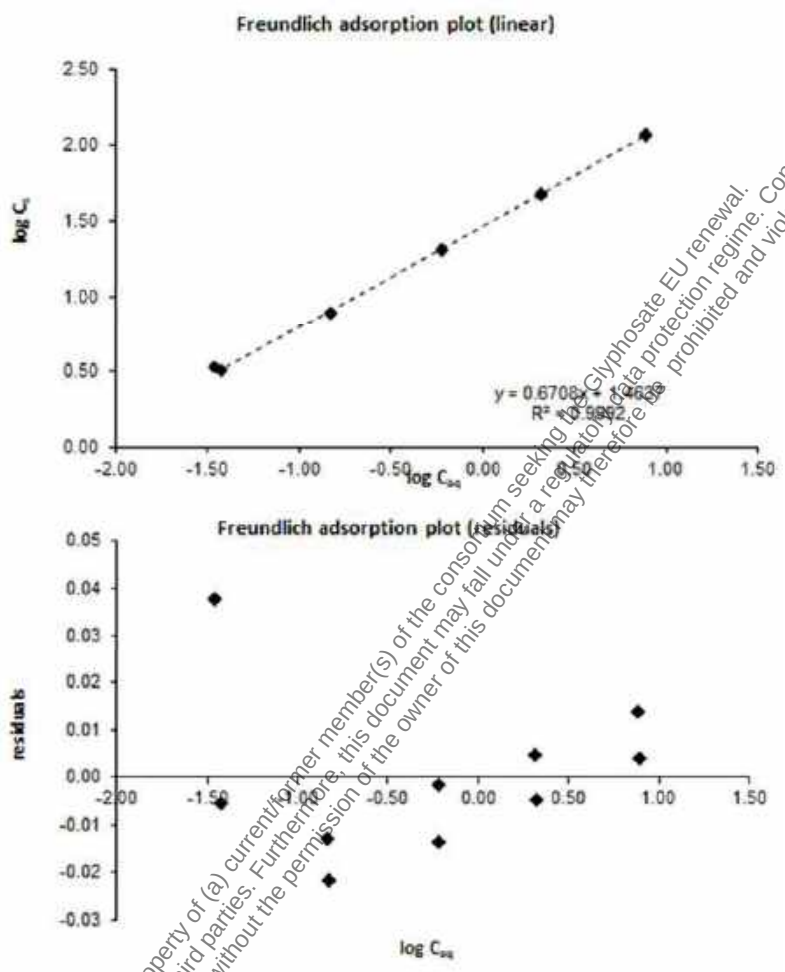


Figure 7.1.3.1.2-5: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 2.1

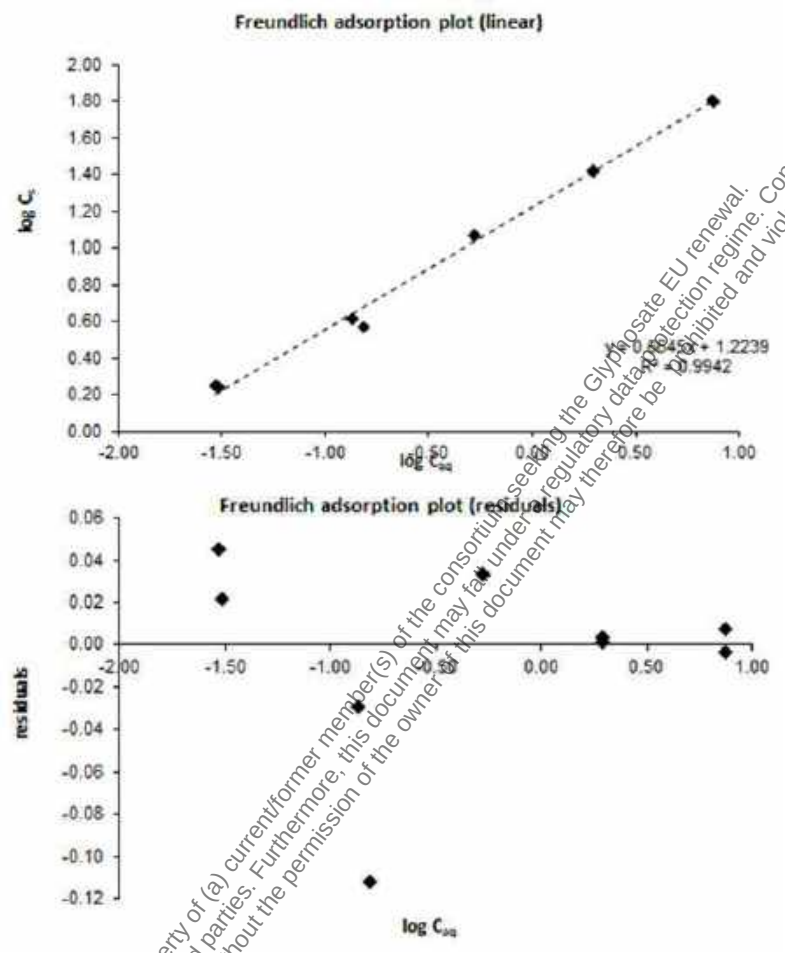
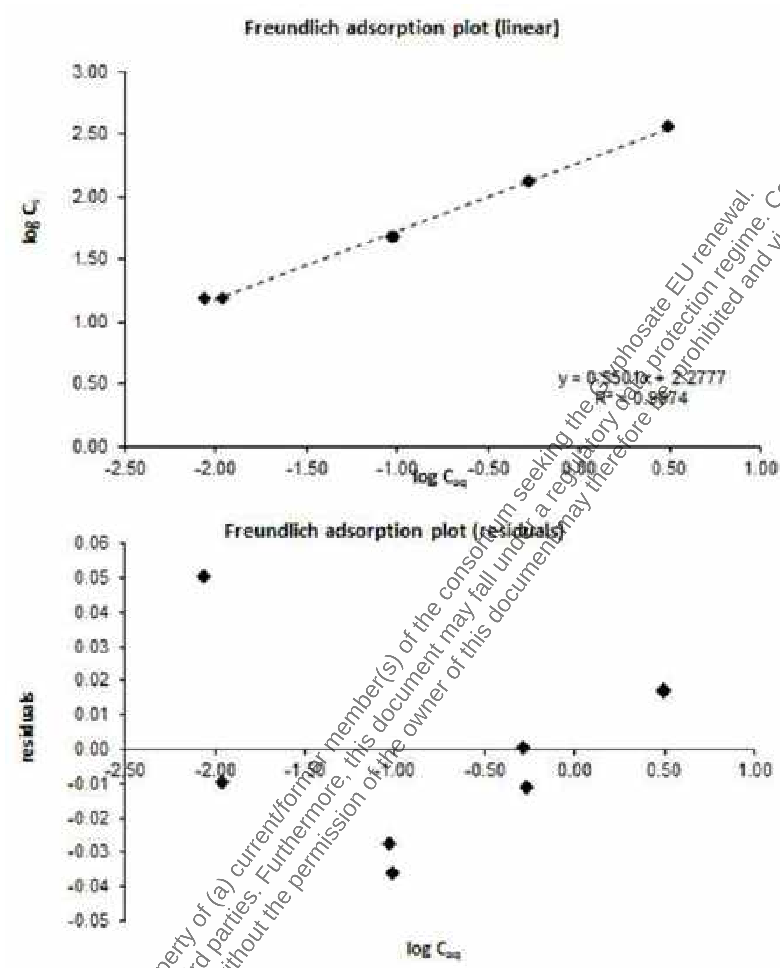


Figure 7.1.3.1.2-6: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 2.2



1. Information on the study

Data point:	CA 7.1.3.1.2/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: adsorption and desorption properties of the major metabolite, AMPA, in soil
Report No	RJ2129B
Document No	
Guidelines followed in study	OECD Guideline 106 U.S. EPA Series 163-1, Leaching and Adsorption/Desorption Studies
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Preliminary test for determination equilibration time performed for soil Visalia - No preliminary test for determination of soil-to-solution ratio - Adsorption of test item to test vessel surface not investigated - No detailed results reported to conclude on stability of test item in terms of parental mass balance
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The adsorption / desorption behaviour of [^{14}C]aminomethylphosphonic acid (AMPA) was studied in five sterilised soils in batch equilibrium experiments in the laboratory at 20 ± 2 °C using the indirect method.

Soil	Origin	Texture (USDA)	pH [1]	OM [2] [%]	OC [3] [%]
Lillyfield	Churt, Surrey, England	Sand	5.7	0.5	0.29
Visalia	Visalia, California, USA	Sandy loam	8.4	1.0	0.28
Wisborough Green	Wisborough Green, Sussex, England	Silty clay loam	5.7	3.9	2.27
Champaign	Champaign, Illinois, USA	Silty clay loam	6.2	3.7	2.15
18 Acres	Bracknell, Berkshire, England	Sandy loam	7.4	3.1	1.80

[1] pH values were derived from a 1:2 soil:water suspension.
[2] OM: Organic matter
[3] Calculated as : OC [%] = OM [%] / 1.72

Analysis of aqueous adsorption and desorption supernatants and soil extracts of the definitive test showed that more than 90 % of the applied radioactivity (% AR) could be assigned to AMPA.

For the definitive phase, the adsorption step of the study was carried out at a soil to solution ratio of 1:10 for 21 hours using pre-equilibrated air-dried soils. Nominal test concentrations of AMPA were 2.0, 1.0, 0.2, 0.1, and 0.05 mg/L. The equilibration solution used was 0.01 M aqueous $CaCl_2$.

The desorption study was conducted by supplying pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ using each soil and each concentration of AMPA with a single desorption cycle.

Mean material balances of radioactivity ranged from 101 to 105 % AR for soil Lillyfield, from 99 to 106 % AR for Visalia soil, from 99 to 104 % AR for Wisborough Green soil, from 95 to 104 % AR for Champaign soil, and from 97 to 102 % AR for 18 Acres soil.

At the end of the adsorption phase, an average of 96.1 % of the applied test material were adsorbed to soil Lillyfield, 61.9 % to soil Visalia, 98.8 % to soil Wisborough Green, 98.0 % to soil Champaign and 93.0 % to soil 18 Acres.

The adsorption coefficients $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the five test soils ranged from 9.97 to 509 mL/g. The Freundlich exponents 1/n were in the range of 0.78 to 0.91, demonstrating a small decrease in adsorption with increasing rate of application, however, there was not saturation of adsorption sites at the highest rate of application. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 1720 and 45900 mL/g. During the single desorption step, calculated $K_{F, OC(des)}$ values varied between 2080 and 71500 mL/g indicating that the adsorption of AMPA is not very reversible.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]Aminomethylphosphonic acid	
Lot No.	Not provided
Specific activity	1.828 GBq/mmol
Radiochemical purity	97 %
Chemical purity	Not provided

2. Test Soils

The soils were air-dried and sieved to a particle size of ≤2 mm. The soils were gamma irradiated with between 25 and 40 kgy to eliminate any living organisms within the soil. The characterisation of test soils used is summarised in the table below.

Table 7.1.3.1.2-98: Physico-chemical properties of test soils

Parameter	Results				
Soil Designation	Lillyfield	Visalia	Wisborough Green	Champaign	18 Acres
Geographic Location					
City	Churt	Visalia	Wisborough Green	Champaign	Bracknell
State	Surrey	California	Sussex	Illinois	Berkshire
Country	England	USA	England	USA	England
Textural Class (USDA)	Sand	Sandy loam	Silty clay loam	Silty clay loam	Sandy loam
Sand (50 µm – 2 mm)	92 %	69 %	8 %	12 %	58 %
Silt (2 µm – 50 µm)	4 %	18 %	60 %	52 %	23 %
Clay (< 2 µm)	4 %	13 %	32 %	36 %	19 %
pH (in 1:2 soil:water suspension)	5.7 %	8.4 %	5.7 %	6.2 %	7.4 %
Organic Matter	0.5 %	1.0 %	3.9 %	3.7 %	3.1 %
Organic Carbon [1]	0.29 %	0.58 %	2.27 %	2.15 %	1.80 %
Cation Exchange Capacity (meq/100 g)	1.8	7.3	11.9	28.3	14.4
Water Holding Capacity					
at 1/3 bar (%)	3.11	10.4	30.9	22.7	17.1
at 15 bar (%)	1.11	4.80	19.8	13.5	10.4

[1] Calculated as : OC [%] = OM [%] / 1.72
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Teflon® centrifuge tubes (50 mL) were used as test systems. The experiments were performed in duplicate.

In a preliminary test, the appropriate adsorption equilibration time was determined for soil Visalia, only. The stability of AMPA (parental mass balance) was investigated in the course of the definitive test.

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:10 (2.0 g soil (dry weight equivalents) / 20 mL solution). Test item was applied at nominal concentrations of 2.0, 1.0, 0.2, 0.1 and 0.05 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 21 hours at 20 ± 2 °C under continuous agitation.

In the desorption phase, pre-adsorbed soil prepared separately for the desorption phase was supplied with fresh aqueous 0.01 M $CaCl_2$ solution. The resultant samples were re-equilibrated for 21 hours at 20 ± 2 °C under continuous agitation.

2. Analytical Procedures

After each adsorption and desorption step, the aqueous supernatant was separated from the soil by centrifugation and radioactivity in the supernatants was determined by liquid scintillation counting (LSC). Aqueous supernatants were analysed by thin layer chromatography (TLC).

Following the adsorption and desorption phase soils were extracted twice by shaking at ambient temperature using ammonium phosphate buffer. Soil extracts were analysed by TLC-radiodetection. The extracted soils were dried and radioactivity was determined by combustion and LSC.

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Mean material balances ranged from 101 to 105 % AR for soil Lillyfield, from 99 to 106 % AR for Visalia soil, from 99 to 104 % AR for Wisborough Green soil, from 95 to 104 % AR for Champaign soil, and from 97 to 102 % AR for 18 Acres soil.

B. STABILITY OF TEST ITEM

Analysis of aqueous supernatants and soil extracts showed that more than 90 % of the applied radioactivity (% AR) could be assigned to AMPA. Mean amounts of non-extractable residues (NER) were 3.8, 7.7, 11.3, 7.6 and 4.6 % AR for soils Lillyfield, Visalia, Wisborough Green, Champaign and 18 Acres, respectively.

C. FINDINGS

At the end of the adsorption phase, an average of 96.1 % of the applied test material were adsorbed to soil Lillyfield, 61.9 % to soil Visalia, 98.8 % to soil Wisborough Green, 98.0 % to soil Champaign and 93.0 % to soil 18 Acres (see Table 7.1.3.1.2-99). The adsorption coefficients $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the five test soils ranged from 9.97 to 509 (see Table 7.1.3.1.2-100). The Freundlich exponents 1/n were in the range of 0.78 to 0.91, demonstrating a small decrease in adsorption with increasing rate of application, however, there was not saturation of adsorption sites at the highest rate of application. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 1720 and 45900. During the single desorption step, calculated $K_{F, OC(des)}$ values varied between 2080 and 71500 indicating that the adsorption of AMPA is not very reversible. At the end of the desorption phase, 2-3 % of the initially adsorbed amount was desorbed in soil Lillyfield, 17-36 % in soil Visalia, 0-1 % in soil Wisborough Green, 1-2 % in soil Champaign and 2-6 % in soil 18 Acres.

Table 7.1.3.1.2-99: [^{14}C]AMPA: Percentage adsorbed and desorbed in soil (mean values)

	Test Concentration [mg/L]									
	Adsorption [1]					Desorption [2]				
Soil	0.05	0.1	0.2	1.0	2.0	0.05	0.1	0.2	1.0	2.0
Lillyfield	97.2	96.5	96.5	95.7	94.6	2.0	2.0	2.0	3.0	3.0
Visalia	70.2	68.8	67.5	53.2	49.7	20.0	17.0	20.0	29.0	36.0
Wisborough Green	99.0	98.9	99.0	98.7	98.6	0.0	1.0	0.0	1.0	1.0
Champaign	98.4	98.3	98.3	97.9	96.9	1.0	1.0	1.0	1.0	2.0
18 Acres	94.5	94.3	94.3	92.3	89.7	2.0	4.0	4.0	4.0	6.0

[1] End of adsorption phase, mean values expressed as percentage of applied radioactivity
[2] End of first desorption phase, mean values expressed as percentage of the initially adsorbed amount

Table 7.1.3.1.2-100: [^{14}C]AMPA: Adsorption / desorption parameters in soil at 20 °C

Soil	Adsorption				Desorption
	$K_{F(ads)}$ [mL/g]	1/n	R^2	$K_{F, OC(ads)}$ [mL/g]	$K_{F, OC(des)}$ [mL/g]
Lillyfield	133	0.86	1.00	45900	71500
Visalia	9.97	0.78	1.00	1720	2080
Wisborough Green	509	0.91	1.00	22500	29600
Champaign	237	0.86	1.00	11100	15000
18 Acres	74.2	0.84	1.00	4130	5130

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the five test soils ranged from 9.97 to 509 mL/g. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 1720 and 45900 mL/g. During the single desorption step, calculated $K_{F, OC(des)}$ values varied between 2080 and 71500 mL/g indicating that the adsorption of AMPA is not very reversible.

3. Assessment and conclusion

Assessment and conclusion by applicant:
On the basis of information provided in the report the results of the study are considered as supportive. The results of the parental mass balance test were not presented in detail while it is stated that >90 % of applied radioactivity was recovered as AMPA in aqueous supernatant and soil extracts for all soils. However, the raw data of the study possibly could provide more detailed information on the results of the parental mass balance in order to evaluate the results according to OECD Guideline 106 and its respective EU Evaluators Checklist.

Since there was no detailed reporting of results of stability and distribution, the study was conservatively assessed as supportive and thus not used for risk assessment.

Assessment and conclusion by RMS:

The evaluation according to EFSA OECD 106 Evaluators Checklist using all available data are provided in the table and figures below for information.

Table 7.1.3.1.2-101: Metabolite AMPA: Results of evaluation according to EU OECD 106 Evaluators Checklist

	Units	Lillyfield	Visalia	Wisborough Green	Champaign	18 Acres
Adsorption method	-	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	g dw:mL	1:10	1:10	1:10	1:10	1:10
Parental mass balance (at 2nd highest conc.)	%	- [1]	- [1]	<90 [2]	- [1]	- [1]
Adsorbed percentage	%	94.7-97.2	50.2-70.6	98.6-99.0	96.9-98.4	89.8-94.6
K_D x (soil:solution ratio)		17.5-34.4	1.0-2.4	70.7-98.5	31.3-60.9	8.7-17.3
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	134.004 (99.736-180.047)	9.974 (8.005-12.428)	531.319 (363.191-777.277)	240.014 (131.592-437.767)	74.216 (52.072-105.778)
$^{ads}1/n$ (95 % confidence interval)	-	0.861 (0.800-0.923)	0.776 (0.696-0.855)	0.917 (0.852-0.981)	0.860 (0.749-0.971)	0.844 (0.761-0.927)
$^{ads}R^2$	-	0.998	0.997	0.999	0.995	0.997
$^{ads}K_{F,OC}$	L/kg OC	44668	1662	23101	11429	4123
K_{FE} / K_F [3]	-	-	-		-	-

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] Results of parental mass balance test not reported.
[2] Formation of NER >10 %.
[3] Cannot be calculated since the f-factor cannot be specified due to missing data of the parental mass balance test.

Figure 7.1.3.1.2-7: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Lillyfield

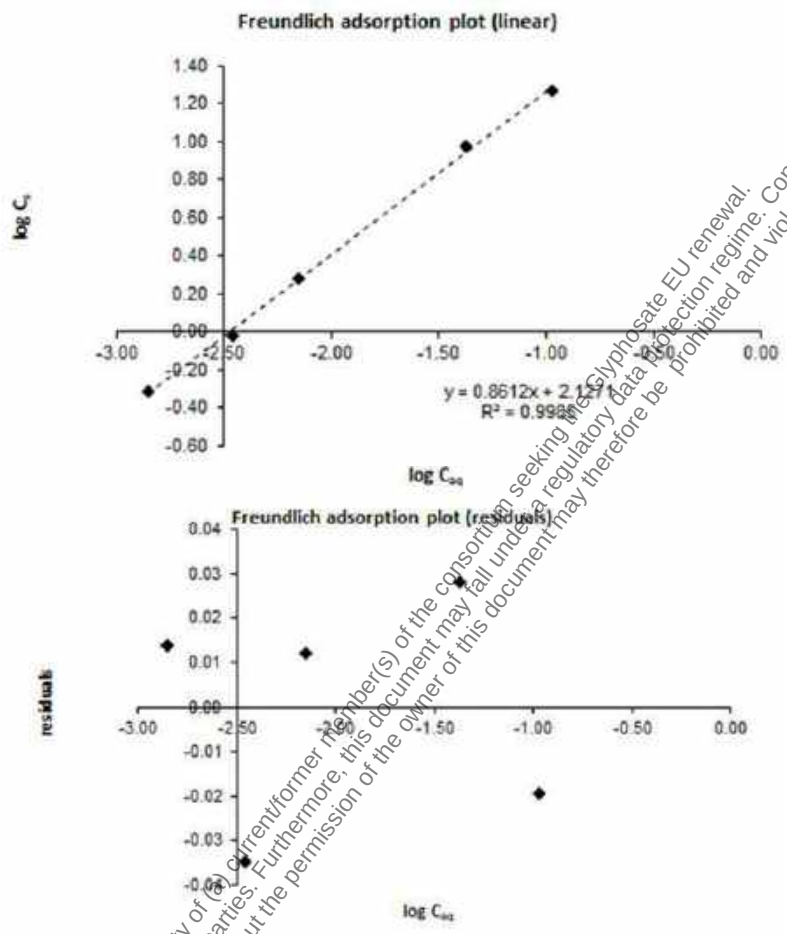


Figure 7.1.3.1.2-8: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Visalia

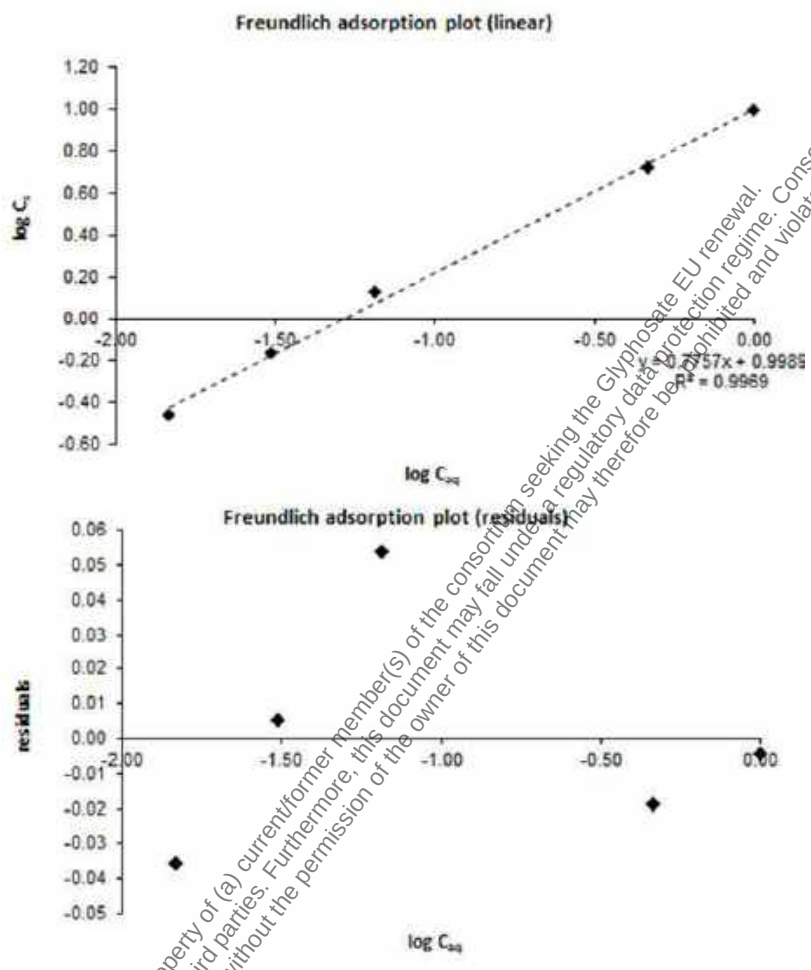


Figure 7.1.3.1.2-9: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Wisborough Green

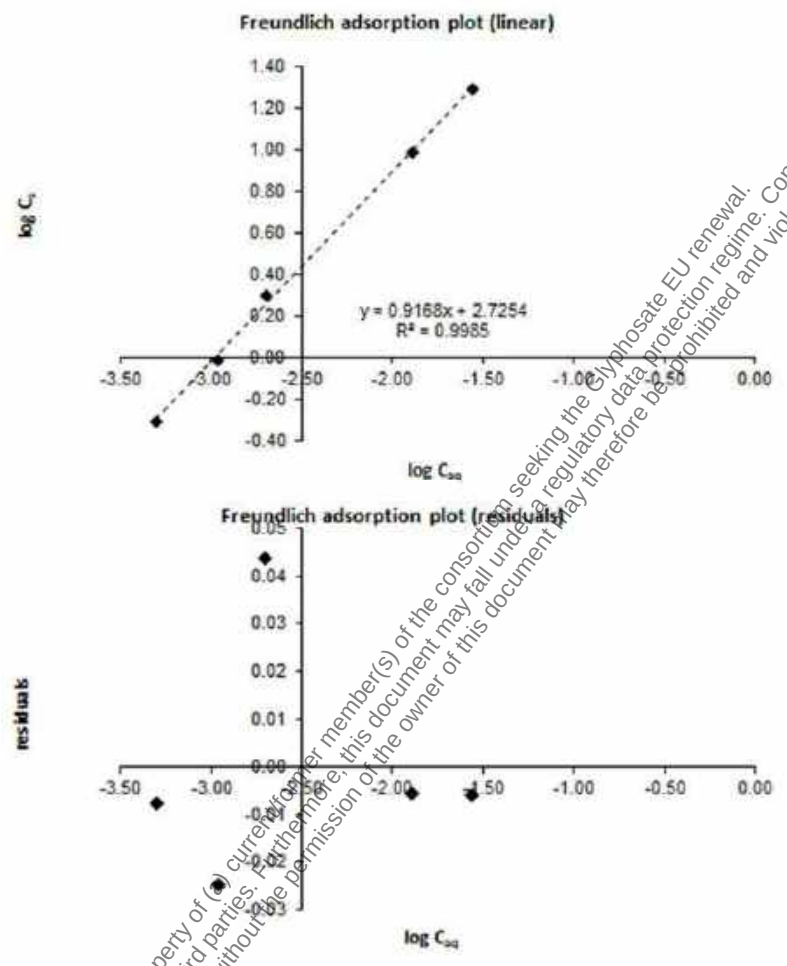


Figure 7.1.3.1.2-10: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil Champaign

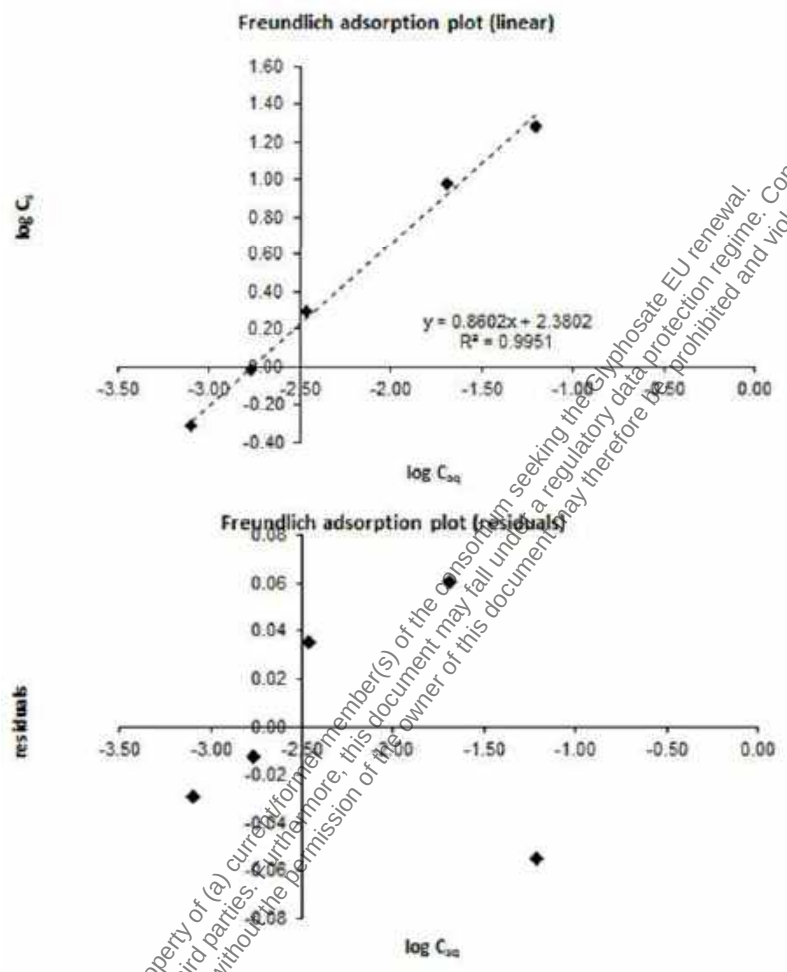
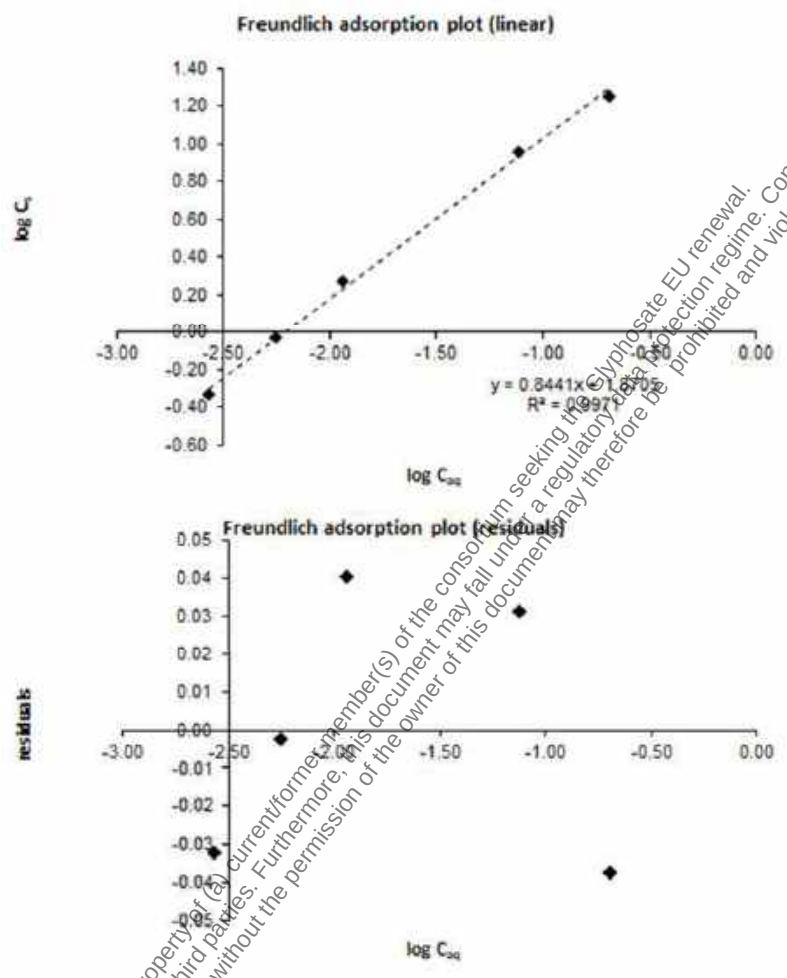


Figure 7.1.3.1.2-11: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil 18 Acres



1. Information on the study

Data point:	CA 7.1.3.1.2/005
Report author	[redacted]
Report year	1993
Report title	Adsorption of aminomethylphosphonic acid to soil particles in six soil types
Report No	IMW-R93/056
Document No	
Guidelines followed in study	Dutch Guideline For Registration Pesticides, Part G1.2
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Aqueous $CaSO_4$ solution used instead of $CaCl_2$ - No preliminary tests on soil:solution ratio, equilibration time or stability of test item - LOD of 0.1 µg/mL, while lowest test concentration was 0.5 µL/mL, i.e. not 1 % of test concentration as required - No pre-equilibration of soil samples
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The adsorption behaviour of aminomethylphosphonic acid (AMPA) was studied in six soils in the laboratory at 20 ± 2 °C.

Soil	Origin	pH [1]	OM [2] [%]	OC [3] [%]
Sandy loam	Heerewaarden, Netherlands	7.5	2.1	1.22
Low humic-content (lhc) sand	Lisse, Netherlands	7.2	1.0	0.58
Gray brown podzol	Caen, France	6.5	2.6	1.51
Sandy (A)	Zeist, Netherlands	4.3	5.6	3.26
Sandy (B)	Zeist, Netherlands	4.4	3.6	2.09
Sandy (C)	Maarn, Netherlands	4.3	4.2	2.44

[1] pH values were derived from KCl suspensions
[2] OM: Organic matter
[3] OC: Organic carbon, calculated as OM / 1.72

For the definitive phase, the adsorption step was carried out at a soil to solution ration of 1:10 for 20 hours using air-dried soils. Nominal concentrations of AMPA were 50.0, 20.0, 10.0 and 5.0 mg/L. The equilibration solution used was 0.01 M aqueous $CaSO_4$.

Values for the Freundlich adsorption coefficients $K_{F(ads)}$ of aminomethylphosphonic acid ranged from 5.8 to 351 mL/g for the six soils tested. Values of the Freundlich coefficient 1/n were in the range of 0.44 to 0.60. The corresponding, calculated $K_{F, OM(ads)}$ values varied between 586 and 7979 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Aminomethylphosphonic acid (non-labelled)	
Lot No.	108 F 3811
Chemical Purity	99 %

2. Test Soils

The soils were sieved to a particle size of ≤2 mm and stored at 3 ± 2 °C prior to use. The characterisation of test soils used is summarised in the table below.

Table 7.1.3.1.2-102: Physico-chemical properties of test soils

Parameter		Results					
Soil Designation		Sandy loam	Sand Low humic-content (lhc)	Gray brown podzol	Sandy (A)	Sandy (B)	Sandy (C)
Geographic Location							
City		Heerewaarden	Lisse	Caen	Zeist	Zeist	Maarn
Country		Netherlands	Netherlands	France	Netherlands	Netherlands	Netherlands
Textural Class (USDA)							
Sand	(50 µm – 2 mm)	64.1	97.0	6.7	89.2	91.4	88.9
Silt	(2 µm – 50 µm)	23.1	0.5	74.0	7.0	4.8	7.0
Clay	(<2 µm)	12.8	2.5	19.3	3.8	3.8	4.1
pH							
- in KCl [1]		7.5	7.2	6.5	4.3	4.4	4.3
- in KCl [2]		7.8	8.0	6.4	4.4	4.7	4.4
Organic Carbon [3]		1.22 %	0.58 %	1.51 %	3.26 %	2.09 %	2.44 %
Organic Matter		2.1 %	1.0 %	2.6 %	5.6 %	3.6 %	4.2 %
$CaCO_3$ (%)		8.0	1.5	0.2	0.1	0.1	0.1

[1] pH values measured at Bedrijfslaboratorium voor Grond- en Gewasonderzoek
[2] pH values measured at IMW-TNO
[3] Calculated as OM / 1.72

B. STUDY DESIGN

1. Experimental Conditions

Scintillation vials (20 mL volume) with screw caps were used as test systems. The experiments were performed in duplicate. A stock solution of 0.2517 g test substance in 500 mL of 0.01 M $CaSO_4$ solution was prepared and used for equilibration. The vials were shaken for 20 h at 20 ± 2 °C in a temperature controlled room.

For the definitive phase, the adsorption step was carried out at a soil to solution ratio of 1:10 for 20 hours by shaking non-pre-equilibrated samples of air-dried soils with a 0.01 M aqueous calcium sulfate solution of AMPA. Nominal concentrations of AMPA were 50.0, 20.0, 10.0 and 5.0 mg/L.

2. Analytical Procedures

The aqueous supernatant after adsorption was separated by centrifugation. AMPA residues in the supernatant and residual soil were analysed by high performance liquid chromatography (HPLC) with fluorescence detection at 254 and 313 nm.

The limit of detection (LOD) and limit of quantitation (LOQ) for HPLC analysis were 0.1 µg/mL (0.1 mg/L) and 0.5 µg/mL (0.5 mg/L), respectively.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. STABILITY OF TEST ITEM

The stability of the test item during the adsorption phase was not investigated (the same accounts for preliminary tests to establish the appropriate soil:solution ratio, equilibration time, and sorption of test item to test vessel surface).

B. FINDINGS

Freundlich adsorption coefficients for aminomethylphosphonic acid ranged from 5.8 to 351 mL/g for the six tested soils. 1/n values were in the range of 0.44 to 0.60. The corresponding, calculated $K_{F, OM(ads)}$ values varied between 586 and 7979 mL/g. A summary of the results of the adsorption isotherms tests is presented in the table below.

Table 7.1.3.1.2-103: AMPA: Distribution between solution and soil (mean values)

Soil	Fraction	Test concentration [mg/L]			
		5	10	20	50
Sandy loam	Solution (µg/mL)	1.65	3.49	8.91	33.51
	Adsorbed (µg/g)	34.60	79.10	112.15	151.55
Sand lhc	Solution (µg/mL)	4.19	8.03	16.59	44.08
	Adsorbed (µg/g)	9.15	33.65	35.40	45.90
Gray brown podzol	Solution (µg/mL)	0.55	1.35	3.74	15.96
	Adsorbed (µg/g)	45.60	100.45	163.85	327.10
Sandy (A)	Solution (µg/mL)	<0.1	<0.1	0.30	1.83
	Adsorbed (µg/g)	51.05 [1]	113.95 [1]	198.25	468.40
Sandy (B)	Solution (µg/mL)	<0.1	<0.1	0.49	2.44
	Adsorbed (µg/g)	51.05 [1]	113.95 [1]	196.35	462.25
Sandy (C)	Solution (µg/mL)	<0.1	0.15	0.74	3.58
	Adsorbed (µg/g)	51.05 [1]	112.45	193.90	450.85

[1] Assumed to be completely adsorbed to the soil.

Table 7.1.3.1.2-104: AMPA: Adsorption parameters in different soils at 20 °C

Soil	Adsorption			
	K_F [mL/g]	1/n	r	$K_{F, OM}$ [mL/g]
Sandy loam	35	0.46	0.93	1678
lhc sand	5.8	0.60	0.83	586
Gray brown podzol	73	0.57	0.99	2812
Sandy (A)	351	0.48	1.0 [1]	6275
Sandy (B)	287	0.53	1.0 [1]	7979
Sandy (C)	245	0.44	0.99 [2]	5835

[1] Two data points
[2] Three data points

III. CONCLUSION

The adsorption coefficients $K_{F(ads)}$ of AMPA acid for the tested soils calculated based on the Freundlich isotherms ranged from 5.8 to 351 mg/L. The respective $K_{F, OM(ads)}$ values ranged from 586 to 7979 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was considered as not valid during review for AIR2 by the RMS.
There were multiple deviations from OECD Guideline 106 including the use of calcium sulfate solution instead of calcium chloride as aqueous phase. In addition, soil samples were not pre-equilibrated. No preliminary tests were performed to establish the appropriate soil:solution ratio, equilibration time, and the stability of the test item. Finally, limits of detection (LOD, 0.1 mg/L) and quantitation (LOQ, 0.5 mg/L) do not fulfil the criterion of 1 % as set by the guideline.
The study and its results were not considered for environmental risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.3.1.2/006
Report author	[redacted]
Report year	1993
Report title	Aminomethylphosphonic acid – Determination of the sorption and desorption properties
Report No	92-8-4390
Document No	
Guidelines followed in study	OECD Guideline 106 U.S. EPA. 1982. Sediment and Soil Adsorption Isotherm CG-1710.
Deviations from current test guideline	From OECD Guideline 106 (January 2000): - Samples of four soils SLI Soil #1, #2, #9 and #11 showed radioactivity material balances and/or parental mass balances <90 %. - Soils were not pre-equilibrated
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The adsorption/desorption behaviour of [[14]C]aminomethylphosphonic acid (AMPA) was studied in six soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 2 °C using the indirect method.

Soil	Texture (USDA)	pH [1]	OC [2] [%]
SLI Soil #1	Clay loam	7.70	2.09
SLI Soil #2	Sand	4.70	18.72
SLI Soil #4	Sand	7.40	1.34
SLI Soil #5	Clay loam	7.60	0.93
SLI Soil #9	Loamy sand	6.30	1.57
SLI Soil #11	Sand	4.60	0.29

[1] pH values were derived from a 1:1 soil:water suspension.
[2] calculated as : OC [%] = OM [%] / 1.72

For the definitive phase, the adsorption step was carried out at a soil to solution ratio of 1:20 (1:100 for SLI Soil #2) for 16 to 48 hours using pre-equilibrated samples of previously air-dried soils. The equilibration solution used was 0.01 M aqueous $CaCl_2$. Approximate nominal concentrations of ^{14}C-AMPA were 5.0, 1.0, 0.2, and 0.04 mg/L. A desorption phase was included in the initial screening test only and is not summarized here.

Mean material balances after 16 to 48 hours of equilibration were 86.7 % of applied radioactivity (AR) for SLI Soil #1, 92.3 % for SLI Soil #2, 90.8 % for SLI Soil #4, 96.6 % for SLI Soil #5, 83.1 % for SLI Soil #9 and 84.6 % for SLI Soil #11.

Parental mass balances were 78.93 % of applied test item (in aq. supernatant and soil extracts) for SLI Soil #1, 15.03 % for SLI Soil #2, 96.99 % for SLI Soil #4, 101.80 % for SLI Soil #5, 68.83 % for SLI Soil #9 and 87.89 % for SLI Soil #11.

The adsorption coefficients $K_{F(ads)}$ of AMPA calculated based on the Freundlich isotherms of the six test soils ranged from 15.7 to 1570 mL/g. The Freundlich exponents 1/n were in the range of 0.752 to 0.904, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range. The corresponding, calculated $K_{F,OC(ads)}$ values varied between 1160 and 24800 mL/g.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

[^{14}C]Aminomethylphosphonic acid	
Lot No.	C-1105.9, C-1521.1, C-1521.2, C-1521.7
Specific activity	3 batches with 23.8 and 1 batch with 26.8 mCi/mmol
Radiochemical purity	≥ 98.7 %
Chemical purity	Not provided

2. Test Soils

The soils were sieved to a particle size of ≤2 mm. The soils were air-dried before application. SLI Soil #I had a previous history indicating the use of phenoxy herbicides within the 12 months prior to collection. The remaining five soils had no pesticides applied in two or more years. The characterisation of test soils used is summarised in the table below.

Table 7.1.3.1.2-105: Physico-chemical properties of test soils

Parameter	Results					
Soil Designation	SLI Soil #1	SLI Soil #2	SLI Soil #4	SLI Soil #5	SLI Soil #9	SLI Soil #11
Geographic Location						
City	Not provided	Not provided	Not provided	Not provided	Not provided	Not provided
State	Not provided	Not provided	Not provided	Not provided	Not provided	Not provided
Country	Netherlands	Netherlands	Netherlands	Netherlands	Netherlands	Netherlands
Textural Class (USDA)	Clay loam	Sand	Sand	Clay loam	Loamy sand	Sand
Sand [%] (50 µm – 2 mm)	20.0	88.0	92.0	22.0	76.0	98.0
Silt [%] (2 µm – 50 µm)	45.3	11.3	5.30	49.3	19.3	1.30
Clay [%] (<2 µm)	34.7	0.70	2.70	28.7	4.70	0.700
pH in 1:1 Soil:Water Suspension	7.70	4.70	7.40	7.60	6.30	4.60
Organic Matter (%)	3.60	32.2	2.30	1.60	2.70	0.500
Organic Carbon [1] (%)	2.09	18.72	1.34	0.93	1.57	0.29
Cation Exchange Capacity (meq/100 g)	32.8	28.3	12.0	31.0	10.2	4.80
Moisture at 1/2 bar (%)	36.9	61.5	9.1	36.6	18.5	7.6

[1] Calculated as : OC [%] = OM [%] / 1.72
USDA: United States Department of Agriculture

B. STUDY DESIGN

1. Experimental Conditions

Glass centrifuge tubes (50 or 200 mL) with Teflon®-lined caps were used as test systems. The experiments for the definitive test were performed in triplicate.

In preliminary tests, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times and the stability of the test item including the parental mass balance were determined .

For the definitive phase, the adsorption step was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:20 (1:100 for SLI Soil #2). AMPA was applied at approximate nominal solution concentrations of 5.0, 1.0, 0.2, and 0.04 mg/L in aqueous 0.01 M $CaCl_2$ solution. The adsorption step was carried out for 16 hours in SLI Soil #2, SLI Soil #9, and SLI Soil #11, for 24 hours in SLI Soil #4 and SLI Soil #5, and 48 hours in SLI Soil #1 in the dark at 20 ± 2 °C under continuous agitation.

2. Analytical Procedures

For stability and determination of the parental mass balance test soil samples (nominal concentration of 4.97 mg/L) were extracted up to two times by shaking at ambient temperature using 0.5 N NH_4OH after the adsorption step. Soil and soil extract were separated by centrifugation and the pH of the soil extracts was adjusted to pH 3 using phosphoric acid. Aqueous supernatants and soil extracts were analysed by HPLC-radiodetection.

After the adsorption step of the definitive test, the aqueous supernatant was separated from the soil by centrifugation and radioactivity in the supernatants was determined by liquid scintillation counting (LSC). Soil samples from the 5.00 or 1.02 mg/L samples, of each soil type from the advanced isotherm test were combusted followed by quantitation using radioassay. The remaining radioactivity in the soil was determined by the combustion of aliquots of soil. This data was used to calculate material balance during the advanced isotherm phase.

Adsorption isotherms were calculated by linear regression analysis of the adsorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Mean material balances after 16 to 48 hours of equilibration were 86.7 % of applied radioactivity (AR) for SLI Soil #1, 92.3 % for SLI Soil #2, 90.8 % for SLI Soil #4, 96.6 % for SLI Soil #5, 83.1 % for SLI Soil #9 and 84.6 % for SLI Soil #11.

B. STABILITY OF TEST ITEM

Parental mass balances were 78.93 % of applied test item (in aq. supernatant and soil extracts) for SLI Soil #1, 15.03 % for SLI Soil #2, 96.99 % for SLI Soil #4, 101.80 % for SLI Soil #5, 68.83 % for SLI Soil #9 and 87.89 % for SLI Soil #11.

C. FINDINGS

The adsorption coefficients $K_{F(ads)}$ of AMPA were derived on the basis of the indirect method to result in Freundlich isotherms for the six test soils and ranged from 15.7 to 1570 mL/g (see Table 7.1.3.1.2-107). The Freundlich exponents 1/n were in the range of 0.752 to 0.904. The corresponding, calculated $K_{F, OC(ads)}$ values varied between 1160 and 24800 mL/g.

Table 7.1.3.1.2-106: Calculated AMPA concentration in soils (mean values)

Soil	Test Concentration [mg/L]			
	Adsorption [1]			
	5	1	0.2	0.04
SLI Soil #1	77.4 ± 0.4	17.2 ± 0.1	3.96 ± 0.01	0.730 ± 0.001
SLI Soil #2	478 ± 1	96.8 ± 0.1	21.6 ± 0.1	4.00 ± 0.01
SLI Soil #4	37.4 ± 2.8	9.87 ± 0.32	2.51 ± 0.09	0.676 ± 0.024
SLI Soil #5	72.4 ± 2.2	17.5 ± < 0.1	3.50 ± 0.08	0.893 ± 0.003
SLI Soil #9	83.3 ± 1.9	19.2 ± 0.1	3.96 ± 0.05	0.727 ± 0.003
SLI Soil #11	72.7 ± 1.8	19.1 ± 0.5	3.84 ± 0.10	0.712 ± 0.006

[1] End of adsorption phase, mean values expressed as mg/kg

Table 7.1.3.1.2-107: Adsorption parameters of AMPA in soil at 20 °C

Soil	Adsorption			
	$K_{F(ads)}$ [mL/g]	1/n	R^2	$K_{F, OC(ads)}$ [mL/g]
SLI Soil #1	77.1	0.786	0.997	3640
SLI Soil #2	1570	0.904	0.998	8310
SLI Soil #4	15.7	0.752	1.00	1160
SLI Soil #5	53.9	0.791	0.998	5650
SLI Soil #9	110	0.769	0.960	6920
SLI Soil #11	73.0	0.788	0.988	24800

III. CONCLUSIONS

The adsorption coefficients $K_{F(ads)}$ of AMPA for the tested soils calculated based on the Freundlich isotherms ranged from 15.7 to 1570 mL/g. The respective $K_{F, OC(ads)}$ values ranged from 1160 to 24800 mL/g.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is considered to be valid for the two soils SLI soil #4 and SLI soil #5.
During review for AIR2, Soil SLI Soil #2 was excluded by the RMS due to its high OC content (18.7 %).

For the three soils SLI #1, #9 and #11, overall balances of radioactivity including parental mass balances were below 90 %.

As a conservative assessment the data of the three soils were not included in the actual risk assessment.

For an evaluation according to the EFSA Evaluators Checklist see below.

Assessment and conclusion by RMS:

All relevant quality checks as part of confirming the acceptability of the study and of the reported endpoints were performed.

For soils SLI Soil #4 and #5, parental mass balances were 97.0-101.8 %, and percentage adsorption was 37.1-89.1 % (see Table 7.1.3.1.2-108). Systematic errors estimated via K_{FE}/K_F were calculated as low (i.e. ≤1.1). The analytical method covered the entire range of test concentrations (lowest test concentration equivalent to approx. 570 Bq per aliquot which is at least 100 fold higher than the typical instrumental LOD of LSC measurements. Furthermore, the lowest test concentration was approx. 300 fold higher than the highest background reported). The use of the indirect method was appropriate based on a K_D x soil/solution ratio >0.3 in all soils. The graphical fits of the Freundlich equation are presented below based on the standard linear regression form using log-log transformed data alongside the associated residual plots (see Figure 7.1.3.1.2-14 and Figure 7.1.3.1.2-15). The R^2 of the standard linear regressions ranged from 0.997 to 0.998 and the visual fit of the standard regression were acceptable.

Table 7.1.3.1.2-108: Results of evaluation according to EU OECD 106 Evaluators Checklist for AMPA

	Units	SLI Soil #1	SLI Soil #2 [1]	SLI Soil #4	SLI Soil #5	SLI Soil #9	SLI Soil #11
Adsorption method	-	indirect	indirect	indirect	indirect	indirect	indirect
Soil:solution ratio	(g dw/mL)	1:20	1:100	1:20	1:20	1:20	1:20
Parental mass balance (at highest conc.)	%	78.9	15.0	97.0	101.8	68.8	87.9
Adsorbed percentage	%	76.4-92.5	93.9-96.5	37.1-71.5	68.4-89.1	76.8-95.7	71.9-92.1
K_D x (soil:solution ratio)		3.4-12.4	16.0-27.9	0.5-2.5	2.1-8.5	3.6-21.8	2.6-11.5
$^{ads}K_F$ (95 % confidence interval)	L/kg dw	77.027 (69.097-85.867)	1564.904 (1359.514-1801.325)	15.668 (14.720-16.677)	53.185 (48.185-58.704)	109.942 (69.335-174.331)	72.676 (56.036-94.256)
$^{ads}1/n$ (95 % confidence interval)	-	0.786 (0.756-0.816)	0.903 (0.871-0.935)	0.751 (0.726-0.776)	0.790 (0.759-0.821)	0.767 (0.653-0.880)	0.785 (0.712-0.857)
$^{ads}R^2$	-	0.997	0.997	0.998	0.997	0.958	0.983
$^{ads}K_{F,OC}$	L/kg OC	3668	8369	1205	5910	6871	24225
K_{FE} / K_F	-	1.4	10	1.1	1.0	1.6	1.2

Note: Values derived from the EFSA evaluators checklist may vary from those in the study reports due to rounding errors.
[1] Soil excluded during previous evaluation due to OC of 18.68 %.

Figure 7.1.3.1.2-12: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil #1

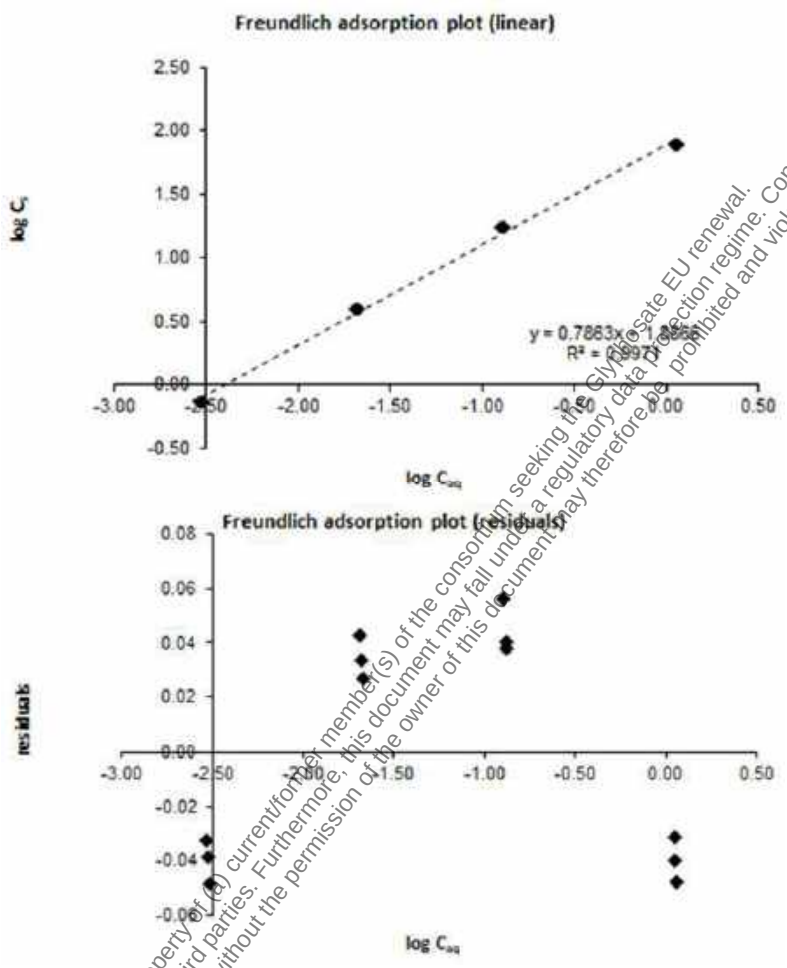


Figure 7.1.3.1.2-13: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil #2

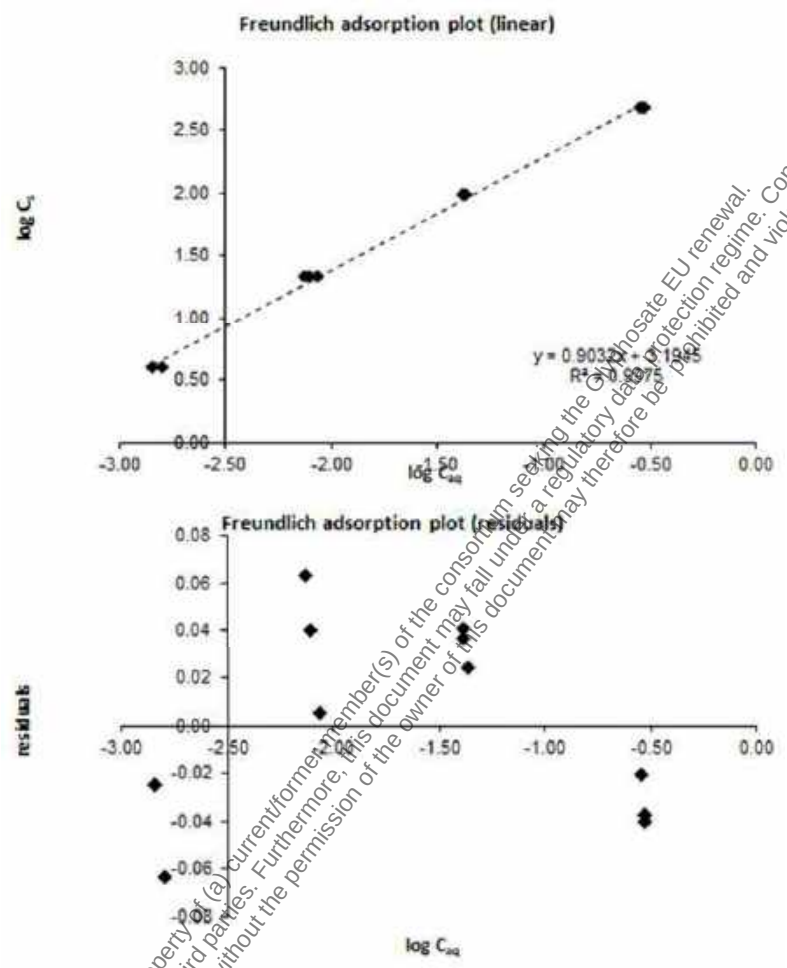


Figure 7.1.3.1.2-14: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil #4

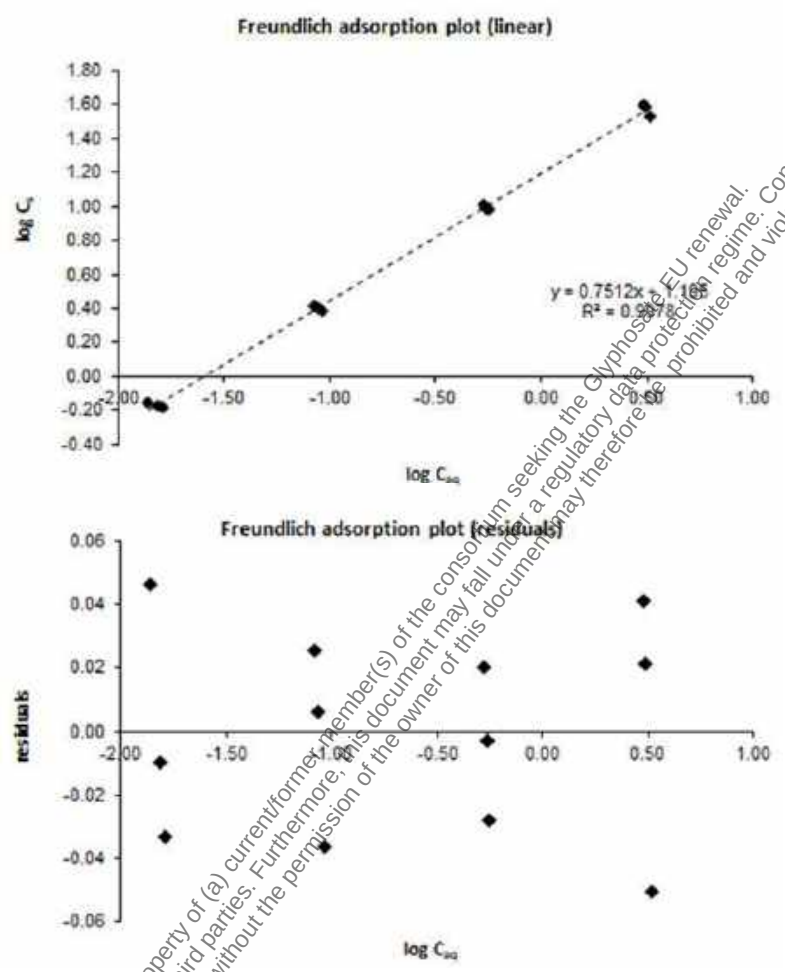


Figure 7.1.3.1.2-15: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil #5

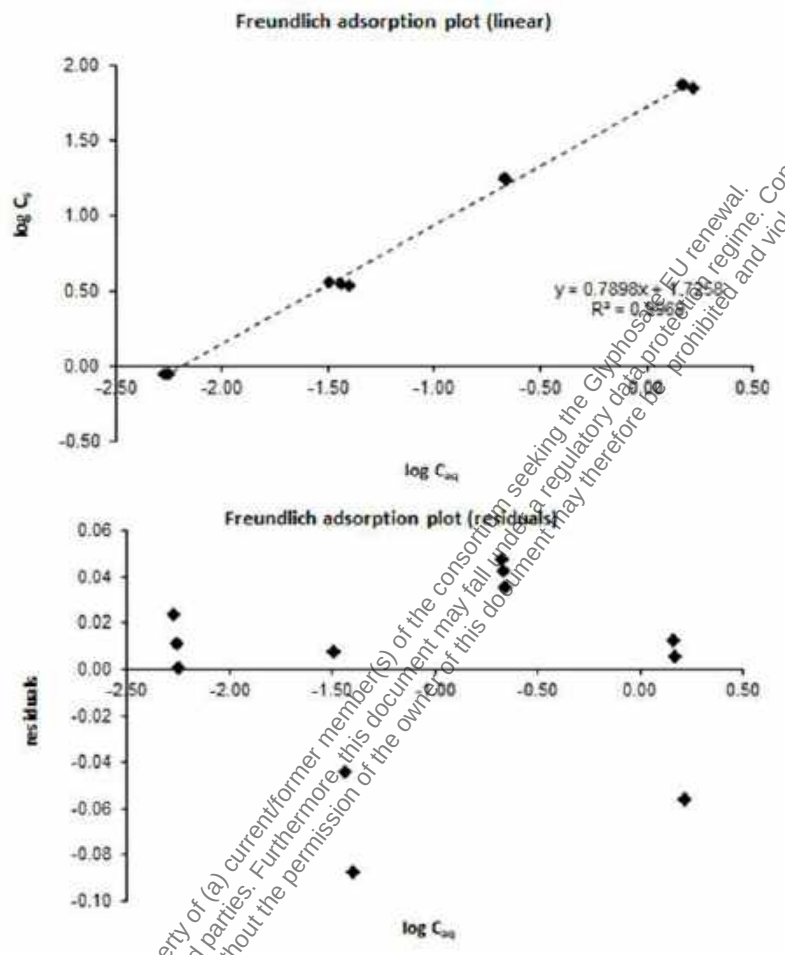


Figure 7.1.3.1.2-16: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil #9

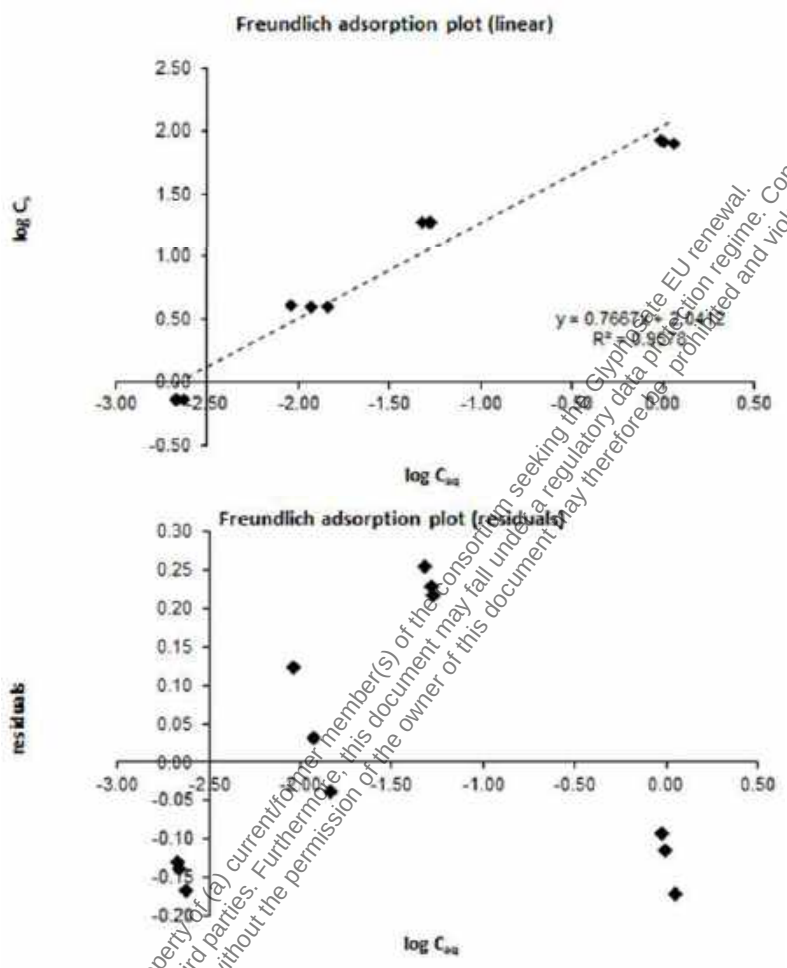
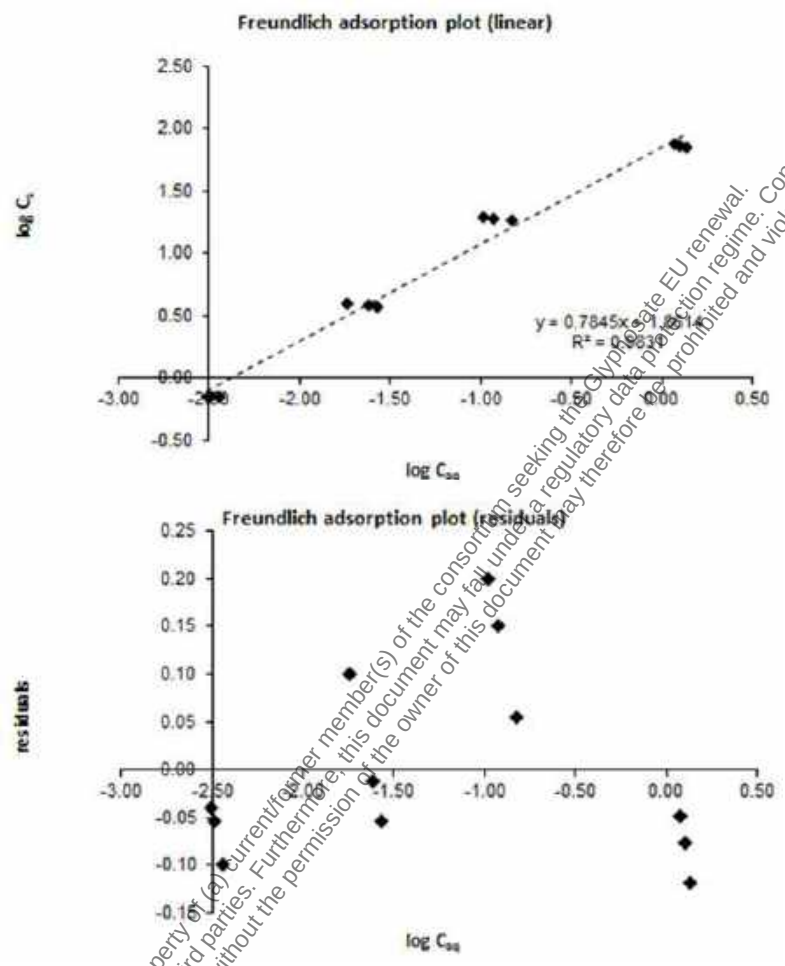


Figure 7.1.3.1.2-17: Graphical Presentation of Freundlich adsorption isotherm analysis (linear regression, top) and the corresponding plot of the residuals (bottom) for soil #11



Relevant articles from literature search

1. Information on the study

Data point:	CA 7.1.3.1.2/007
Report author	Skeff, W. *et al.*
Report year	2018
Report title	Adsorption behaviors of glyphosate, glufosinate, aminomethylphosphonic acid, and 2-aminoethylphosphonic acid on three typical Baltic Sea sediments
Document No	DOI 10.1016/j marchem.2017.11.008 E-ISSN-1872-7581
Guidelines followed in study	None
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found potentially relevant for multiple data points. The summary is provided under CA 7.1.3.1.1/016.

1. Information on the study

Data point:	CA 7.1.3.1.2/008
Report author	Sidoli, P. *et al.*
Report year	2016
Report title	Glyphosate and AMPA adsorption in soils: laboratory experiments and pedotransfer rules
Document No	DOI 10.1007/s11356-015-5796-5 E-ISSN 1614-7499
Guidelines followed in study	OECD Guideline 106 (January 2000)
Deviations from current test guideline	OECD Guideline 106 (January 2000) Insufficient information reported to assess validity of results
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found potentially relevant for multiple data points. The summary is provided under CA 7.1.3.1.1/023.

Assessment of pH dependency of adsorption parameters of AMPA

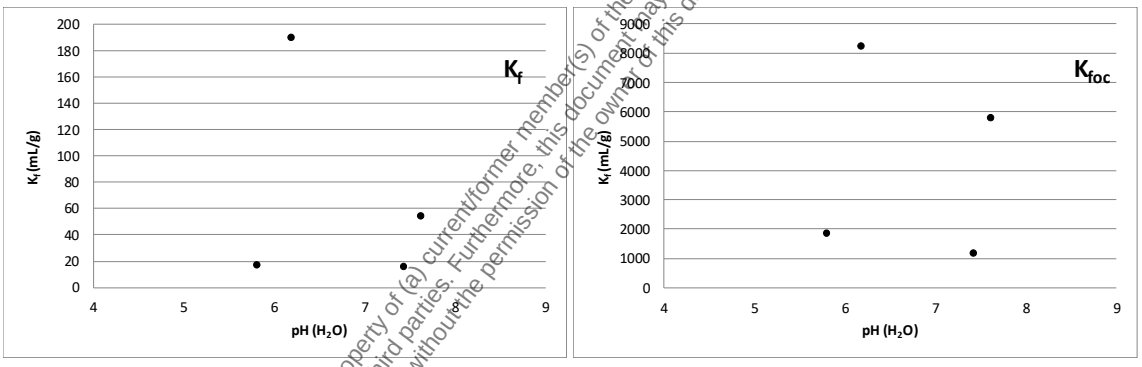
The pH dependency of the adsorption parameters $K_{F(ads)}$ and $K_{F, OC(ads)}$ was assessed using the German Input Decision Tool 3.3 (Holdt, G. *et al.*, 2012) for the soils SLI Soil #4 and SLI Soil #5 of [redacted] (1993) and Lufa 2.1 and Lufa 2.2 of [redacted] (2020). If pH values measured in H_2O were not available, they were converted from pH values measured in $CaCl_2$ by formula implemented in Input Decision Tool 3.3. For AMPA, there is no significant correlation between the pH-value and the adsorption coefficients $K_{F(ads)}$ and $K_{F, OC(ads)}$.

Therefore, it is concluded that the adsorption behaviour of AMPA is not pH-dependent.

Table 7.1.3.1.2-109: AMPA: Correlation parameters for $K_{F(ads)}$ and $K_{F, OC(ads)}$ values and pH_{H2O} values

Compound	Parameter	Kendall tau (stringency of the correlation)	p (level of significance)
AMPA	$K_{F(ads)}$	0.000	1.000
	$K_{F, OC(ads)}$	0.000	1.000

Figure 7.1.3.1.2-18: AMPA: Correlation between $K_{F(ads)}$ values and pH_{H2O} as well as $K_{F, OC(ads)}$ and pH_{H2O}



CA 7.1.3.2 Aged sorption

A study on aged sorption is not required and was not conducted.

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

Reliable adsorption coefficients of the active substance were obtained by adsorption/desorption studies and, consequently, column leaching studies are not required (please refer to CA 7.1.3.1). However, three column leaching studies ([redacted] 1992, CA 7.1.4.1.1/002, [redacted], 1992, CA 7.1.4.1.1/003, and [redacted] 1991, CA 7.1.4.1.1/005) and two aged column leaching studies ([redacted] 1996, CA 7.1.4.1.1/001, [redacted] 1992, CA 7.1.4.1.1/004) with glyphosate or glyphosate-trimesium are available and are considered as supportive information. For studies performed with glyphosate-trimesium only the results for the glyphosate (PMG) anion are considered for evaluation and further assessment.

Overall, less than 2 % of the applied radioactivity were found in the leachates of the individual experiments. Concentrations of glyphosate and AMPA were generally < 1.0 µg/L with the exception of one replicates sample where a glyphosate concentration was 2.6 µg/L while for the other replicate the concentration was < 1 µg/L. Based on these results, it is concluded that glyphosate and its metabolite AMPA possess a very low potential for leaching.

In the scientific literature research for glyphosate (2010-2019), two articles were identified to provide further information relevant to the data point. The reliability of the articles was assessed as "reliable with restrictions". Thus, no new endpoints were derived, and the articles are considered as supportive information. Gjettermann *et al.* (2011, CA 7.1.4.1.1/008) showed in desorption experiments with soil particles mobilized from two soil columns after application of glyphosate that ≤ 20 % of particle-bound radioactivity in leachate desorbed within 20 min. In the article of Gjettermann *et al.* (2011, CA 7.1.4.1.1/009), 0.21 to 0.31 % of radioactivity applied as glyphosate were recovered in the leachates of soil columns from field plots cultivated with reduced tillage and conventional tillage, respectively.

Table 7.1.4.1.1-1: Column leaching studies

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.4.1.1/001	[redacted] 1996	Column Leaching	Glyphosate	Supportive	Leaching of aged residues
CA 7.1.4.1.1/002	[redacted] 1992	Column Leaching	Glyphosate	Supportive	
CA 7.1.4.1.1/003	[redacted] 1992	Column Leaching	Glyphosate-Trimesium	Supportive	
CA 7.1.4.1.1/004	[redacted] 1992	Column Leaching	Glyphosate-Trimesium	Supportive	Leaching of aged residues
CA 7.1.4.1.1/005	[redacted] 1991	Column Leaching	Glyphosate	Supportive	
CA 7.1.4.1.1/006	[redacted] 1978	Column Leaching	Glyphosate	Invalid	
CA 7.1.4.1.1/007	[redacted] 1972	Column Leaching	Glyphosate	Invalid	

Table 7.1.4.1.1-2: Column leaching – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.4.1.1/008	Gjettermann *et al.*, 2011	Column Leaching	Glyphosate	Reliable with restrictions	
CA 7.1.4.1.1/009	Gjettermann *et al.*, 2011	Column Leaching	Glyphosate	Reliable with restrictions	

1. Information on the study

Data point:	CA 7.1.4.1.1/001
Report author	[redacted]
Report year	1996
Report title	[14C]-Glyphosate: Determination of the mobility of aged residues in one soil
Report No	96-121-1020
Document No	
Guidelines followed in study	SETAC procedures for assessing the environmental fate and ecotoxicity of pesticides, Annex of FAO revised guideline on environmental criteria for the registration of pesticides, BBA Guideline Part IV, 4-2

Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
To evaluate the transport of the herbicide glyphosate and its soil metabolites, a sandy soil (Speyer 2.1) was treated with [^{14}C]-glyphosate. The test compound was incorporated into dry soil at a rate of 3.33 mg/kg equivalent to a field application rate of 2.5 kg/ha assuming penetration of glyphosate into the top 5 cm soil layer and a soil bulk density of 1.5 g/cm^3. The incubation temperature was 20 ± 2 °C. The treated soil samples were aged for 8 days in a soil metabolism apparatus and then an aliquot was applied on top of untreated triplicate columns filled with the same soil type. The soil columns were percolated continuously with 0.01 M $CaCl_2$ corresponding to 200 mm of rainfall in amounts of 100 mm per day over two days. After the percolation period, the amount and nature of radioactivity in the daily eluates and the 5 soil layers of 6 cm each were determined.

After eight days of aerobic incubation, an average of 72.4 % of the applied radioactivity was extractable by 0.35 M H_3PO_4/0.09 M $CaCl_2$. The rest of the radioactivity consisted of $^{14}CO_2$ (19.5 %) and non-extractable radioactivity (1.4 %). Organic volatiles did not contribute for more than 0.1 % of applied radioactivity. HPLC analysis of the combined extracts showed the presence of two radioactive components: [^{14}C]-glyphosate and its degradation product AMPA. The concentration of the parent compound was on average 50.7 % of applied radioactivity. AMPA was found at an average 21.7 % of applied radioactivity.

After percolation, 101.2 % and 101.3 % of the radioactivity applied onto column A and B, respectively, was retained by the soil. The major part of the ^{14}C-radioactivity (97.9 %-99.1 % of AR) was associated with the top column soil layer (0-6 cm). The subsequent soil layers (6-12 cm) contained 2.0 % to 3.3 % of applied radioactivity. All other soil layers (12-30 cm) did not contain more than 0.1 % of the applied radioactivity. The perculate did not contain more than 0.1 % of the applied radioactivity. Extractions of the top 2 segments (0 to 6 cm and 6 to 12 cm depth) showed that most of the radioactivity was extractable with 0.35 M H_3PO_4/0.09 M $CaCl_2$. Non-extractables did not exceed 2.4 and 2.2 % of the 0 to 6 cm layer radioactivity and 0.1 % of the 6 to 12 cm layer radioactivity (column A and B, respectively). HPLC analysis of the 0 to 6 cm layer extracts showed that 52.5 % and 44.7 % of the total applied radioactivity was glyphosate and 18.2 % and 24.7 % AMPA (columns A and B, respectively). These values are very similar to the values found prior to the leaching experiment. The 6 to 12 cm layer extracts were not characterised due to the low radioactivity levels. However, the total segment radioactivity was very low at 2.0 and 3.3 % of the applied radioactivity.

In conclusion, the results of this laboratory study indicate, that glyphosate and its major soil metabolite are immobile in the representative sandy soil used in this study. No residues penetrated deeper than 12 cm into the soil column and radioactivity in the leachates did not exceed 0.1 % of the applied radioactivity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate
Lot No.: Not indicated
Specific activity: 316 µCi/mg
Radiochemical purity: 99.6 %

2. Soil:

After receipt at Springborn Laboratories (Europe) AG, Horn, Switzerland on 23 May 1995, the soil was placed outside in the Springborn holding area and kept in a wooden box seeded with Phacelia and irrigated if necessary to provide natural conditions. An amount of the test soil was collected from the Springborn soil holding area located outside of the facility on 15 October 1995 and sieved to 2 mm. The soil moisture content was determined and adjusted to the approximate incubation moisture. Thereafter, the soil was stored under test conditions in closed plastic boxes. During storage, the soil was moistened, if necessary and thoroughly mixed daily to provide aerobic conditions for the soil microflora.

Table 7.1.4.1.1-3: Soil physicochemical properties

Parameter	Results
Soil type	Sand
Common name	Speyer 2.1
Batch number	F 12095
Country	Germany
Sand (50 µm – 2 mm) (%)	88.4
Silt (2 µm – 50µmm) (%)	9.8
Clay (< 2 µm) (%)	1.9
pH ($CaCl_2$)	5.9
Organic carbon (%)	0.62
Cation exchange capacity (meq/100 g)	5.0
Maximum Water Holding Capacity (%)	31
Bulk Density (disturbed) (g/cm^3)	Assumed: 1.5
Microbial biomass (mg C/100g)	Study start: 46 Study end: 71

B. STUDY DESIGN

1. Experimental conditions

The application rate was calculated as 3.33 mg/kg dry soil corresponding to a field application rate of 2.5 kg/ha. The radiolabelled test compound was isotopically diluted with the analytical standard of the test compound, yielding a specific activity of the application solution of 80.1 µCi/mg.

Prior to application, the soil moisture was adjusted to the approximate target moisture. Thereafter, a 200 µL aliquot of the application solution containing 0.335 mg of the diluted ^{14}C-test substance was added drop by drop to each 100 g (equivalent dry weight) soil sample by means of a Hamilton syringe.

The control soil samples were adjusted with deionised water to the target moisture of the respective soils. The aerobic incubation part of the study was carried out in all-glass metabolism flasks equipped with a trapping system. Ethylene glycol was used to trap organic volatiles, 0.5 M sodium hydroxide was used to trap $^{14}CO_2$.The metabolism flasks were continuously ventilated with CO_2 free and moistened air.

The aged leaching part of the study was conducted with 40 cm long all-glass column equipped with a porous glass-filter plate at the bottom. The inner diameter of the column was 4.8 cm. The water supply to the column was by means of a peristaltic pump. The leaching experiment was performed in duplicate. The columns were packed with untreated, pre-weighed, air-dried soil up to 28 cm. Thereafter, the soil columns were saturated with 0.01 M $CaCl_2$ (approximately 236 mL Aliquots of the dried treated soil were then packed on top of the untreated soil columns. Leaching was performed with a total of 380 mL of 0.01 M $CaCl_2$ solution per column over 2 days. This corresponds to an irrigation rate of 200 mm per 48 hours.

2. Sampling

Samples were taken immediately after dosing and after 5 and 8 days of aerobic incubation. A total of 10 samples were incubated aerobically. Five samples were used to monitor the degradation of the test compound up to its DT_{50}. Aliquots of 3 aged samples were used to confirm the DT_{50} and to conduct the aged leaching experiment. Aliquots from volatility traps for organic compounds and $^{14}CO_2$ were collected on days 1, 2, 4, 5, 7 and 8 post-treatment. Trapped radioactivity was measured by LSC.

Leachates were collected after 24 and 48 h from soil columns. After the percolation period, soil columns were sacrificed and sectioned in 5 soil layers of 6 cm each.

3. Analytical procedures

Radioactivity in traps for volatiles and leachates were measured by LSC. Soils of segment 1were extracted exhaustively with total 125 mL of 0.35 M H_3PO_4/0.09 M $CaCl_2$ per 50 g dry weight soil. Soils were extracted three times at room temperature using the soil to solvent ratio of approximately 1:2.5 (w:v). This procedure was done by shaking the samples on an overhead shaker. After centrifugation of each individual extract, the radioactivity in extracts was determined by LSC.

Non-extractable radioactivity of extracted wet or air dried soil was measured by post-extraction combustion followed by radio assay.

Extractable radioactivity of glyphosate and its radioactive degradation products was qualitatively and quantitatively analysed by HPLC without any further clean-up (direct injection, column: Nucleosil 5 SB 20 cm x 0.4 cm id; flow rate: 1 mL/min) with radiometric detection (RAM). One dimensional, radio-TLC (Thin-Layer Chromatography on silica gel 60 F 254, 0.25 mm Merck) plates with selected samples helped to tentatively characterise AMPA using solvent system consisting of 40 mL methanol, 20 mL water, and 3 mL of 25 % aqueous ammonia.

II. RESULTS AND DISCUSSION

A. DATA

The material balance and degradation product pattern of [^{14}C]-glyphosate in soil Speyer 2.1 is presented in the table below. The values are presented in % of AR, at start of the ageing period and after 5 and 8 days of incubation.

Table 7.1.4.1.1-4: Material balance of [^{14}C]glyphosate in soil Speyer 2.1 during 8 days of incubation

Radioactive residues (%)	Incubation time (days)					
	0	5	8 (mean)	8 (1) [1]	8 (2) [1]	8 (3) [1]
Volatiles						
Carbon dioxide	n.d.	12.0	**19.5**	19.7	19.3	19.6
Organic volatiles	n.d.	< 0.1	**< 0.1**	< 0.1	< 0.1	< 0.1
Total	**n.d.**	**12.0**	**19.5**	**19.7**	**19.3**	**19.6**
Extractables						
Glyphosate	92.1	63.9	**50.7**	55.9	51.9	51.2
AMPA	4.1	19.7	**21.7**	16.0	20.6	21.6
Total	**96.3**	**83.6**	**72.7**	**71.9**	**72.5**	**72.8**
Non-extractables	0.3	0.8	**1.4**	1.2	1.4	1.5
Recovery	96.6	96.5	**93.3**	92.8	93.3	93.9
Mean recovery	**94.4 ± 1.7**					

[1] 8(1) and 8(2) stand for the aged samples of which aliquots were applied on top of untreated Speyer 2.1 columns A and B, respectively. 8(3) was applied on top of column C (reserve).
n.d. = Not determined

The vertical distribution of aged soil residues of [^{14}C]-glyphosate in Speyer 2.1 sand after percolation of 200 mm artificial rain and the radioactive residues in soil columns are presented in Table 7.1.4.1.1-5 and Table 7.1.4.1.1-6, respectively.

Table 7.1.4.1.1-5: Vertical distribution of aged soil residues of [^{14}C]glyphosate in Speyer 2.1 soil (sand)

	Speyer 2.1 Column A		Speyer 2.1 Column B	
	(%)[1)]	(%)[2)]	(%)[1)]	(%)[2)]
Leachate Day 1	< 0.1	< 0.1	< 0.1	< 0.1
Leachate Day 2	< 0.1	< 0.1	< 0.1	< 0.1
Total leachate	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**
CO_2 Headspace	3.2	2.4	3.1	2.3
Organic volatiles headspace	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles headspace	**3.2**	**2.4**	**3.1**	**2.3**
Column segment 1 (top)	99.1	72.4	97.9	72.4
Column segment 2	2.0	1.5	3.3	2.5
Column segment 3	< 0.1	< 0.1	< 0.1	< 0.1
Column segment 4	< 0.1	< 0.1	< 0.1	< 0.1
Column segment 5 (bottom)	< 0.1	< 0.1	< 0.1	< 0.1
Total column segments	**101.2**	**73.9**	**101.3**	**74.9**
Recovery	104.4	76.3	104.4	77.2

[1)] Values were calculated in percent of radioactivity applied to each column.
[2)] Values were calculated in percent of radioactivity applied to each soil sample prior to aging and leaching

Table 7.1.4.1.1-6: Radioactive residues in the top segment of the duplicate soil columns

Radioactive residues (%)		Column A Segment 1 (Top)			Column B Segment 1 (Top)		
		(%)[1)]	(%)[2)]	(%)[3)]	(%)[2)]	(%)[1)]	(%)[3)]
Extractables	Glyphosate	72.5	71.8	52.5	71.0	69.6	44.7
	AMPA	25.2	24.9	18.2	24.7	24.2	24.7
	Total	97.6	96.7	70.7	95.7	93.7	69.3
Non-extractables		2.4	2.4	1.7	2.2	2.2	1.6
Recovery		100.0	99.1	72.4	97.9	95.9	70.9

[1)] Radioactive residues related to extractable and non-extractable radioactivity per sample.
[2)] Values were calculated in percent of radioactivity applied to each column.
[3)] Values were calculated in percent of radioactivity applied to each soil sample prior to aging and leaching.

B. MASS BALANCE

The overall recovery over the incubation period amounted to 94.4 % AR. Regarding the leaching experiment, the results demonstrated that 101.2 and 101.3 % of the applied radioactivity applied onto the duplicate soil columns was retained by the column. The majority (99.1 % and 97.9 %) of radioactivity was found in the 0 to 6 cm segment. Significantly less radioactivity was found in the 6 to 12 cm soil layer: 2.0 % and 3.3 % of total column A and B radioactivity, respectively. Leached radioactivity did not exceed 0.1 % of the total column radioactivity. Head volatiles contributed 3.2 and 3.1 %. The total recovery for both columns A and B amounted to 104.4 %. This value corresponds to 76.3 % and 77.2 % of the radioactivity which had been applied to each individual metabolism flask.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

During aerobic incubation almost complete extraction of radioactivity was observed, since 96.6 % of the applied radioactivity was found in the extracts and 0.3 % in the extracted soil. Thereafter a constant and significant decrease of extractable radioactivity was seen during the eight days of incubation: Extractables accounted for a range of 71.9 % to 72.8 % which corresponds to a mean of 72.7 % of the applied radioactivity. At the same time, non-extractable radioactivity accounted on average at 1.5 %.

Following leaching, 97.6 % and 95.7 % of the 0 to 6 cm soil segment radioactivity (column A and B, respectively) was extractable. Non-extractable radioactivity amounted to 2.4 % (column A) and 2.2 % (column B).

D. VOLATILE RADIOACTIVITY

Volatiles increased constantly and significantly during aerobic incubation. By far, most of the volatile radioactivity was $^{14}CO_2$. Organic volatiles contributed less than 0.1 % of the applied radioactivity. The total amount of volatiles during sample incubation was between 19.3 % and 19.7 % which corresponds to a mean value of 19.5 %.

E. TRANSFORMATION OF THE TEST ITEM

In the 0 to 6 cm segment 72.5 % and 71.0 % of the extractable segment radioactivity was characterised as glyphosate. These values correspond to 52.5 % and 44.7 % of total applied radioactivity to one metabolism system. In the extracts from the top segment AMPA appeared at 25.2 % and 24.7 % of the column layer radioactivity corresponding to 18.2 and 24.7 % of the total applied radioactivity. The dpm level of the 6 to 12 cm layers of both columns was very low. As a consequence, a characterization was not feasible.

III. CONCLUSIONS

The results indicate that glyphosate and its major soil metabolite are immobile in the representative sandy soil used in this study. No residues penetrated deeper than 12 cm into the soil column and radioactivity in the leachates did not exceed 0.1 % of the applied radioactivity.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The mobility of glyphosate was assessed via aged column leaching experiments. Only small amounts of radioactivity were found in the leachate, while the majority of the test substance remained in the topmost soil segments or was mineralised during aging. The results demonstrate that glyphosate is not prone to leaching in soil. The study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.1.1/002
Report author	[redacted]
Report year	1992
Report title	Leaching characteristics of formulated [^{14}C]glyphosate in three soils
Report No	281430
Document No	
Guidelines followed in study	Biologische Bundesanstalt Deutschland (BBA) Richtlinien Teil IV, 4.2 Dezember 1986
Deviations from current test guideline	From OECD 312: - No mass balance given in study report
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The leaching characteristics of formulated [^{14}C]-glyphosate were investigated in three German standard soils Speyer 2.1 (sand), Speyer 2.2 (loamy sand) and Speyer 2.3 (sandy loam). For this purpose, the ^{14}C-1abelled test article formulated as the commercial product GLYPHOSATE 360 diluted with water was applied dropwise onto the top of untreated soil columns at an amount of 3.47 kg a.s./ha, corresponding to 0.680 mg a.s. per soil column. Leaching was then performed for 2 days by artificial rainfall of 200 mm (393 ml). The collected leachates were analysed for total radioactivity.

Only a low proportion of the applied radioactivity was found in the leachates. Total mean values of the leachates from two columns found in the 0-48 hours interval were 1.45 % (Speyer 2.1), 0.12 % (Speyer 2.2) and 0.63 % (Speyer 2.3). In terms of parent equivalents, for the 0-48 hour interval, 0.025 mg/kg, 0.002 mg/kg and 0.011 mg/kg were found for soils Speyer 2.1, 2.2 and 2.3, respectively. The radioactivity leached originated mainly from polar unknown fractions.

Based on these results, it is concluded that glyphosate when applied as GLYPHOSATE 360 at a rate of 10 L/ha does not represent a potential danger to groundwater reservoirs.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Identification: Formulated [^{14}C]glyphosate
Lot No.: CFA.745 C5
Specific activity: 11.1 MBq/mg (299 µCi/mg)
Radiochemical purity: 99.0 % and 98.3 % as determined before and after conduct of the test

and

Identification: Glyphosate
Lot No.: 185-ff-131
Chemical purity: 99.5 % (0.1 %NaCl, 0.1 %H_2O)

2. Soil

The study was performed with three German standard soils: Speyer 2.1, 2.2 and 2.3. The soils were air dried and sieved through a 1 mm sieve. The moisture content of the soils was adjusted to the field capacity.

Characteristics of the test soils are presented in the table below.

Table 7.1.4.1.1-7: Characteristics of test soils

Parameter	Results		
Soil	Speyer 2.1	Speyer 2.2	Speyer 2.3
Textural Class	Loamy sand	Sandy loam	Sandy loam
Sand (> 0.2 mm)	67.6	48.4	44.5
Sand/Silt (20 µm – 200 µm) [2] (%)	23.3	39.6	31.2
Silt (2 µm – 20 µm) [2] (%)	3.8	7.1	13.4
Clay (< 2 µm) [2] (%)	5.3	4.9	10.9
pH [1]	6.0	6.0	6.6
Organic carbon (%)	0.48	2.55	0.74
Cation exchange capacity (meq/100 g)	3.6	7.2	4.5
Bulk density (g/cm^3) [3]	1.65/1.66	1.45/1.43	1.46/1.43/1.41

[1] Medium not indicated
[2] 2-20 µm corresponding to fine and medium silt, 20-200 µm corresponding to coarse silt and fine sand and > 200 µm corresponding to medium and coarse sand according to German DIN 4022
[3] Determined for each column separately

B. STUDY DESIGN

1. Experimental conditions

Glass columns (5.0 cm inner diameter, 40.0 cm length), corresponding to a cross-sectional area of 19.6 cm^2, were filled with the air-dried untreated soils up to 30 cm, a paper filter was placed on top of the soil and thereafter saturated with water overnight. The bulk density in the soil columns ranged from 1.41 to 1.66 g/cm^3, depending on the soil type. Two replicate soil columns were treated per soil type. Additionally, one column was filled with Speyer 2.3 soil and not treated. Leachates obtained from this column served as control samples.

A stock solution was prepared from radio-labelled and un-labelled material, and determined to have a concentration of 1.42 mg/ml. The formulation solution, consisting of isopropylamine, Berol, ethylene glycol and bi-distilled water, and the stock solution were used to prepare the application solution with a

content of 0.680 mg/ml. A total volume of 1000 µl was applied, which corresponded to 0.680 mg a.s./column or a field rate of 3.47 kg a.s./ha. The applied amount was slightly lower than the target value of 3.60 kg a.s./ha. The test article was applied onto the top layer of the saturated soils in an aqueous formulation solution, dropwise following a spiral movement about 0.5 cm away from the column walls, to avoid preferred paths of flow.

Thereafter, a paper filter was placed on top of the column and then the rain-simulation was started using bi-distilled water. Artificial rain of about 200 mm (= 196 ml daily or 393 ml total as target volume) was delivered within 48 hours by means of a peristaltic pump at a flow rate of about 0.14 ml/min. The leaching study was performed at room temperature in the dark for two days.

2. Sampling

The total leachate was collected in Erlenmeyer flasks from 0-24 hours and 24-48 hours. After completion of the leaching period, the columns were sectioned into 6 cm segments and stored at -20 °C.

3. Analytical procedures

The radioactivity was determined on a Packard scintillation counter.

For the characterisation of radioactivity in leachate one-dimensional TLC was performed on pre-coated plates of cellulose with a layer thickness of 0.5 mm and on RP-18 F 254 plates with a layer thickness of 0.25 mm. 150 ml of leachates obtained were concentrated by lyophilisation. The residues were suspended in 3 ml of bi-distilled water, centrifuged and chromatographed. SS 11 (n-Propanol/water/acetic acid/ammonia solution 25 % (40+20+10+5)) and SS 16 (Methanol/water/ammonia solution 25 % (40+10+0.5)) were used as solvent systems. Co-chromatography was performed by mixing the solutions containing the radioactive material 1:1 with a solution containing the analytical standard (6 mg/mL).

The radioactive zones on TLC-plates were detected by scanning with an Automatic TLC Linear Analyser. Un-labelled parent compound was visualised by spraying with ammonium molybdate (1 % in water) followed by spraying 1 % tin(II)-chloride (dissolved in 10 % HCl) and heated for 5 minutes at 100 °C.

The radiochemical purity of the test article was determined by TLC in two solvent systems SS 11 and SS 16. The results obtained indicated that the purity of 99.0 % agreed well with that given by the sponsor, e.g. 99 % using a HPLC method. A further purity check performed on September 19, 1991, using a HPLC method confirmed the radiochemical stability of the test article resulting in 99.3 % (RCC-Project 271618). Concentrations and stability of stock and application solutions was confirmed via LSC.

II. RESULTS AND DISCUSSION

A. DATA

The radioactivity levels found in the leachates is presented for both columns in the following tables.

Table 7.1.4.1.1-8: Leached water (ml) and radioactivity levels (in % of AR) in the leachate from soil Speyer 2.1 treated with formulated [^{14}C]glyphosate

Parameter	Time interval: 0 – 24 h		Time interval: 24 – 48 h		Total: Time interval: 0 – 48 h	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Leached water (ml)	190.9	193.1	201.0	201.3	391.9	394.4
Mean	**192.0**		**201.2**		**393.2**	
Radiocativity (%)	0.16	0.10	0.81	1.82	0.97	1.92
Mean (%)	**0.13**		**1.32**		**1.45**	

Table 7.1.4.1.1-9: Leached water (ml) and radioactivity levels (in % of AR) in the leachate from soil Speyer 2.2 treated with formulated [^{14}C]-glyphosate

Parameter	Time interval: 0 – 24 h		Time interval: 24 – 48 h		Total: Time interval: 0 – 48 h	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Leached water (ml)	193.5	177.1	201.3	194.6	394.8	371.7
Mean	**185.3**		**198.0**		**383.3**	
Radiocativity found (%)	< 0.01	0.01	0.07	0.16	0.07	0.17
Mean (%)	**0.01**		**0.11**		**0.12**	

Table 7.1.4.1.1-10: Leached water (ml) and radioactivity levels (in % of AR) in the leachate from soil Speyer 2.3 treated with formulated [^{14}C]-glyphosate

Parameter	Time interval: 0 – 24 h		Time interval: 24 – 48 h		Total: Time interval: 0 – 48 h	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Leached water (ml)	195.4	190.4	202.5	205.5	397.9	395.9
Mean	**192.9**		**204.0**		**396.9**	
Radiocativity found (%)	0.01	0.04	0.75	0.47	0.76	0.51
Mean (%)	**0.02**		**0.61**		**0.64**	

Table 7.1.4.1.1-11: Total concentration of radioactivity (mg a.s./kg) found in the leachate [1] of soils Speyer 2.1, 2.2 and 2.3 treated with formulated [^{14}C]-glyphosate (0 - 48 h)

Parameter	Speyer 2.1		Speyer 2.1		Speyer 2.1	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
Concentration (mg/kg)	0.017	0.033	0.001	0.003	0.013	0.009
Mean	**0.025**		**0.002**		**0.011**	

[1] 1 liter of leachate was taken as equivalent to 1 kg

B. CHARACTERISATION OF LEACHATES

The mean total leached volume per column amounted to 393.2 mL, 383.3 mL and 396.9 mL for soils Speyer 2.1, 2.2 and 2.3, respectively. These values compared well with the target value of 393 ml per column.

In soil Speyer 2.1, the leachates from two columns for 0-24 hours contained 0.13 % of the applied radioactivity, whereas the second fraction (24-48 hours) contained 1.32 %. The mean total radioactivity detected (0-48 hours) was 1.45 % of the total applied radioactivity. In terms of mg a.s./kg the highest concentration of parent equivalents found was 0.033 mg/kg. A mean total of 0.025 mg/kg was obtained for these two columns.

Soil Speyer 2.2 contained a higher amount of organic carbon and thus its adsorption capacity was larger. For the 0-24 hour interval only 0.01 % of the applied radioactivity was found in the two columns. In the 24-48 hour interval, this value increased to 0.11 %. The mean total radioactivity in the 0-48 hour interval amounted to 0.12 % of the total applied radioactivity. In terms of mg a.s./kg, for each column, not more than 0.003 mg/kg were detected.

In soil Speyer 2.3 also low levels of radioactivity were found in the leachates of the two columns used. In the time interval from 0 to 24 hours, only 0.02 % of the applied radioactivity was found. In the following 24 hours, an increase to 0.61 % took place. Hence, in the 0-48 hour period, a mean total radioactivity of 0.63 % was found.

The highest total amount of parent equivalents per column was 0.013 mg/kg. Thus, a mean total of 0.011 mg/kg was obtained for these two soil columns in the 0-48 hour leaching period.

For soil Speyer 2.1 at least three radioactive fractions could be detected by TLC, whereby the presence of parent molecule besides two unknown polar fractions seems probable. However, the total concentration of [^{14}C]-glyphosate equivalents in the leachate of soil Speyer 2.1 did not exceed 0.011 mg/kg. Similar results were obtained for the other two soils.

III. CONCLUSIONS

The radioactivity levels found in the leachates from soils Speyer 2.1, 2.2 and 2.3 amounted to 1.45 %, 0.12 % and 0.63 % of the total applied radioactivity, respectively. These levels represented 0.025 mg/kg, 0.002 mg/kg and 0.011 mg/kg in the leachates from soils Speyer 2.1, Speyer 2.2 and Speyer 2.3, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The mobility of glyphosate was assessed via column leaching experiments. Only negligible amounts of applied radioactivity were encountered in the leachate. Soil columns were not analysed. The results confirmed the low leaching potential of glyphosate in soil. Due to the limited information provided in the study report and in view of the fact that adequate batch equilibrium data is available, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.1.1/003
Report author	[redacted]
Report year	1992
Report title	Glyphosate-trimesium: Leaching of formulated material in soil column
Report No	RJ1247B
Document No	
Guidelines followed in study	Guidelines for the Official Testing of Plant Protection Products Part IV, December 1986 4-2. Seepage Behaviour of Plant Protection Products (formerly BBA Memorandum No. 37). Federal Biological Research Centre for Agriculture and Forestry Federal Republic.
Deviations from current test guideline	From OECD 312: - The simulation of rain was done for 40-45 h and afterwards the columns were left for 1-3 h to allow the water to drain off - Soil column segments were not analysed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The mobility of glyphosate-trimesium, formulated as a suspension concentrate, was determined by leaching in soil columns (35 cm long). Three LUFA standard soils, one coarse sand, one loamy sand and one sandy loam (1.4 %. 5.1 % and 2.5 % organic matter, respectively) were used. Glyphosate was applied at a rate equivalent to 4 kg a.s./ha to saturated soil columns and then eluted with 393 mL (corresponding to 200 mm) of water over approximately 48 hours. Residues of glyphosate (N-phosphonomethylglycine, PMG) and trimesium (trimethylsulphonium cation, TMS+) both derived from glyphosate-trimesium were below the limit of determination (i.e. <25 µg/L for glyphosate and <10µg/L for TMS+). The total amount of glyphosate-trimesium in the leachate was less than 2 % of that applied.

It is concluded that the normal agricultural use of glyphosate-trimesium is unlikely to result in any contamination of ground water.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate-trimesium in concentrated solution
Formulated product:	YF7712A
Nominal concentration:	480 g/L glyphosate-trimesium
Measured concentration:	97 %

2. Soil

Three German standard soils, Speyer 2.1, Speyer 2.2 and Speyer 2.3, were used. The physical and chemical characteristics of the soils were determined by Natural Resource Management Ltd, Jealott's Hill Research Station, Jealott's Hill, Bracknell, Berkshire, RG12 6EY as presented in the table below.

Table 7.1.4.1.1-12: Soil physicochemical properties

Parameter	Results		
Soil	Speyer 2.1	Speyer 2.2	Speyer 2.3
Textural Class (USDA)	Sand	Loamy sand	Sandy Loam
Sand (50 µm – 2 mm) (%)	89	84	71
Silt (2 µm – 50µmm) (%)	7	11	18
Clay (< 2 µm) (%)	4	5	11
pH [1]	5.4	5.7	6.7
Organic matter (%)	1.4	5.1	2.5
Organic carbon (%) [2]	0.81	2.96	1.45
Cation exchange capacity (meq/100 g)	3.5	8.2	8.3

[1] Medium not indicated
[2] Calculated from organic matter according to OC = OM × 0.58

B. STUDY DESIGN

1. Experimental conditions

The soil columns were made of glass tubing (35 cm length) with an internal diameter of ≤5 cm. A glass funnel of internal diameter 5.2 cm was attached to the bottom of the column by glass fusion. The funnel stem was plugged with glass wool and the funnel filled with acid-washed quartz sand. The columns were uniformly packed with air-dried 1 mm sieved soil to a depth of 30 cm. The soil was added in small

increments (approximately 1 cm depth). The initial 5 cm soil added was weighed and used to determine the total weight of soil required to fill the column (30 cm). This was used as an additional check to ensure a uniform density was achieved. The average air dried weight of Speyer 2.1, 2.2 and 2.3 soils added to the columns was 1000 g, 923 g and 904 g, respectively. The top 5 cm of the glass column contained no soil and a glass wool pad was placed on top of the soil to assist uniform distribution of water to the soil surface. Triplicate columns containing each soil type were prepared, two of which were to be treated with glyphosate-trimesium and a third to be used as an untreated control.

The columns were clamped in a vertical position and a flask placed under each to collect the leachate. Before application of the pesticide, the soil columns were maintained at a constant temp 22 ± 5 °C and were leached for between 40 and 45 hours with deionised water applied at a rate of 0.15 – 0.17 cm^3/min using a peristaltic pump. After this time, during which the soil in the columns had become saturated, the water flow was stopped and the columns left for 1-3 hours to allow excess water to drain off.

The rate of application to the soil surface of each column was 4 kg a.s./ha concentrated solution. Formulated glyphosate-trimesium (10 cm^3) was diluted in 100 cm^3 of ultra-pure water. An aliquot (10 cm^3) of this solution was further diluted to 100 cm^3 with ultra-pure water. An aliquot of this diluted suspension (170 µL) was evenly applied to the soil surface of each column (except untreated controls) using a syringe. A 5 mm band of soil was placed around the circumference of the column to minimise the risk of leaching between the soil and glass interface. After treating the columns, the glass wool pad was replaced on top of the soil and the water flow re-started. A total of 393 cm^3 of deionised water (equivalent to 200 mm rain) was applied dropwise to the top of each column within a period of 48 hours using a peristaltic pump.

2. Analytical procedures

After completion of leaching, the observed volume, odour and colour of each leachate was recorded. Each leachate was analysed by two analytical procedures, one to determine the concentration of glyphosate (N-phosphonomethylglycine) and one to determine trimesium (trimethylsulphonium ion (TMS+)) concentration.

For glyphosate (PMG) analysis, the method involved an aliquot of the leachate was diluted 1:10 with deionised water, and percolated through cation exchange resin. An aliquot was evaporated to dryness, dissolved in 0.1 M disodium-hydrogen borate and derivatised with 9-fluorenylmethyl chloroformate. Final quantitative determination of the derivative was by high performance liquid chromatography (HPLC) using fluorescence detection. For trimesium analysis an aliquot of the leachate was heat treated with base to dealkylate the trimesium to form dimethylsulfide (DMS). The amount of DMS was then determined by gas chromatography using flame photometric detection in the sulphur mode. For both glyphosate and trimesium analysis, residues were quantified using external standards and corrected for recovery values generated by analysis of fortified control samples if < 100 %.

The mean recovery value for trimesium in spiked leachate was found to be 103 % (coefficient of variation = 13 %, n = 6). The mean recovery value for glyphosate in spiked leachate was found to be 93 % (coefficient of variation = 14 %, n = 6).

For glyphosate and trimesium the limit of determination in leachate was set at 25 µg/L and 10 mg/L, respectively.

II. RESULTS AND DISCUSSION

A. DATA

Residues of glyphosate and the trimesium cation in the leachate obtained from soil columns treated with the formulation YF7712A, are given in the following table, together with the volume, colour and odour of the leachate.

Table 7.1.4.1.1-13: Results of leachate analysis of glyphosate (PMG) and trimesium (TMS+)

Application Rate (kg a.s./ha)	Volume (ml)	Odour	Colour	Residues	
				PMG (µg/L)	TMS+ (µg/L)
Leachate Type : Speyer 2.1					
Control	387	Rich Earth	Clear light, amber	< 25	< 10
4.0	395	Rich Earth	Clear light, amber	< 25	< 10
4.0	397	Rich Earth	Clear light, amber	< 25	< 10
Leachate Type : Speyer 2.2					
Control	395	Damp Earth	Amber	< 25	< 10
4.0	397	Damp Earth	Amber	< 25	< 10
4.0	400	Oamp Earth	Amber	< 25	< 10
Leachate Type : Speyer 2.3					
Control	180 [1]	Fresh Earth	Very light, amber clear	< 25	< 10
4.0	390	Fresh Earth	Very light, amber clear	< 25	< 10
4.0	385	Fresh Earth	Very light, amber clear	< 25	< 10

[1] After 48 hours only 180 ml of leachate had passed through the column containing Speyer 2.3 control. This was due to the soil being packed so tightly that the flow of water was impeded.

B. CHARACTERISATION OF LEACHATES

From an application of glyphosate-trimesium at 4 kg a.s./ha onto coarse sand, loamy sand and sandy loam soils, residues of glyphosate and trimesium both derived from glyphosate-trimesium were below the limit of determination (i.e. < 25 µg/L for glyphosate and < 10 µg/L for the trimesium-cation). The total amount of glyphosate-trimesium in the leachate was thus less than 2 % of that applied.

III. CONCLUSIONS

Normal agricultural use of glyphosate-trimesium is unlikely to result in any contamination of ground water.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The mobility of glyphosate was assessed via column leaching experiments. While column soils were not analysed for the test substance, only small amounts were found in the leachate. The results demonstrate that glyphosate is not prone to leaching in soil. The study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.1.1/004
Report author	[redacted]
Report year	1992
Report title	(14C)-Glyphosate-Trimesium: Aged soil Leaching
Report No	7113-38/172
Document No	
Guidelines followed in study	OECD 312
Deviations from current test guideline	From OECD 312: - The mass balance during incubation was incomplete - Incubation temperature fell do 14 °C on three days during the 1st week of incubation, due to failure of the water bath heater, temperature was not recorded for two days
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The mobility of [^{14}C]-glyphosate-trimesium and its degradation products has been investigated following ageing for 30 days in Speyer 2.1 soil at 20 ± 2 °C and 40 % MWHC.

[^{14}C]glyphosate-trimesium, radiolabelled in either the glyphosate (phosphonomethylglycine anion, PMG) or the trimesium (trimethylsulphonium cation, TMS) moiety, was applied to pre-equilibrated soil samples (100 g dry weight equivalent) at an application rate equivalent to 4 kg a.s./ha. The treated soil was aged under aerobic conditions in Erlenmeyer flasks. Carbon dioxide-free air was drawn through the units and passed through two ethanolamine traps to collect liberated $^{14}CO_2$.

Duplicate soil samples treated separately with [^{14}C]-anion and [^{14}C]-cation labelled test article were analysed for glyphosate and trimesium, respectively, immediately after application and at the end of the ageing period.

In the day 0 samples a large proportion (> 95 %) of applied radioactivity was recovered in extracts of soil. This declined to about 52 % and 10 % for anion and cation labelled test article, respectively, after 30 days. During the ageing period significant quantities of $^{14}CO_2$ were formed from both anion (about 33 %) and cation (about 57 %) labelled forms of glyphosate-trimesium. Unextracted residues accounted for about 12 % and 21 % of applied radioactivity in 30 day soil samples treated with anion and cation labelled test article, respectively.

Immediately after application of glyphosate-trimesium the extractable radioactivity was predominantly parent compound. After 30 days glyphosate and trimesium accounted for about 15 and 5 % of applied radioactivity, respectively. In soil treated with anion labelled glyphosate-trimesium, aminomethylphosphonic acid (AMPA) was a significant degradation product comprising about 26 % of applied radioactivity.

On completion of the ageing period, duplicate soil samples treated with each form of the test article were transferred to the top of two pre-conditioned soil columns (28 cm lengthx 5 cm inner diameter) of Speyer 2.1 soil and leached with deionised water (ca. 393 mL; equivalent to 200 mm rain) over a 48 h period.

The mean percentage of applied radioactivity present in the leachates was 0.1 and 0.5 % for anion and cation labelled glyphosate-trimesium, respectively, and therefore neither the test article nor its degradates are likely to move through soil to ground water supplies.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate-trimesium, anion labelled
Lot No.: 91-J19
Specific activity: 2.07 GBq/mmol
Radiochemical purity: > 99 %

Identification: [^{14}C]glyphosate-trimesium, cation 887eionize
Lot No.: 91-70
Specific activity: 2.02 GBq/mmol)
Radiochemical purity: 98 %

2. Soil:

A mildly humus sand (Speyer standard soil 2.1) was supplied by ICI Agrochemicals, Jealott's Hill, Bracknell, Berkshire. The soil was stored outside undercover prior to use. Deionised water was added regularly to prevent dehydration.

After sieving (2 mm) the soil was characterised for organic matter content, particle size distribution, cation exchange capacity, moisture holding capacity at 1/3 and 15 bar, and pH (H_2O) by ICI Agrochemicals, Jealott's Hill, Bracknell, Berkshire. Maximum water holding capacity (MWHC) was determined at the Soil Survey and Land Research Centre, Shardlow, Derbyshire.

Characteristics of the test soils are presented in the table below.

Table 7.1.4.1.1-14: Soil physicochemical properties

Parameter	Results
Soil	Speyer 2.1
Textural Class (USDA)	Sand
Sand (50 µm – 2 mm) (%)	89
Silt (2 µm – 50µmm) (%)	8
Clay (< 2 µm) (%)	3
pH (water)	6.9
Organic matter (%)	1.8
Organic carbon (%) [1]	1.04
Cation exchange capacity (meq/100 g)	2.6
Maximum Water Holding Capacity (%)	30.44
Water Holding Capacity at 0.33 bar (%)	4.16
Water Holding Capacity at 15 bar (%)	2.98

[1] Calculated from organic matter according to OC = OM × 0.58

B. STUDY DESIGN

1. Experimental conditions

Eighteen portions of 2 mm sieved Speyer 2.1 soil (100 g dry weight equivalent) were weighed into Erlenmeyer flasks (250 mL) and adjusted to 10 % of the MWHC. Moistened carbon dioxide-free air was drawn over the surface of each sample except when condensation within the units caused the moisture content of the soil to rise. When this occurred, un-moistened carbon dioxide free air was drawn through the units until the soil moisture content returned to the correct level. The moisture content of the soil samples was determined every two to three days and any moisture loss was replaced with 888eionized water. The units were incubated in the dark at 20 ± 2 °C in a thermostatically controlled water bath. Flasks were pre-incubated for 36 days prior to test article application to permit the soils to equilibrate.

[^{14}C]anion labelled glyphosate-trimesium (0.394 mg/mL; 1 mL) [^{14}C]cation labelled glyphosate-trimesium (0.366 mg/mL; 1 mL) in HPLC grade water were each applied to eight soil samples dropwise using a glass pipette. Two units were not treated with test article. After test article application the flasks were shaken to ensure thorough mixing of the samples.

Following test article addition, air drawn through the units was passed through a series of three traps, the first empty trap acting as a security trap and the second and third containing ethanolamine to trap liberated $^{14}CO_2$. The ethanolamine was changed 7, 14, 23 and 30 days after test article application.

For the preparation of the soil columns six glass columns (ca. 35 cm length x 5 cm inner diameter) were used. The column outlets were plugged with glass wool and acid washed sand was placed in the conical part of the columns. Air dried soil, 1 mm sieved, was added to the columns with mechanical shaking to a depth of 28 cm. Shaking was continued until the surface of the column had settled. A Whatman GFA glass fibre filter paper disc was placed on the top of each column. The soil was then saturated by adding water dropwise to the surface of the column until seepage water percolated through the foot of the column. The application of the treated, aged soils took place within seven hours of saturation.

After 30 days of incubation two samples were transferred to the top of separate saturated soil columns after removing the glass fibre filter paper. Two samples of untreated incubated soil were transferred to the top of the remaining two columns. Leachate from these columns was used as blank material for liquid scintillation counting (LSC). A quantitative transfer was achieved using a small volume of water. The added soil was pressed down and a glass fibre filter paper placed on the top of each soil column. Light was excluded from the columns and collecting vessels by surrounding them with aluminium foil. The leaching was conducted at room temperature.

Each column was eluted with the equivalent of 200 mm of deionised water (ca. 393 mL) over a period of 48 h. Steady leaching rates were achieved using a calibrated multichannel peristaltic pump. An additional volume of water (18.3 mL), equivalent to the quantity of water required to raise the moisture content of 100 g dry weight equivalent of Speyer 2.1 soil from 40 % MWHC to 100 % MWHC, was then applied to each column.

2. Sampling

Duplicate soil samples treated with each form of test article were sampled immediately after application and after 30 days of incubation.

Leachates were collected over the entire 48 h period forming one merged sample. Additional leachates collected after the 48 h period were assayed radioactivity separately.

3. Analytical procedures

The total soil sample in each incubate was extracted, on the day of sampling, three times with ammonia solution (0.5 M; 100 mL) for glyphosate analysis, and with ammonium formate (1 M; 100 mL) for trimesium analysis, for 30 minutes with mechanical shaking. Extracts were separated from soil by centrifugation and were kept cool and dark between successive extractions. The total weight of extract was determined and a weighed subsample (ca. 10 g) was taken for further analysis. The remaining extract was

stored at ca. -18 °C. Following centrifugation subsamples of extract were passed through a series of filters. The filters were rinsed with small volumes of ammonia solution (0.5 M) and formic acid (1 M). The rinsings were pooled with the filtrate. The filtered extracts were neutralised with concentrated formic acid, total weights determined and weighed aliquots were radio-counted. Combined filtered and neutralised extracts were freeze-dried and re-suspended in formic acid (1 M, 5 to 10 mL). The suspensions were transferred to vials, to provide samples for chromatography. The original flasks were rinsed with formic acid (1 M, 20 mL) and the rinsings were weighed and counted. Prior to TLC, the reconstituted extracts were basified with ammonia (about 1 to 2 mL) and thoroughly mixed to produce a very fine suspension. Aliquots (100 µl) of this suspension were radio-counted to determine recovery. Prolonged storage before chromatography of the basified extracts was avoided.
The following solvents and plates were used for thin layer chromatography (TLC).

Table 7.1.4.1.1-15: Solvents and plates used for TLC

Compound	No.	Solvent	Plate
[^{14}C]anion labeled Glyphosate-trimesium	1	Methanol : Ammonia (10 %) : Trichloroacetic acid solution :Water 12:3:1:6 (v/v/v/v)	Analtech Silica HLF
	5 [1]	Methanol : Ethanol : Ammonia (s.g.880) : water 3:3:2:2	Analtech Silica HLF
[^{14}C]cation labeled Glyphosate-trimesium	3	4 % Ammonium Formate solution : Methanol 1:1 (v/v)	Macherey Nagel Silica
	4	Isopropanol : Formic acid : Water 20:1:5 (v/v/v)	Whatman K_2F Cellulose

[1] Solvent 2 was replaced by solvent 5 (amendment to protocol and deviations)

For determination of glyphosate, aliquots (ca. 15 µL) of appropriate extracts were chromatographed with non-radiolabelled glyphosate-trimesium and AMPA. Radiolabelled compounds were detected and quantified by linear analysis. Non-radiolabelled glyphosate and AMPA were detected on each TLC plate by spraying with ninhydrin solution.

For determination of trimesium, aliquots (ca. 10 to 25 µL) of appropriate extracts were chromatographed with non-radiolabelled glyphosate-trimesium. Non-radiolabelled compounds were detected by spraying with Dragendorffs reagent.

For determination of radioactivity weights or volumes of all samples were measured where appropriate in duplicate and determined by LSC.

Triplicate portions of air dried, extracted soil samples (ca. 0.1 g) were combusted and radioactivity was determined by LSC.

II. RESULTS AND DISCUSSION

A. DATA

The recovery of applied radioactivity during the ageing period in the extracts, in the combusted soil and in ethanolamine traps for [^{14}C]glyphosate and [^{14}C]trimesium and its degradation products determined in different solvents is presented in the tables below.

Table 7.1.4.1.1-16: Percent of applied radioactivity in soil extracts, combusted soil and volatiles from [^{14}C]glyphosate (PMG)

		Day 0 [1]			Day 30 [2]		
		Rep.1	**Rep.2**	**Mean**	**Rep.1**	**Rep.2**	**Mean**
[^{14}C]anion and degradates in Extracts	Glyphosate	75.10	69.54	72.32	14.01	12.30	13.16
	AMPA	2.49	2.79	2.64	25.43	27.09	26.26
	Other	1.46	1.60	1.53	1.51	2.57	2.04
	Origin material	2.52	1.80	2.16	0.81	1.21	1.01
	Unresolved background	0.99	1.07	1.03	1.98	1.59	1.79
	Procedural loss	14.68	18.90	16.79	8.89	6.42	7.66
Total		97.24	95.70	96.47	52.63	51.18	51.91
[^{14}C]anion and degradates in Soil residues (combusted)		3.46	3.61	3.54	12.00	12.87	12.44
[^{14}C]anion and degradates in Ethanolamine traps		-	-	-	30.95	35.43	33.19
Total		100.70	99.31	100.01	95.58	99.48	97.53

[1] Values from solvent system 5
[2] Values from solvent system 1

Table 7.1.4.1.1-17: Percent of applied radioactivity in soil extracts, combusted soil and volatiles from [^{14}C]trimesium (TMS)

		Day 0 [1]			Day 30 [2]		
		Rep.1	**Rep.2**	**Mean**	**Rep.1**	**Rep.2**	**Mean**
[^{14}C]cation and degradates in Extracts	TMS	87.17	82.01	84.59	5.58	6.76	6.17
	Origin material	n.d.	n.d.	n.d.	0.73	0.53	0.63
	Unresolved background	2.27	1.76	2.02	0.43	0.31	0.37
	Procedural loss	8.66	11.42	10.04	2.23	2.87	2.55
Total		98.10	95.19	96.65	8.97	10.47	9.72
[^{14}C]cation and degradates in Soil residues (combusted)		3.52	3.19	3.36	22.16	18.89	20.53
[^{14}C]cation and degradates in Ethanolamine traps		-	-	-	59.07	54.92	57.00
Total		101.62	98.38	100.00	90.20	84.28	87.24

[1] Values from solvent system 3
[2] Values from solvent system 4
n.d. = Not detected

The radioactivity leached from Speyer 2.1 soil aged for 30 days treated with [^{14}C]anion and [^{14}C]cation labelled Glyphosate-trimesium is presented in the table below.

Table 7.1.4.1.1-18: Percent of applied radioactivity in the leachate

Column Identification		Percent of radioactivity applied to soil prior to ageing present in :			Leachate Volume (mL)	Additional Leachate Volume (mL)	Concentration in total Leachate (µg/mL)
		Initial. Leachate	Additional Leachate	Total Leachate			
[^{14}C]Anion	Rep.1	0.045	0.003	0.048	392	29.2	< 0.001
	Rep.2	0.136	0.009	0.145	384	24.9	0.001
	Mean	**0.091**	**0.006**	**0.097**	**388**	**27.1**	**< 0.001**
[^{14}C]Cation	Rep.1	0.254	0.012	0.266	390	23.7	0.003
	Rep.2	0.644	0.011	0.655	378	25.2	0.007
	Mean	**0.449**	**0.012**	**0.461**	**384**	**24.5**	**0.005**

B. MASS BALANCE

Thirty days after test article application overall recoveries of applied radioactivity ranged from 96 to 99 % and 84 to 90 % for anion and cation labelled glyphosate-trimesium, respectively. The incomplete recovery of applied radioactivity with the cation labelled form of the test article may be due to the formation of volatile compounds, e.g. dimethyl sulphide and methane which were not absorbed by the trapping reagent employed in this study.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

Immediately after test article application, the majority of applied radioactivity (> 95 %) was extractable. After 30 days the percentage of applied radioactivity recovered in the soil extract declined to about 52 and 10 % for anion and cation labelled test article, respectively.

D. VOLATILE RADIOACTIVITY

In soil treated with both anion and cation labelled glyphosate-trimesium significant quantities of $^{14}CO_2$ were formed, namely about 33 and 57 % of applied radioactivity, respectively. Levels of unextracted radioactivity increased to about 12 % (anion) and 21 % (cation) after 30 days.

E. TRANSFORMATION OF THE TEST ITEM

Glyphosate and trimesium were the only major components detected when day 0 soil extracts were analysed by TLC. Small quantities (each less than 4 % of the applied radioactivity) of AMPA, polar material and unidentified degradates (observed on TLC) were also present in extracts of soil treated with [^{14}C]anion labelled glyphosate-trimesium. After 30 days of incubation, AMPA was the major component in [^{14}C]anion test article treated soil extracts accounting for about 26 % of applied radioactivity. Glyphosate, polar material and unidentified degradates comprised about 15, 1 and 2 %, respectively. Trimesium was the major component in extracts of [^{14}C]cation test article treated soil (about 5 % of applied radioactivity).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The mobility of glyphosate was assessed via aged column leaching experiments. The mean percentages of the applied radioactivity recovered in the leachates were 0.1 for anion labelled glyphosate-trimesium. This is considerably less than the 2 % of applied radioactivity that would trigger analysis of the leachates. The results illustrate that neither glyphosate, nor its degradation product AMPA are likely to leach into groundwater. The study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.1.1/005
Report author	[redacted]
Report year	1991
Report title	Behavior of glyphosate in water and soil, Part 4 Leaching behaviour, second performance
Report No	PR90/002
Document No	
Guidelines followed in study	BA-guideline for testing of pesticides Part IV 4-2
Deviations from current test guideline	From OECD 312: - No analysis of soil columns - Report is lacking important information on the study conduct
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

Soil columns containing the standard soils of Speyer 2.1, Speyer 2.2 and Speyer 2.3 were saturated with water. Then 50 µL (equivalent of 360 µg glyphosate / 20 cm^2 = 1.8 kg a.s./ha) of the solution was distributed on top of each soil column. The soil columns were leached with water for about 48 hours. Leachates were collected and analysed. The test was performed twice.

Concentrations of glyphosate and AMPA in the Speyer 2.2 soil were < 1.0 µg/L to 2.6 µg/L, and for Speyer 2.1 and 2.3 soil, glyphosate concentrations of < 1.0 µg/L were obtained.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate
Formulated product: Taifun 360
Nominal concentration: 360 g/L Glyphosate
Sample No.: 10/08/90

2. Soil:

Soils were received from landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA), Speyer. Characteristics of the test soils are presented in the table below.

Table 7.1.4.1.1-19: Characteristics of test soils

Parameter	Results		
Soil	**Speyer 2.1**	**Speyer 2.2**	**Speyer 2.3**
Textural Class (DIN)	Sand	Loamy sand	Sandy loam
Sand (0.63 – 2.0 mm)	4.5 ± 0.6	2.0 ± 0.6	3.2 ± 0.6
Medium sand (0.2 – 0.63 mm)	62.9 ± 2.4	52.6 ± 3.3	32.5 ± 3.2
Fine sand (0.063 – 0.2 mm)	20.0 ± 2.8	27.4 ± 5.0	28.4 ± 2.9
Coarse silt (0.02 – 0.063 mm)	4.7 ± 2.0	7.4 ± 3.5	16.4 ± 3.3
Medium silt (0.006 – 0.02 mm)	2.5 ± 0.7	3.5 ± 1.4	7.5 ± 1.2
Fine silt (0.002 – 0.006 mm)	1.9 ± 0.8	2.1 ± 0.7	3.9 ± 0.5
Clay (< 2 µm) (%)	3.5 ± 1.6	5.1 ± 1.4	8.3 ± 1.4
pH [1)]	5.7	5.6	6.4
Organic carbon (%) [1]	0.70 ± 0.07	2.29 ± 0.37	1.34 ± 0.14
Organic matter (%)			
Cation exchange capacity (meq/100 g)	4.9 ± 0.8	9.7 ± 0.3	9.5 ± 0.9
Maximum Water Holding Capacity (%)	31.9 ± 0.6	44.3 ± 1.1	34.9 ± 1.6

[1] Medium not indicated

B. STUDY DESIGN

1. Experimental conditions

Soil columns containing the standard soils of LUFA, Speyer, 2.1, Speyer 2.2 and Speyer 2.3 were saturated with water. Then 50 µL (equivalent of 360 µg glyphosate / 20 cm^2 = 1.8 kg a.s./ha) of the solution was distributed on top of each soil column. The soil columns were leached with water for about 48 hours.

2. Sampling and analytical procedure

Leachates were collected and analysed using a GC-ECD method.

The test was performed twice. In a first test, only leachate from Speyer soil 2.2 was analysed, in a repeat test leachates all three Speyer soils were analysed.

II. RESULTS AND DISCUSSION

A. DATA

Residues for glyphosate and AMPA are presented in the table below.

Table 7.1.4.1.1-20: Residues (µg/L) in leachates

Test	Soil	Leachate (ml)	Residues (µg/L)	
			Glyphosate	**AMPA**
1	Speyer 2.1	396	n.a.	n.a.
	Speyer 2.2	401	< 1.0	< 1.0
	Speyer 2.3	400	n.a.	n.a.
2	Speyer 2.1	407	< 1.0	n.a.
	Speyer 2.2	396	2.6	n.a.
	Speyer 2.3	422	< 1.0	n.a.

n.a. = Not analysed

B. CHARACTERISATION OF LEACHATES

In the first test, measured concentrations of glyphosate and AMPA in the Speyer 2.2 soil were < 1.0 µg/L, in the second test glyphosate measured concentrations were < 1.0 µg/L in Speyer 2.1 and 2.3 soil and 2.6 µg/L in Speyer 2.2 soil.

III. CONCLUSIONS

For all investigated cases the quantity of active ingredient on drainage water was < 2 % of the original amount given on top of the columns. Glyphosate does not show any significant leaching behavior.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The mobility of glyphosate and AMPA was assessed via column leaching experiments. Results confirm the low leaching potential of glyphosate and AMPA. Due to the fact that some residues in soil columns were not analysed and in view of the limited information given in the report, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.1.1/006
Report author	[redacted]
Report year	1978
Report title	Solubility, volatility, adsorption and partition coefficients, leaching and aquatic metabolism of MON 0573 and MON 1010
Report No	MSL-0207
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From OECD 312: - Inner diameter of columns is below 4 cm (3.8 cm) - Water instead of artificial rain was used - Irrigation conditions differ from current guideline requirements
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary
The leaching behavior of glyphosate applied as mixture of radioactive and unlabeled MON 0573 at a rate of 8.97 kg a.s./ha (8 lbs/acre) was examined in a rapid leaching test on seven soil types and in an aged leaching test on three soil types.

There was no leaching of either compound when aged on soil columns before leaching. The greatest leaching was observed when glyphosate was applied to soil columns of Leon fine sand and leached immediately with 20 inches of water. However, in this case only 20 % of the compound was leached beyond 10 cm.

Besides column leaching, other parameters were also assessed. However, this summary only refers to column leaching.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled Test Material:
Identification: [^{14}C]glyphosate (MON-0573)
Specific activity: 10.12 mCi/mM
Radiochemical purity: 94.0 %

Non-radiolabelled test compound
Identification: [^{14}C]sodium sesquiglyphosate (MON-0101)
Specific activity: not indicated
Radiochemical purity: not indicated

2. Soil:

All soils were air dried and sieved to 2 mm. Characteristics of the test soils are presented in the table below.

Table 7.1.4.1.1-21: Characteristics of test soils

Parameter	Results						
Soil	**Ray**	**Drummer**	**Spinks**	**Lintonia**	**Leon**	**Hilo**	**Molokai**
Textural Class (USDA)	Silt loam	Silty clay loam	Sandy loam	Sandy loam	Fine sand	Volcanic ash	Lava
Sand (50 µm – 2 mm) (%)	4.6	2.4	75.1	86.0	94.0	54.0	18.0
Silt (2 µm – 50 µm) (%)	84.2	68.8	17.8	11.0	5.0	20.0	30.0
Clay (< 2 µm) (%)	10.0	25.3	4.8	1.8	1.0	26.0	52.0
pH [1]	8.1	6.2	4.7	6.5	4.8	5.7	7.0
Organic carbon [2] (%)	0.70	1.97	1.39	0.41	0.58	5.51	1.74
Organic matter (%)	1.2	3.4	2.4	0.7	1.0	9.5	3.0
Cation exchange capacity (meq/ 100 g)	10.4	24.6	11.3	5.1	7.2	60.0	20.0
Maximum Water Holding Capacity (%)	23.9	28.8	17.9	15.6	-	-	-

USDA: United States Department for Agriculture
[1] Medium not indicated
[2] Calculated from organic matter according to OC = OM × 0.58

B. STUDY DESIGN

1. Experimental conditions

Glass columns of 3.8 cm inner diameter (1.5 inches) were constructed from 15 segments of 2 cm length and an upper segment of 10 cm length. The bottom segment was packed with glass wool and placed in a Coors funnel (4.3 cm inner diameter). The columns were uniformly packed with air-dried soil. The total weight of soil used for each column was recorded. Water was added to the soil columns that were aged before leaching so the moisture content of these columns was 15 to 20 % at the time chemical was added. An aqueous solution of glyphosate (MON-0573) or sodium sesquiglyphosate (MON-0101) diluted with [^{14}C]-MON-0573 equal to 1.2 x $10^7$to 2.6 x 10^7 dpm, equivalent to 8.97 kg a.s./ha (8 lbs/acre) was applied to the surface of the soil columns.

The following table presents the soils and compounds used for the rapid and the aged leaching part of the study.

Table 7.1.4.1.1-22: Overview of soils and compounds used for rapid and aged leaching

Soil column	Study type	Compound applied
Ray	aged	Sodium sesquiglyphosate (MON-0101)
Ray	aged	Glyphosate (MON-0573)
Hilo	aged	
Molokai	aged	
Ray	rapid	Glyphosate (MON-0573)
Lintonia	rapid	
Drummer	rapid	
Spinks	rapid	
Florida	rapid	
Hilo	rapid	
Molokai	rapid	

For the rapid leaching, test duplicate columns were set up for each soil with the exception of Hilo and Molokai. After application of the chemical, the soil columns were allowed to stand for 30 minutes before water was added.

Those columns that were set up to evaluate leaching of chemical aged on soil were immediately topped with a sidearm and coupled with an Ascarite trap. $^{14}CO_2$ evolution was measured throughout the entire ageing period. Duplicate columns of Ray, Molokai, and Hilo soils were treated with [^{14}C]-glyphosate for this study. Leaching of [^{14}C]-sodium sesquiglyphosate was determined only on Ray soil as there has been no indication that glyphosate and sodium sesquiglyphosate were significantly different except for their solubility.

In both types of columns water was added at a rate slower than the infiltration capacity of the soil. The columns that were leached rapidly required 540 ml H_20 corresponding to 508 mm (20 inches) of rainfall. The aged columns were allowed to stand 30 days before biweekly leaching with the equivalent of 13 mm (½ inch) of rainfall per day.

2. Sampling
The eluants from the rapid leaching columns were measured and aliquoted for LSC.

The eluants from the aged columns were pooled and stored for analysis after completion of the 45 day leaching period. Then they were concentrated, filtered, and submitted to analyses. The Ascarite towers were changed periodically and analysed tor $^{14}C0_2$.

The 2 cm soil segments were separated, immediately after leaching was complete, frozen, lyophilized, and analysed for ^{14}C-content.

3. Analytical procedures
The eluants, which varied in volume from 360 to 415 ml, were analysed by LSC and TLC. The soil segments were separated immediately after leaching was completed; subsequently each soil segment was frozen, lyophilized, and analysed by combustion and LSC. An aliquot of 2.0 g, of the uppermost segment of all columns was extracted 2 times with 10 ml of 0.5 N NH_40H. The extract was concentrated and analysed by TLC. The total recovery of ^{14}C-activity applied was calculated, and the distribution was recalculated based on 100 % recovery. The distribution (% of AR) of glyphosate and AMPA was determined in the eluants and in the extracts from the uppermost segments from all columns.

II. RESULTS AND DISCUSSION

A. DATA

The total recovery and the distribution of ^{14}C-activity (combusted segments) from rapidly leached soil columns and from soil columns after ageing is presented in the tables below.

Table 7.1.4.1.1-23: Overview of soils and compounds used for rapid and aged leaching: Distribution of ^{14}C-activity in rapidly leached soil columns (combusted segments)

Soil Segment	Lintonia	Ray	Spinks	Florida	Drummer	Hilo	Molokai
1	33.29	24.53	72.12	21.18	80.03	99.47	98.57
2	25.28	24.30	24.65	19.79	14.26	0.17	0.93
3	17.30	17.98	1.85	15.47	2.35	0.15	0.13
4	10.44	14.48	0.38	15.41	0.85	0.05	0.30
5	4.84	6.84	0.21	10.40	0.42	0.04	0.01
6	2.27	2.37	0.13	6.67	0.28	0.02	0.01
7	0.80	1.35	0.09	4.10	0.20	0.02	-
8	0.44	0.74	0.07	2.39	0.12	0.01	-
9	0.19	0.31	0.05	1.90	0.31	0.01	-
10	0.14	0.14	0.04	0.75	0.06	0.01	-
11	0.11	0.10	0.03	0.34	0.06	-	-
12	0.11	0.09	0.03	0.27	0.05	-	-
13	0.11	0.07	0.16	0.15	0.03	-	-
14	0.11	0.07	0.04	0.13	0.06	0.01	-
15	0.09	0.07	0.10	0.05	0.04	0.01	-
Eluant	4.38	6.56	0.10	1.00	0.88	0.03	0.05
Totally recovered	**78.71**	**90.53**	**95.48**	**99.87**	**88.95**	**98.68**	**101.66**

Table 7.1.4.1.1-24: Overview of soils and compounds used for rapid and aged leaching: Distribution of ^{14}C-activity in soil columns (combusted soil segments) after ageing

Soil	Ray	Ray	Hilo	Molokai
Segment / Compound applied	MON 0101	MON 0573	MON 0573	MON 0573
1	30.38	30.30	40.39	97.53
2	0.87	1.07	0.20	0.03
3	0.47	0.49	0.05	0.03
4	0.25	0.27	0.07	0.01
5	0.22	0.24	0.07	0.01
6	0.19	0.17	0.04	0.01
7	0.36.	0.10	0.04	0.01
8	0.13	0.12	0.02	-
9	0.11	0.09	0.02	-
10	0.08	0.11	0.02	-
11	0.14	0.08	0.02	0.01
12	0.18	0.05	0.02	0.01
13	0.03	0.06	0.02	-
14	0.14	0.08	0.01	-
15	0.07	0.04	0.01	0.01
Total in soil	**33.67**	**33.26**	**41.00**	**97.66**
Total in eluent	1.16	1.56	0.22	0.02
$^{14}CO_2$, evolved	65.17	65.18	58.97	2.12
Total recovery	**95.75**	**98.40**	**84.62**	**98.94**

The following tables summarise the analysis of the leachates and the analysis of the extracts from the uppermost soil segments.

Table 7.1.4.1.1-25: Overview of soils and compounds used for rapid and aged leaching: Analysis of glyphosate and AMPA in the leachates

Soil column	Study type	Compound applied	Radioactivity in the leachates (% AR)	% of radioactivitiy in leachates	
				Glyphosate	AMPA
Ray	aged	Sodium sesquiglyphosate (MON-0101)	1.2	1.0	0.1
Ray	aged	Glyphosate (MON-0573)	1.5	0.8	0.7
Hilo	aged		0.1	-	-
Molokai	aged		0.2	-	-
Ray	rapid	Glyphosate (MON-0573)	6.6	5.8	0.8
Lintonia	rapid		4.4	3.9	0.5
Drummer	rapid		0.9	0.6	0.3
Spinks	rapid		0.1	-	-
Florida	rapid		1.0	0.6	0.4
Hilo	rapid		0.1	-	-
Molokai	rapid		0.1	-	-

Table 7.1.4.1.1-26: Overview of soils and compounds used for rapid and aged leaching: Analysis of glyphosate and AMPA in the extracts from the uppermost soil segments

Soil column	Study type	Compound applied	Extractables (% AR)	% of total radioactivity in extracts	
				Glyphosate	AMPA
Ray	aged	Sodium sesquiglyphosate (MON-0101)	51.1	34	66
Ray	aged	Glyphosate (MON-0573)	52.4	26	84
Hilo	aged		8.4	94	6
Molokai	aged		47.0	22	78
Ray	rapid	Glyphosate (MON-0573)	72.3	76	24
Lintonia	rapid		78.9	80	20
Drummer	rapid		77.8	86	14
Spinks	rapid		95.7	90	10
Florida	rapid		99.1	93	7
Hilo	rapid		15.1	94	6
Molokai	rapid		50.3	86	14

B. MASS BALANCE

The rate of leaching varied with the soil; e.g. Leon fine sand was leached in 8 hours while Drummer silty clay loam was leached in 44 hours. The total recovery of applied ^{14}C-activity is less than 100 % in those soils which required longer to leach and in those soils in which degradation of glyphosate (MON-0573) to $^{14}C0_2$ occurred rapidly (Drummer, Ray and Lintonia). Total recovery of the ^{14}C-activity applied was 95 % or greater on all of the aged soil columns.

The leachate contained 1.0 %, or less, of the applied ^{14}C-activity with the exception of Ray and Lintonia which contained 6.6 and 4.4 % of the ^{14}C-activity applied, respectively. Glyphosate (MON-0573) showed very little mobility on any of the soils after 508 ml (20 inches) of water was applied immediately. The greatest mobility observed was on Leon fine sand, and even in this case only 20 % of the ^{14}C-activity applied, leached more than 10 cm.

Only 0.1 to 1.5 % [^{14}C] of applied radioactivity was found in the eluents. In leachates [^{14}C]glyphosate ranged from 0.8 to 1.0 and [^{14}C]AMPA ranged from 0.1 to 0.7 % of applied AR.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

Extractable residues in the top segment reached from in the rapid leaching study ranged from 15.1 % to 99.1 % (Ray) of applied radioactivity. For the aged columns 8.4 to 52.4 % (Molokai) to 1.56 % were extracted from the uppermost soil segment.

D. VOLATILE RADIOACTIVITY

Degradation of [^{14}C]glyphosate to $^{14}CO_2$ was negligible on Hilo volcanic ash (2.12 % of AR), but rapid degradation occurred on Molokai and Ray soils (58.97 and 65.10 % of AR, respectively).

E. TRANSFORMATION OF THE TEST ITEM

TLC analysis of the NH_40H extract of the uppermost segment showed 14 to 24 % degradation of [^{14}C]glyphosate to AMPA in these same soils. Analysis of the extracts of the uppermost segment resulted in 85 % AMPA (MON-0453) in Ray soil and 78 % AMPA in Molokai soil, as would be expected based on the degradation to $^{14}CO_2$.

The data from the aged soil columns indicated that there was no leaching of AMPA (MON-0435), the degradation product of glyphosate and sodium sesquiglyphosate, or the compounds themselves.

3. Assessment and conclusion

Assessment and conclusion by applicant: Column leaching experiments with several different soils were conducted to assess the leaching behavior of aged and freshly applied glyphosate. Only small amounts of AR were found in the leachate, while the majority of the test substance was encountered in the soil or in CO_2 traps in case of aged substance, demonstrating the low leaching potential of glyphosate. In view of the irrigation regime used, i.e. freshly applied columns received 540 ml at a rate slower than the infiltration capacity and the aged columns received 13 mm of rainfall daily over a two weeks period, the study is not fit for the purpose to describe the leaching behavior of glyphosate. Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.1.1/007
Report author	[redacted]
Report year	1972
Report title	MON-0573, Residue and Metabolism, Part 2: The photolysis, run-off and leaching of MON-0573 on or in soil
Report No	258
Document No	
Guidelines followed in study	United States Department of Agriculture's guidelines for studies to determine the impact of pesticides on the environment as stated in PR Notice 70-15, June 23, 1970
Deviations from current test guideline	From OECD 312: - Soil thin layer chromatography study is not in line with pertinent guideline
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

Soil thin layer chromatography (TLC) was utilized to investigate the vertical mobility of glyphosate in soil. Separate soil TLC plates (20 cm x 20 cm) with a soil thickness of 0.76 mm were prepared using a light (Norfolk sandy loam), medium (Ray silt loam) and heavy soil type (Drummer silty clay loam).

Glyphosate was strongly absorbed by all three soils. 97-100 % of the applied radioactivity had an RF of less than 0.09.

Besides thin layer chromatography, other parameters were also assessed. However, this summary only refers to thin layer chromatography.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]MON-0573 (N-(phosphonomethyl-^{14}C)-glycine)
Specific activity: 8.03 mCi/mmol
Radiochemical purity: 97.0 %

2. Soil:

Characteristics of the three test soils are presented in the table below.

Table 7.1.4.1.1-27: Overview of soils and compounds used for rapid and aged leaching: Characteristics of test soils

Parameter	Results		
Soil	Ray	Drummer	Norfolk
Textural Class (USDA)	Silt loam	Silty clay loam	Sandy loam
Sand (50 µm – 2 mm) (%)	6.0	2.0	86.0
Silt (2 µm – 50µmm) (%)	83.2	55.4	11.0
Clay (< 2 µm) (%)	9.6	36.8	2.3
pH [1]	6.5	7.0	5.7
Organic carbon (%) [2]	0.58	3.48	0.58
Organic matter (%)	1.0	6.0	1.0

[1] Medium not indicated
[2] Calculated from organic matter according to OC = OM × 0.58

B. STUDY DESIGN

1. Experimental conditions

Soil thin layer chromatography (TLC) was utilized to investigate the vertical mobility of glyphosate in soil. Separate soil TLC plates (20 cm x 20 cm) with a soil thickness of 0.76 mm were prepared using a light (Norfolk sandy loam), medium (Ray silt loam) and heavy soil type (Drummer silty clay loam).

10 ml of a solution of 46.75 mg of [^{14}C]glyphosate dissolved in 46.75 ml of 0.1 M NH_4CO_3 was applied to a 2 cm band located 3 cm from the bottom of each soil TLC plate (origin). The soil TLC plates were developed with distilled water in a horizontal position in a water saturated chamber. TLC plates were connected to the development by a paper-towel wick. The development time for the solvent front to migrate 16 cm beyond the origin was 9, 0.7 and 1.3 hours for the sandy loam, silt loam and silty clay loam soils, respectively. Following development, the plates were dried and the distribution of radioactivity between the origin and final solvent front was determined for each band by autoradiography. After evaluation of the first development, the plates were developed a second time with water and analysed as before. The mobility of radioactivity (Rf) was calculated as the distance of the leading edge of radioactivity from the origin divided by the distance of the solvent front from the origin following each development.

The total ^{14}C-activity present in soil samples was determined by combustion of homogenised and lyophilised samples. Combustion analysis was performed using a Peterson automatic combustion apparatus followed by liquid scintillation counting of the resulting $^{14}CO_2$ (PACA/LSC). The total ^{14}C-activity present in aqueous samples was determined by liquid scintillation counting using Packard Insta-Gel scintillation fluid.

II. RESULTS AND DISCUSSION

Glyphosate was so strongly adsorbed by all three soils used to investigate its vertical mobility by TLC so that 97-100 % of the applied ^{14}C-activity had an Rf of less than 0.09. Similarly, 95-99 % of the applied

^{14}C-activity remained at an Rf of less than 0.09 after the second development. In no case any of the radioactivity showed an Rf value greater than 0.18.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The mobility of glyphosate was assessed using soil thin layer chromatography. The test substance was almost immobile, in line with the low leaching potential of glyphosate. As the methodology used in the study is not in line with current requirements, the study is not considered fit for the purpose to describe the mobility of glyphosate in soil.
Therefore, the study is considered invalid.

Assessment and conclusion by RMS:

Relevant articles from literature search

1. Information on the study

Data point:	CA 7.1.4.1.1/008
Report author	Gjettermann, B. *et al.*
Report year	2011
Report title	Kinetics of Glyphosate Desorption from Mobilized Soil Particles
Document No	DOI 10.2316/sssaj2010.0198 ISSN 1435-0661
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (Study not sufficiently described to check validity of results)

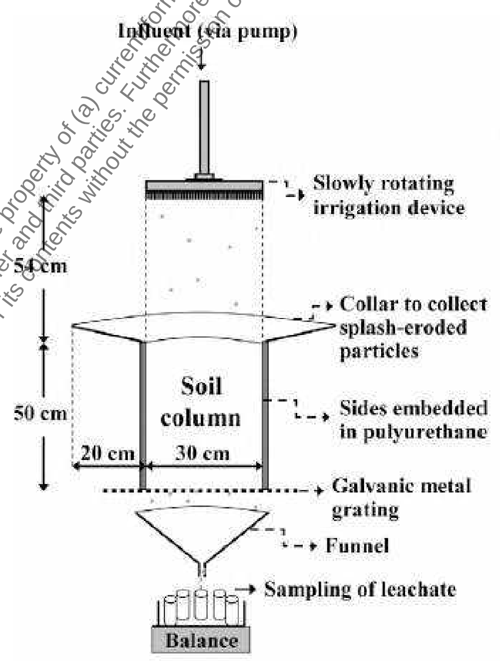
2. Full summary
Desorption kinetics of chemical compounds can be important both for their mobility in soil and for the significance of particle–facilitated transport. We studied desorption of glyphosate [N–(phosphonomethyl) glycine] on mobilized particles from two soil columns (50–cm height, 30–cm diameter), i.e., particles leached by free drainage from the bottom and particles mobilized by splash erosion and collected next to the top of the column. Leaching and splash erosion were driven by three, 30–mm irrigation events following surface application of ^{14}C–labeled glyphosate. Fresh leachate samples were investigated within 30 min of sampling, and desorption from splash–eroded particles in suspension (100 mg solid/L) was followed for 48 h (starting 2.0 min after immersion). Glyphosate concentrations were determined by measuring the ^{14}C activity using liquid scintillation counting. Similar fractional amounts of glyphosate (on average, 10–20 % in 20 min) desorbed from leached and from splash–eroded particles (>20 nm) shortly after leaching or immersion, respectively, indicating that the processes of desorption from the different sources of particles were similar. In leachate, about 45 to 79 % remained particle bound after 20 min, while calculated values at equilibrium were 20 % or less. Equilibrium was established after about 5 to 10 h in suspensions with splash–eroded particles, except for one sample. These direct observations, supported by estimated values of the Damköhler number, lead to the conclusion that desorption kinetics are important for evaluating the significance of dissolved and particle–facilitated transport of glyphosate. To quantify particle–facilitated glyphosate transport, the water and solid phases in the leachate should consequently be separated within a few minutes after leaching.

Materials and methods

Experimental Setup

The investigated sandy loam soil and parts of the experimental setup have previously been described in detail by Gjettermann *et al.* (2009), who focused on the influence of soil structure on particle–facilitated pesticide leaching. Two undisturbed cylindrical soil columns (B1 and B2) were carefully excavated from a recently tilled (plowed and drilled) experimental plot, encapsulated with polyurethane foam, and trimmed at the bottom end (50–cm cylinder length, 30–cm diameter). As reference, two columns (A1 and A2) were excavated from an untilled plot. The coding of the columns used in the glyphosate experiments by Gjettermann *et al.* (2009) has been kept to facilitate comparison. Particular care was observed not to disturb the surfaces of the columns or to block large macropores while trimming the bottom ends. The investigated sandy loam soil (an Agrudalf) is located at the University of Copenhagen research farm Roerrendegaard at Taastrup, Denmark, and has previously been described by Petersen *et al.* (2001). The contents of coarse sand (200–2000 µm), fine sand (20–200 µm), silt (2–20 µm), clay (<2 µm), and organic C in the upper 30 cm were 29, 40, 18.5, 12.5, and 1.2 %, respectively. A schematic presentation of the experimental setup is given in Figure 7.1.4.1.1-1. All irrigation water applied to the columns (influent) had a composition similar to rainwater (Miljøstyrelsen, 1996) containing 0.017 mmol/L $CaCO_3$, 0.018 mmol/L KNO_3, 0.021 mmol/L $MgSO_4$, 0.126 mmol/L NaCl, and 0.94 mmol/L NH_4Cl. The pH was 6.32 and the electrical conductivity was 0.047 mS/cm. Water was applied to the column with a pump (FMI Pump QG 150, Fluid Metering Inc., Syosset, NY) through a motor–driven, slowly rotating sprinkling device with 90 syringe needles (Trumo, 25G), to ensure a uniform application rate of 15.0 mm/h (1.06 L/h). The sprinkling device was placed 54 cm above the surface of the column. The drop size was determined at the used intensity by sampling and weighing about 20 drops (10 repetitions). The mass of a drop was 6.4 ± 0.5 mg, corresponding to a (spherical) drop diameter of 2.3 ± 0.9 mm.

Figure 7.1.4.1.1-1: Schematic illustration of the experimental setup for the leaching experiments and for the collection of splash–eroded soil particles



The columns were rewetted at the start of the experiment by irrigation, which was stopped 35 min after the first appearance of leachate. One day later, ^{14}C–labeled glyphosate mixed with the commercial glyphosate product Roundup Bio (Monsanto Europe, Antwerp, Belgium) was applied uniformly to the surface. The glyphosate stock solution had a specific concentration activity of 0.80 MBq/mg and contained 4.4 % ^{14}C-labeled glyphosate, 93.5 % unlabeled glyphosate, and 2.1 % aminomethylphosphonic acid (AMPA).

The applied dose of glyphosate (12.5 mg/column) was comparable to current agricultural practice. A total of 13 mL of solution (stock solution and rinse water) was applied during the glyphosate application.

Three irrigations were applied to the soil columns 5, 8, and 12 d after rewetting, respectively. Each event lasted 2.0 h and had a constant intensity of 15.0 mm/h. Water drained freely from the column during and after each irrigation event. The mass of drainage water (leachate) was measured continuously. The leachate was sampled continuously, yielding a total of 21 samples, each containing 30 to 50 mL of leachate. The top ends of the columns were covered with plastic whenever possible to minimize evaporation.

A new plastic collar was mounted around the tops of the columns before each irrigation event to collect water splashes and soil particles (splash–eroded particles) that were eroded by drops and thrown over the sides (height up to 1 cm above the soil surface) with the droplets. All of these water droplets were collected on the collar. The water droplets generally evaporated within a few hours. The particles left were scraped off the collar 24 h after the irrigation event, allowed to air dry, and sieved through a 100-µm sieve. The mass of air–dry particles <100 µm was determined. This material was used for the desorption experiments.

Measurements
The ^{14}C activity of unfiltered and filtered leachate samples was measured with a Wallac 1414 (Perkin Elmer Corp., Waltham, MA) liquid scintillation counter (LSC) using 10 mL of scintillation cocktail (InstaGel, PerkinElmer) to a 9–mL sample. Using ^{14}C–labeled pesticides has the consequence that also metabolites, for example the major metabolite of ^{14}C–glyphosate (^{14}C–AMPA) were measured by LSC. The detection limit of the ^{14}C LSC analysis was 16.4 disintegrations min^{-1} (0.038 µg ^{14}C–glyphosate/L) and the method had trueness for quantification on ^{14}C standard buttons (PerkinElmer) of 100.2 ± 0.8 %. The effect of quenching was automatically adjusted by the LSC, and increasing quench induced by increasing particle concentration was accurately measured. Gjettermann *et al.* (2009) found good agreement between these determinations and direct chemical measurements of glyphosate plus AMPA, and they showed that AMPA constituted only a minor part (up to 17.5 %) in leachate samples from the investigated columns.

Particle concentration in the leachate was determined indirectly from the measured turbidity. Turbidity was measured with a turbidity meter (Tintometer GmbH, Dortmund, Germany). Samples were shaken and immediately transferred to glass vials. Turbidity was then measured after exactly 60 s. With the chosen procedure, isolated soil particles were <30 to 50 µm (equivalent spherical diameter), assuming a particle density of 1600 to 2650 kg/m^3. The mass of particles was estimated in 70 randomly selected leachate samples of known volume to establish a relationship between turbidity and concentration of particles. The samples were centrifuged (30 min at 4100 × *g*) and washed twice with deionized water. Finally, the particles were dried at 105°C before determining the mass. The correlation between turbidity T (in nephelometric turbidity units [NTU]) and the concentration of soil particles in the leachate was used to calculate the concentration of particles: concentration of particles (mg/L) = 110 ln(T) − 241 (R^2 = 0.74, 70 samples). For turbidity <20 NTU, equivalent to particle concentrations of less than approximately 88 mg/L, the relationship was poor and this limit was therefore used as the detection limit.

Desorption was investigated in five leachate samples from each of the tilled soil columns (Samples 2, 8, and 14 from the first irrigation event and Sample 21 from the other two events; see Table 1). The samples were selected to illustrate the development in glyphosate levels during the three irrigation events. Only one leachate sample (Sample 2, first irrigation) from each of the untilled columns was investigated, the amount of sediment being too small (considerably below the detection limit) and the uncertainty of the determination on individual samples too high during the later phases of the drainage events (Gjettermann *et al.*, 2009). Approximately 40 mL of leachate sample was collected and a stopwatch was activated. A 10-mL sample was immediately filtered (0.02–µm inorganic, anopore filter, Frisenette, Knebel, Denmark) into a clean glass and the time (about 1.5 min) was recorded. Nine milliliters of the filtrate was later extracted for ^{14}C–activity measurement. After 5 min, another 10 mL of leachate was extracted and filtered for activity measurement. This was repeated after another 5 to 10 min and, if the amount of the original sample allowed it, after approximately 30 min. The so–called reaction time associated with each filtration, t_r, was assigned as the time span from the midpoint [(t_{beg} + t_{end})/2] of the sampling interval to when filtration had just been performed.

The concentrations of soil particles in the leachate used for the desorption experiments were not measured but estimated as the average of measured concentrations in the directly preceding and succeeding samples (first irrigation) or as the concentration measured in the directly preceding sample (second and third irrigations). The sample size did not allow combined determination of both particle concentration and desorption, and larger samples would have compromised the need for fast separation of colloids from the water phase. The uncertainty associated with this procedure was estimated from concentration difference between consecutive samples measured by Gjettermann *et al.* (2009), the absolute average concentration difference being assigned as *D*.

Detectable splash erosion occurred from both of the tilled columns (B) in all events, but not from the untilled columns (A). Sieved and air–dried, splash–eroded particles generated during each irrigation event from the tilled columns were immersed (at time zero) in stirred artificial rainwater (irrigation water) yielding a suspended particle concentration of $C_{particle}$ = 100 mg/L. Samples of 10 mL were extracted and filtered, and the ^{14}C activity of the filtered samples was determined five or six times, typically 2.0, 10.0, 60, 120, 1440, and 2880 min after immersion (equivalent to t_r) as described above for the leachate.

Table 7.1.4.1.1-28 summarizes all analytical results available on the leachates.

Table 7.1.4.1.1-28: Overview of analyses conducted on leachate samples from each of the three irrigation events

Sample no.	First irrigation	Second and third irrigation
1	pesticide (colloidal and soluble fractions), ph, conductivity and turbidity	
2	sorption and desorption kinetics of pesticide	total concentration of pesticide, colloidal and soluble organic c, ph, conductivity and turbidity.
3–7	pesticide (colloidal and soluble fractions), ph, conductivity and turbidity	
8	sorption and desorption kinetics of pesticide	total concentration of pesticide, colloidal and soluble organic c, ph, conductivity and turbidity.
9–13	pesticide (colloidal and soluble fractions), ph, conductivity and turbidity	
14	sorption and desorption kinetics of pesticide	total concentration of pesticide, colloidal and soluble organic c, ph, conductivity and turbidity.
15–20	pesticide (colloidal and soluble fractions), ph, conductivity and turbidity	
21		sorption and desorption kinetics of pesticide

Data Analyses

The specific activity of the applied glyphosate stock solution was checked by LSC analysis at each application time. The measured specific activity was used to convert the measured ^{14}C activity into glyphosate concentration units. The concentration of particle–bound glyphosate (C_p) in the leachate was defined as the difference between the measured concentration in the suspension (total concentration, Cs) and the measured concentration in the filtrate (dissolved glyphosate, Cd). The particle–bound fraction of leached glyphosate was calculated as C_p/C_s. Rates of change of the particle–bound fraction were estimated by least squares linear regression, i.e., from fitting experimental data to the simple approach presented by

[1]
$$\frac{100C_p}{C_s} = \alpha t_r + \beta$$

where the rate α (% min^{-1}) and β (%) are constants (positive α values indicate desorption) and t_r is the time of reaction (min).

Equation [1] was also fitted to the first data points from experiments with splash–eroded particles in an attempt to obtain similar time scales of desorption from the different sampling types of particles (leached and splash eroded). In this analysis, C_s was obtained as the sum of particle–bound and dissolved concentrations at equilibrium ($C_{p,eq}$ and $C_{d,eq}$, respectively), and C_p was calculated as the difference between

C_s and C_d.

At long experimental periods ($0 < t_r \leq 48$ h), however, the desorption from splash–eroded particles was not linear and the points were therefore also described according to

[2]

$$\frac{dC_d}{dt} = -k\left(C_d - C_{d,eq}\right)$$

where C_d (µg/L) is the dissolved concentration (<20 nm), t is the time (h), $C_{d,eq}$ (µg/L) is the "equilibrium" dissolved concentration, and k is a rate constant (h^{-1}). This approach has been termed a linear driving force approximation (LeVan *et al.*, 1997) or a first–order mass transfer model (Lick *et al.*, 1997). Assuming that C_d at time $t = 0$ h when the particles were suspended $[C_d(0)] = 0$, Eq. [2] can be integrated into

[3]

$$C_d = C_{d,eq}\left[1 - \exp(-kt_r)\right]$$

where t_r is the time of reaction. The Solver function in Excel (Wraith and Or, 1998) was used to adjust the model parameters $C_{d,eq}$ and k by minimizing the difference between predicted and measured C_d values (maximizing R^2). Because the chemical or physical processes involved in the desorption are expected to be similar in the two data sets, the choice of a linear vs. an exponential model is based solely on the number of data points available and the time scale used to observe the different particles.

For the splash–eroded particles, the total content of glyphosate in the sample was not measured (the results had to be discarded due to an error in the laboratory). It therefore had to be estimated. Gjettermann *et al.* (2009) reported a K_d value of 503 L/kg for the bulk topsoil (and 496 L/kg for AMPA). It has previously been shown that particles larger than about 0.1 mm are not present in drainage from the investigated field site (Holm *et al.*, 2003), indicating that coarse sand and parts of the fi ne sand fraction either are not mobile or are immobilized on the way through the soil column. For this soil, it may generally be expected that the Fe and Al oxides that sorb glyphosate is mainly present in the fraction <20 µm. Hence, K_d for the investigated leached particles will be larger than that for the bulk soil. Based on the texture of the topsoil, 40 % of the constituents were >0.100 mm. Hence, an estimate of K_d was obtained as 503 L/kg / 0.60 = 8.4 × 102 L/kg. This is a conservative estimate because it assumes no sorting of particles below the 0.1–mm limit within the soil columns. Estimates of the concentration of particle–bound glyphosate at equilibrium, $C_{p,eq}$ (µg/L) were obtained from the fitted $C_{d,eq}$, the soil/water ratio (particle concentration $C_{particle}$, kg/L), and K_d as $C_{p,eq} = C_{particle}K_dC_{d,eq}$. Hence, in the absence of direct measurements, the total glyphosate concentration was calculated as

[4]

$$C_s = C_{d,eq} + C_{p,eq} = C_{d,eq}\left(1 + C_{particle}K_d\right)$$

The Damköhler number, D_a, is a measure of the relative importance of kinetics to equilibrium processes in transport (Bold *et al.*, 2003). The D_a is defined as the ratio between the transport and the reaction time scales, and can be calculated as

[5]

$$D_a = \frac{k}{(U/L)}$$

where L (cm) is the transport distance (e.g., length of column, cm) and U (cm/h) is the water velocity in the soil.

Results and Discussion

Desorption in Leachate from Tilled Soil

Measured dissolved glyphosate concentrations in the leachate from the tilled soil generally increased with time (Figure 7.1.4.1.1-2), and the particle–bound fraction decreased (Figure 7.1.4.1.1-3). Thus, one immediate finding of the experiment is that considerable amounts of glyphosate desorbed from leached soil particles (>20 nm) during the investigated period (about 20 min). Desorption was particularly large for the first irrigation on Column B1 (Figure 7.1.4.1.1-2), probably reflecting leaching of highly pesticide–enriched particles. Thus, the initially (about 1.5 min after sampling) measured concentration of glyphosate on particles was 19 to 24 mg/kg for this irrigation event, while it was between 7.7 and 3.5 mg/kg for the other irrigations on Column B1 and all irrigations on Column B2. The data do not indicate that concentrations of dissolved glyphosate reached stable levels.

The concentration of leached particles, $C_{particle}$ (Table 7.1.4.1.1-29), could be a critical factor for the α values describing glyphosate desorption. Higher concentrations of particles should result in lower desorption rates due to a higher final equilibrium value (c f. Eq. [4]). The particle concentrations showed little variation from sample to sample within events (Table 7.1.4.1.1-29, D ≤24 mg/L), although it often varied significantly from the beginning to the end of an irrigation event (Gjettermann *et al.*, 2009). Thus, the uncertainty associated with the estimated $C_{particle}$ values in Table 7.1.4.1.1-29 is probably on the order of 24 mg/L or less. The concentrations ranged between 123 and 292 mg/L and were higher for Column B1 than for Column B2. The expected dissolved mass fraction at equilibrium, $C_{d,eq}/C_s$ can be estimated by rearranging Eq. [4] and inserting the measured particle concentrations from Table 7.1.4.1.1-29. According to this calculation, the mass of sorbed glyphosate at equilibrium in the leached samples will account for 20 % or less of the mass in solution. Hence, with the investigated range of particle concentrations and the high initial fractions of particle–bound glyphosate (Figure 7.1.4.1.1-3), the samples are far from equilibrium and particle concentrations should not be important for the relative amount of desorbed pesticide or the desorption rates.

Figure 7.1.4.1.1-2: Concentration of dissolved glyphosate (C_d) in leachates from two soil columns, B1 (left) and B2 (right), at different reaction times (t_r, 0–30 min): (a) and (b) data for the first irrigation event (Samples 2, 8, and 14); (c) and (d) data for the second and third irrigation events (Sample 21)

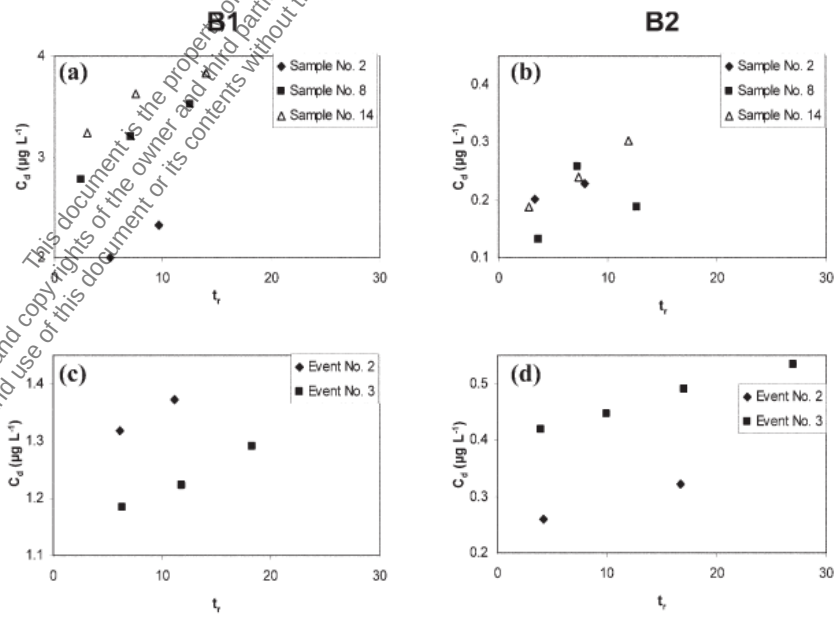


Table 7.1.4.1.1-29: Relative desorption rate (α), intercept parameter (β), concentration of soil particles ($C_{particle}$) in the investigated leachate, and average absolute particle concentration difference between consecutive samples (D) derived from experiments on leachates; α, β, and coefficient of determination (R^2) obtained by fitting data from different soil columns (B1, B2, A1, and A2), irrigation events (1–3), and samples (2, 8, 14, and 21) to Eq. [1]

Column	Irrigation no.	Sample no.	α	β	R^2†	$C_{particle}$	D
			% min^{-1}	%		— mg L^{-1} —	
B1	1	2	0.94	78		292	
	1	8	0.80	72	0.98	276	9 (6)‡
	1	14	0.56	67	0.92	268	
	2	21	0.41	53		212	13 (9)
	3	21	0.33	58	0.99	263	6 (5)
B2	1	2	0.48	85		169	
	1	8	0.39	87	0.11	161	14 (20)
	1	14	0.98	89	1.00	161	
	2	21	0.69	67		133	14 (13)
	3	21	0.51	60	0.89	166	11 (13)
A1	1	2	0.37	21		264	23 (21)
A2	1	2	−1.03	8	1.00	190	21 (26)

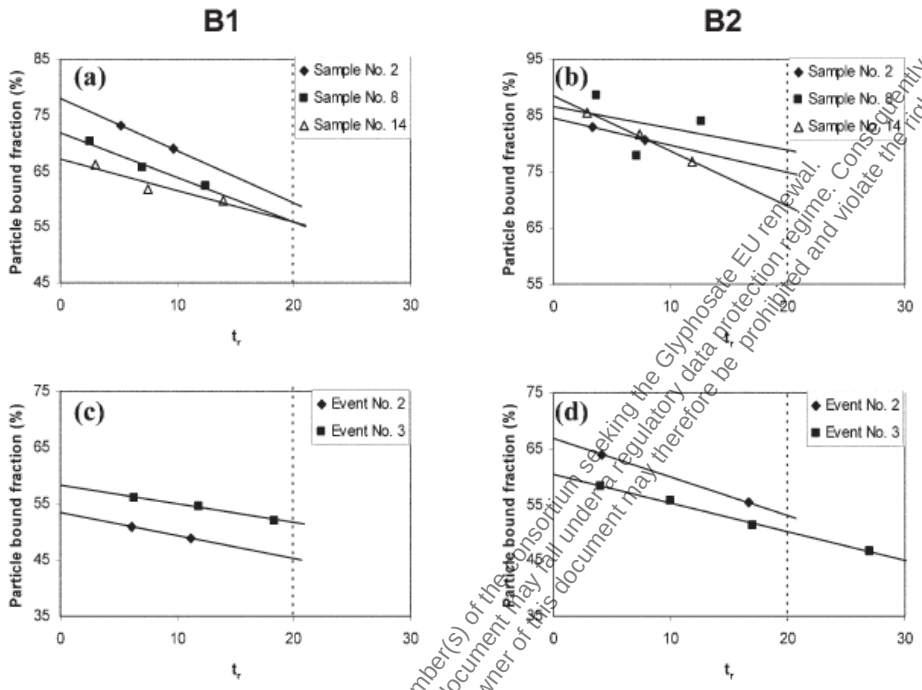
† Not applicable when the number of samples was <3.
‡ Standard deviation in brackets ($14 < n < 21$).

In general, Eq. [1] fitted well to the measured fractions of particle–bound glyphosate (Figure 7.1.4.1.1-3). The coefficients of determination were high ($R^2 \geq 0.92$), except for Sample 8 from the first irrigation on Column B2 (Table 7.1.4.1.1-29). By using this equation with the parameter values from Table 7.1.4.1.1-29, it was estimated that 7 to 20 % (on average, 12 %) of the leached glyphosate was desorbed from soil particles (>20 nm) within the first 20 min after sampling, corresponding approximately to the time scale of the observations. The reaction time (t_r) associated with the first filtration varied somewhat between events due to differing lengths of the sampling intervals. The 20–min relative desorption may be overestimated if the last measureements were close to the equilibrium concentrations (which was probably not the case according to the above calculations), and it may be underestimated if desorption took place much faster before the first filtrations (1.5 min after sampling).

The reaction time, defined as the time from the midpoint of the sampling interval, can be considered as an estimate of the time span after leaching. Hence, an estimate of the particle–bound fraction of glyphosate at a given time after leaching can be obtained from Eq. [1] by using parameters from Table 2. The data indicate that 45 to 79 % of the leached glyphosate was still particle bound 20 min after leaching (Figure 3). Thus, the rates of desorption measured shortly after sampling could not fully account for the amounts of glyphosate being desorbed 20 min after leaching.

The particle–bound fraction measure in leachate from the tilled soil 1.5 min after sampling varied between 51 and 89 % (Figure 7.1.4.1.1-3). This is in accordance with results reported by Gjettermann *et al.* (2009). It is probable that such figures depend considerably on the conditions that eventually lead to bypass flow and leaching. The applied methods were chosen to minimize desorption in the leachate before sampling and phase separation.

Figure 7.1.4.1.1-3: Particle (>20–nm) bound fraction of glyphosate in leachates from two soil columns, B1 (left) and B2 (right), at different reaction times (t_r, 0–30 min): (a) and (b) data for the first irrigation event (Samples 2, 8, and 14); (c) and (d) data for the second and third irrigation events (Sample 21)



The particle–bound fractions of glyphosate measured in the leachate from the two untilled soil columns (A1 and A2) 1.5 min after sampling were 19 and 14 %, respectively. This conforms to previously reported results that the fraction of particle–bound glyphosate in recently produced leachate can be much smalller with a minimally disturbed soil structure than with a tilled structure (Gjettermann *et al.*, 2009). The particle–bound fraction decreased with time after sampling in the A1 sample, indicating desorption, whereas it increased in the A2 sample, indicating sorption (Table 7.1.4.1.1-29). By inserting the estimated K_d (= 8.4 × 10^2 L/kg) in Eq. [4], the fractions of particle–bound glyphosate at equilibrium were estimated to be 18 and 14 % for the A1 and A2 samples, respectively. Hence, leached glyphosate from the untilled soil columns appears to have been close to equilibrium, which is probably why both sorption and desorption may have occurred, as indicated by the measurements.

The individual desorption rates are relatively uncertain, being based on only two to four measured particle–bound fractions (Figure 7.1.4.1.1-3). More observations could have been obtained, but only if the sample sizes had been increased correspondingly. This would have increased the time of reaction and hence desorption taking place before the measurements. The trends observed are similar for all samples, however, corroborating the conclusion that the particle–bound glyphosate in the solution leaching from the tilled columns was not in equilibrium with the surrounding water phase.

Desorption from Splash–Eroded Particles

Noticeable splash erosion occurred during all irrigation events involving the tilled columns. The amounts of (air–dry) splash–eroded particles varied between 31 and 70 mg per event independent of irrigation number and column. All fine–earth particle sizes were present, in accordance with earlier findings that eroded material is typically unsorted (Heilig *et al.*, 2001; Hairsine and Rose, 1991; Al–Durrah and Bradford, 1982). Larger particles were removed by using the 100–µm sieve in consequence of the earlier reported finding that particles smaller than about 0.1 mm are not present in drainage water from the investigated field site (Holm *et al.*, 2003). The fine particles released considerable amounts of glyphosate after being suspended. Hence, dissolved glyphosate concentrations increased with time, with gradually

decreasing rates (Figure 7.1.4.1.1-4 a and b). The rates were still relatively high after 1 h. After a few hours, concentrations were high compared with concentrations measured in most leachates (Figure 7.1.4.1.1-2), except from the first irrigation on Column B1. An equilibrium concentration of dissolved glyphosate appeared to be reached after about 5 to 10 h, except for the first irrigation event on Column B2; equilibrium was not attained within 48 h in this case. Glyphosate desorption decreased successively with irrigation event number.

Equation [3] fitted well to the measured dissolved concentration as a function of time (0–48 h) (Figure 7.1.4.1.1-4 a and b; Table 7.1.4.1.1-30). Coefficients of determination, R^2, varied between 0.87 and 0.98. The rate constant of desorption, *k*, was found to be in the range 0.57 to 1.19 h^{-1}, largest for the first irrigation event on Column B1. The equilibrium concentration, $C_{d,eq}$, was in the range 1.56 to 4.1 µg/L, decreasing successively with each additional irrigation event.

Table 7.1.4.1.1-30: Parameters and key data derived from experiments on splash-eroded particles. Rate constants (k), dissolved concentrations at equilibrium ($C_{d,eq}$), and coefficient of determination (R^2) obtained by fitting Eq. [3] to all data (reaction time t_r 0–48 h) from the two columns (B1 and B2) and three irrigation events. Relative desorption rate (α), intercept parameter (β), and R^2 obtained by fitting Eq. [1] to data for relatively short time scales: results based on the first three data points (t_r = 2, 10, and 60 min) and desorption rate based on the first two data points (t_r = 2 and 10 min)

Column	Irrigation no.	From Eq. [3], 0–48 h			From Eq. [1] 2–60 min			From Eq. [1] 2–10 min
		k	$C_{d,eq}$	R^2	α	β	R^2	α
		h^{-1}	µg L^{-1}		% min^{-1}	%		% min^{-1}
B1	1	1.19	4.0	0.98	0.56	71	0.94	1.62
	2	0.75	2.19	0.91	0.54	79	0.98	1.09
	3	0.59	1.83	0.94	0.48	82	0.98	1.06
B2	1	0.57	4.1	0.92	0.41	81	0.99	0.79
	2	0.58	2.59	0.95	0.55	87	1.00	0.40
	3	0.99	1.56	0.87	0.50	73	0.98	1.10

The equilibrium concentrations, $C_{d,eq}$, the previously estimated K_d = 8.4 × 102 L/kg, and the constant particle concentration $C_{particle}$ = 100 × 10^{-6} kg/L were used when calculating total concentrations (Eq. [4]), particle–bound fractions, and relative desorption rates (Eq. [1]). Accordingly, the fitted dissolved concentrations at equilibrium (Table 7.1.4.1.1-30) represent about 92 % of the total concentrations. The linear relationship Eq. [1] fitted well to the three data points representing the particle-bound fraction vs. time (2 to 60 min) after dissolving the splash–eroded particles, R^2 being in the range 0.94 to 1.00 (Figure 7.1.4.1.1-4 c and d; Table 7.1.4.1.1-30). The relative desorption rates (α) were estimated to be in the range 0.41 to 0.56 % min^{-1} (average 0.51 % min^{-1}), with no systematic dependence on irrigation event or column (Table 7.1.4.1.1-30). Hence, at these rates, 8 to 11 % (average 10 %) would be desorbed during a 20–min time period. The rates tended to be slightly smaller than desorption rates measured in the leachate at a similar or somewhat shorter time scale (α for Columns B1 and B2 in Table 7.1.4.1.1-29, average value 0.61 % min^{-1}). For the period 2 to 10 min, the measured relative desorption rates were in the range 0.40 to 1.62 % min^{-1} (Table 7.1.4.1.1-30; average value 1.01 % min^{-1}), i.e., generally somewhat larger than for the period 2 to 60 min. This was expected also from the good fit of all the data to Eq. [3]. The rates obtained for the 2– to 10–min period were of the same order of magnitude, although generally larger than desorption rates measured in the leachate at a similar or somewhat longer time scale (α for Columns B1 and B2 in Table 7.1.4.1.1-29). Calculated from rates obtained for the 2– to 10–min period, 8 to 32 % (average 20 %) would desorb in 20 min right after the first fractionation. Overall, similar desorption rates were found for leached and splash–eroded particles when determined at similar time scales. This indicates that similar desorption processes were involved for the two types of particles.

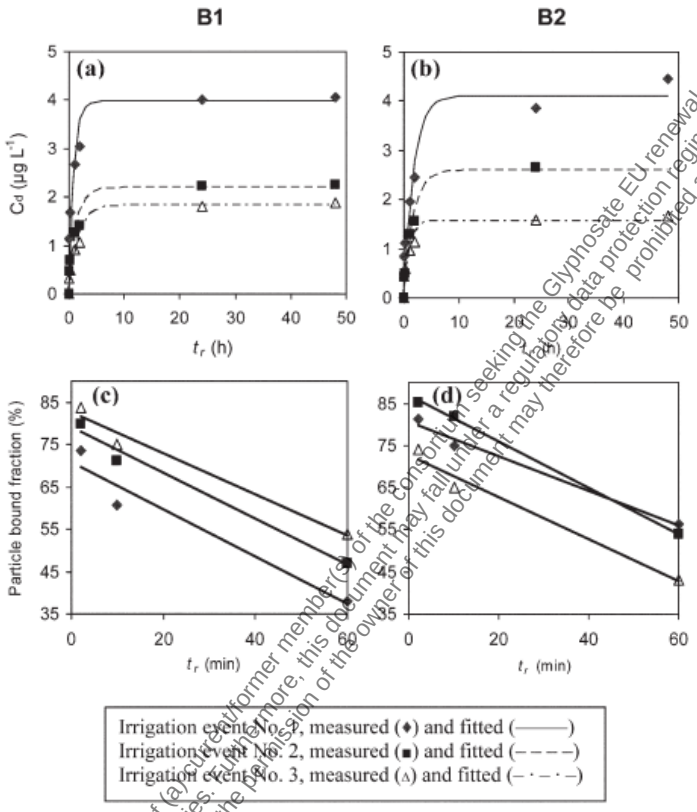
The initial glyphosate concentrations (mg/kg) were somewhat higher on splash–eroded particles than on

leached particles. For the first measurements on leached particles made 1.5 min after sampling, the range of concentrations was 4 to 24 mg/kg; for measurements on splash–eroded particles made 2.0 min after immersion, the range of estimated concentrations was 13 to 36 mg/kg. The concentrations decreased systematically with succeeding irrigation event for both types of particles. The splash–eroded particles may have been enriched with glyphosate when the water droplets evaporated on the collar after irrigation; however, concentrations on splash–eroded particles from the first irrigations (about 44 mg/kg according to Table 7.1.4.1.1-30 and Eq. [4]) were within a realistic range for the uppermost soil layer shortly after spraying. Thus, by assuming that the applied glyphosate was distributed in the uppermost 2– to 5–mm soil layer having a bulk density of 1.6 g/cm^3, an expected average glyphosate concentration of 55 to 22 mg/kg can be calculated for the layer.

The splash–eroded particles were air dry, and the particle–bound fraction of the glyphosate must therefore have been close to 100 % right before the particles were immersed in water (at t_r = 0). At t_r = 2.00 min, however, the particle–bound fraction had already decreased to between 74 and 85 % (Figure 7.1.4.1.1-4 c and d). It is difficult to model this very rapid decrease as a function of time when seen at the shorter time scales. It indicates that a fraction (up to 26 %) of the glyphosate could have been very weakly bound. Physical effects of the immersion may also have affected the rapid glyphosate release.

From the linear models shown in Figure 7.1.4.1.1-4 c and d, it can be calculated that about 60 to 76 % of the glyphosate was still particle bound 20 min after immersion of the particles in water. The values are of a similar magnitude as the estimated particle–bound fractions in the leachate 20 min after leaching (45-79 %, cf. above). In the study on leachate, it is probable that desorption had taken place in wet fractions of the soil columns before leaching and from leached particles in the leachate before the first fractionation. This may to some extent have reduced the initially measured fraction of particle–bound glyphosate and the measured desorption rates.

Figure 7.1.4.1.1-4: Desorption of glyphosate in suspensions containing splash–eroded soil particles from two soil columns, B1 (left) and B2 (right): (a) and (b) concentration of dissolved glyphosate (C_d) monitored at long time scales of reaction ($0 < t_r \leq 48$ h), the curves represent least squares fits of Eq. [3] to data points; (c) and (d) particle–bound fraction monitored at short time scales (2–60 min), the lines represent least squares fits of Eq. [1] to data points



Can Desorption Kinetics be Ignored in Glyphosate Transport?

Bold *et al.* (2003) investigated the significance of kinetics in contaminant transport using an intraparticle diffusion model to account for the kinetic contaminant–particle interaction. They showed by sensitivity analysis that kinetic limitations of contaminant–particle interactions have to be taken into account for $0.01 < D_a < 100$. They also concluded that for $D_a < 0.01$, desorption of contaminants from particles is so slow that it can be neglected.

A range of possible outcomes of D_a for the present column experiments was estimated based on desorption rate coefficients obtained in the 0– to 48–h experiments on splash–eroded particles (k values in Table 3). The fluid velocity inside the column depends on whether it is moving through macropores or the matrix. Two extreme boundaries could be: (i) transport exclusively through a water–filled continuous macropore from the surface to the bottom of the column, and (ii) transport exclusively thought the soil matrix. For extreme (i), a continuous macropore with a diameter of 0.6 cm (area = 0.28 cm^2), and steady–state condition, the irrigating rate (1060 cm^3/h) would give rise to an average fluid velocity of approximately 3700 cm/h. For extreme (ii), a homogeneous soil matrix (column area = 707 cm^2), steady-state condition, and a water content equal to field capacity (about 30 %), the irrigation would give rise to an average fluid velocity of approximately 5 cm/h. For extreme (i), the D_a would be in the range of 0.01 to 0.02 (cf. Eq. [4]), depending on the value of k. For extreme (ii), the D_a would be in the range of 6 to 12. These intervals, even the one for homogeneous matrix flow, are within the critical range estimated by Bold *et al.* (2003), indicating that kinetic limitations of glyphosate–particle interactions have to be taken into account in describing the transport. In reality, bypass flow and glyphosate transport below the 25–cm depth took place

almost exclusively in earthworm channels in the size range 2 to 8 mm (Gjettermann *et al.*, 2009), indicating conditions much closer to extreme (i) than (ii). Although we realize that the measured rates are not necessarily representative of the conditions throughout the soil columns, the results of this analysis indicate that particle mobilization and particle–facilitated transport could play a critical role in pesticide leaching under such conditions.

The interaction between contaminants and mobile particles has, in many studies, been described as an instantaneous equilibrium process (e.g., Prechtel *et al.*, 2002; Villholth *et al.*, 2000). To our knowledge, no study has described the importance of desorption kinetic behavior of contaminants in structured soil with special attention to facilitated transport. Turner *et al.* (2006), however, revealed that Cs desorption from illite particles was slower than Sr desorption and demonstrated that this difference in desorption kinetics resulted in greater colloid–facilitated transport of Cs in columns packed with a quartz porous medium. They estimated D_a to be in the range of 0.00035 to 0.086 for Cs and 0.97 to 2.0 for Sr. Van de Weerd and Leijnse (1997) also found that desorption of Am from humic particles was a slow process that could only be described by taking into account a kinetic interaction between Am and humic particles. These findings combined with the current investigations show that it is important to consider desorption kinetics as an integral part of the transport process when considering particle–facilitated transport of glyphosate and other non-instantaneously desorbing contaminants.

Conclusion

Glyphosate desorbed with similar fractional rates from leached and from splash–eroded particles (>20 nm) when investigated at similar relatively short time scales. Thus, 7 to 20 % of the total amount of leached glyphosate (average 12 %) desorbed in 20 min shortly after leaching, while on average between 10 and 20 % desorbed from splash-eroded soil particles in suspension in 20 min shortly after immersion. The similarities support the view that the particles investigated and the processes of desorption were similar for the two types of material. Concentrations of glyphosate on leached particles were always somewhat lower than concentrations on splash–eroded particles.

Equilibrium concentrations were generally obtained within 5 to 10 h in suspensions containing splash-eroded particles. Hence, depending on the time of fractionation of the collected samples (in the interval 0–10 h), very different relative amounts of particle–bound glyphosate may be found; to quantify particle–facilitated glyphosate transport, the water and solid phases should be separated immediately after leaching. Furthermore, an analysis of the Damköhler number indicates that desorption kinetics is important for glyphosate transport and for the significance of particle–facilitated transport.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a leaching experiment with glyphosate in soil columns. The desorption of glyphosate from soil particles and its effect on interpretation of leaching experiments was in the focus of the study and desorption kinetics of particle-bound glyphosate are postulated to influence glyphosate transport strongly. Not all necessary information was reported to check the validity of the results (no mass balances, study set-up not clearly described, insufficient information on soil properties and soil origin, test item not sufficiently described, temperature not provided, molecular identity of desorbed radioactivity not determined).
The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.1.1/009
Report author	Gjettermann, B. *et al.*
Report year	2011
Report title	Evaluation of Sampling Strategies for Pesticides in a Macroporous Sandy Loam Soil
Document No	DOI 10.1080/15320383.2011.620049 E-ISSN 1549-7887
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (Not sufficient information available to check validity of the results, scope of the study is not on leaching of glyphosate itself but on evaluating the usage of a dye to improve sampling strategies)

2. Full summary

It is not straightforward to sample and demonstrate the presence and transport of pesticides in heterogeneous soil. Following leaching experiments with four differently structured 50–cm–long soil columns (tilled and untilled soil), the objective of this study was to investigate the extent that visual tracing of the dye Brilliant Blue could support in soil sampling for two strongly sorbing pesticides (^{14}C–labeled glyphosate and pendimethalin). About 830 samples were collected. No pesticide was found below 10-25 cm depth by random sampling, even though 0.21–0.31 % of the applied amounts were leached, and 0.18 % of the soil volume was sampled. With similar sampling efforts, the pesticides could generally be traced throughout the columns by sampling from stained soil volumes, only. None of the two particular sampling strategies for pesticides produced accurate mass balances or balances that were obviously better than the other. No pesticide was detected outside stained soil volumes, except for glyphosate in one sample. Below 30 cm, stained soil comprized on average 5 % of the total soil volume, leaving 95 % as expectedly pesticide–free. The results suggest that much more efficient sampling for sorbing pesticides can be obtained by using the dye and focusing on stained soil volumes.

Materials and methods

Soil Columns

The macroporous Rørrendegaard sandy loam soil investigated in this experiment is developed on till from the Weichselian glaciation. The contents of coarse sand (200–2000 µm), fine sand (20–200 µm), silt (2-20 µm), and clay (<2 µm) in the upper 30 cm is 29 %, 40 %, 18.5 %, and 12.5 %, respectively, and the organic C content is 1.2 %. At 50 cm depth, the number of vertically oriented earthworm channels (diameter: 3–8 mm) is typically in the range 200–600 m^{-2}. The soil has previously been described in detail by Petersen *et al.* (2001) and Gjettermann *et al.* (2009).

Undisturbed soil columns (diameter: 30 cm; 0–60 cm soil depth) were sampled in late autumn from two experimental plots with different tillage treatments (A and B). For each of the previous nine years the same cereal crop (wheat or barley) had been grown in the plots. Plot A had not been tilled for one year, and it had not been subjected to deep (>4–6 cm), loosening tillage for eight years. Plot B had been under traditional tillage (including annual ploughing) for at least nine years. It had been ploughed and drilled for wheat one month before sampling, and a new wheat crop had just been established. Treatment A (untilled) gave rise to a relatively stable soil structure with vertically oriented earthworm channels from the partially covered surface (old wheat stubble, weeds, and moss) to the bottom of the columns. Treatment B (tilled) did result in a more variable structure with stubbles being heterogeneously incorporated in the plough layer (0–25 cm). Fewer vertically oriented earthworm channels penetrated all the way to the surface. Columns

(two per treatment) were manually excavated and encapsulated with polyurethane to stabilize and seal the walls. Care was observed not to disturb the surface structure. The columns were trimmed to 50 cm length from the bottom end, avoiding sealing macropores, and placed on a galvanized metal grating. The columns were sealed with plastic foil at the upper end and stored at 2–3°C whenever not used in the experiments.

Leaching Experiments
The leaching experiments have been described in detail by Gjettermann *et al.* (2009) and were in brief as follows: Columns were rewetted by irrigation one day before pesticide application. Each pesticide was applied uniformly to the surface of one column per treatment. The pesticides were applied in doses similar to the ones used in agriculture, i.e. 12.51 mg glyphosate/column and 14.24 mg pendimethalin/column. Glyphosate was taken from a stock solution made from the commercial product Roundup Bio, ^{14}C-glyphosate, and blank formulation (all from Monsanto). The stock solution had a specific concentration activity of 0.80 MBq/mg. It contained both glyphosate and its major metabolite, AMPA (2.034 g/L in total) distributed on ^{14}C–glyphosate (4.4 %), unlabeled glyphosate (93.5 %), and AMPA (2.1 %). Pendimethalin was taken from another stock solution made from the commercial product Stomp mixed with ^{14}C-pendimethalin (both from BASF). This stock solution had a specific concentration activity of 4.55 MBq/mg. It contained ^{14}C–pendimethalin (4.2 %) and unlabeled pendimethalin (95.8 %).

Leaching was driven by irrigation water having a composition similar to rain water (Gjettermann *et al.*, 2009). The water was applied uniformly at a fixed intensity (15 mm/h) to the top of the columns through a rotating irrigation device. Each column received three 2.0 hours irrigation events 5, 8, and 12 days after rewetting, respectively. Thus 30 mm of irrigation water was applied in each event, corresponding approximately to 7.6 % of the total soil pore volume. A 15 mm/h rain event in 2 h may be considered as an extreme for Danish conditions expected to occur about once every 10 years, even though short–time rain intensities are frequently much higher (Madsen *et al.*, 2009). Water was allowed to drain freely from the bottom of the columns. Pesticide contents in the leachate were determined by measuring the ^{14}C–activity with liquid scintillation counting. Brilliant Blue was applied to the four columns (one per combination of soil treatment and pesticide) after the pesticide–leaching experiments. The dye was applied in aqueous solution (4.0 g/L) as a standard irrigation (i.e. 15 mm/h in two hours) after rewetting.

Sampling
Samples were obtained from 9 or 10 separate column sections prepared 1–2 days after dye application. Initially, the columns were sectioned into 7 or 8 depth intervals (cylindrical slices). All columns were cut at 15, 20, 25, 30, and 40 cm depth using a steel thread or a narrow–bladed saw to minimize smearing. Two more cross-sections were made at depths below 15 cm in three columns, whereas only one cross–section was obtained in one column representing treatment B. All cross–sections were carefully cleaned for traces of soil materials and dye being smeared during the cutting procedure. They were then subjected to intensive diffuse light and photographed using a 3.0 Mpx camera.

Soil sampling within the slices was conducted according to three different strategies: (1) 5–10 soil samples were taken from different, intensively blue–colored soil volumes in the vicinity of dyed (flow active) macropores; (2) 10 core samples were taken randomly within non–colored areas (as determined at the top-end); and (3) 10 completely randomized core samples were collected. Thus, typically 25–30 soil samples were taken per slice. However, due to extensive staining making it difficult to avoid blue soil, sampling according to strategy 2 was not performed above 5–10 cm depth. Furthermore, special procedures were followed in the uppermost slice. Following strategy 3, 10 samples were taken randomly in the 0-0.5 cm and the 0.5–1.5 cm depth intervals (the uppermost 1.5 cm was not included when sampling below). With strategy 1, sampling started at 0.5 cm depth (treatment A) or 1.5 cm depth (treatment B) because it was not possible to identify flow active macropores in the uppermost layer. Sampling according to strategy 1 was accompliced by scraping 1–2 mm of stained soil from the inside of biopores or cracks using a spatula. Sampling following strategies 2 and 3 was supported by coordinates generated by a random number generating program. It was done using a drill (diameter: 4.0 mm) throughout the entire soil layer. Hence with 10 samples per layer, roughly 0.18 % of the total soil volume was sampled. A total of 185–240 soil samples were obtained per column. Similar samples, according to the sampling strategy, were pooled within each soil layer.

Analyses
The pooled soil samples were air–dried, grounded using a ball–mill (350 rpm for 1 min), and mixed carefully. ^{14}C–activity was measured by LSC after heating of 250 mg soil to 800°C in a constant flow of oxygen (Packard Sample Oxidizer Model 507) followed by ^{14}C–CO_2 absorption by Carbosorb E (Packard) and Permafluor E+ (Packard). Two replicates were analyzed from each pooled soil sample. The concentrations of pesticides in soil were calculated from the specific concentration activity of the ^{14}C-labeled pesticides and the relationship between labeled and unlabeled pesticide in the applied pesticide solutions. The detection limits for glyphosate and pendimethalin in soil were 0.01 and 0.005 mg/kg, respectively.

All the blue-stained representations of flow patterns appearing on photos of the cross–sections were manually transferred to transparent plastic sheets, and the new binominal representations (images showing either color or no color) were digitized using the procedures described by Petersen *et al.* (1997). The only distinction made in this process was whether or not blue dye was visible on the photos as evaluated by one person. The photos were handled in systematic order governed by a random serial number assigned to each. The digitized images were then scaled in two mutually perpendicular directions, and the fractional dye-stained area (DC, %) was determined using the image processing program ImageJ (Collins, 2007). The thickness of the uppermost completely dyed in soil layer (maximum depth with DC = 100 %) was measured. Fractional volume of dyed soil in a given soil layer was calculated as the average of DC observed at the top and bottom ends.

Mass balances for the pesticides were established based on sampling strategy 1 and 3, respectively. For strategy 1, measured pesticide concentration in soil was multiplied with fractional volume of dyed soil and by the mass of soil to get the pesticide content of a given soil layer. Concentrations obtained with strategy 3 were applied in the uppermost 0.5 cm (treatment A) or 1.5 cm (treatment B) layers in the lack of strategy 1 observations. For strategy 3, the pesticide content of a given soil layer was obtained by multiplying the measured concentration by the mass of soil. A dry bulk density of 1.60 g/cm^3 (average value for all columns) was applied throughout in these calculations.

Results and Discussion
Distribution of Pesticides
Significant pesticide concentrations (above the detection limits) could be traced all the way through the columns by sampling strategy 1, except for glyphosate in treatment B at 25–50 cm depth. The concentrations generally decreased with depth (Figure 7.1.4.1.1-5). Below 30 cm depth, the pendimethalin concentrations approached the detection limits.

No significant amounts of the pesticides were found using sampling strategy 2, except in one sample (glyphosate in treatment A at 20–25 cm depth, cf. Figure 7.1.4.1.1-5). Thus, as a rule, pesticides were not detected by sampling outside blue-stained areas, not even at 5–10 cm depth, where considerable amounts of pesticide were found with the other sampling strategies. This is particularly noteworthy because three irrigations were carried out after pesticide application prior to application of the dye solution. Stronger sorption of the pesticides than of the dye may be part of the explanation. For the investigated (top–) soil, Gjettermann *et al.* (2009) reported soil–water partition coefficients (K_d–values) of 503 L/kg for glyphosate and 242 L/kg for pendimethalin. Hence, both pesticides sorb strongly to the soil material. For Brilliant Blue, Flury and Flühler (1995) have reported much smaller K_d–values in the range 0.19–5.78 L/kg. Somewhat stronger sorption than measured by Flury and Flühler has been found for soils rich in clay minerals (German–Heins and Flury, 2000; Ketelsen and Meyer–Windel, 1999). It should be noticed that the sampling strategy being based on coring from upper surfaces of the slices does not completely exclude the inclusion of blue-stained soil material.

It was not possible in any case to trace the pesticides all the way through the columns by using strategy 3. With treatment A, glyphosate was not found below 10 cm depth and pendimethalin not below 20 cm. The pesticides tended to be found at slightly greater depths with treatment B. However, no significant concentrations were found below 25 cm depth. Hence with completely randomized sampling, pesticides were not found in significant amounts in the soil below 10–25 cm depth even though significant amounts (0.21–0.31 % of applied) were leached (Table 1) and 0.18 % of the soil volume was sampled. It was noticed

that the strategy generally led to the inclusion of some blue–colored soil in the pooled samples when applied above 15–20 cm. Pesticide concentrations decreased strongly with depth in the 0–5 cm depth interval (Figure 7.1.4.1.1-5). By far the highest concentrations were found in the uppermost 5 mm of soil being completely dyed–in for both tillage treatments. Glyphosate concentrations measured in this layer were 8.59 and 10.5 mg/kg for the A and B treatment, respectively, while the corresponding numbers for pendimethalin were 24.6 and 14.1 mg/kg. Significant pesticide concentrations measured according to strategy 1 were always higher than concentrations obtained at the corresponding depths by strategy 3 (Figure 7.1.4.1.1-5). The differences between the two repeated measurements of pesticide concentrations of the soil samples were negligible (not shown).

Figure 7.1.4.1.1-5: Glyphosate and pendimethalin concentration (C_G and C_P, respectively) as a function of soil column depth obtained by the sampling strategies 1 (dyed), 2 (non–dyed), and 3 (random). Data for the two tillage treatments (A and B, average of 2 repeated measurements). Notice the broken 2nd axes

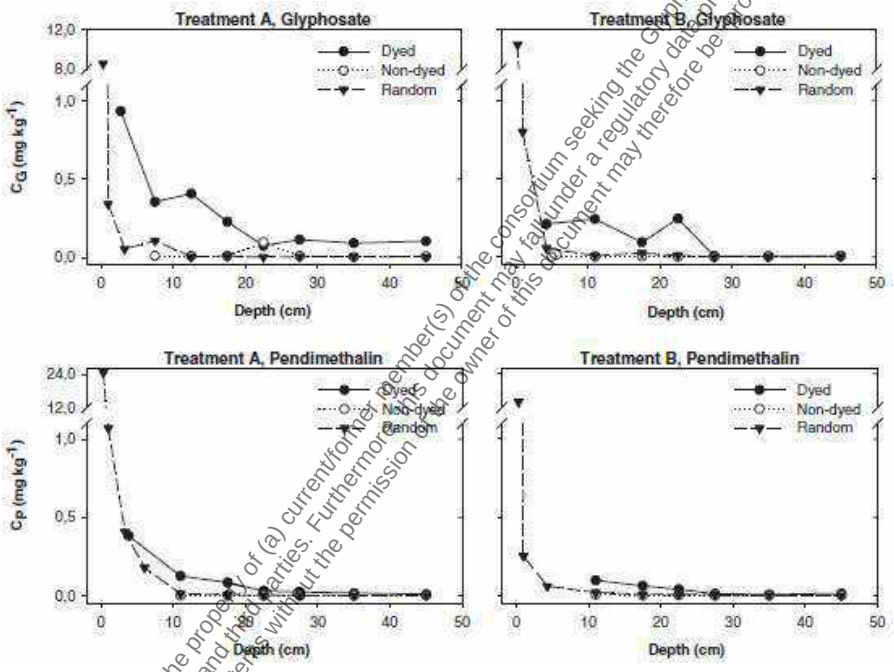


Table 7.1.4.1.1-31: Amounts of pesticides retrieved in columns estimated from two different column sampling strategies (1 and 3), and amounts lost with leachate (% of applied)

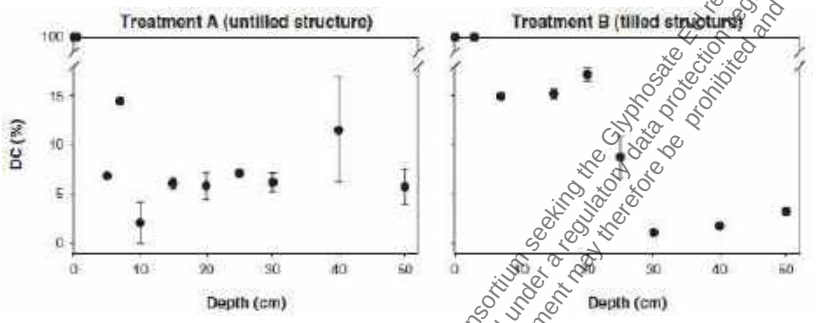
	Treatment A		Treatment B	
	Strategy 1	Strategy 3	Strategy 1	Strategy 3
Glyphosate found in column	63	50	67	61
Glyphosate leached	0.31	0.31	0.21	0.21
Pendimethalin found in column	110	123	65	63
Pendimethalin leached	0.23	0.23	0.21	0.21

Applied amounts per column, Glyphosate: 12.5 mg; Pendimethalin: 14.2 mg

Dye Patterns

The thickness of the uppermost completely dyed–in soil layer was about 0.5 cm for treatment A and about 3 cm for treatment B. Thus, the fractional volume of dyed soil was 100 % above 0.5–cm depth in columns subjected to treatment A and above 3 cm in columns subjected to treatment B. The fractional area covered with dye (DC) rapidly decreased with depth right below these depths. In the topsoil, DC tended to be larger for treatment B than for treatment A, whereas the opposite trend was observed in the subsoil (Figure **7.1.4.1.1-6**).

Figure 7.1.4.1.1-6: Fractional area covered with dye (DC) at different soil depths. Average values for each of the two tillage treatments (A and B, n = 2) with the range shown by the bars. Notice the broken 2nd axis



Below 30–cm depth, the stained flow pathways were mainly concentrated around vertically oriented earthworm channels comprising a relatively small fraction of the total soil volume. The dye had typically penetrated less than 1–2 cm into the soil matrix from these flow active macropores (4–10 per column). This is more than previously reported from studies conducted under field conditions (Petersen *et al.*, 1997), probably due to the wet conditions prevailing in the columns with drainage at atmospheric pressure from 50–cm depth. For treatment A, a considerable fraction of the stained soil volume was found at the column walls in connection with large flow–active macropores that were cut during the excavation process. On average for all columns, the fractional volume of dyed soil below 30–cm depth comprized 5 %, leaving about 95 % as unstained and expectedly pesticide free.

Mass Balances

Under typical field conditions, half–lives (DT_{50} values) for glyphosate and pendimethalin are about 12 and 90 days, respectively (PPDB, 2010). However, under the low temperatures prevailing in the columns, both pesticides are expected to be slowly degradable. Furthermore, any non–volatile metabolites containing the ^{14}C would be included in the measurements. The columns were sealed with plastic foil, except when used in the experiments. Hence, losses due to degradation and evaporation are expected to be very small. Also, the fraction of applied pesticide (^{14}C) being leached was small (0.21–0.31 %) and unimportant for the mass balance (Table 7.1.4.1.1-31). Consequently, we expected a recovery close to 100 % based on the soil sampling alone. We found between 50 and 123 % with sampling strategy 3, and between 63 and 110 % with strategy 1, respectively (Table 7.1.4.1.1-31). Hence, none of the sampling strategies resulted in the expected (slightly less than) 100 % recovery in the columns although the balances tended to be better for strategy 1 than 3.

Both methods of constructing a mass balance obviously had large uncertainties. The largest concentrations (and amounts) of pesticide were found in the uppermost 0.5 cm of the profile, and the major uncertainty appears to be the sampling of this top layer. If, for instance, the actual depth of sampling was 6 mm rather than 5, the error to the mass balance would be 8–19 % for the investigated columns. However, any difference in mass recovery obtained with the two sampling strategies is not related to this uncertainty, since the same sampling of this uppermost thin soil layer was used in both cases. The method based on sampling strategy 1 does correctly include some pesticide from lower parts of the columns. However, it may be biased if sampling for the pesticides did not fully represent the stained soil volumes. The mass recovery was of the same order of magnitude or considerably better than that obtained by Flury *et al.* (1995)

working with a systematic very dense two–dimensional sampling scheme for herbicides in structured field soil.

Tracing Pesticides in Macroporous Soil
The magnitude of preferential flow contributing to the leaching of pesticide is difficult to quantify from studies on soil samples. Prichard *et al.* (2005) investigated the predominant source of pesticide residues detected in domestic wells located in an area with cracking clay soil. Although preferential flow through macropores within the field was a potential pathway, pesticide residues were retained in the top 15 cm of the soil. They deduced that the contribution of preferential transport to leaching was insignificant, despite the fact that lack of correlation of pesticide data between soil and water samples has previously been documented for soils with preferential flow (e.g. Sanchez *et al.*, 2006; Laabs *et al.*, 2000; Malone *et al.*, 2000). Sanchez *et al.* (2006) found high concentrations of methidathion in the upper 25 cm of a soil profile but very low concentrations below this depth. They attributed high concentrations found sometimes in leachates from deeper layers to preferential flow processes. Laabs *et al.* (2000) similarly suggested that absence of pesticide residues in soil at depths below 25 cm combined with observed leaching indicated non–chromatographic transport of these substances in the soil profile. Malone *et al.* (2000) concluded that a sampling strategy including the mixing of horizontal slices with dimension 3.75 × 30 × 30 cm was not well suited to trace the movement of pesticides in the subsoil of structured soils. The present study support the interpretations made by Laabs *et al.* (2000), Malone *et al.* (2000), and Sanchez *et al.* (2006).

As expected, visible traces of Brilliant Blue were indicators for the occurrence of the pesticides in the soil. The measurements strongly suggest that both pesticides were transported exclusively within some fraction of the stained soil volume. With transport concentrated on a few macropores as in the subsoil of the present study, it should be possible to sample virtually all dye (and pesticide) at a given depth. Further developed, strategy 1 could therefore be used as the basis for better quantification of strongly sorbing pesticides in macroporous subsoil profiles. It is likely that the dye tracer should be applied under similar conditions (e.g. soil structure, soil moisture content, irrigation/precipitation amount and intensity) as the pesticides themselves.

Conclusions
Visible traces of Brilliant Blue transported under similar conditions as the two pesticides indicated the occurrence of both pesticides in the soil. The results suggest that efficient sampling for sorbing pesticides can be obtained by using the dye and focusing on stained soil volumes. None of the investigated sampling strategies led to mass balances that were accurate enough to detect amounts of pesticide leaching.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a leaching experiment on soil columns with a dye and glyphosate (as well as pendimethalin). Glyphosate was only transported within a fraction of the stained soil volume. Some important information about study conditions are missing: agricultural use of the soil, temperature, soil parameters, details on analytics and on substance identification, sample storage conditions before analysis. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

Reliable adsorption coefficients of soil metabolites were obtained by adsorption/desorption studies and, consequently, column leaching studies are not required (please refer to CA 7.1.3.2).

CA 7.1.4.2 Lysimeter studies

A lysimeter study is not required and none was conducted.

During the scientific literature research for glyphosate (2010-2019), three articles were identified to provide further information relevant to the data point. The reliability of the articles was assessed as "reliable with restrictions". Thus, no new endpoints were derived, and the articles areconsidered as supportive information.

The article of Napoli *et al.* (2015, CA 7.1.4.2/001) reported concentrations of glyphosate and AMPA in leachates of 1 m² lysimeters filled with silty clay soil columns from an Italian vineyard of up to 13.5 and 24.9 µg/L, respectively, following annual twofold spring application of about 0.72 kg a.s./ha over three years. Glyphosate was detected in 3 % of the soil leachates at concentrations ranging from 0.2 to 1 µg/L and in 16 % of the leachates at concentrations ranging from 1 to 13.5 µg/L, while AMPA was detected in 13 % of the leachates from soil at concentrations ranging from 0.2 to 1 µg/L and in 20 % of the leachates from soil at concentrations ranging from 1 to 24.9 µg/L. The annual recovery of glyphosate and AMPA in the leachates of the 1st, 2nd and 3rd year related to the applied amount of glyphosate was 0.19, 0.31 and 0.12 % for glyphosate and 0.49, 0.78 and 0.48 % for AMPA, respectively. The use of clay soil is considered not appropriate for lysimeter experiments (low- to non-permeable soil), and from the information given in the article it cannot be excluded that the leaching may have been caused by preferential flow rather than percolation through the soil column (also see chapter CA 7.1.4.3 on field leaching below regarding the behaviour of glyphosate in low-permeable soils). Further, there is no information available whether, apart from application, lysimeters were handled according to normal agricultural practice. From the information reported it is not possible to calculate annual mean concentrations in leachate for glyphosate and AMPA which would however be required in view of risk assessment.

In the article of Al-Rajab & Hakami (2014, CA 7.1.4.2/002) the leaching behaviour of glyphosate was investigated in three agricultural soils in micro-lysimeters (10 cm diameter, 35 cm length) under outdoor conditions. Leaching was found to be very low with less than 0.3 % of the initially applied radioactivity in the leachate after 100 days.

In experiments with micro-lysimeters (29.5 cm diameter, 118 cm length) by Bergstrom *et al.* (2011, CA 7.1.4.2/002), 0.009 % of the initial amount of glyphosate applied were recovered in the leachate of a sand soil after 100 days. In a clay soil, 0.019 % were recovered in the leachate. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil.

Table 7.1.4.2-1: Lysimeter experiments – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.4.2/001	Napoli *et al.*, 2015	Lysimeter	Glyphosate	Reliable with restrictions	
CA 7.1.4.2/002	Al-Rajab & Hakami, 2014	Lysimeter	Glyphosate	Reliable with restrictions	Summary under CA 7.1.2.1.1/015
CA 7.1.4.2/003	Bergstrom *et al.*, 2011	Lysimeter	Glyphosate	Reliable with restrictions	Summary under CA 7.1.2.1.1/017

1. Information on the study

Data point:	CA 7.1.4.2/001
Report author	Napoli, M. *et al.*
Report year	2015
Report title	Leaching of Glyphosate and Aminomethylphosphonic Acid through Silty Clay Soil Columns under Outdoor Conditions
Document No	DOI 10.2134/jeq2015.02.0104 E-ISSN 1537-2537
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (Not sufficiently described to check validity against current guideline)

2. Full summary

Glyphosate [*N*-(phosphono-methyl)-glycine] is the main herbicide used in the Chianti vineyards. Considering the pollution risk of the water table and that the vineyard tile drain may deliver this pollutant into nearby streams, the objective of the present study was to estimate the leaching losses of glyphosate under natural rainfall conditions in a silty clay soil in the Chianti area. The leaching of glyphosate and its metabolite (aminomethylphosphonic acid [AMPA]) through soils was studied in 1-m-deep soil columns under outdoor conditions over a 3-yr period. Glyphosate was detected in the leachates for up to 26 d after treatments at concentrations ranging between 0.5 and 13.5 µg/L. The final peak (0.28 µg/L) appeared in the leachates approximately 319 d after the first annual treatment. Aminomethylphosphonic acid first appeared (21.3 µg/L) in the soil leachate 6.8 d after the first annual treatment. Aminomethylphosphonic acid detection frequency and measured concentration in the leachates were more than that observed for the glyphosate. Aminomethylphosphonic acid was detected in 20 % of the soil leachates at concentrations ranging from 1 to 24.9 µg/L. No extractable glyphosate was detected in the soil profile. However, the AMPA content in the lowest layer ranged from 13.4 to 21.1 mg/kg, and on the surface layer, it ranged from 86.7 to 94 mg/kg. Overall, these results indicate that both glyphosate and AMPA leaching through a 1-m soil column may be potential groundwater contaminants.

Materials and methods

Borate buffer (0.05 M) was prepared by dissolving 1.9 g disodium-tetraborate-decahydrate in 100 mL ultra-pure water. The FMOC-Cl solutions (1 g/L) were prepared by dissolving 10 mg FMOC-C1 in 10 mL acetonitrile. Glyphosate and AMPA working standard (30 µg/L) were prepared by dissolving glyphosate and AMPA in ultra-pure water. Working standards were stored at 4°C for no more than 1 wk.

In summer 2006, three lysimeters were installed at a lysimeter station in Montepaldi, San Casciano Val di Pesa, Tuscany, Italy. Each lysimeter consisted of a cube-shape casing (1-m edge) made of 4-mm-thick stainless steel sheet. At the bottom end of each lysimeter, a polyethylene corrugated drainage pipe was installed to collect the leachate. During the summer of 2006, the containers were filled with a silty clay soil collected from a nearby Chianti vineyard that had been mechanically weeded over the previous 3 yr. The soil was taken from the 0- to 100-cm layer of three randomly selected vine interrows. The soil was then taken and placed in the lysimeter, taking care to maintain the profile's natural order of layers. During the monitoring period, hourly temperature and rainfall data were measured by a meteorological station located 300 m from the experimental site. The annual mean temperature and precipitation at the study site were 14.6°C and 914 mm/yr, respectively.

The commercial formulations of glyphosate (360 g/L a.i.) were applied in the study area at a dose of 2 L/ha per application. There were one to two spring applications along each vine row, covering a strip of ~1 m. This implies that along the treated strip, the concentration of the active ingredient ranged from 70 to 150 mg/m^2 depending on the number of spring applications. For the lysimeter study, the concentration data associated with the two spring applications was modeled. Therefore, in the middle of March and in the middle of May, glyphosate was applied to each lysimeters. An aqueous solution of herbicide was sprayed onto the surface of the soils to simulate an application rate of 0.72 kg/ha a.i.

Drainage water was collected after each rainfall event from 1 Mar. 2007 to 28 Feb. 2010. To ensure limited degradation, leachate volumes were determined gravimetrically and then preserved in the dark at −20°C for a maximum of 25 d until analysis. On 26 Feb. 2007 and then at the end of each year (i.e., the last week of February), the soil was sampled in triplicate for each of the lysimeters, which were separated into six layers (0–5, 5–20, 20–40, 40–60, 60–80, and 80–100 cm), air-dried, weighed, and sieved. The chemical and physical analyses were performed on air-dried, 2-mm fractions taken from each layers. The soil characteristics are listed in Table 7.1.4.2-2.

Table 7.1.4.2-2: Principal chemical and physical properties of study soil; organic matter and carbonates in percentage of the weight of the 2-mm sieved soil; soil electric conductivity (EC) and cation-echange capacity (CEC) are reported

Layer	Particle-size distribution (USDA)						Bulk density	Organic matter	Total carbonates	EC	CEC
	Gravel	Fine earth									
		Coarse sand	Fine sand	Coarse silt	Fine silt	Clay					
	%						kg m^{-3}	%		dS m^{-1}	cmol kg^{-1}
0–5 cm	18.2	7.7	8.6	14.7	28.5	40.5	1332	0.81 ± 0.21	14.8 ± 0.2	0.21	23.6 ± 0.5
5–20 cm	19.6	13.1	14.4	13.3	16.2	43.8	1347	0.64 ± 0.22	14.7 ± 0.2	0.2	22.6 ± 0.5
20–40 cm	20.7	15.0	15.2	13.7	14.8	40.3	1410	0.43 ± 0.17	14.7 ± 0.2	0.2	22.1 ± 0.5
40–60 cm	19.9	20.0	18.3	14.5	14.0	33.2	1421	0.37 ± 0.19	14.8 ± 0.2	0.21	20.5 ± 0.5
60–80 cm	22.3	25.0	19.7	14.2	13.4	27.7	1469	0.35 ± 0.07	14.9 ± 0.2	0.2	20.6 ± 0.5
80–100 cm	21.6	26.1	19.5	14.4	13.1	26.9	1488	0.33 ± 0.08	15.1 ± 0.2	0.2	20.2 ± 0.5

Water samples were filtered through 1-mm glass-fiber filters. The liquid was immediately derivatized. The herbicide residues in the sediment, along with the residues in the soil samples, were extracted first by ultrasonic extraction in methanol after which the derivatization procedure was used. To reduce the sorption of glyphosate and AMPA from the methanol-extracted solutions onto glassware surfaces, water and soil samples were dispensed in parallel into plastic vials. Methanol (50 mL) was added to soil samples (50 mg) that had been dried and sieved. The soil suspension was mixed for 60 min and then left at 20°C for 24 h to allow complete solvent evaporation. Then, 15 g of soil was added to 40 mL of solvent and sonicated at 30 to 40 kHz for 30 min. Extracts were filtered through Whatman 40 filter paper, and the filtrate was evaporated on a rotary vacuum evaporator at 40°C to dryness. The residue of herbicide extract was dissolved in 5.0 mL of water and then collected in plastic vials for the derivatization procedure. Sediment extraction was performed as depicted for soil samples.

Following Le Bot *et al.* (2002), 3-mL samples were derivatized by adding 0.5 mL borate buffer and, after mixing, 500 µL FMOC-Cl solution. Then, samples were shaken for 1 h and incubated, allowing the reaction to take place for 15 h at room temperature (20°C). Derivatization was performed in the dark. The reaction was stopped by adding formic acid at about pH 3.0. The samples were washed with 2 mL diethyl ether to eliminate excess derivatization reagent.

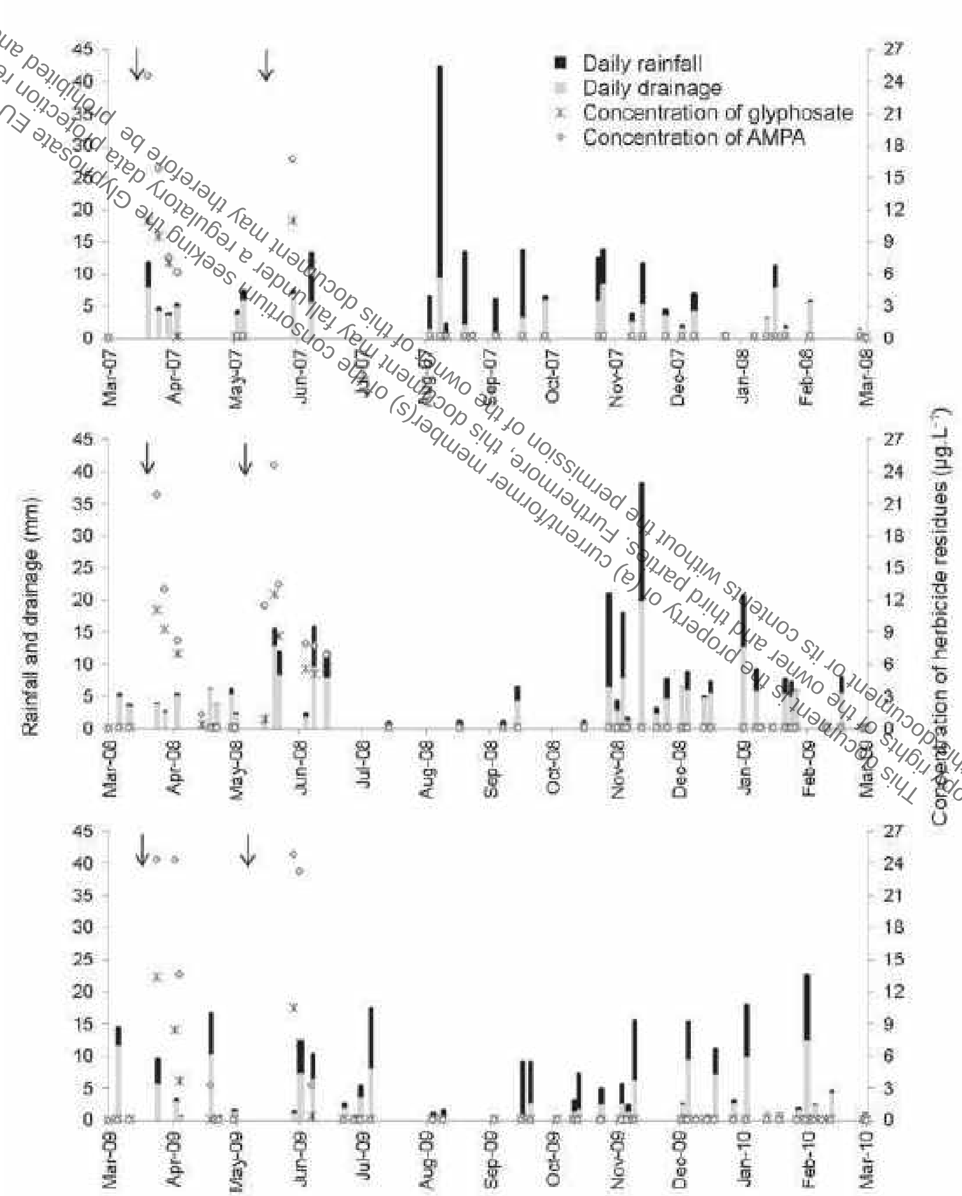
The solid-phase extraction (SPE) was performed by means of a Dionex AutoTrace 280 SPE autosampler (Thermo Scientific). Glyphosate and AMPA were analyzed by liquid chromatography–electrospray ionization–tandem mass spectrometry (TSQ Vantage triple quadrupole mass spectrometer, Thermo Scientific), which comprise an analytical column (Syncronis C8, 2.1 by 150 mm, 5 mm, Thermo Scientific) and a column guard (Syncronis C8, 2.1 by 10 mm, 5 mm, Thermo Scientific). Each standard and sample (3 mL) were injected onto the analytical column and then eluted in gradient mode using a binary solvent

mix comprising 99 % 5 mM ammonium acetate and 1 % acetonitrile (mobile phase A) and 99 % acetonitrile and 1 % 5 mM ammonium acetate (mobile phase B). The mobile phase flow rate was 0.3 mL/min. Analyses were performed in negative ionization mode with a spray voltage of 3.5 kV. The source temperature and the ion transfer tube temperature were 325°C and 250°C, respectively. The minimum detectable level (MDL) was 0.1 µg/L for glyphosate and AMPA in leachates and 10 µg/kg in soil.

Results

The daily rainfall and the glyphosate and AMPA concentrations in the leachates are presented in Figure 7.1.4.2-1.

Figure 7.1.4.2-1: Daily rainfall and drainage and concentrations of glyphosate and AMPA measured in the leachates of the vineyard soil from March 2007 to March 2010. Herbicide dates of application are indicated with arrows



The cumulative rainfall amounts for the period from 1 March to 28 February of the subsequent year were 524, 751, and 1429 mm during the first, second, and third year of the experiment, respectively. During the monitoring period, glyphosate was detected in 3 % of the soil leachates at concentrations ranging from 0.2 to 1 µg/L and in 16 % of the leachates at concentrations ranging from 1 to 13.47 µg/L. Glyphosate appeared at high concentrations (12.1 ± 1.3 µg/L) in the soil leachates 9.3 ± 4 d after each treatment.

Glyphosate was detected in the leachates for 25.8 ± 8.3 d after treatments at concentrations exceeding 0.5 µg/L. During the latter, average drainage of 15.5 ± 2.9 mm was measured, corresponding to 22.9 ± 6.7 mm of measured rainfall. Thereafter, the glyphosate concentration in leachates decreased to 0.1 µg/L. At the end of each trial year, the final glyphosate peaks appeared in the leachates between late January and early February (about 318.9 ± 8 d after the first annual treatment) at an average concentration of 0.3 µg/L.

Similar to the results for glyphosate, AMPA first appeared at an average concentration of 21.3 ± 6.2 µg/L in the soil leachate approximately 6.8 ± 1.2 d after each treatment. Aminomethylphosphonic acid was detected more frequently than glyphosate; it was detected in 13 % of the leachates from soil at concentrations ranging from 0.2 to 1 µg/L and in 20 % of the leachates from soil at concentrations ranging from 1 to 24.9 µg/L.

The amounts of water drained from the soil for the period from 1 March to 28 February of the following year, were 113.8, 187.4, and 130.5 mm, respectively, during the first, the second and the third year of the experiment. Approximately 0.19, 0.31, and 0.12 % of the amount of glyphosate distributed in the first, second, and third year of the experiment, respectively, were recovered in the leachates as glyphosate, whereas 0.49, 0.78, and 0.48 %, respectively, were recovered as AMPA.

On the basis of the analysis, the number of days from the treatment (DN) showed the highest negative correlation with the glyphosate and AMPA concentrations in leachate ($p \leq 0.001$). In contrast, the daily mean temperature (T_{med}) and the daily rainfall (R) showed a positive role in determining the herbicide concentration ($p \leq 0.05$). Since these variables were not autocorrelated, they were selected as independent variables X_1, X_2, and X_3, respectively, for the multiregressive model (Eq. [1]). The multiregression analysis led to the set up of Eq. [2] and [3] for the estimation of glyphosate and AMPA concentration in leachate, respectively:

$$Y_{\text{glyphosate}} = 0.0508T_{\text{med}} - 0.3445\text{DN} - 0.0179R + 13.2308 \quad [2]$$

$$Y_{\text{AMPA}} = 0.1937T_{\text{med}} - 0.6727\text{DN} - 0.1412R + 25.2585 \quad [3]$$

The glyphosate and AMPA concentrations were computed using data measured during the second and the third year of the experiment.

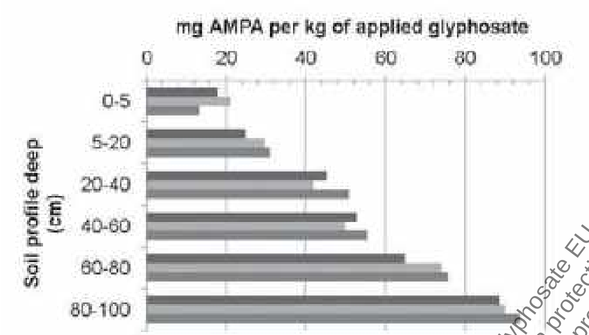
At least for the current study, climatic conditions and for this soil type, Eq. [4] and [5] can be used to determine the number of days free of rain (NR) necessary to ensure a safe threshold for distributing the herbicide.

$$\text{NR}_{\text{glyphosate}} = -2.9028Y_{\text{glyphosate}} + 0.1475T_{\text{med}} - 0.0519R + 38.4058 \quad [4]$$

$$\text{NR}_{\text{AMPA}} = -1.4866Y_{\text{AMPA}} + 0.2879T_{\text{med}} - 0.2099R + 37.5479 \quad [5]$$

No extractable glyphosate was detected in the soil profile. Aminomethylphosphonic acid was found as deep as 100 cm in the soil column. The concentration of AMPA increased with increasing depth, thus indicating a gradual accumulation of AMPA in the lower profile during the 3-yr experimental period. On the contrary, AMPA was distributed throughout the soil columns as shown in Figure 7.1.4.2-2.

Figure 7.1.4.2-2: Distribution profile of aminomethylphosphonic acid (AMPA) in the soil 1 year after the application of glyphosate for the first (dark gray), the second (light gray), and the third (medium gray) year of experiment



The AMPA content in the surface soil layer ranged between 0.0013 and 0.0021 %, based on the amount of glyphosate applied. The AMPA content in the lowest layer ranged between 0.0089 and 0.0094 %, based on the amount of glyphosate applied. During the 3 yr, a continuous increase in the concentration of AMPA in the lower layers of the profile was measured; however, there are no statistical data to attribute this to an accumulation effect, but rather to different weather conditions. Finally, at the end of each year of experimentation, the total amount of AMPA recovered in the soil profiles was about 0.03 %, based on the amount of glyphosate applied.

The amounts of glyphosate and AMPA, in terms of applied glyphosate, measured in the leachates and in the soil profiles were summed on a yearly basis (Table 7.1.4.2-3).

Table 7.1.4.2-3: Mass balance of glyphosate and aminomethylphosphonic acid (AMPA) in leachates and soil profles (in percentage based on the amount of glyphosate applied) for the three experimentation years

Year	Herbicide leachates		Herbicide in soil		Total residue
	Glyphosate	AMPA	Glyphosate	AMPA	
	%				
First year	0.19	0.49	0	0.03	0.70
Second year	0.31	0.78	0	0.03	1.11
Third year	0.12	0.48	0	0.03	0.63

Conclusion
After a 3-yr experimental period under outdoor conditions, the present work has demonstrated that both glyphosate and AMPA may be transported in leachates through 100 cm of soil profile, thus confirming the high mobility of this herbicide. The mean annual percentage of glyphosate and AMPA, as a percentage of applied glyphosate, recovered in leachates were about 0.2 and 0.58 %, respectively. Moreover, results suggested that preferential, flow along with rains that occurred within 2 wk after the treatment, can cause the leaching of glyphosate and AMPA in high concentration. At least in this environment and for this soil, a multiregressive equation was found to determine the number of days free of rain necessary to ensure a safe herbicide distribution. Soil analyses indicated that glyphosate was below detection in 1 yr. On the contrary, the total amount of AMPA, based on the amount of glyphosate applied, recovered in the soil profiles was around 0.03 % at the end of each year of experimentation. Overall, these results suggest that

when applied to shallow soils, herbicides can pose a risk of groundwater contamination, and, when applied to pipe-drained crops, contaminated leachate can be transported by the pipe drain to surface waters.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a lysimeter study with glyphosate using three lysimeters from the Chianti region in Italy. The study is well described, however, there is some information missing to check the validity of the study against current guidelines. The use of clay soil (low- to non-permeable soil) is considered not appropriate for lysimeter experiments, and from the information given in the article it cannot be excluded that the leaching may have been caused by preferential flow rather than percolation through the soil column. Further, there is no information available whether, apart from application, lysimeters were handled according to normal agricultural practice. From the information reported it is not possible to calculate annual mean concentrations in leachate for glyphosate and AMPA which would however be required in view of risk assessment.
The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.2/002
Report author	Al-Rajab, A., Hakami, O.M.
Report year	2014
Report title	Behavior of the non-selective herbicide glyphosate in agricultural soil
Document No	DOI 10.3844/ajessp.2014.94.101 E-ISSN 1558-3910
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (some deviations from study guidelines, not all necessary data reported to derive comprehensive DT_{50} values, preferential flow in the soil column)

The article was found relevant for multiple data points. The summary is provided under CA 7.1.2.1.1/015.

1. Information on the study

Data point:	CA 7.1.4.2/003
Report author	Bergström, L. *et al.*
Report year	2011
Report title	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil
Document No	DOI 10.2134/jeq2010.0179 E-ISSN 1537-2537
Guidelines followed in study	OECD 106 Guideline
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (Not all validity criteria of the studies were met)

The article was found relevant for multiple data points. The summary is provided under CA 7.1.2.1.1/017.

CA 7.1.4.3 Field leaching studies

A field leaching study is not required and none was conducted.

During the scientific literature research for glyphosate (2010-2019), five articles were identified to provide further information relevant to the data point. The reliability of the articles was assessed as "reliable with restrictions". Thus, no new endpoints were derived, and the articles are considered as supportive information.

The article of Ulen *et al.* (2014, CA 7.1.4.3/001) investigated the spatial variation of leaching from a Swedish experimental tile-drained field with marine clay soil during two winter periods. It was shown that 0.08 % (dissolved form) and 0.16 % (particulate form) of the glyphosate applied was leached in two consecutive winters with high spatial variations. Levels of glyphosate and AMPA in drain water were on all occasions below their no-effect concentrations. Ulen *et al.* (2012, CA 7.1.4.3/002) focused on the particulate-bound and dissolved leaching of glyphosate via tile drains in a field experiment in Sweden. It was shown that structure liming of the topsoil could reduce total glyphosate leaching losses compared with unlimed soils, while shallow tillage may not be a suitable way to mitigate particulate-facilitated transport of glyphosate and phosphorous via tile drains from this type of clay soil. In an article of Aronsson *et al.* (2011, CA 7.1.4.3/003), the leaching of glyphosate, nitrogen and phosphorous was investigated in two different experimental tile-drained soil plots planted with ryegrass as a catch crop in Sweden. It was shown, that the soil type had a higher impact on the leaching of glyphosate than the experimental treatments of undersowing ryegrass. Glyphosate was not leached from the sand at all, while it was found at average concentrations of 0.25 µg/L in drainage water from the clay soil. An article of Kjaer *et al.* (2011, CA 7.1.4.3/004) investigated transport modes of glyphosate through structured tile-drained soils in an 8-month field experiment in Denmark. Glyphosate was found in drainage water at an average concentration of 3.5 µg/L. The particle-facilitated transport accounted for only 13 to 16 % of the observed leaching. Glyphosate entered the drainage system through drain–connected macropores (above or in the vicinity of the drains) as well as the macropores situated between the drains and connected to underlying fractures. Candela *et al.* (2010, CA 7.1.4.3/005) studied the transport of glyphosate, AMPA and bromide through weathered granite soils under irrigated and non-irrigated conditions in the Mediterranean Maresme area of Spain, north of Barcelona by analysis of soil concentrations in different depths. 69 days after application, residues of glyphosate up to 73.6 µg/g soil were detected to a soil depth of 0.5 m under irrigated conditions, AMPA, analyzed only in the irrigated plot, was detected to a depth of 0.5 m. This was attributed to the low

content of organic matter and clay along with irrigation and heavy rain, possibly leading to preferential solute or colloidal mediated transport. Glyphosate was not detected in groundwater samples along all the monitoring periods.

Table 7.1.4.3-1: Field leaching experiments – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.1.4.3/001	Ulen *et al.*, 2014	Field leaching	Glyphosate	Reliable with restrictions	
CA 7.1.4.3/002	Ulen *et al.*, 2012	Field leaching	Glyphosate	Reliable with restrictions	
CA 7.1.4.3/003	Aronsson *et al.*, 2011	Field leaching	Glyphosate	Reliable with restrictions	
CA 7.1.4.3/004	Kjaer *et al.*, 2011	Field leaching	Glyphosate	Reliable with restrictions	
CA 7.1.4.3/005	Candela *et al.*, 2010	Field leaching	Glyphosate	Reliable with restrictions	

1. Information on the study

Data point:	CA 7.1.4.3/001
Report author	Ulen, B.M. *et al.*
Report year	2014
Report title	Spatial variation in herbicide leaching from a marine clay soil via subsurface drains
Document No	DOI 10.1002/ps.3574 E-ISSN 1526-4998
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (essential parameters to derive endpoint missing)

2. Full summary

Subsurface transport via tile drains can significantly contribute to pesticide contamination of surface waters. The spatial variation in subsurface leaching of normally applied herbicides was examined together with phosphorus losses in 24 experimental plots with water sampled flow-proportionally. The study site was a flat, tile-drained area with 60 % marine clay in the topsoil in southeast Sweden. The objectives were to quantify the leaching of frequently used herbicides from a tile drained cracking clay soil and to evaluate the variation in leaching within the experimental area and relate this to topsoil management practices (tillage method and structure liming).

In summer 2009, 0.14, 0.22 and 1.62 %, respectively, of simultaneously applied amounts of MCPA, fluroxypyr and clopyralid were leached by heavy rain five days after spraying. In summer 2011, on average 0.70 % of applied bentazone was leached by short bursts of intensive rain 12 days after application. Peak flow concentrations for 50 % of the treated area for MCPA and 33 % for bentazone exceeded the Swedish no-effect guideline values for aquatic ecosystems. Approximately 0.08 % of the glyphosate applied was leached in dissolved form in the winters of 2008/2009 and 2010/2011. Based on measurements of glyphosate in particulate form, total glyphosate losses were twice as high (0.16 %) in the second winter.

The spatial inter-plot variation was large (72–115 %) for all five herbicides studied, despite small variations (25 %) in water discharge.

The study shows the importance of local scale soil transport properties for herbicide leaching in cracking clay soils.

Materials and Methods

The field site is located in a flat valley with a clay soil of marine origin in eastern Sweden. The experimental field was tile-drained in 2006 to 0.9 m depth. Twenty-four of these plots were used in the present experiment. The plots are situated in two rows of 14 plots at varying distance from an open ditch that acts as the recipient of drainage water from the surrounding valley. Three management practices were randomly assigned to the plots: Conventional autumn ploughing, shallow autumn tillage and structure-liming (i.e. liming carried out to reduce phosphorus leaching and to improve crop yield by improving soil structure). Soil pH and total organic carbon (OC) content are given in Table 7.1.4.3-2. There were no significant differences ($P > 0.05$) in soil pH and OC between treatments.

Table 7.1.4.3-2: Mean values and standard deviations (SD) of soil pH and concentrations (%) of organic carbon (OC) at the start of the project in the autumn of 2007 and five years later in the spring of 2012 after repeated different tillage treatments and after structure liming first year

Mean values and standard deviations (SD) of soil pH and concentrations (%) of organic carbon (OC) at the start of the project in the autumn of 2007 and five years later in the spring of 2012 after repeated different tillage treatments and after structure liming first year

			Shallow tillage		Structure-limed		Conventional ploughing	
Sampling time	Property	Depth (cm)	Mean	SD	Mean	SD	Mean	SD
2007	pH	0–2	6.4	0.1	6.3	0.1	6.4	0.1
2012	pH	0–2	6.1	0.2	6.5	0.5	6.1	0.1
2007	OC (%)	0–23	2.4	0.4	2.5	0.5	2.5	0.5
2007	OC (%)	23–60	1.4	0.3	1.5	0.4	1.5	0.3
2007	OC (%)	60–90	0.6	0.1	0.6	0.1	0.6	0.1
2012	OC (%)	0–2	2.7	0.2	2.7	0.3	2.6	0.3

Pesticide leaching

We studied the leaching of seven different pesticides with contrasting properties (Table 7.1.4.3-3). It should be noted that both pesticide half-lives and adsorption partitioning coefficients are dependent on soil properties and the values presented in Table 2 may, therefore, not be representative for the clay soil at this site. Pesticide leaching was studied in two different crop rotations (Table 7.1.4.3-4), both with oats and peas during the last two years (2010–2011). In crop rotation I (20 plots), conventional autumn ploughing was compared with shallow autumn tillage and the effects of previous structure-liming in autumn 2007 were examined. Glyphosate was applied before sowing in spring 2008 to control couchgrass in eight shallow-tilled plots in crop rotation I (Table 7.1.4.3-5). In early summer the same year, the low-dose substances thifensulfuron-methyl and tribenuron-methyl were applied in both rotations. In autumn 2008 glyphosate was applied after harvest (four plots) in crop rotation II in order to control couchgrass and volunteer cereals. The three pesticides clopyralid, fluroxypyr and MCPA (all ingredients in the same commercial product) were sprayed for weed control on 9 June 2009 in crop rotation I (20 plots) and on 23 June 2010 in both rotations (24 plots). Glyphosate was applied after harvest in September 2010 and bentazone was applied on 11 June 2011 in both rotations (24 plots). Most applications were made in the evening, with no wind and always in the recommended dose. The total loads of herbicides applied (Table 7.1.4.3-5) were similar to those reported from agricultural catchments within the Swedish National Pesticide Monitoring Programme. Precipitation was measured at the site with unheated tilting bucket equipment and collected in a data logger.

Table 7.1.4.3-3: Herbicide properties and potential data taken from the Pesticide Properties Database (PPDB, 2010) Substance

Herbicide properties and leaching potential data taken from the Pesticide Properties Database (PPDB, 2010)[11] Substance

	DT_{50}lab[a](days)	DT_{50}field[b](days)	K_{oc}[c] ($cm^3 g^{-1}$)	GUS[d]	
Bentazone	13	14	55.3	2.30	3.28
MCPA	24	25	74[f]	2.94	3.73
Fluroxypyr	1	51	195[f]	0	2.94
Clopyralid	34	11	5.0	5.98	2.01
Glyphosate	12	12	1435	2.19	2.34
Thifensulfuron-methyl	4	4	28.3	1.81	4.4
Tribenuron-methyl	14	14	35	4.88	4.7

[a] Degradation half-life for aerobic conditions measured in the laboratory.
[b] Degradation half-life for aerobic conditions measured in the field.
[c] Adsorption distribution coefficient to organic carbon.
[d] Groundwater ubiquity score.
[e] Acid dissociation constant.
[f] Freundlich adsorption coefficient to organic carbon.

Table 7.1.4.3-4: Year, crop, date and commercial brand name of herbicides applied in 2008-2011 in crop rotations I and II (number of conventionally ploughed plots/total number of treated plots)

Year, crop, date and commercial brand name of herbicides applied in 2008-2011 in crop rotations I and II (number of conventionally ploughed plots/total number of treated plots)

Year	Rotation I Crop	Date	Herbicide (16/20 plots)	Rotation II Crop	Date	Herbicide (4/4 plots)
2008	Spring barley	24/4	Glypro Bio[a, b]	Winter wheat	26/6	Harmony 50T Plus[c]
	Spring barley	26/6	Harmony 50T Plus[c]	After W wheat	16/8	Glypro Bio[b]
2009	Spring barley	9/6	Ariane S[d]	Winter wheat	6/5	Harmony 50T Plus[c]
2010	Oats	23/6	Ariane S[d]	Oats	23/6	Ariane S[d]
	Oats	22/9	Glypro Bio[b]	After oats	22/9	Glypro Bio[b]
2011	Pea	11/6	Basagran[e]	Pea	11/6	Basagran[e]

[a] Only in eight shallow-tilled plots.
[b] Active ingredient glyphosate (49%).
[c] Active ingredients thifensulfuron-methyl (25%) and tribenuron-methyl (17%).
[d] Active ingredients MCPA (20%), fluroxypyr (4%) and clopyralid (2%).
[e] Active ingredient bentazone (87%).

Table 7.1.4.3-5: Year, date of application, substance anylsed in drainage water, crop and applied dose of detected substance, together with the general dose (in g/ha) applied in Swedish monitored small catchments in 2008-2011

Year, date of application, substance analysed in drainage water, crop and applied dose of detected substance, together with the general dose (in g ha^{-1}) applied in Swedish monitored small catchments in 2008–2011

Year	Date	Substance	Crop	Dose (g ha^{-1})	General dose (g ha^{-1})
2008	24/4	Glyphosate	Before barley	707	748
2008	26/4	Thifensulfuron-methyl	Spring barley	4	4
		Tribenuron-methyl	Spring barley	2	3
2008	16/8	Glyphosate	After winter wheat	1060	1116
2009	26/5	Thifensulfuron-methyl	Winter wheat	6	6
		Tribenuron-methyl	Winter wheat	3	3
2009	9/6	Clopyralid	Spring barley	52	48
		Fluroxypyr	Spring barley	104	81
		MCPA	Spring barley	520	590
2010	23/6	Fluroxypyr	Oats	104	75
		MCPA	Oats	520	510
		Glyphosate	After oats	1060	1110
2011	11/6	Bentazone	Pea	435	500

Water sampling and analysis

Water discharge from each plot was measured with tilting vessels in an underground basement where sampling of drainage water also took place. The water was sampled flow-proportionally, with every subsample representing 0.003 mm discharge in summer and 0.04 mm discharge in the rest of the year. The bulk samples were collected weekly (or for the first flow events following application more frequently). The concentration of thifensulfuron-methyl and tribenuronmethyl (in 2008) was determined with solid-phase extraction followed by liquid chromatography and mass spectrometry (LCMS) and the concentration of clopyralid, fluroxypyr and MCPA (in 2009) by the same solid-phase extraction and by derivatisation and gas chromatography/mass spectrometry (GC/MS). Fluroxypyr and MCPA (in 2010) and bentazone (in 2011) were analysed by mass spectrometric determination (LC-MS/MS). Dissolved glyphosate (DissGly) and its main metabolite AMPA were analysed in winter 2008/2009 and 2010/2011, which involved ion exchange and derivatisation, followed by final identification and quantification by GC/MS. In winter 2010/2011, glyphosate analysis included particulate glyphosate (PartGly), which was trapped using a cellulose acetate filter with pore size 0.45 µm.

Table 7.1.4.3-6: Monthly precipitation (Prec) and total snow accumulation (Snow acc) in winter periods (October-April current year and January-April following year), water discharge (Flow) and ratio Flow/Prec dfor the experimental years and long-term (1988-2011) average

Monthly precipitation (Prec) and total snow accumulation (Snow acc) in winter periods (October–April current year and January–April following year), water discharge (Flow) and ratio Flow/Prec for the experimental years and long-term (1988–2011) average

Year		May	June	July	August	September	October–April	Snow acc (cm)
2008	Prec (mm)	30	5	44	42	8	385	14
	Flow (mm)	10	1	0	4	5	395	
	Flow/Prec	0.33	0.20	0	0.03	0.63	0.99	
2009	Prec (mm)	45	95[a]	94	54	35	384	50
	Flow /mm)	3	43	3	1	0	370	
	Flow/Prec	0.07	0.45	0.03	0.02	0	0.85	
2010	Prec (mm)	53	39	155[b]	95	51	430	75
	Flow (mm)	16	3	8	87	42	370	
	Flow/Prec	0.30	0.08	0.05	0.92	0.82	0.85	
2011	Prec (mm)	40	70	26	138	72	358	3
	Flow (mm)	3	14	0	0	4	350	
	Flow/Prec	0.08	0.20	0	0	0.06	0.96	
1988–2011	Prec (mm)	40	67	82	69	61	338	32
	Flow (mm)	7	12	2	18	9	360	
	Flow/Prec	0.15	0.18	0.02	0.21	0.16	0.97	

[a] Maximum intensity 46 mm day^{-1} in the middle of the month.
[b] Maximum intensity 79 mm day^{-1} at the end of the month.

Results and Discussion

Concentrations of pesticides in drain water

The sulphonylureas (thifensulfuron-methyl and tribenuronmethyl) were not detected above LOD in 2008. Because of the fast dissipation of these substances, they were not analysed for in subsequent years. Unlike these low-dose substances, detectable levels of all other herbicides were found every year in drain flow in the first 1–2 months after early summer application. Detectable concentrations of fluroxypyr and MCPA were also observed 31 days after application (Table 7.1.4.3-7) in the samples taken after flooding of the measuring station. Detection of pesticides in the first few rainfall/drainage events after application is consistent with the flow was the simultaneous arrival of clopyralid and fluroxypyr on 14–16 June 2009, despite large differences in K_{oc} values (Table 7.1.4.3-3). However, since only five days had passed between application and rainfall, the substances might not have been in equilibrium with the soil solid material due to slow kinetics. Dissolved glyphosate was detected in consecutive events in autumn 2008. Both particle-bound glyphosate and dissolved glyphosate were detected in the discharge from all fast-flow events in autumn 2010. Levels above the $C_{no\ effect}$ concentrations were observed in 50 % of the plots for MCPA and in 33 % for bentazone (Table 7.1.4.3-7). Levels of glyphosate, AMPA, clopyralid and fluroxypyr were on all occasions below their $C_{no\ effect}$ concentrations. The coefficient of variation in the most important leaching event for the substances studied varied between 72 and 115 % between all different plots (including different treatments) and increased in the order bentazone < clopyralid < fluroxypyr < PartGly < MCPA < DissGly. These highly variable pesticide concentrations were not significantly correlated to the basic soil factors pH value, clay content and organic matter content in the topsoil, which only showed minor variance (2, 17 and 10 %, respectively).

Table 7.1.4.3-7: Year, date of application of substance (including glyphosate metabolite AMPA) and glyphosate in dissolved (diss) form and total glyphosate, numbers of plots (Plots), number of days (No. days) until major rain event, Swedish guideline values for no effect ($C_{no\ effect}$), maximum (Max) and mean concentration in the main drainage event, ratio of number of plots with concentration exceeding $C_{no\ effect}$ to total number of plots treated (Ratio $C_{no\ effect}$) and total period (days) after application when values exceeding were $C_{no\ effect}$ detected

Year, date of application of substance (including glyphosate metabolite AMPA) and glyphosate in dissolved (diss) form and total glyphosate, numbers of plots (Plots), number of days (No. days) until major rain event, Swedish guideline values for no effect ($C_{no\ effect}$), maximum (Max) and mean concentration in the main drainage event, ratio of number of plots with concentration exceeding $C_{no\ effect}$ to total number of plots treated (Ratio $C_{no\ effect}$) and total period (days) after application when values exceeding $C_{no\ effect}$ were detected

Year	Date	Substance	No. days	$C_{no\ effect}$ (µg L^{-1})	Maximum (µg L^{-1})	Mean (µg L^{-1})	Ratio $C_{no\ effect}$	Period (days)
2008	16/8	Glyphosate diss.	47	100	1.2	0.48	0/4	—
		AMPA	47	500	0.3	—	—	—
2009	9/6	Clopyralid	5	50	5.5	3.3	0/20	—
		Fluroxypyr	5	100	1.7	0.63	0/20	—
		MCPA	5	1	5.5	3.0	10/20	5–14
2010	23/6	Fluroxypyr[a]	31	100	0.3	0.081	0/24	—
		MCPA[b]	31	1	0.04	0.007	0/24	—
2010	22/9	Glyphosate diss.	33	100	3.9	0.58	0/24	—
		Glyphosate total	33	100	9.4	2.2	0/24	—
		AMPA	33	500	0.7	—	—	—
2011	11/6	Bentazone	12	30	63	23.9	8/24	12–16

[a] Generally only analysed in dissolved form.
[b] Late collection of sample, as the measuring station was flooded.

Leaching losses of pesticides

The amount of pesticide leached in summer periods from conventionally ploughed plots sprayed simultaneously with the same herbicide in 2009–2011 varied between 0.2 and 3.3 g/ha (0.1–1.6 % of amount applied) (Table 7.1.4.3-8). Leaching losses above 1 % are generally associated with large rainfall amounts shortly after application. However, for our case the hydrological conditions did not represent 'worst-case' leaching conditions and hence the large leaching losses demonstrate the great potential for preferential transport in this soil. Losses exceeding 0.1 % took place from 22 to 24 plots (92–100 % of the experimental area) for clopyralid and bentazone, while the relative losses of MCPA exceeding 0.1 % represented 42 % of the area. The relative leaching losses of the substances studied here are presented in Table 7. Surprisingly, autumn application of glyphosate in 2008 and 2010 resulted in quite similar losses in dissolved form in the following winters (0.9 g/ha corresponding to 0.08 % of applied amounts; Table 7.1.4.3-8), irrespective of whether the main discharge took place after autumn rain followed by a mild winter (2008) or in connection with snowmelt after a cold winter with continuous snow cover (2011). Due to slow degradation during the winter of 2010/2011 owing to long-lasting snow cover, glyphosate was available for leaching during the main snowmelt event, which was fast and probably resulted in preferential transport.

Table 7.1.4.3-8: Year, date, applied substance, including the sum of the three components in the commercial product Ariane S, mean loss from all ploughed plots with standard deviation (SD), mean losses relative to applied amount, range of the relative losses and area with relative losses exceeding 0.1 g/ha. Glyphosate was analysed in both dissolved (diss.) and particulate (part.) form in 2010

Year, date, applied substance, including the sum of the three components in the commercial product Ariane S, mean losses from all ploughed plots with standard deviation (SD), mean losses relative to applied amount, range of the relative losses and area with relative losses exceeding 0.1 g ha $^{-1}$. Glyphosate was analysed in both dissolved (diss.) and particulate (part.) form in 2010

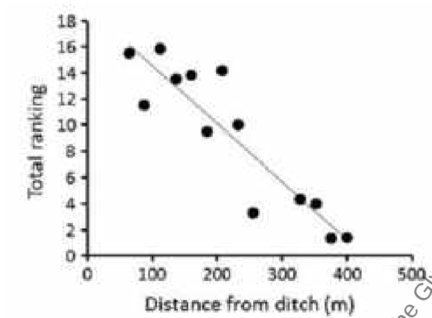
Year	Date	Substance	Mean (g ha^{-1})	SD	Relative losses (%)	Range of relative losses (%)	Area (%)
2008	16/8	Glyphosate diss.	0.89	0.64	0.084	0.02–0.17	25
2009	9/6	Clopyralid	0.84	0.70	1.62[c]	0.09–1.5	92
		Fluroxypyr	0.24	0.36	0.22	0.02–0.5	60
		MCPA	0.71	0.89	0.14	0.004–0.4	42
		Sum	1.81	3.03	0.34	0.02–0.96	60
2010	23/6	Fluroxypyr[a]	> 0.03	0.02	> 0.03		—
2010	22/9	Glyphosate diss.[b]	0.90	0.32	0.085	0.05–0.12	—
		Glyphosate part.	0.82	0.25	0.064	0.08–0.10	—
		Glyphosate total	1.72	1.47	0.15	0.12–0.23	100
2011	11/6	Bentazone	3.31	2.92	0.70[b]	0.42–2.16	100

[a] From late sample after flooding of the measuring station.
[b] Period 22 September–15 April and calculated from the same four plots as treated in 2008.
[c] Relative losses of clopyralid were significantly greater ($P < 0.01$) than losses of fluroxypyr applied simultaneously.

Herbicide correlation with particulate phosphorus and plot position

In spite of the small variation in the amounts of water discharge between plots, there was a large variation in herbicide losses for all substances resulting from the highly varying concentrations in drainage water. Similar relationships have previously been reported between total glyphosate and PP for the same field. Due to their strong sorption, both glyphosate and PP are considered to leach mainly through preferential transport in macropores. Our results suggest that preferential transport dominates leaching also for the weakly sorbed substances at this site. In addition, since the pesticides which were applied at the soil surface were leaching with a similar pattern as PP, this suggests that the topsoil was the major source of leached PP. We did not observe any surface runoff in the direction of the Recipient ditch during the experimental period. Lateral flows below the soil surface and e.g. on a plough pan were also unlikely to occur, since there was no distinct plough pan at the site. There was no correlation between the topsoil (0–23cm) pH and the plot position. However, topsoil OC clearly increased with decreasing distance between plot mid-point and the recipient ditch ($R^2 = 0.70$ %, $P < 0.001$) and pH in the deeper subsoil (60–90 cm) decreased ($R^2 = 78$ %, $P < 0.001$). The concentration of all pesticides tended to increase with decreasing distance between plot position and the recipient ditch. The relationship was significant for bentazone, and was also significant from a total ranking of all pesticides detected (Figure 7.1.4.3-1).

Figure 7.1.4.3-1 Total ranking of mean concentration of clopyralid, fluroxypyr, MCPA, bentazone, dissolved glyphosate and particulate glyphosate related to the distance between the ditch and the centre of the respective plot. The estimates were made for the observed concentrations in the major event for every substance. The slope of the regression line is significantly different from zero (P < 0.001).



Effects of soil management, soil structure, pH and organic matter

There was a general tendency for larger losses of all substances from shallow-tilled plots than from ploughed plots, with or without previous structure liming (Table 7.1.4.3-9). The apparent differences, which were not significant for any single substance, increased in the order clopyralid < MCPA < bentazone < fluroxypyr < total glyphosate. However, estimated for all five substances lumped together (paired *t*-test), the difference between shallow-tilled and ploughed structure-limed plots was significant ($P < 0.05$), both before and after adjustment to the effect of plot position in relation to the ditch. From soils where preferential flow and transport are important, ploughing is generally considered to reduce pesticide leaching by interrupting continuous macropores. For our case the larger losses from the shallow-tilled plots may also have been an effect of shallow and uneven accumulation of crop residues in these plots which resulted in uneven infiltration and preferential herbicide transport along straw residues. At the study site, it has been demonstrated that structure liming (quicklime) significantly improves soil aggregate stability measured as a decrease in readily dispersed clay. Improved aggregate stability should influence the transport of glyphosate which adsorbs strongly to clay particles. However, the improved aggregate stability did not result in any significantly smaller losses of glyphosate from structure limed plots compared to conventionally tilled plots.

Table 7.1.4.3-9: Year of application, mean and standard deviation (SD) of transported masses of the applied substances in g/ha) from tilled, structure-limed (+ ploughed) and conventionally ploughed plots

Year of application, mean and standard deviation (SD) of transported masses of the applied substances (in g ha^{-1}) from tilled, structure-limed (+ ploughed) and conventionally ploughed plots

		Shallow tillage Mean			Structure-limed Mean			Conventional ploughing Mean		
Year	Substance	Un-adjusted	Adjusted	SD	Un-adjusted	Adjusted	SD	Un-adjusted	Adjusted	SD
2009	Clopyralid	1.16	1.14	0.77	1.07	1.13	0.95	0.73	0.71	0.59
2009	Fluroxypyr	0.36	0.35	0.29	0.25	0.26	0.28	0.22	0.21	0.25
2009	MCPA	1.11	1.09	1.03	0.86	0.88	1.25	0.63	0.61	0.82
2011	Bentazone	4.78	4.73	2.84	3.52	3.75	3.81	3.40	3.32	2.53
2010	Glyphosate[a]	3.81	3.77	2.58	0.85	0.90	1.13	1.59	1.56	1.56

Note: Mean transported losses are given both as unadjusted values and values adjusted for the distance to the ditch.
[a] Total glyphosate in both particulate and dissolved form in the period 22 September 2010–15 April 2011.

For ionisable pesticides, leaching is also affected by soil pH, with weaker sorption at higher pH. Based on the p*K*a values of the substances studied here and the small differences in pH between treatments (Table

7.1.4.3-9), any pH effects on leaching were probably minor. The topsoil OC content is often higher under long term shallow tillage than under conventional tillage, which has consequences for pesticide sorption and degradation. However, in our case the OC content was not significantly different between treatments and there were no significant differences in subsoil OC between plots with different management regimes. The coefficient of variation in relative leaching losses between all substances for the shallow-tilled plots varied between 40 and 92 %. The coefficient of variation in the relative leaching losses from all plots and for all substances combined (92–156 %) varied even more. In conclusion, the variation in relative leaching losses between plots within the same treatment was larger than that between different substances. This finding also demonstrates that the differences in transport pathways through the soil between plots have a larger effect on pesticide concentrations than the differences in pesticide properties.

Conclusions

Concentrations of the herbicides bentazone, clopyralid, fluroxypyr, MCPA and glyphosate were measured in subsurface drain discharge from a clay field during a four-year study. Despite hydrological conditions not representing a worst case scenario for leaching, the relative leaching losses of all herbicides studied were large compared to values reported in the literature. Measured concentrations of bentazone and MCPA exceeded Swedish guideline values based on predicted no effect on aquatic ecosystems for 50 and 33 % of the plots for MCPA and bentazone, respectively. All substances studied (except sulphonyl ureas which were not detected), irrespective of sorption strength, showed similar leaching patterns. These observations clearly demonstrate that preferential transport in macropores is the dominant transport process at this site. The variation in relative leaching losses between plots within the same treatment was greater than that between different substances. Crack stabilisation by gyttja, especially in the deeper subsoil, was suggested as an important explanatory factor for this large spatial variation in pesticide leaching, although it was not possible to investigate differences in gyttja content between plots. Continuous macropores connecting the soil surface to the subsoil may be a factor contributing to the generally large pesticide losses observed after shallow tillage. However, careful studies of soil macropore systems, including topsoil and subsoil properties, are needed to explain the unpredictability in leaching at this site.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes a leaching experiment from a tile-drained Swedish marine clay soil with agricultural land use. Glyphosate among other herbicides was considered in analysis. Preferential transport in macropores was the dominant process for all investigated substances at the test site. Glyphosate losses in total were up to 0.23 %. The study provides supportive information but not all parameters to derive endpoints are reported.

The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.3/002
Report author	Ulen, B.M. *et al.*
Report year	2012
Report title	Particulate-facilitated leaching of glyphosate and phosphorus from a marine clay soil via tile drains
Document No	DOI 10.1080/09064710.2012.697572 E-ISSN 1651-1913
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (Not enough information provided to check validity)

2. Full summary

Losses of commonly used chemical pesticides from agricultural land may cause serious problems in recipient waters in a similar way to phosphorus (P). Due to analytical challenges concerning determination of glyphosate (Gly), transport behaviour of this widely used herbicide is still not well known. The objective of the present study was to quantify and evaluate leaching of Gly in parallel with P. Leaching losses of autumn-applied Gly (1.06 kg/ha) via drainage water were examined by flow-proportional sampling of discharge from 20 drained plots in a field experiment in eastern Sweden. Samples were analysed for Gly in particulate-bound (PGly) and dissolved (DGly) form. The first 10 mm water discharge contained no detectable Gly, but the following 70 mm had total Gly (TotGly) concentrations of up to 6 µg/L, with 62 % occurring as PGly. On average, 0.7 g TotGly/ha was leached from conventionally ploughed plots, compared with 1.7 g TotGly/ha from shallow-tilled plots (cultivator to 12 cm working depth). Higher Gly losses occurred in snowmelt periods in spring, but then with the majority (60 %) as DGly. All autumn concentrations of PGly in drainage water were significantly correlated ($p < 0.001$) to the concentrations of particulate-bound phosphorus (PP) lost from the different plots (Pearson correlation coefficient 0.84), while PP concentrations were in turn significantly correlated to water turbidity (Pearson correlation coefficient 0.81). Leaching losses of TotGly were significantly lower (by 1.3 g/ha; $p < 0.01$) from plots that had been structure-limed three years previously and ploughed thereafter than from shallow-tilled plots. Turbidity and PP concentration also tended to be lowest in discharge from structure-limed plots and highest from shallow-tilled plots. This difference in TotGly leaching between soil management regimes could not be explained by differences in measured pH in drainage water or amount of discharge. However, previously structure-limed plots had significantly better aggregate stability, measured as readily dispersed clay (RDC), than unlimited plots. The effects of building up good soil structure, with strong soil aggregates and an appropriate pore system in the topsoil, on mitigating Gly and P losses in particulate and dissolved form should be further investigated.

Materials and Methods

Experimental plots and soil characteristics

The experiment was done on 20 drained plots in an experimental field with a sub-surface drainage water collection system constructed on a flat plain close to the Lake Bornsjön reservoir. Drainage water flows to a sampling and measuring station and is recorded with tilting vessels and data logger. The data logger controls the flow proportional sampling by means of small tube pumps in the basement of the station. After a certain volume of water has passed, the suction tube is first cleaned by reverse pumping and thereafter a small volume is sampled. The flow-proportional (composite) sampling took place in dark glass vessels at relatively cold temperature and in darkness for a maximum of one week prior to freezing the water samples and transport to the laboratory before analysis.

Clay content (60 %), is high throughout the profile (Table 7.1.4.3-10), with small spatial variation in both topsoil and sub-soil (variance less than 0.5 %). pH and soil concentration of P are uniformly distributed in the experimental area (variance less than 15 %). In the soil profile, the pH (dry soil samples) varies between 5.2 and 6.9, with the lowest values occurring in the 70-100 cm layer, which includes the tile drains at approximately 90 cm depth. Under wet conditions the pH in the upper sub-soil is higher than that under dry conditions (6.9 compared with 6.6). Overall, the soil profile generally demonstrates a high ability to sorb P to the soil matrix.

The soil horizon has a strongly aggregated structure, especially in the deeper part, with approximately 10 cm wide and 10-20 cm prismatic aggregates in the layer 43-100 cm. Water retention is very high. In an adjoining field with an old drainage system, the deeper soil horizon is very wet, the aggregates similarly very prismatic and the structure is easily destroyed by digging.

Table 7.1.4.3-10: Selected physical and chemical properties of the soil at the study site.

Properties	Soil depth (cm) 0–10	10–30	30–50	50–70	70–100	Reference for the method
Particle size distribution						
<0.002 mm (clay) (%)	60	60	59	61	54	Eriksson et al. (1998)
0.002–0.02 mm (%)	31	30	30	31	34	Eriksson et al. (1998)
0.02–0.2 mm (%)	9	10	11	8	12	Eriksson et al. (1998)
Organic matter (%)	3.9	0.9	0.1	0.0	0.0	Eriksson et al. (1998)
pH_{H_2O}	6.9	6.2	6.6	6.5	5.2	ISO (2005)
P_{Olsen} (mmol kg^{-1})[a]	0.59	0.53	0.13	0.17	0.68	Olsen and Sommers (1982)
P_{AL} (mmol kg^{-1})[a]	1.4	1.0	0.3	0.4	1.0	Egnér et al. (1960)
Al_{ox} (mmol kg^{-1})[a]	130	106	71	77	88	Schwertmann (1964)
Fe_{ox} (mmol kg^{-1})[a]	165	169	158	181	118	Schwertmann (1964)
Al_{AL} (mmol kg^{-1})[a]	10.3	9.9	9.8	9.6	16.1	Ulén (2006)
Fe_{AL} (mmol kg^{-1})[a]	9.1	10.1	8.8	9.4	12.5	Ulén (2006)
PSI_2 (mmol kg^{-1}),[a]	7.9	7.8	7.3	7.2	10.5	Börling et al. (2004)
P_{Olsen}/PSI_2[a] (%)	8.1	6.8	1.8	2.4	6.4	Börling et al. (2004)
DPS_{AL}[a] (%)	8.7	6.2	2.0	2.5	4.3	Ulén (2006)

[a]Data from Andersson et al. (2012).

Glyphosate application and cultivation practices.

No Gly had been applied to the actual experimental plots for the previous three years. Quicklime (CaO) had been applied in dry conditions on the stubble in four plots in 2007 Phosphorus fertilization was 11 kg (ha year), always applied in mineral form in spring. This is a moderate load, since the area has special restrictions. When starting the experiment the aim was to avoid P limitation of the crop and therefore 20 kg/(ha year) were applied in 2007-2011 for all plots except four. Glyphosate was applied on 22 September 2010 as the commercial product Glypro Bio, at a rate equal to 1.06 kg/ha active substance. Twelve days later, the conventional and structure-limed plots were stubble-harrowed (Table 2) and eight plots were shallow-tilled (12 cm) twice and reconsolidated with a rib-roller. After a further 10 days, the conventionally ploughed plots (8) and the structure-limed plots (4) were mould-board-ploughed and the soil was inverted to a depth of 23 cm.

Table 7.1.4.3-11: Management regime in the different treatments (A-E) in 2010, where A+B (eight plots) represent regular conventional autumn ploughing, C (four plots) represents previous structure liming and D+E (eight plots) represent regular shallow tillage in autumn

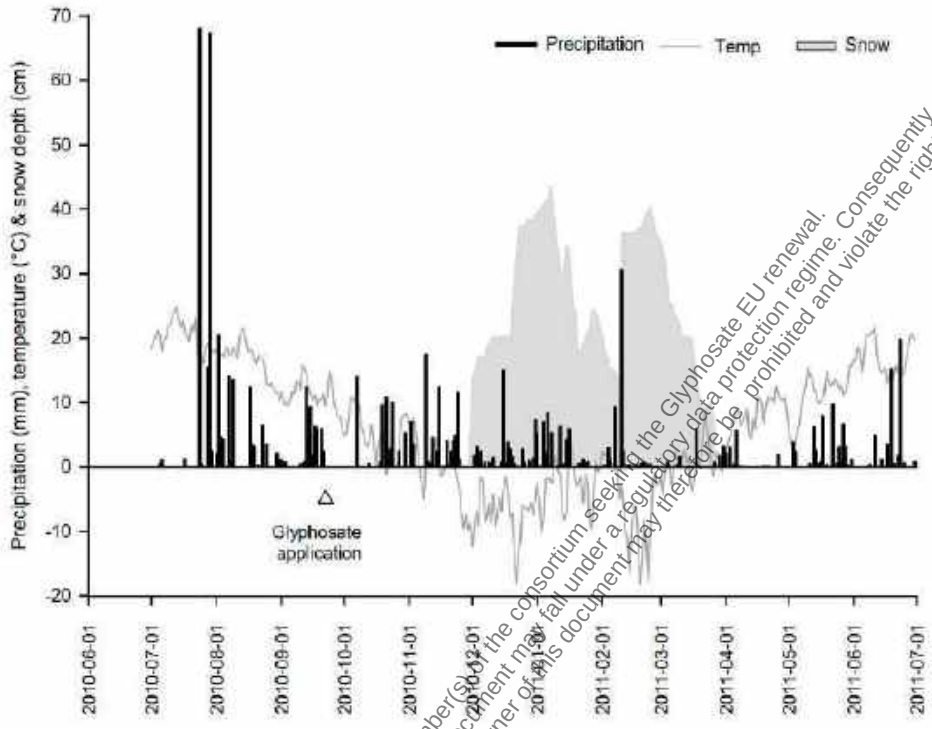
Treatment	Management	Date
A+B, C, D+E	Harrowing (0–5 cm)	16 May
A+B, C, D	Fertilization, seed drilling[a]	17 May
E	Fertilization (broadcasting)	17 May
A+B, C, D+E	Sowing (oats)	17 May
A+B, C, D+E	Harvesting	27 August
A+B, C, D+E	Glyphosate application ($1.06\ kg\ ha^{-1}$)	22 September
A+B, C	Stubble harrowing	4 October
D+E	Cultivation (8 cm) twice	4 October
A, B, C	Conventional ploughing (23 cm)	14 October

[a]No P fertilization to B plots.

Weather, discharge and water sampling procedure

Autumn 2010 was short, with permanent snow from the end of November (Figure 7.1.4.3-2). Owing to the thickness of the snow cover, soil freezing was limited despite low air temperatures. The main snowmelt took place in late March and the first two weeks in April. The glass vessels with flow-proportional samples in the station basement were observed regularly (at least weekly) and when at least 300 mL turbid water had been collected from most plots, sub-samples were taken from every plot for Gly analysis. When there was a moderate amount of water or less turbid water in the glass vessel, sampling was performed only for analysis of P and turbidity for reasons of economy. Such sampling occurred in total on five sampling occasions. On 28 March, 186 days after glyphosate application in autumn, turbidity was observed once again in the flow-proportionally sampled water and additional water was collected for Gly analysis, which was performed on the 14 most turbid samples.

Figure 7.1.4.3-2: Temperature (°C), precipitation (mm) and snow cover (mm) on the experimental field in 2010-2011



Water analysis

Total P was analysed as soluble molybdate-reactive P after acid oxidation with $K_2S_2O_8$ (ECS, 1996). DRP was analysed after pre-filtration using filters with pore diameter 0.45. Particulate P (PP) is the absolute dominant P fraction, while non-mineral forms of dissolved P are very small, and accordingly the difference between TotP and DRP was taken as PP. The concentration of particles was analyzed from thawed samples as turbidity on a HACH 2100 turbidimeter. Before analysing Gly, each thawed sample was thoroughly shaken by hand, centrifuged and filtered. The filtered water was used for analysis of DGly, including AMPA, after pH adjustment (pH 7-8) with either diluted HCl or NaOH. After a few more rounds of extraction, centrifugation and filtration, the pH of the samples was adjusted to 2 in order to precipitate any humic acids and to harmonize with the method used for stream and lake sediment. After dilution, the pH was readjusted to 7-8.

The same analytical procedure was used for both PGly and DGly and involved ion-exchange and derivatization, using a modified version of Mogadati *et al.* (1996), followed by final identification and quantification by gas chromatography-mass spectrometry (GC-MS).

Soil aggregate stability

Soil samples from plots with structure liming, conventional ploughing and reduced tillage were analysed in the laboratory for aggregate stability, expressed as readily dispersed clay (RDC). Slightly moist samples were collected from the topsoil (0-20 cm) on 27 August 2010, before post-harvest stubble cultivation, and gently transported to the laboratory. Four sub-samples representing 12 aggregates (8-10 mm) were prepared for each plot and gently wet-sieved (0.6 mm mesh opening) with a slow oscillating movement. After 4 hours sedimentation (to allow all particles larger than clay to settle; Sheldrick & Wang, 1993), the content of dispersed clay still in solution was determined by turbidometer.

Data calculations and statistical analyses

The mean and standard deviation were calculated for the experimental parameters determined in all flow-proportional samples (four or eight parallel samples) from replicate plots for the different treatments. If no residue of Gly or AMPA was detected in a given sample, the value 0 was used for calculating the mean. Pearson correlation and regression linear relationships were determined between the parameters total glyphosate (TotGly_PGly_DGly), TotP, PGly, PP and turbidity for the autumn period (27 September – 15 November) and between TotP and turbidity for the spring period (21 March - 11April). Any differences in glyphosate concentrations between the different soil treatments were analysed using Bonferroni post test assuming equal variance and a significance level of $p < 0.05$. Leaching losses from the different plots in the autumn period were calculated by multiplying discharge by measured flow-proportional concentrations in the periods between sample collections. In the spring period, transport of TotGly was estimated from measured values from 14 plots on 28 March.

Results and Discussion

Glyphosate and phosphorus concentrations in water

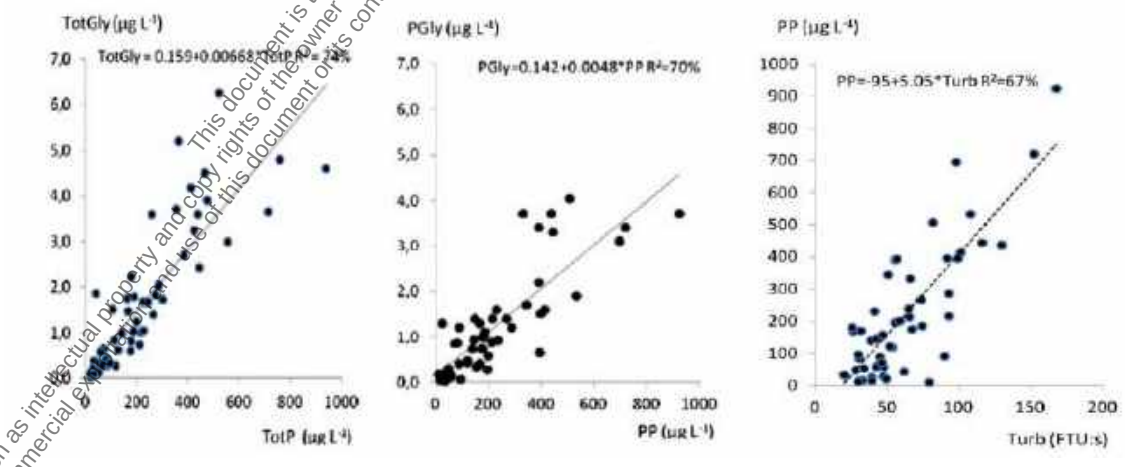
One week after Gly application in autumn, when 10 mm discharge had passed through the tile drainage system, no Gly or AMPA was present in detectable quantities in the discharge (Table 7.1.4.3-12). In the following 7-8 weeks, representing 70 mm water discharge, relatively high and quantifiable concentrations of both DGly and PGly were detected in practically all water samples and, in addition, dissolved AMPA was frequently observed. The concentrations varied greatly from plot to plot and TotGly concentrations of up to 5-6 µg/L were recorded for some plots. High PGly concentrations were generally associated with high DGly concentrations and the two forms of Gly were significantly correlated to each other (Pearson correlation 0.35; $p < 0.002$). Hence, more DGly seemed to leach with mobilized soil particles with high Gly content. Mean DGly concentration in discharge in the autumn (22/9-15/11) was 1.03 µg/L for plots with shallow tillage; 0.43 µg/L for plots with conventional ploughing and 0.36 µg/L for plots with structure liming (differences not statistically significant).

Similar to TotGly, the majority of TotP was lost in particulate form. The proportion of PP was higher (90 %) than the proportion of PGly (60 %). The present study site Gly was tilled down (10 or 23cm depth) in autumn after spraying which would facilitate the dispersion of Gly. A clear and positive correlation between TotGly and TotP concentrations and between PGly and PP concentrations was recorded (Figure 7.1.4.3-3). In turn, PP concentrations could be quite well predicted from turbidity (Figure 7.1.4.3-3). In contrast, DRP concentrations were generally low (0.018-0.027 mg/L) and DGly concentrations were more weakly correlated to DRP concentrations ($r = 0.65$; $p < 0.001$). Glyphosate is commonly suggested to compete with phosphate ions for adsorption sites, but at the present site, with high sorption capacity of the soil particles, this seemed not to be the case, since the correlation was positive. Mean PGly concentrations in the autumn were 1.73 µg/L in discharge from shallow-tilled plots; 0.62 µg/L for conventional ploughed plots; and 0.36 µg/L for structure-limed plots, all differences being statistically significantly different ($p < 0.001$). This implies that colloid P, colloid glyphosate and dissolved pesticides, although mobilized with different mechanisms (de Jonge *et al.*, 2009), may be transported via macropore flow.

Table 7.1.4.3-12: Discharge, pH (in stored composite samples) and flow-proportional concentrations of dissolved glyphosate (DGly), AMPA, particulate glyphosate (PGly), dissolved reactive phosphorus (DRP), particulate P (PP) and turbidity (Turb) in five periods 2010 - 2011 (n.d. = not detected)

Period	22/9–27/9	28/9–25/10	26/10–8/11	8/11–15/11	21/3–28/3
Conventional ploughing					
Discharge (mm)	8.2±3.0	9.2±4.3	25.5±11.1	33.5±13.1	72.8±30.2
pH	6.5	7.2	7.0	6.7	6.6
DGly (µg L^{-1})	n.d.	0.43±0.32	0.43±0.34	0.39±0.21	0.31±0.34
AMPA (µg L^{-1})	n.d.	0.05	0.04	0.03	n.d.
PGly (µg L^{-1})	n.d.	0.67±0.63	0.61±0.67	0.60±0.34	0.22±0.43
DRP (mg L^{-1})	0.021±0.011	0.021±0.011	0.018±0.007	0.020±0.003	0.048±0.019
PP (mg L^{-1})	0.132±0.068	0.122±0.010	0.161±0.166	0.168±0.184	0.124±0.039
Turb (NTU)	64±26	36±7	62±24	60±20	30±16
Structure liming					
Discharge (mm)	10.4±4.1	13.5±5.4	29.1±11.4	30.1±9.1	74.4±25.5
pH	6.9	7.3	7.2	6.9	6.6
DGly (µg L^{-1})	n.d.	0.24±0.20	0.30±0.21	0.21±0.27	0.23±0.25
AMPA (µg L^{-1})	n.d.	0.03	e.d.	0.05	0.08
PGly (µg L^{-1})	n.d.	0.40±0.48	0.41±0.53	0.33±0.58	0.16±0.35
DRP (mg L^{-1})	0.018±0.007	0.017±0.008	0.015±0.005	0.020±0.006	0.047±0.027
PP (mg L^{-1})	0.075±0.066	0.066±0.074	0.093±0.151	0.100±0.106	0.078±0.032
Turb (NTU)	46±30	34±11	64±48	46±31	28±6
Shallow tillage					
Discharge (mm)	10.8±5.3	15.6±6.6	28.0±10.8	29.7±6.4	76.4±23.5
pH	6.8	7.2	7.1	6.8	6.6
DGly (µg L^{-1})	n.d.	1.15±0.89	1.03±1.42	0.99±0.64	0.82±0.93
AMPA (µg L^{-1})	n.d.	0.05	0.23	1.3	0.02
PGly (µg L^{-1})	n.d.	1.99±1.64	1.82±1.44	1.89±1.48	0.57±0.84
DRP (mg L^{-1})	0.024±0.007	0.024±0.007	0.023±0.008	0.027±0.007	0.047±0.021
PP (mg L^{-1})	0.142±0.078	0.236±0.181	0.411±0.355	0.275±0.151	0.136±0.029
Turb (NTU)	88±44	50±17	99±45	81±31	52±43

Figure 7.1.4.3-3: Regression equation for the relationship between concentrations of: (a) total glyphosate (TotGly) and total phosphorus (TotP); (b) particulate glyphosate (PGly) and particulate P (PP); and (c) PP and turbidity (NTUs) in the period 27 September - 15 November 2010. Corresponding Pearson correlations (0.86, 0.84 and 0.82, respectively) were all significant (p < 0.001)



Glyphosate and phosphorus concentrations and losses in spring versus autumn period

As with Gly and P, pH was measured in the cumulative flow-proportionally sampled water and may have changed in the glass vessel. However, measured pH generally did not differ between the three treatments

and pH in discharge from the previously structure-limed plots was similar to that in discharge from the unlimited plots (Table 7.1.4.3-12). The pH tended to be lower (6.6) in the snowmelt period (Table 7.1.4.3-12). The measured drop in (logarithmic-based) pH value is equal to 75 % less H^+ ions, which may have influenced both the electrical charge of Gly and the hydrogen bonds of the minerals, and which may explain the high concentrations of DGly in snowmelt. The snowmelt water had low electric conductivity and DRP concentrations that were twice as high as those in the autumn discharge water. The PGly concentrations found in snowmelt in the present study were generally lower than the DGly concentrations and remained at nearly the same level as in autumn. Consequently, the relative proportions of DGly and PGly were reversed from autumn to spring (snowmelt) (Table 7.1.4.3-13). However, the latter case is based on a more limited number of analyses (n = 14).

Table 7.1.4.3-13: Number of samples analysed (n), relative proportions of dissolved glyphosate (DGly) and particulate glyphosate (PGly) in total glyphosate (TotGly) and relative proportions of dissolved reactive P (DRP) and particulate P (PP) in total phosphorus (TotP) in autumn (28/9 - 15/11 2010) and in a snowmelt period in spring (21 - 28/3 2011), based on flow-proportional concentrations

Glyphosate			Phosphorus		
Fraction	Autumn	Spring	Fraction	Autumn	Spring
n	80	14	No.	80	20
DGly/TotGly (%)	40	60	DRP/TotP (%)	10	32
PGly/ TotGly (%)	60	40	PP/TotP (%)	90	68

In practice, half-life degradation rate may be several months. However, as indicated here; ratio PGly/turbidity was only 20-40 % lower in March than in November. Simultaneously, PGly/PP ratio decreased by 30 % on average (from 0.54 % in autumn to 0.15 % in spring). Correspondingly the topsoil colloids may be more depleted of P in spring than in autumn, since the ratio PP to turbidity was lower and had a lower slope in snowmelt than in autumn.

Therefore, there may be similarities between Gly and P transport behavior in spite of the fact that P exists in a large P pool in topsoil and that yearly net P load to the soil in recent years has been six-fold higher than the glyphosate load.

Since the major water discharge took place during the snowmelt period, glyphosate losses tended to be higher in spring than in autumn (Table 7.1.4.3-14 and Table 7.1.4.3-15). In relation to applied amount, losses were approximately 0.1 % in spring and 0.05 % in autumn for the conventionally ploughed plots. The main reason for the high spring discharge was the intensive snowmelt taking place after a winter with much snow accumulation. These results indicate the importance of such a snowmelt period for Gly losses, confirming findings by Laitinen *et al.* (2009). Snow accumulation also had great consequences for P losses.

Table 7.1.4.3-14: Discharge and transport of dissolved glyphosate (DGly), particulate glyphosate (PGly), total glyphosate (TotGly), dissolved reactive phosphorus (DRP), particulate P (PP) and total P (TotP) from conventionally ploughed, structure-limed (and ploughed) and shallow-tilled plots in the period 28/9 - 15/11 2010

Period 28/9–15/11	Conventional	Structure-limed	Shallow-tilled
Discharge (mm)	69±28	74±23	72±22
DGly (g ha^{-1})	0.25±0.13	0.12±0.10	0.65±0.54
PGly (g ha^{-1})	0.45±0.53	0.19±0.19	1.01±0.75
TotGly (g ha^{-1})	0.70±0.60	0.31±0.31**	1.65±0.96
DRP (kg ha^{-1})	0.012±0.004	0.012±0.003	0.018±0.007
PP (kg ha^{-1})	0.104±0.082	0.048±0.044	0.192±0.117
TotP (kg ha^{-1})	0.117±0.084	0.060±0.044	0.209±0.114

**Significantly lower ($p < 0.05$) than in shallow-tilled plots

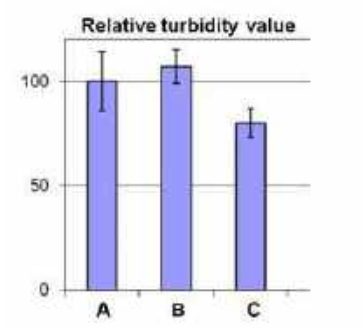
Table 7.1.4.3-15: Discharge (mm) and leaching losses of dissolved glyphosate (DGly), particulate glyphosate (PGly) and total glyphosate (TotGly) as a percentage of original amount applied from conventionally ploughed, structure-limed (and ploughed) and shallow-tilled plots based on measurements in autumn (28/9 - 15/11 2010) and more rough estimates in the most intensive spring snowmelt period (31/3 - 11/4)

	Conventional		Structure-limed		Shallow-tilled	
	Autumn	Snowmelt	Autumn	Snowmelt	Autumn	Snowmelt
Discharge (mm)	69	170	74	169	72	160
DGly (%)	0.024	–	0.011	–	0.061	–
PGly (%)	0.041	–	0.018	–	0.095	–
TotGly (%)	0.066	0.09	0.029	0.05	0.156	0.19

Glyphosate and phosphorus losses under different soil management regimes

In the autumn period, TotGly leaching losses were on average 0.70 g/ha from the conventionally ploughed plots (Table 7.1.4.3-15). TotGly losses from structure-limed plots were significantly lower ($p < 0.05$) than from shallow-tilled plots, expressed in absolute terms (Table 7.1.4.3-14), and also as a percentage of applied amount of Gly (Table 7.1.4.3-15). Fewer particles with attached Gly and P are expected to mobilize from soil aggregates that are less prone to dispersion. The structure-limed plots had significantly ($p < 0.05$) better aggregate stability (lower RDC values) in autumn than the conventionally ploughed and shallow-tilled plots (Figure 7.1.4.3-4), which may explain the clear tendency for lower losses of both PGly and PP from this treatment (Table 7.1.4.3-14).

Figure 7.1.4.3-4: Readily dispersed clay (RDC) in the topsoil from (A) conventionally ploughed, (C) structure-limed and (B) shallowtilled plots. The soil was sampled in September 2010, three years after structure liming



Leaching losses of both PGly and PP tended to be highest from the regular shallow-tilled plots (Table 7.1.4.3-14). Any enhanced amounts of stubble residues in the topsoil, combined with higher potential biological activity and organic matter content, did not seem to have improved aggregate stability from plots (Figure 7.1.4.3-4). However, significantly higher organic matter content was not expected, since such major changes may take at least 10 years. Sorption of Gly is generally not increased in the presence of more straw residues as a consequence of reduced tillage. Therefore, the straw may have facilitated water transport rather than providing new sorption sites after the mixing and re-consolidation of the soil surface. In addition, shallow and uneven accumulation of crop residues on the shallow-tilled plots possibly resulted in uneven infiltration and rapid lateral water movement compared with annually ploughed plots. This is a factor that should be further investigated.

There was no major difference in amount of discharge between the different treatments (Table 7.1.4.3-15). Topsoil structure should be further explored in connection with topsoil susceptibility to preferential flow and transport under different agricultural management regimes. In addition, there was a great variation in concentrations between different plots. Both 'gyttja' (cohesive matter of organic origin settled in marine or lake sediment) and oxidized iron (rust) have been frequently observed in soils at the present site. Such material might strengthen the crack walls and make them into permanent pathways, which could explain the general fast transport of particulate-bound glyphosate and P at the present site.

The source of the Gly leaching in this study was the tilled topsoil (0-12 or 0-23 cm), which was possibly the main source of P leaching too. Besides the total amount applied, risk assessment of leaching is often based on the sorption/desorption properties of the actual substance. However, according to the results of the present study, factors such as soil structure, macropore topology and macropore flow may be of great importance.

Conclusion

This study has demonstrated that a significant proportion of glyphosate (Gly) leaching losses may occur in particulate form from clay soils with high amounts of sorption sites available. Crack stabilization by gyttja, especially in the deeper sub-soil, might be an important explanatory factor for fast vertical transport of Gly and phosphorus (P) at the study site. The crack might also be an important explanatory factor for the great spatial variability in Gly and P, in both particulate and dissolved form, at the study site. Structure liming of the topsoil was demonstrated to reduce total Gly leaching losses compared with unlimited soils, while shallow tillage may not be a suitable way to mitigate particle-facilitated transport of Gly and P via tile drains from this type of clay soil. Proper agricultural management and improved topsoil structure can counteract fast macropore flow in this type of clay soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a field leaching experiment with glyphosate in Sweden on an agriculturally used soil. At this particular site with a clay soil, vertical transport of glyphosate through macropores (preferential flow) is the main transport process. The article provides no information to check the validity against current standards. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.3/003
Report author	Aronsson, H. *et al.*
Report year	2010
Report title	Leaching of N, P and glyphosate from two soils after herbicide treatment and incorporation of a ryegrass catch crop
Document No	DOI 10.1111/j.1475-2743.2010.00311.x ISSN 0266-0032
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (experiment not sufficiently described to evaluate the validity of the results)

2. Full summary

During 2005–2007, studies were carried out in two field experiments in southwest Sweden with separately tile–drained plots on a sandy soil (three replicates) and on a clay soil (two replicates). The overall aim was to determine the effects of different cropping systems with catch crops on losses of N, P and glyphosate. Different times of glyphosate treatment of undersown ryegrass catch crops were examined in combination with soil tillage in November or spring. Drainage water was sampled continuously in proportion to water flow and analysed for N, P and glyphosate. Catch crops were sampled in late autumn and spring and soil was analysed for mineral N content. The yields of following cereal crops were determined. The importance of keeping the catch crop growing as long as possible in the autumn is demonstrated to decrease the risk of N leaching. During a year with high drainage on the sandy soil, annual N leaching was 26 kg/ha higher for plots with a catch crop killed with glyphosate in late September than for plots with a catch crop, while the difference was very small during 1 yr with less drainage. Having the catch crop in place during October was the most important factor, whereas the time of incorporation of a dead catch crop did not influence N leaching from either of the two soils. However, incorporation of a growing catch crop in spring resulted in decreased crop yields, especially on the clay soil. Soil type affected glyphosate leaching to a larger extent than the experimental treatments. Glyphosate was not leached from the sand at all, while it was found at average concentrations of 0.25 µg/L in drainage water from the clay soil on all sampling occasions. Phosphorus leaching also varied (on average 0.2 and 0.5 kg/(ha x yr) from the sand and clay, respectively), but was not significantly affected by the different catch crop treatments.

Materials and methods

Experimental fields

The study was conducted over 2 years (2005–2007) in field experiments with a similar treatment design, but located at different sites, Lanna and Lilla Böslid in southwest Sweden. In both experiments, leaching was measured in separately tile–drained experimental plots where drainage flow was measured continuously and water was sampled in proportion to flow. Precipitation and air temperature were recorded at both sites.

Lanna site, clay soil.

Lanna research station (58°20′N, 13°07′E) is situated in a region which has a mean annual temperature of 6.1°C and mean annual precipitation of 558 mm (Lanna, 1961–1990). The experimental field, which was established in 2001, consists of 10 plots (790 m^2). Each plot was separately tile–drained at ca. 1 m depth and the drains were backfilled with 10–cm gravel at the bottom and then with the soil. The soil at Lanna consists of 47 % clay (<2 µm) in the topsoil (0–0.3 m depth) and 55–60 % clay in the subsoil (0.3–0.9 m depth). During the study, the topsoil had an organic matter content of 4.4 % and a mean pH of 6.6. The mean amount of ammonium lactate soluble P was 3.4 mg⁄100 g dry soil which is considered as low P status. The soil contains numerous cracks and macropores in the upper 1.0 m of the profile. More details on this soil are given by Bergström *et al.* (1994). At Lanna, the same plots were used during the two experimental years, with the same treatment being applied on each plot during the two consecutive years (with two replicates).

Lilla Böslid site, sandy soil.

Lilla Böslid experimental farm (56°35′N, 12°56′E) is located ca. 240 km south of Lanna. The mean annual temperature is 7.2°C and the mean annual precipitation is 803 mm (Halmstad, 1961–1990). The sandy soils in this region are commonly drained as the groundwater levels are often high because of a clay layer under the sand deposits. This experimental field was constructed in 2002, and consists of 36 separately tile-drained plots, each 320 m^2. The tile drains are at 0.9–m depth. The soil is an unstructured sand with 9 % clay in the topsoil (0–0.3 m depth) and 1–2 %in the subsoil (0.3–0.9 m depth). At the time of study, the topsoil had a mean organic matter content of 4.9 % and a pH value of 6.1. The mean amount of ammonium lactate soluble P was 12.8 mg⁄100 g dry soil. This value indicates that this soil is rich in P and that reduced P application rates are recommended for spring cereals. At Lilla Böslid, the experimental lay–out allowed two experimental years on different plots by dividing the field into two sections and using one section each year (with three replicates).

Experimental design and management practices

During the year before the experiment started, a spring cereal was grown at Lilla Böslid and winter wheat at Lanna. The experiments started in 2005 by undersowing a catch crop of ryegrass (*Lolium perenne L.*) in a cereal crop in three of five treatments at Lanna and in all treatments at Lilla Böslid (Table 7.1.4.3-16). At Lanna, glyphosate was applied in four treatments at the beginning of October, and in one in spring. Glyphosate treatment in October was combined with tillage in November (mouldboard ploughing, 25–cm depth) or in April (stubble cultivation, 6–cm depth). At Lilla Böslid, different times of glyphosate treatment in autumn were tested in combination with mouldboard ploughing (25–cm depth) in November or April. There was also one treatment without use of herbicide and spring ploughing, which was considered as a control treatment representing the best scenario for low N leaching. At Lilla Böslid, the soil was tine–cultivated to ca. 10 cm depth just before ploughing. Dates of tillage and glyphosate treatment are shown in Table 7.1.4.3-16. At Lanna, glyphosate was applied as Glyphomax Bio at a dose of 3.5 or 4.0 L⁄ha and at Lilla Böslid as Round–up Bio, 3.5 L⁄ha. The crop following incorporation of the catch crop was a spring cereal (oats or barley). It was fertilized with 100–110 kg N⁄ha at Lanna and with 90 kg N⁄ha at Lilla Böslid. A dose of 10 kg⁄ha of mineral P was applied at Lilla Böslid in 2006 and the same amount at Lanna in 2007.

Sampling and analyses of water, soil and crops

Drainage water from the plots at both sites was led to an underground monitoring station with temperatures never >15°C and <10°C during the main drainage periods when discharge rates were recorded using tipping buckets connected to a data logger which stored accumulated daily drainage volumes from each plot. Flow–proportional water samples of 15 mL were taken using a peristaltic pump after every 0.2 mm discharge. The samples for each plot were collected in individual polyethylene bottles which were emptied every

2 weeks during drainage periods for analysis of total–N, NO_3–N, total–P and PO_4–P. During sampling, the bottles were prepared with sulphuric acid for conservation of glyphosate. Glyphosate and the degradation product of aminomethylphosphonic acid (AMPA) were analysed for the same samples on 5–6 occasions during each of the two drainage seasons. At Lanna, glyphosate was analysed in samples from treatments A–D and at Lilla Böslid, in treatments F–J (Table 7.1.4.3-16). These events were primarily chosen to represent periods when drainage started in autumn with high flow periods. The first samples were taken before glyphosate treatment to ensure that any leaching detected originated from the experimental treatments. During the first year, the samples from replicates were pooled for analyses of glyphosate because of the high cost of analyses, but during the second year, all samples were analysed individually. Prior to analysis, the water samples were pretreated with a C18 ion exchange column for removal of non–polar substances, which also caused some filtration of particles (unknown size). Then glyphosate was derived with trifluoroacetic acid/trifluorethanol before combined gas chromatograph/mass spectrometer (GC/MS) analyses. The partitioning between particle–bound and dissolved glyphosate was not examined and some particles were also filtered before analysis. Thus, the analysis mainly covered the amount of dissolved glyphosate, but it is also likely that some particle–bound glyphosate was included as water samples were acidified during storage, which may have resulted in some dissolution of particle–bound glyphosate.

Table 7.1.4.3-16 The different experimental treatments at the two sites during the 2 years, with planned and actual time of glyphosate treatment and catch crop incorporation

		Time of glyphosate treatment			Time of incorporation		
	Catch crop	Plan	Act yr 1	Act yr 2	Plan	Act yr 1	Act yr 2
Lanna, clay soil							
A	Per. ryegr.	1 Oct	4 Oct 2005	4 Oct 2006	10 Nov	11 Nov 2005	10 Nov 2006
B	Per. ryegr.	1 Oct	4 Oct 2005	4 Oct 2006	1 Apr	28 Apr 2006	12 Apr 2007
C	Per. ryegr.	1 Mar	14 Apr 2006	29 Mar 2007	1 Apr	28 Apr 2006	12 Apr 2007
D	–	1 Oct	4 Oct 2005	4 Oct 2006	10 Nov	11 Nov 2005	10 Nov 2006
E	–	1 Oct	4 Oct 2005	4 Oct 2006	1 Apr	28 Apr 2006	12 Apr 2007
Lilla Böslid, sandy soil							
F	Per. ryegr.	20 Sep	26 Sep 2005	26 Sep 2006	10 Nov	24 Nov 2005	24 Nov 2006
G	Per. ryegr.	20 Sep	26 Sep 2005	26 Sep 2006	1 Apr	12 Apr 2006	2 Apr 2007
H	Per. ryegr.	5 Oct	4 Oct 2005	10 Oct 2006	10 Nov	24 Nov 2005	24 Nov 2006
I	Per. ryegr.	5 Oct	4 Oct 2005	10 Oct 2006	1 Apr	12 Apr 2006	2 Apr 2007
J	Per. ryegr.	20 Oct	31 Oct 2005	22 Nov 2006	1 Apr	12 Apr 2006	2 Apr 2007
K	Per. ryegr.	–	–	–	1 Apr	12 Apr 2006	2 Apr 2007

Per. ryegr., perennial ryegrass.

Calculations and statistical analysis

Analysis of variance was carried out by the Mixed procedure in SAS 9.1 (SAS Institute Inc. 2003: SAS/Stat 9.1 Users' Guide. Cary, NC, USA) for the statistical analysis of differences in yields, catch crop biomass and N and P contents, soil mineral N, leaching of N and P and concentrations of glyphosate between treatments. The t–test at P = 0.05 was used for pairwise comparisons by the PDIFF statement. Block was used as the random variable in analysis of a single year. For the Lanna site, the average for the 2 years was analysed by calculating an average per plot and by using block as random variable. For the Lilla Böslid site, where the experiment was carried out in separate plots during the 2 years, year was used as random variable when analysing the average for the 2 years.

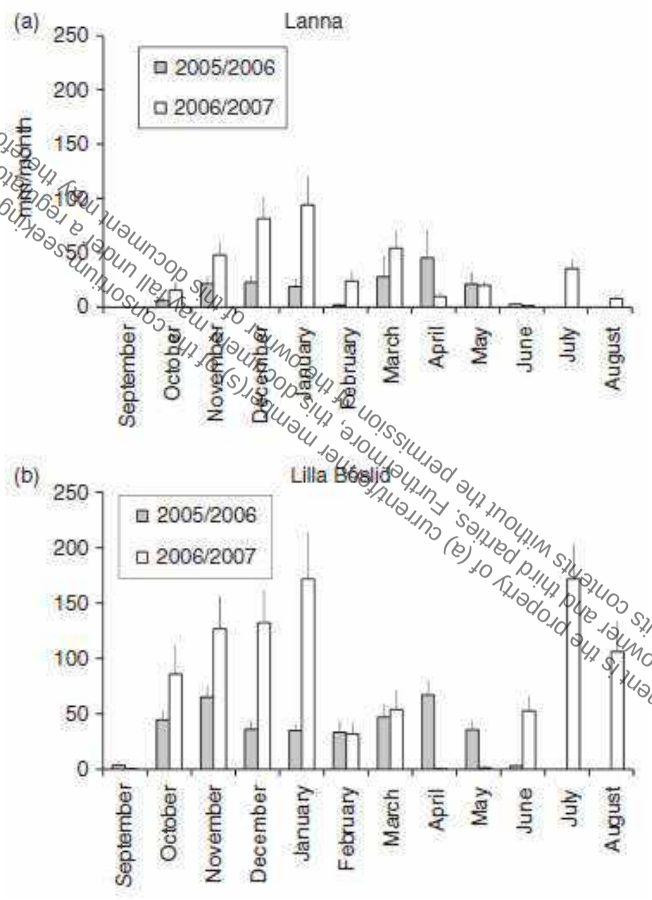
Results

Drainage and climate conditions

The two experimental years represented varying climate and drainage conditions, 1 yr with a cold winter with relatively small drainage amounts, and one mild winter with high drainage. At Lanna, the mean temperature during December 2005–March 2006 was –3.5°C, while it was +2.1°C during the same period 2006–2007. For Lilla Böslid, corresponding values were –2.6°C and +4.3°C. At Lanna, the measured precipitation was 480 mm during 2005–2006 and 759 mm during 2006–2007 (1 September–31 August).

At Lilla Böslid, the corresponding figures were 542 mm and 950 mm, respectively. During the first year, the precipitation was considerably lower than the long–term mean value for both sites, but higher during the second year. Different precipitation and temperature conditions during winter clearly affected drainage and the N and P leaching during the two experimental years. The high rainfall resulted in much drainage during the autumn and winter of 2006–2007. During summer 2007, southwest Sweden was exposed to several large low pressure cells, which resulted in extremely rainy conditions and major drainage events. There was some variation in drainage water totals between individual plots as shown in Figure 1 where standard deviations for all plots are included. These differences could not be attributed to different experimental treatments except for 2005–2006 at Lilla Böslid, when treatment K had higher drainage than most of the other treatments (P = 0.03).

Figure 7.1.4.3-5: Mean monthly drainage (mm) from all plots during the two experimental years at the two sites Lanna (a) and Lilla Böslid (b). Standard deviations are shown with narrow bars



Management practices, catch crop growth and crop yields

The planned time of glyphosate treatment in September and beginning of October corresponded quite well to the actual time at both sites (Table 7.1.4.3-16). From field observations, the catch crop was still intact 1 week after treatment, but after 3 weeks, it was totally killed in both years. However, glyphosate treatment in late October at Lilla Böslid was delayed by up to 4 weeks because of bad weather conditions, especially in 2006 (Table 7.1.4.3-16) when the catch crop was treated in late November. This resulted in a poor effect of the glyphosate, and only 50 % of the catch crop was killed 3 weeks after treatment. Glyphosate treatment in spring (treatment C at Lanna) resulted in problems with the timing. Obtaining an effect of the herbicide, while simultaneously being able to cultivate this heavy clay soil, was a challenge. In spring 2007, when there was a very dense catch crop, it was particularly difficult to incorporate the catch crop material in this

treatment, and about 20–40 % of the catch crop was estimated to be still growing at harvest of the following crop. Shallow cultivation in spring worked much better after glyphosate treatment in autumn (treatments B and E) with respect to incorporation of plant material, although this tillage practice is not common for this type of soil.

Leaching of glyphosate

At the sandy soil at Lilla Böslid, drainage water was analysed for glyphosate on eight occasions during the experimental period (November 2005, December 2005, April 2006, October 2006, November 2006, December 2006, January 2007 and March 2007). Glyphosate was only detected twice and occurred at trace levels, that is at concentrations above the detection limit (ca. 0.01 µg/L), but under the limit for determination of the concentration (ca. 0.05 µg/L). These occasions were in treatments F and I at sampling on 20 December 2006 and in treatment J on 8 January 2007.

AMPA was not found at all. As a result of bad weather conditions during October–November 2006, glyphosate application in treatment J was not possible until 22 November. If there had been a risk of glyphosate transport, it would probably have arisen during conditions like these, but the risk seemed to be very small for this soil. The adsorption of glyphosate in the sandy soil was probably very efficient, probably because of Al/Fe–oxides, the same as for P. At the Lanna clay soil, glyphosate was found at concentrations above the determination limit in all samples except two during the experimental period (Table 7.1.4.3-17). Thus, application of glyphosate both in autumn and in spring resulted in some transport to drainage water, but with this experimental design, it was possible that application of glyphosate during 2005–2006 also affected to some extent the results from 2006 to 2007. Even at sampling in spring 2005, before the start of the experiment at Lanna, traces of glyphosate were found in drainage water. This probably originated from autumn 2004 when glyphosate was applied to borders between the experimental plots. Concentrations were low, on average 0.25 µg/L, and only exceeded 1 µg/L on one occasion (January 2007 in treatment D). The concentrations of glyphosate measured at Lanna were similar to those found in monitoring of streams in agricultural catchments in southern Sweden (Adielsson *et al.*, 2007).

Table 7.1.4.3-17: Measured concentrations of glyphosate and its metabolite AMPA

	A		B		C		D	
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
Glyphosate treatment	4 Oct 2005 4 Oct 2006		4 Oct 2005 4 Oct 2006		14 Apr 2005 20 Mar 2006		4 Oct 2005 4 Oct 2006	
24 Apr 2005	Trace*	nd*	Trace*		–	–	Trace*	nd*
15 Nov 2005	0.39	nd	0.86	Trace	–		0.85	0.40
27 Nov 2005	nd	nd	0.19	nd	–		0.01	ed
5 Apr 2006	0.23	nd	0.48	Trace	Trace*	Trace*	0.16	Trace
2 May 2006	–	–	–	–	0.29	Trace	–	–
1 Jun 2006	Trace	nd	0.08 (0.05)	nd	–	–	0.04 (0.01)	nd
1 Nov 2006	0.55[a] (0.09)	Trace	0.66[a] (0.48)	Trace	0.19[a] (0.04)	Trace	1.04[a] (0.37)	Trace
15 Nov 2006	0.21[a] (0.11)	Trace	0.44[b] (0.02)	0.10	0.10[a] (0.06)	Trace	0.51[a] (0.07)	0.10
8 Jan 2006	0.15[a] (0.01)	Trace	0.66[b] (0.12)	Trace	0.12[a] (0.06)	Trace	0.18[a] (0.06)	Trace
15 Jan 2006	0.13[a] (0.01)	Trace	0.50[b] (0.01)	Trace	0.10[a] (0.02)	Trace	0.20[a] (0.09)	Trace
13 Mar 2007	0.16[a] (0.04)	Trace	0.31[b] (0.02)	Trace	0.07[a] (0.02)	nd	0.13[a] (0.03)	Trace
21 May 2007	Trace	Trace	0.06[a] (0.04)	Trace	0.14[a] (0.07)	Trace	0.05[a] (0.03)	Trace

Standard deviations are shown in brackets. Different superscript letters indicate significant differences between treatments ($P = 0.01$). *Samples taken before treatment with glyphosate. nd, not detected. Trace, between detection and determination limit, ca. between 0.02 and 0.05 µg/L.

Discussion

Results from this study indicate that soil texture was the dominant factor in influencing both P and glyphosate losses, whereas different treatments had small or no effects. For glyphosate, this was not surprising, as soil structure and transport pathways have been shown to be of major importance for glyphosate leaching (Vereecken, 2005; Borggaard & Gimsing, 2008). The immediate detection of glyphosate in drainage water from the clay soil at Lanna clearly shows that there are rapid pathways for

water and solutes in this soil, as reported previously by Larsson & Jarvis (1999). The glyphosate analyses did not distinguish between dissolved and particle–bound glyphosate; however, as 70–80 % of the P losses were in particle–bound form, this might also be an important transport form for glyphosate. In studies on two soils in Denmark, the contribution of colloid–facilitated transport was up to 27 % and 52 % for a sandy loam and a sandy soil, respectively (de Jonge *et al.*, 2000). It is probable that total leaching of glyphosate, especially from the clay soil, was underestimated in this study as it is uncertain of the extent to which particle–bound glyphosate was included in the analyses. Soil tillage practices affect transport pathways through the soil. For example, conservation tillage has been shown to increase the amount of macropores and related preferential flow paths (Shipitalo *et al.*, 2000), but time of ploughing may also affect the partitioning between different types of losses. Spring ploughing instead of autumn ploughing protects the surface against destruction of soil aggregates over winter and is highly relevant in minimizing particle–bound P losses by erosion (Kronvang *et al.*, 2005), especially in combination with a catch crop (Ule´ n, 1997). In contrast, losses of dissolved compounds may increase when the soil is not cultivated in autumn. This was reported in studies of glyphosate losses in Norway (Stenrød *et al.*, 2007) and Denmark (Lærke Baun *et al.*, 2007) where tillage in autumn increased the leaching of particulate–bound glyphosate, while there was increased leaching of dissolved glyphosate when the soil was not tilled in autumn. These findings are supported by the results from Lanna, where there are indications of higher losses of total–P after ploughing in autumn, but differences in concentrations or yearly transport are ns. Spring tillage at Lanna (treatment B) gave significantly higher concentrations of glyphosate in drainage water than the other treatments on four occasions ($P = 0.01$) in 2006–2007, which may indicate that spring tillage conserved transport pathways through the topsoil during winter. However, it is not possible to draw conclusions about the partitioning between dissolved and particle–bound glyphosate. Another study on the Lanna soil in lysimeters shows that losses of particle–bound glyphosate were negligible and that almost all leached glyphosate was in dissolved form (Bergström *et al.*, 2010). There were no indications of increased transport of dissolved P in spring–ploughed plots, with or without a catch crop over winter. However, catch crop plant material may constitute a risk of dissolved P leaching if exposed to freezing, as shown by Bechmann *et al.* (2005).

In the sandy soil at Lilla Böslid, glyphosate was efficiently sorbed, which was also true for P. The high P status of this soil did not seem to increase the risk of P losses, although studies have shown a relationship between high P content of the soil and P leaching (Heckrath *et al.*, 1995). The larger proportion of dissolved P at Lilla Böslid, compared with Lanna, could be an indication of enhanced P desorption because of high soil P content, but this is probably not the case as P concentrations in drainage water were consistently low and stable. There is also considered to be an increased risk of glyphosate transport in soils with high P content, as PO_4–P and glyphosate may compete for the same surface binding sites on soil mineral particles (Gimsing & Borggaard, 2002). However, the P and glyphosate sorption capacity of the subsoil and the degree of saturation of sorption sites have a large impact on actual P losses, and there was no indication of saturated conditions in the sandy soil at Lilla Böslid.

The results from the sandy soil at Lilla Böslid show that the time available for catch crop growth and N uptake during autumn significantly affected the accumulation of N in the soil and the risk of N leaching during the following winter, although it is somewhat surprising that there is no clear correlation between soil mineral N in autumn and N leaching. The results also show that glyphosate treatment in September or early October resulted in fast release of N available for leaching. This confirms the findings by Snapp & Borden (2005) that N mineralization increases when the catch crop is treated with glyphosate 8 days before incorporation, compared with no treatment before incorporation. The time of catch crop incorporation after chemical kill–off in autumn seems to be of minor importance according to the results from both sites. This is somewhat surprising for the sandy soil, as several studies have shown that time of tillage in autumn clearly influences N mineralization and N leaching from this type of soil (e.g. Wallgren & Lindén, 1994; Djurhuus & Olsen, 1997; Stenberg *et al.*, 1999). In the present study, glyphosate treatment obviously had a similar effect to incorporation on N release in the soil, at least during the second year. For the clay soil at Lanna, the results are similar to those found in a study in an adjacent field (Aronsson & Stenberg, 2010), where time of tillage in autumn or spring did not affect N leaching to any large extent.

Growing a catch crop may affect the yield of the main crop because of inter–plant competition, although this effect is often small or negligible (Ohlander *et al.*, 1996). The catch crop may also affect the following

crop after incorporation.

This effect can be positive as a result of the fast remineralization of catch crop N (Lyngstad & Borresen, 1996). It may also be negative as a result of the immobilization of catch crop N or depletion of soil mineral N content in spring as a result of N uptake by the catch crop. This pre–emptive effect (Thorup–Kristensen, 1993) probably contributed to decreased yields at Lanna together with regrowth of the catch crop and at Lilla Böslid. Incorporation of a living catch crop in November–December or in February–March instead of April would probably have been more suitable for remineralization of catch crop N, as suggested by Torstensson & Aronsson (2000). To improve synchronization with the N requirements of the following crop, the N mineralization dynamics must be considered rather than increasing N fertilization rates after incorporation of catch crops.

Conclusion
To develop recommendations to achieve decreased nutrient leaching and pesticide contamination of water, the leaching and contamination risks need to be considered in relation to each other, and crop production aspects also need to be considered. It is clear from this study that N leaching was considerably lower from the clay soil (2–22 kg N/ha/yr) than from the sandy soil (15–53 kg N/ha/yr). It was also clear that spring incorporation of a catch crop could not be recommended on the clay soil as it negatively affected crop yields. Glyphosate treatment causes some risk of contamination of percolating water on the clay soil, irrespective of time of application, while time of incorporation does not affect leaching of N and P on either clay soil or sandy soil. This suggests that clay soil should not be given special priority for the use of catch crops with chemical treatment or for the use of excluded tillage in autumn. The reasons are that the overall risk of N leaching is relatively low and that the beneficial effects on N leaching may be counteracted by some risk of glyphosate leaching. Moreover, there is no reduction in P losses.

For the sandy soil, N leaching was much higher than from the clay soil and keeping the soil covered with a catch crop until November or until spring considerably reduced N leaching during high–flow conditions compared with chemical kill–off in September or mid-October. It was difficult to achieve a good herbicide effect with delayed chemical treatment, indicating that there always has to be a compromise between N leaching and successful weed control. Despite the low risk of glyphosate and P leaching, the results suggest that for a sandy soil, glyphosate treatment should be excluded during autumn when growing catch crops to maximize the reduction in N leaching. Incorporation of the catch crop as late as possible in autumn or in very early spring probably reduces the risk of decreased crop yields of the following crop as a result of N uptake by the catch crop.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a 2-years leaching experiment in Sweden on two agricultural soils (one soil and one sand) with glyphosate. The method is not sufficiently described to evaluate the validity of the results. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.3/004
Report author	Kjaer, J. *et al.*
Report year	2011
Report title	Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils
Document No	DOI 10.1016/j.chemosphere.2011.03.029 E-ISSN 1879-1298
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Geological Survey of Denmark and Greenland)
Acceptability/Reliability:	Reliable with restrictions (Substance properties not sufficiently described, no evaluation of the residues in soil layers after finalization of the study was conducted, duration of the study too short)

2. Full summary

Leaching of the strongly sorbing pesticides glyphosate and pendimethalin was evaluated in an 8–month field study focussing on preferential flow and particle–facilitated transport, both of which may enhance the leaching of such pesticides in structured soils. Glyphosate mainly sorbs to mineral sorption sites, while pendimethalin mainly sorbs to organic sorption sites. The two pesticides were applied in equal dosage to a structured, tile–drained soil, and the concentration of the pesticides was then measured in drainage water sampled flow–proportionally. The leaching pattern of glyphosate resembled that of pendimethalin, suggesting that the leaching potential of pesticides sorbed to either the inorganic or organic soil fractions is high in structured soils. Both glyphosate and pendimethalin leached from the root zone, with the average concentration in the drainage water being 3.5 and 2.7 µg/L, respectively. Particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of the observed leaching (13–16 % for glyphosate and 16–31 % for pendimethalin). Drain–connected macropores located above or in the vicinity of the drains facilitated very rapid transport of pesticide to the drains. That the concentration of glyphosate and pendimethalin in the drainage water remained high (>0.1 µg/L) for up to 7 d after a precipitation event indicates that macropores between the drains connected to underlying fractures were able to transport strongly sorbing pesticides in the dissolved phase. Lateral transport of dissolved pesticide via such discontinuities implies that strongly sorbing pesticides such as glyphosate and pendimethalin could potentially be present in high concentrations (>0.1 µg/L) in both water originating from the drainage system and the shallow groundwater located at the depth of the drainage system.

Materials and methods

Chemicals

Glyphosate [N–(phosphonomethyl)glycine] – the active ingredient in Roundup – is a broad–spectrum, post–emergence, non–selective herbicide that is one of the most used herbicides worldwide. In Denmark, glyphosate is the herbicide sold in the largest quantities; in 2003, glyphosate sales for agricultural purposes accounted for 44 % of all herbicide sales. By 2008, this had increased to 52 % (Danish Environmental Protection Agency, 2004, 2009). Pendimethalin [N–(1–ethylpropyl)–2,6–dinitro–3,4–xylidine] is a selective herbicide used to control most annual grasses and certain broadleaf weeds both pre–emergence (i.e. before weed seeds have sprouted) and early post–emergence. Pendimethalin ranks fourth among the herbicides used in Demark, accounting for 5 % of all herbicide sales for agricultural use in 2003 and 6 % in 2008 (Danish Environmental Protection Agency, 2004, 2009). Solubility of glyphosate is 10500 mg/L while that of pendimethalin is 0.33 mg/L pesticide properties database available at

http://sitem herts.ac.uk/aeru/footprint/index2 htm.

Site description
The study was conducted at the Estrup field research site in Denmark, a virtually flat systematically tile–drained loamy field located on glacial till with a cultivated area of 1.26 ha. The tile drains are located at an average depth of 1 m b.g.s., and the water table is relatively shallow, located 1–3 m b.g.s. The uppermost meter of the soil is heavily fractured and bioturbated, with plough layer containing 100–1000 biopores/m^2 (Lindhardt *et al.*, 2001). The geological structure is complex, comprising a clay till core with deposits of different age and composition. Of three pedological profiles available for the site, one is classified as Aquic Argiudoll, one as Abruptic Argiudoll and one as Fragiaquic Glossudalf. Details on soil properties are reported in Table 1 and geological properties are further described in Lindhardt *et al.* (2001).

Agricultural management
After maize (Zea mays L.) had been harvested on 13 October 2005, glyphosate (1.44 kg/ha active ingredient; 4.0 L/ha Round–up Bio) and pendimethalin (1.44 kg/ha active ingredient; 3.6 L/ha Stomp) were applied simultaneously together with 30.0 kg/ha of potassium bromide as tracer on 9 November 2005. On 12 April 2006 the field was ploughed to a depth of 18 cm. Spring barley was sown on 27 April 2006. Whereas glyphosate had been applied previously (13 October 2000 and 2 September 2002) the field had been treated with pendimethalin 7 October 1997. The minor residues of glyphosate (0.01–0.03 µg/L) found in the drainage water before the current application of pesticides (Figure 7.1.4.3-7) is thus likely to derive from these previous treatment.

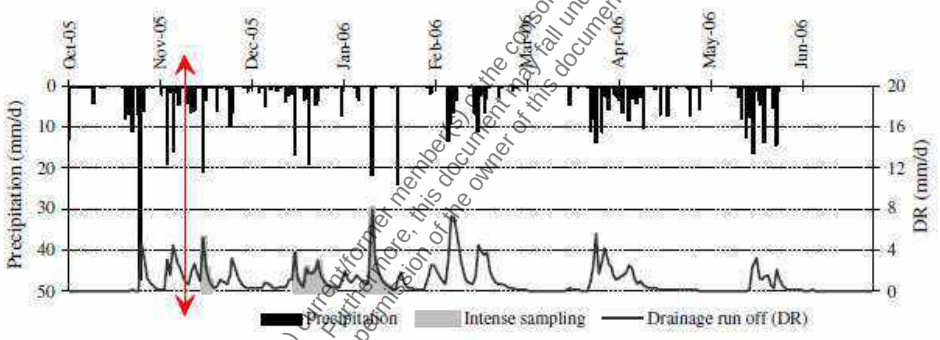
Monitoring and sample preparation
For a period of 8 months following application of the glyphosate and pendimethalin the concentration of the pesticides and bromide was measured on a weekly basis in drainage water sampled flow–proportionally. In addition, more intense sampling of drainage water was performed in connection with three flow events triggered by precipitation on 14 November 2005, 16 December 2005 and 11 January 2006 (Figure 1) in order to enable detailed description of the transport of water and pesticides. Sampling lasted for 2, 13 and 9 d, respectively. Flow events are characterised by an initial rapid rise in the hydrograph followed by a less rapid drop (tailing). During these events, drainage water subsamples were collected for every 2 mm of drainage runoff using a refrigerated Isco sampler (Teledyne Isco, Inc., US) containing eight 2–L borosilicate bottles. Within 24 h of the onset of the flow events, each bottle from the Isco sampler was shaken thoroughly to resuspend the sediment. The particles in the individual samples were then separated by centrifugation at 3500 rpm using Teflon vials. The time required for separation of particles ≥0.24 µm was calculated according to Gimbert *et al.* (2005). The supernatant was removed using a pipette, cleaned with 20 % HCl. The supernatant of samples to be analysed for pendimethalin was placed in glass bottles and preserved by adjusting to pH 2.0 with sulphuric acid. The pellets were flushed into a glass bottle using demineralised water and preserved using sulphuric acid. The samples to be analysed for pendimethalin were stored at 2°C until analysis. The supernatant of samples to be analysed for glyphosate and AMPA was pipetted into polypropylene (PP) bottles and adjusted to pH 2.0 with sulphuric acid. The pellets were flushed into PP bottles and adjusted to pH 2.0 with sulphuric acid. The latter two types of sample were stored at –18°C until analysis. As the flow event on 16 December 2005 occurred at a weekend, it was not possible to conduct particle separation on the samples. With all the samples collected on a weekly basis and the intensive samples collected following the flow event on 16 December 2005, pesticide concentrations were measured on the entire water sample. Thus the reported concentrations refer to the total concentration of both dissolved and particle–bound pesticide. With samples collected intensively following the flow events on 14 November 2005 and 11 January 2006, pesticide concentrations are reported for both particle–bound pesticide (concentration in the pellets) and dissolved pesticide (concentration in the supernatant). Furthermore, measurements of turbidity, chloride concentration and conductivity were conducted on all water samples obtained from the Isco sampler.

Table 7.1.4.3-18: Physical and chemical properties of the soil

Profile[a]	Horizon	Depth	Clay[b] (%)	Silt[b] (%)	Sand[b] (%)	OM (%)	C/N	CEC (meq 100 g^{-1})	pH_{CaCl2}	Fe (mg kg^{-1})	Al (mg kg^{-1})
Estrup 2											
	Ap	0–26	13.8	12.7	70.8	2.7	13	12.1	6.5	2044	808
	Bt(g)	26–45	36.3	15.3	47.8	0.5	6	13.9	6.3	4144	1748
	Bt(g)2	45–121	33.0	15.9	50.9	0.2	4	16.8	6.6	2294	1034
	Cc[c]	121–150	31.1	24.9	7.5	0.5	6	19.7	7.5	2290	568
Estrup 3											
	Ap	0–28	9.9	7.1	77.5	5.5	17	15	7.0	1648	1024
	Bs/Bhs	28–58	8.8	4.7	85.7	0.8	12	10.2	6.6	1730	1368
	Bt(g)	58–115	12.2	4.3	83.1	0.4	8	9.2	4.2	1702	1016
	2C	115–185	38.9	24.1	26.9	10.1	39	40.5	4.5	1576	2934

nd.: Not determined; OM: Organic matter determined as 1.72 total organic carbon; Fe and Al: oxalate extractable Fe and Al determined by the methods of McKeague and Day (1966).
a Profiles are classified as Abruptic Argiudoll (Estrup 2) and Fragiaquic lossudalf (Estrup 3).
b Clay: <2 µm; Silt: 2–20 µm; Sand: 20–2000 µm.
c Contains 36.1 % $CaCO_3$. Contains 20.0 % $CaCO_3$.

Figure 7.1.4.3-6: Precipitation (hanging bars on primary axis) and drainage runoff (solid line on secondary axis). The red vertical arrow indicates the date of application. The shaded grey area beneath the solid line indicates the flow events that were intensively monitored



Methods of analysis

Glyphosate

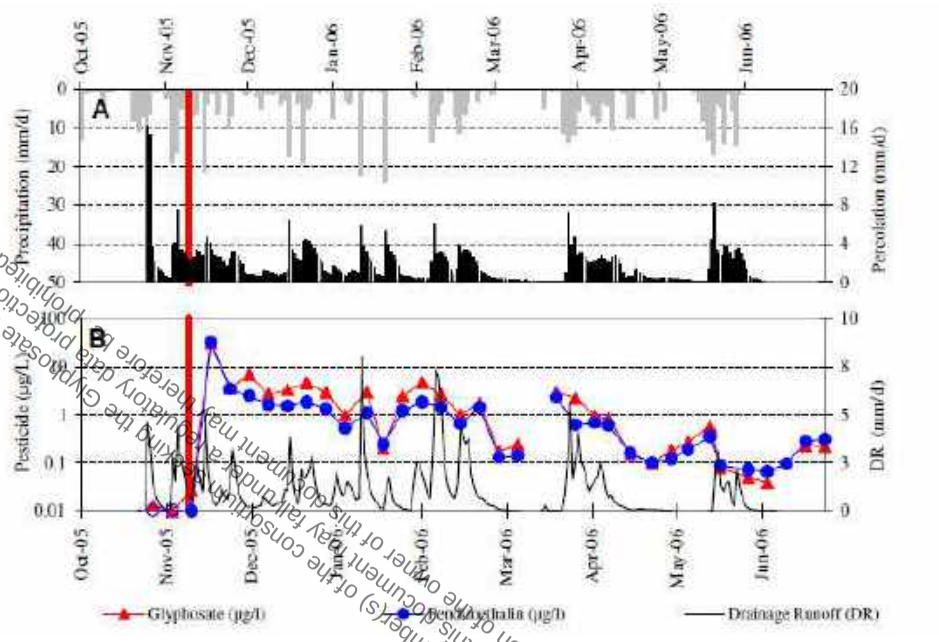
The preserved water samples were first concentrated on a column of Chelex 100 resin, iron form 100–200 mesh from Bio–Rad. After washing with 0.1 M HCl, the analytes were eluted with 6 M HCl. The eluate was further cleaned on a column of AG 1–X8 resin, chloride form 200–400 mesh. The eluate was evaporated to dryness under nitrogen and redissolved in 200 µL of water–methanol–HCl (160:40:2.7). Derivatisation was carried out with 1 mL of trifluoroacetic anhydride–2,2,3,3,4,4,4–heptafluoro–1–butanol (2:1). The derivatives of glyphosate were measured by GC–MS using a 5 % phenyl methylsiloxane GC-column (HP–5) with the MS in electron impact (EI) mode. 2 µL sample was injected by splitless injection at 280°C with oven temperature at 65°C. After 2 min the oven temperature was raised to 310°C at 20°C min^{-1} and held at 310°C for 4 min. The glyphosate derivatives were identified by MS using m/z 612, 611 and 584. The calculations were made using the internal standard procedure with glyphosate–$^{13}C^{15}N$ as the internal standard. The LOD (limit of detection) was below 0.01 µg/L. The preserved pellet samples were treated with 1 M ammonia prior to analysis in order to extract the glyphosate from the solids. The extract was then diluted with water, adjusted to pH 2.0 with HCl, and analysed as described above for the water samples.

Inorganic analysis

The water samples were analysed for turbidity, conductivity and chloride concentration. Turbidity was measured with an infrared LED light source using a pHotoFlex Turb photometer (WTW Gmbh, Weilheim, Germany). Conductivity was measured using a Cond 340i conductivity pocket meter (WTW Gmbh,

Weilheim, Germany). Chloride concentration was measured using a FIAstar™ 5000 flow injection analyser (Foss Analytical AB, Höganäs, Sweden).

Figure 7.1.4.3-7: Precipitation and simulated percolation (A) together with concentration of pendimethalin and glyphosate (B) in the drainage runoff (DR on secondary axis). The red vertical lines indicate the date of application. The open circles indicate concentrations below the LOD (0.01 µg/L)



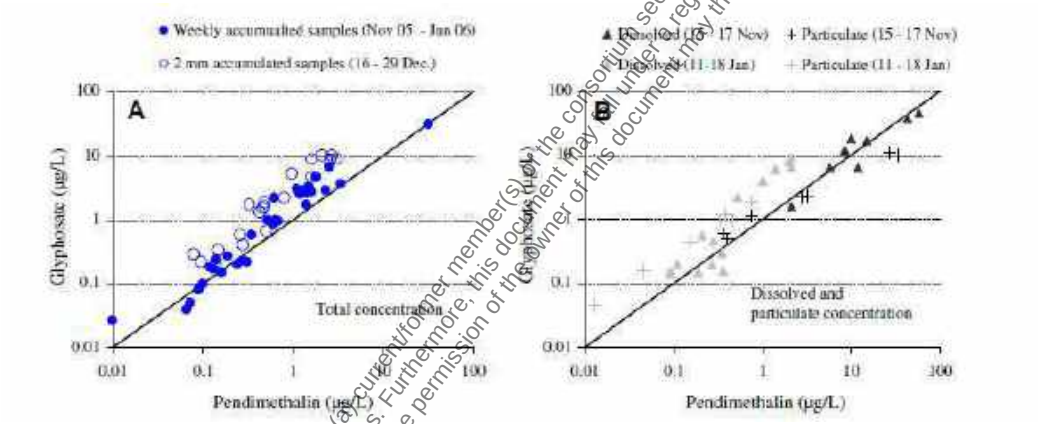
Results and Discussion

Leaching of glyphosate and pendimethalin

The leaching pattern of glyphosate resembled that of pendimethalin (Figure 7.1.4.3-7), thus suggesting (i) that the leaching potential of strongly bound pesticides from structured soil is high both with pesticides that bind to soil organic matter (e.g. pendimethalin) or to the inorganic fraction (e.g. glyphosate) and (ii) that the pathways governing the transport of these two pesticides are similar. Both glyphosate and pendimethalin leached from the root zone in average concentrations considerably exceeding the EU limit value for groundwater (0.1 µg/L) during the 8–month drainage flow period (Figure 7.1.4.3-7). The average concentration of glyphosate and pendimethalin in the drainage water was 3.5 and 2.7 µg/L, respectively. Both pesticides were found in all of the weekly drainage water samples. Among the 32 samples collected after pesticide application, the concentration exceeded 0.1 µg/L in 29 (Figure 7.1.4.3-7). The similarity of the leaching patterns of the two pesticides was reflected in the close correlation between the measured concentration of pendimethalin and glyphosate (Figure 7.1.4.3-8). R^2 for measured total concentration (both dissolved and particle–bound) in samples collected (i) on a weekly basis during the entire monitoring period (32 samples) and (ii) for every 2 mm of drainage runoff occurring during a 13–d period in December (20 samples) was 0.962 and 0.963, respectively (Figure 7.1.4.3-8A). A similar tendency was found when comparing the particulate and dissolved concentrations of pendimethalin and glyphosate measured during two individual flow events, R^2 being 0.943 for dissolved pesticide (19 samples) and 0.928 for particle–bound pesticide (10 samples) (Figure 7.1.4.3-8B). Pesticide leaching was governed by preferential transport, as evidenced by the soil hydraulic properties (Kjær *et al.*, 2005) and fast solute transport. Piston flow through the low–permeable soil matrix would entail a transport time to the drainage system of about 98 d (Kjær *et al.*, 2007). However, glyphosate and pendimethalin were detected in drainage water samples as early as 8 d after application. This finding is thus consistent with previous transport studies conducted at the Estrup site (Kjær *et al.*, 2005, 2007), as well as other field studies demonstrating rapid macropore–mediated transport of pesticides (for a review see Jarvis (2007)). As both glyphosate and pendimethalin

leached in high concentrations following the same transport pathways, the difference in sorption characteristics of glyphosate, which sorbs strongly to the inorganic soil fraction, and pendimethalin, which sorbs strongly to the soil organic fraction, had little impact on leaching in this structured soil. Our finding is in line with previous studies showing that differences in the leaching of pesticides that differ widely in sorption properties are significantly reduced in the presence of macropore flow (Larsson and Jarvis, 1999). Likewise, Flury (1996) concluded from the transport studies of Kladivko *et al.* (1991), Traub–Eberhard *et al.* (1995) and Flury *et al.* (1995) that part of the various pesticides applied simultaneously to the soil surface moved through structured soil in an identical manner irrespective of their chemical properties.

Figure 7.1.4.3-8: **Measured concentration of glyphosate and pendimethalin in drainage water samples collected after pesticide application. A (left): Total concentration (both dissolved and particle–bound) in samples collected on either a weekly basis during the entire monitoring period (closed circles) or for every 2 mm of drainage runoff during a 13–d period in December 2005. B (right): Concentration of dissolved (triangles) and particle–bound (crosses) pesticide in samples collected for every 2 mm of drainage runoff during two selected flow events in November 2005 (black) and January 2006 (grey). Sampling periods are indicated in parentheses**



Quantitative impact of particle–facilitated transport on total leaching

Measured concentration of particle–bound pesticides were marked lower than that of dissolved pesticides, ratio between measured concentration of dissolved pesticides and particle–bound ranging between 4–14 and 1.2–30 for glyphosate and pendimethalin respectively. Intensive monitoring of two individual flow events (Figure 7.1.4.3-9) suggested that particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of the observed leaching (13–16 % of the leached mass of glyphosate and 16–31 % of the leached mass of pendimethalin). These values are in line with the few available field studies quantifying particle–facilitated transport of strongly sorbing pesticides. In Danish drainage water studies using a cut–off size of 0.7 µm, Petersen *et al.* (2003) found that 9 % of the leached pesticide was particle bound. Correspondingly, Vilholdt *et al.* (2000), using a cut–off size of 0.24 µm, found that 6 % of the leached pesticide was particle bound. In laboratory experiments with undisturbed 20–cm soil columns, de Jonge *et al.* (2000) found that particle–facilitated transport (particles >0.24 µm) accounted for <1–27 % of total glyphosate leaching. In a study by Gjettermann *et al.* (2009) using intact soil columns from ploughed and minimal tillage cultivation systems, colloid–facilitated glyphosate leaching (cut–off size >0.02 µm) accounted for 68 ± 10 % of total glyphosate leaching from the ploughed system as compared to only 17 ± 12 % from the minimal tillage system. That leaching of particle–bound glyphosate from the ploughed soil was markedly greater than that seen in our study and previous studies may be attributable to differences in experimental conditions, e.g. ploughing before or after pesticide application and precipitation intensity. In our field study the total amount of precipitation and maximum precipitation intensity were 12 mm within 11 h and 2.1 mm h^{-1} (11 January 2006) and 18 mm within 10 h and 4.6 mm h^{-1} (14 November 2005). In the study of Gjettermann *et al.* (2009) glyphosate was applied to the soil 1 d after the last of two rewettings and

the soil then irrigated twice for 2 h using 15 mm h^{-1} on days 5, 8 and 12 following the last rewetting. In Denmark such high precipitation intensity is rare during the period relevant for autumn application of glyphosate (September–November). Analysis of precipitation data collected in a national grid of approximately 60 automatic climate stations run by the Danish Meteorological Institute revealed that there had only been 8 precipitation events exceeding 15 mm h^{-1} during the preceding 10 years (Birgit Sørensen, personal communication). The combination of wet, loose soil and very intensive precipitation shortly after the application of pesticide is likely to result in greater contact between pesticide and soil particles and enable greater mobilisation of soil particles. In soils having had time to consolidate, such as the minimal tillage soil studied by Gjettermann *et al.* (2009) and in the present study (ploughed 7 months before pesticide application) fewer particles will be available for contact with the pesticide.

Figure 7.1.4.3-9: Hourly precipitation (grey hanging bars in A and E) together with turbidity (B and F), particulate (crosses) and dissolved (circles) glyphosate (C and G), particulate (crosses) and dissolved (circles) pendimethalin (D and H) in the drainage runoff (DR on the secondary axis) following flow events on 14 November 2005 (right) and 11 January 2006 (left)

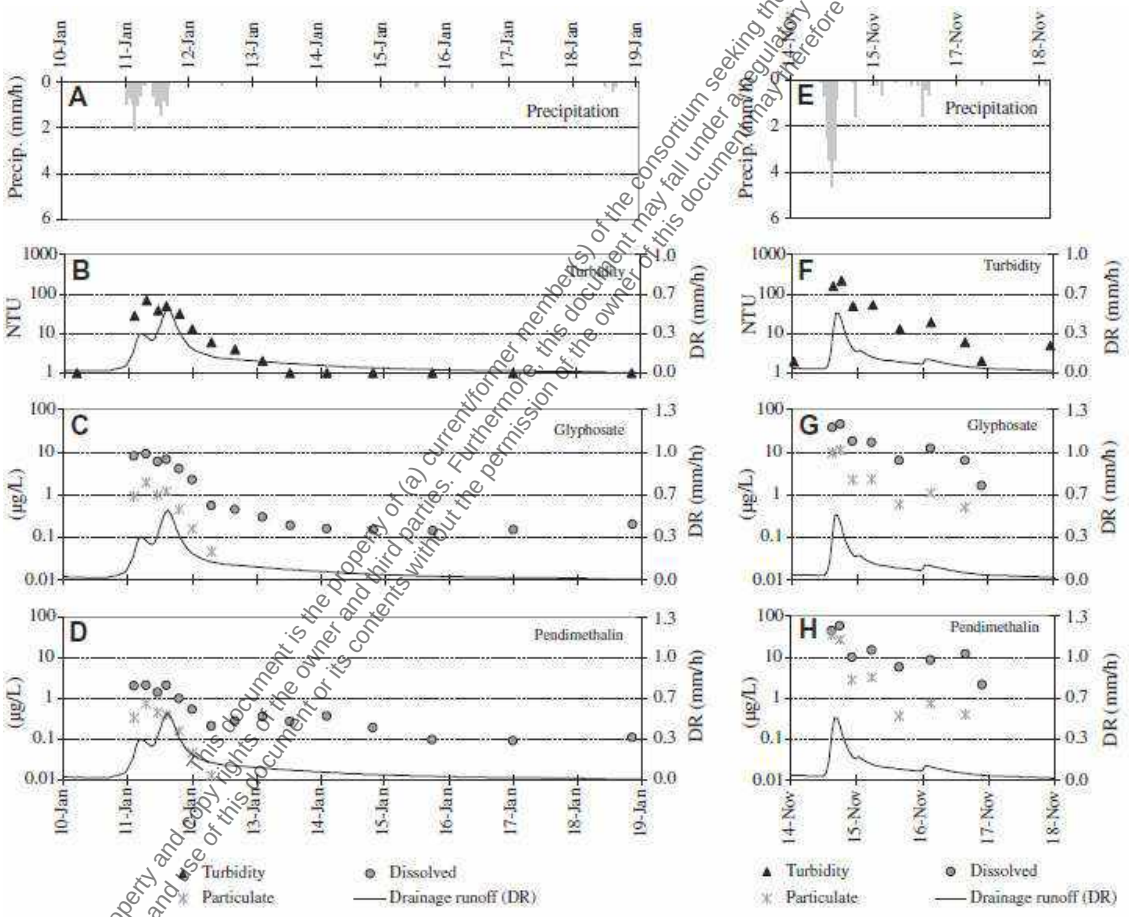
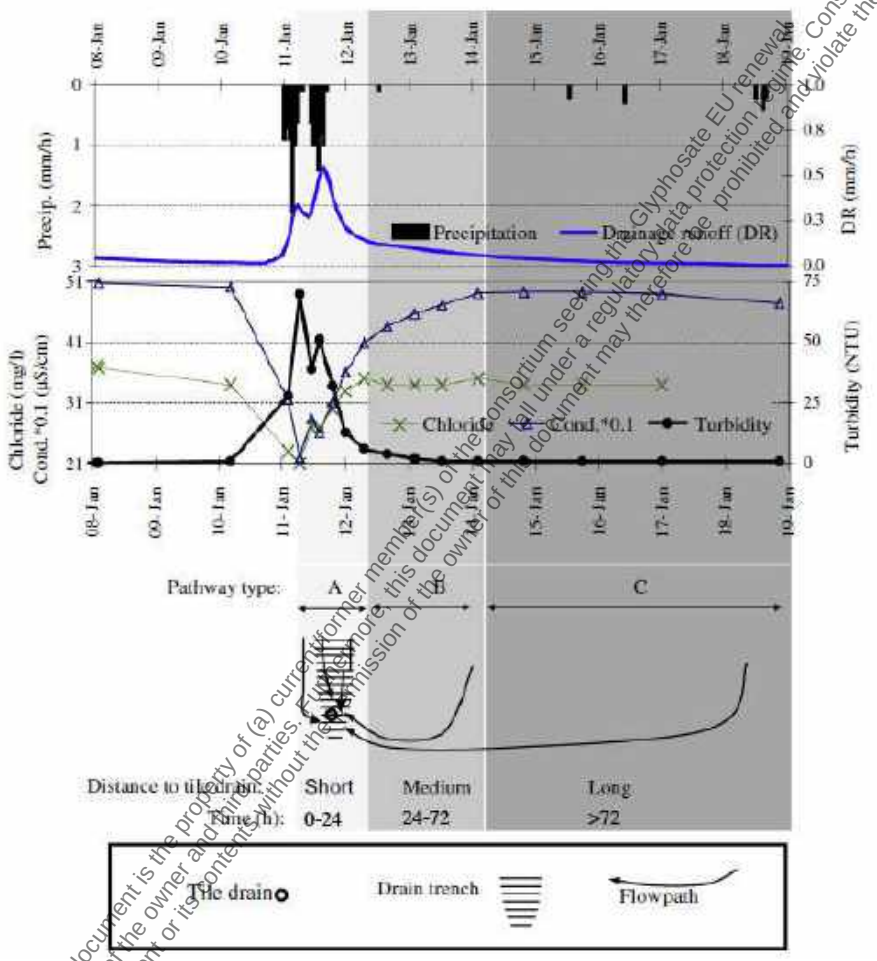


Figure 7.1.4.3-10: Hourly precipitation and drainage runoff together with measured chloride concentration, conductivity and turbidity (lower graph). The shaded areas indicate the dominant transport pathways (types A–C) feeding into the sampled drainage water during the flow event. While ‘‘time’’ and ‘‘pathway types’’ are classified directly from measured data, ‘‘distance to tile drain’’ and shown water flow pathways are indicative providing our interpretation of measured data. (see Section “*Through which pathways do strongly sorbing compounds enter the drainage system?*”)



Water flow pathways

Drainage water consists of a mixture of water of different origins, with the dominant flow pathways varying over the course of time (Jacobsen and Kjær, 2007). Knowledge of the dominant transport pathways is thus important for the interpretation of measured pesticide concentrations. The transport pathways during the flow event of 11 January 2006 are indicated by the measured turbidity, chloride concentration and conductivity (Figure 7.1.4.3-10). Thus the chloride concentration and conductivity decreased markedly during the first precipitation event, while the turbidity increased. This indicates rapid transport of precipitation with low chloride concentration and conductivity, probably through drain–connected macropores. During the 24–h period from the end of the first precipitation event the turbidity decreased, while the chloride concentration and conductivity increased. This indicates that water entered the drainage system not only from macropores connected directly to the drains but also from the vicinity of the drain pipe, i.e. the trench dug when installing the tile drain system. Transport pathways is likely also to involve a lateral component in the shallow saturated zone through natural macro pores aided by gradients generated by inter–drain mounding of the water table during the high–flow condition following the rain event

(Figure 7.1.4.3-10). Water from here would have a relative short travelling distance before entering the drainage system. These transport pathways characterised by having a short flow path to the drain and being active during the first 24–h period are designated type A (Figure 7.1.4.3-10). From 24 to 72 h the turbidity remained low and below the detection level of 1 NTU (nephelometric turbidity units), the chloride concentration plateaued out and the conductivity continued to increase. That the chloride concentration returned to the ''background level'' indicates cessation of the rapid entrance of precipitation low in chloride. Instead the length of the pathway increased with drainage water entering from in between the drain trench, designated pathway B in Figure 5. During these longer transport pathways, the particles are filtered by the soil causing the turbidity to decrease below the detection limit, while the longer retention time allows the infiltrating water to interact with the soil matrix causing the conductivity to increase. By 72 h after the end of the first precipitation event the conductivity, chloride concentration and turbidity had returned to their background levels, and the drainage water is dominated by the longer transport pathways (designated type C). These transport pathways are likely to comprise precipitation that has infiltrated vertically some distance from the drain trench and subsequently been transported laterally to the drain via the saturated layer.

Pesticide transportation pathway

During the 24–h period following cessation of the first precipitation event on 11 January 2006 the leaching pattern was similar for both particles, particle–bound pesticide and dissolved pesticide (Figure 7.1.4.3-9), thus indicating that all three follow the same transport pathways, presumably involving drain–connected macropores located above or in the vicinity of the drains or rapid lateral transport near the drain line (type A in Figure 7.1.4.3-10). Thereafter the leaching of particles and particle–bound pesticide ceased, whereas dissolved pesticide continued to leach in high concentrations (>0.1 µg/L) for up to 7 d after the precipitation had stopped (Figure 7.1.4.3-9). This ''tailing'' of dissolved pesticides indicates that the transport pathways involve transport through macropores between drains followed by lateral transport to the drains (types B and C; Figure 7.1.4.3-10). Moreover, it indicates that while particles (indicated by elevated turbidity) and particle–bound pesticide seems to be retained in the soil during the lateral transport in between the drain, dissolved pesticide can be transported laterally through the saturated zone to the drainage system. The leaching pattern following the flow event on 14 November 2005 (Figure 7.1.4.3-9) was very similar to that observed after the flow event on 11 January 2006, although sampling conditions precluded the recording of transport occurring through pathways B and C. The flow event on 11 January 2006 was characterised by high precipitation (12 mm) followed by 7 d virtually free of precipitation (1 mm in total). Such conditions are ideal for describing variation in flow pathways over time and capturing the transport involving all three pathways (A–C; Figure 7.1.4.3-10). In contrast, the flow event on 14 November 2005 was characterised by one major (18 mm on 14 November) and several minor precipitation events (6 mm in total), and sampling was performed for just 2 d (Figure 7.1.4.3-9. E–H). The conditions were ideal for descryibing transport pathway A, but inadequate for describing pathways B and C (Figure 7.1.4.3-10). The fact that turbidity remained high for a much longer period (approx. 24 h) during the November 2005 event than during the January 2006 event (Figure 7.1.4.3-9 E–H) is attributable to the minor precipitation events on 16 and 17 November 2005 and resultant rapid preferential transport of leachate via pathway A (Figure 7.1.4.3-10). The direct transport from surface layers to drains via macropores (pathway A in Figure 7.1.4.3-10) reported here is in line with previous observations. Thus several studies report that the soil surface can be in direct contact with drains through macropores comprised of old root channels or earthworm burrows (Nielsen *et al.*, 2010; Nuutinen and Butt, 2003; Shipitalo and Gibbs, 2000). The same pathways were also responsible for the leaching of colloid–size particles (Nielsen *et al.*, 2011) and the strongly sorbing pesticides (both dissolved and particle–bound) pendimethalin (Petersen *et al.*, 2003) and prochloraz (Vilholdt *et al.*, 2000) on drained, loamy soils. The observed transport pathway involving transport through macropores located between drains followed by lateral transport to the drains (pathways B and C; Figure 7.1.4.3-10) is presumably attributable to connectivity between the vertical biopores and the three–dimensional fracture system in the soil, which enables rapid, lateral transport in the soil (Rosenbom *et al.*, 2008; Nilsson *et al.*, 2000, 2001; McKay *et al.*, 1999). Studies of the transport of two fluorescent tracers in clayey till (Rosenbom *et al.*, 2008) indicate that during periods of continuous drainage runoff the extent of rapid macropore transport in the soil between the drain lines is determined by the degree of connectivity between root zone biopores and high–permeability fractures. Evidence that such connectivity enables leaching of solutes from the surface of fractured till is also provided by forced gradient tracer experiments conducted at three different locations (Ringe, Avedøre and Lillebæk) in Denmark (Nilsson *et al.*, 2000, 2001; McKay *et al.*,

1999). These transport studies were all performed with conservative or slightly sorbing tracers (chloride, bromide, bacteriophage tracer PRD–1, colloidal tracer, sulforhodamine B, and acid yellow). Similar studies addressing the potential of pathways B and C (Figure 7.1.4.3-10) to transport strongly sorbing pesticides are very limited, however. Transport of the strongly sorbing pesticides pendimethalin and prochloraz in drained structured soil has been studied by Vilholdt *et al.* (2000) and Petersen *et al.* (2003). However, the study design, while suitable for describing vertical transport from the top soil to the vicinity of the drain line (pathways A and B), was unsuitable for describing transport involving vertical infiltration between the tile drains followed by a subsequent lateral transport to the drain (pathway C). In Vilholdt *et al.* (2000), pesticide sampling was performed 2.5 m either side of the drain trajectory up to 7.5 h following a precipitation event. The study of Petersen *et al.* (2003) was conducted on a very well drained soil with limited lateral water flow (drainage runoff accounting for only 2–10 % of total precipitation input during the sampling period) with most pesticide samples being collected within 36 h of the precipitation event. Under such conditions, lateral transport of pesticides (pathway C) is unlikely. The leaching pattern found in our study indicate that while particles (indicated by elevated turbidity) and particle–bound pesticides were retained in the soil during lateral transport, dissolved glyphosate and pendimethalin were transported through the saturated zone to the drainage system. Similar findings suggesting that dissolved, strongly sorbing pesticide can be transported over long distances via discontinuities are provided by Gooddy *et al.* (2007), who studied the concentration of dissolved and particle–bound diuron and its metabolites in chalk groundwater sampled 30 m b.g.s. Most of the pesticide–colloid complexes (particles >0.1 µm) formed in the soil were removed during migration of the water through the 30 m deep, unsaturated zone and/or the saturated zone, whereas pesticides in soluble form was detectable in the groundwater 30 m b.g.s. Moreover, in a study of the transport of brilliant blue, bromide and micropheres along macropores in sandy loam, Nielsen *et al.* (2011) found that while colloid–size particles were trapped in the bottom of the biopores, dissolved tracer (brilliant blue and bromide) migrated further into the soil.

Conclusion

Pesticides leaching from the unsaturated zone may eventually pose a risk to the aquatic environment. The present 8–month study of a loamy field demonstrates that: Strongly bound pesticides, whether bound to the organic or inorganic soil fraction, may leach from the root zone and enter the aquatic environment in average concentrations exceeding 0.1 µg/L. Particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of observed leaching (13–16 % for glyphosate and 16–31 % for pendimethalin). The pathway by which these strongly sorbing compounds entered the drainage system involved transport through drain–connected macropores (above or in the vicinity of the drains) as well as the macropores situated between the drains and connected to underlying fractures. Particle–bound pesticide (particles >0.24 µm) was transported solely by vertical transport in macropores and rapid lateral transport occurring nearby the drain line, whereas dissolved pesticide was also transported laterally over larger distances through the saturated zone via discontinuities in the soil. This newly identified transport pathway whereby dissolved pesticides are transported laterally via discontinuities in the soil needs to be taken into account when assessing the risk posed by pesticides to the aquatic environment. Our findings imply that strongly sorbed pesticides such as glyphosate and pendimethalin may be present in high concentrations (>0.1 µg/L) in both the water flowing from the drainage system and in the shallow groundwater located at the depth of the drainage system.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a leaching experiment with glyphosate and pendimethalin in a Danish tile-drained agricultural soil over eight months. The substance properties are sufficiently reported. Pesticide leaching from the unsaturated soil zone may occur as particle-facilitated transport via drain-connected macropores as lateral flow with strongly bound pesticides. With regard to the data requirement, the study is too short for a comprehensive evaluation of the leaching behavior. In addition, no residues were determined in different soil layers after finalization of the study, and sample storage stability prior to analysis was not established. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.1.4.3/005
Report author	Candela, L. *et al.*
Report year	2010
Report title	Glyphosate transport through weathered granite soils under irrigated and non–irrigated conditions–Barcelona, Spain
Document No	DOI 10.1016/j.scitotenv.2010.03.006 E-ISSN 1879-1026
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (Experimental conditions not sufficiently described to evaluate validity of the results)

2. Full summary

The transport of Glyphosate ([N–phosphonomethyl] glycine), AMPA (aminomethylphosphonic acid, CH_6NO_3P), and Bromide (Br^-) has been studied, in the Mediterranean Maresme area of Spain, north of Barcelona, where groundwater is located at a depth of 5.5 m. The unsaturated zone of weathered granite soils was characterized in adjacent irrigated and non–irrigated experimental plots where 11 and 10 boreholes were drilled, respectively. At the non–irrigated plot, the first half of the period was affected by a persistent and intense rainfall. After 69 days of application, residues of Glyphosate up to 73.6 µg/g were detected till a depth of 0.5 m under irrigated conditions, AMPA, analyzed only in the irrigated plot was detected till a depth of 0.5 m. According to the retardation coefficient of Glyphosate as compared to that of Br^- for the topsoil and subsoil (80 and 83, respectively) and the maximum observed migration depth of Br^- (2.9 m) Glyphosate and AMPA should have been detected till a depth of 0.05 m only. Such migration could be related to the low content of organic matter and clays in the soils; recharge generated by irrigation and heavy rain, and possible preferential solute transport and/or colloidal mediated transport.

Materials and methods

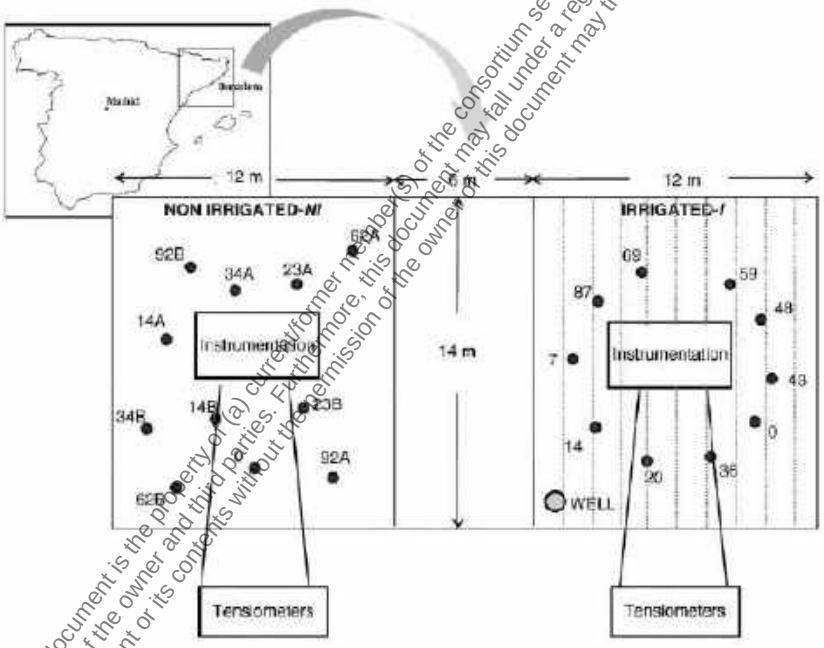
Experimental site

The experimental site was located in a narrow coastal strip composed of weathered granite in the IRTA agricultural station of the Maresme region, located 30 km North of Barcelona– Spain (Figure 7.1.4.3-11). The area, under no–tillage farming, was only covered by a wheat crop to protect the soil from erosion for

more than ten years. Groundwater was at a depth of 5.5 m; the hydrology of the study site has been described in detail by Guimerà *et al.* (1995).

Two individual plots of approximately 168 m^2 each, separated by a control area of 84 m^2, were selected. Initially, the weeds covering both plots were manually removed to allow installation of the irrigation and vadose zone monitoring equipment. Subsequently, the wheat cover was allowed to redevelop, prior to herbicide application. The upward–downward flux of water in the unsaturated zone was monitored by 7 tensiometers (Soilmoisture®). At the beginning of the experiment duplicate tensiometer sets were installed by manual drilling, in the middle of the plots, at a depth of 0.30, 0.60 and 0.90 m 106 and one tensiometer was installed at a depth of 1.20 m (Figure 7.1.4.3-11). Instrumentation remained in place until the end of the experimental activities.

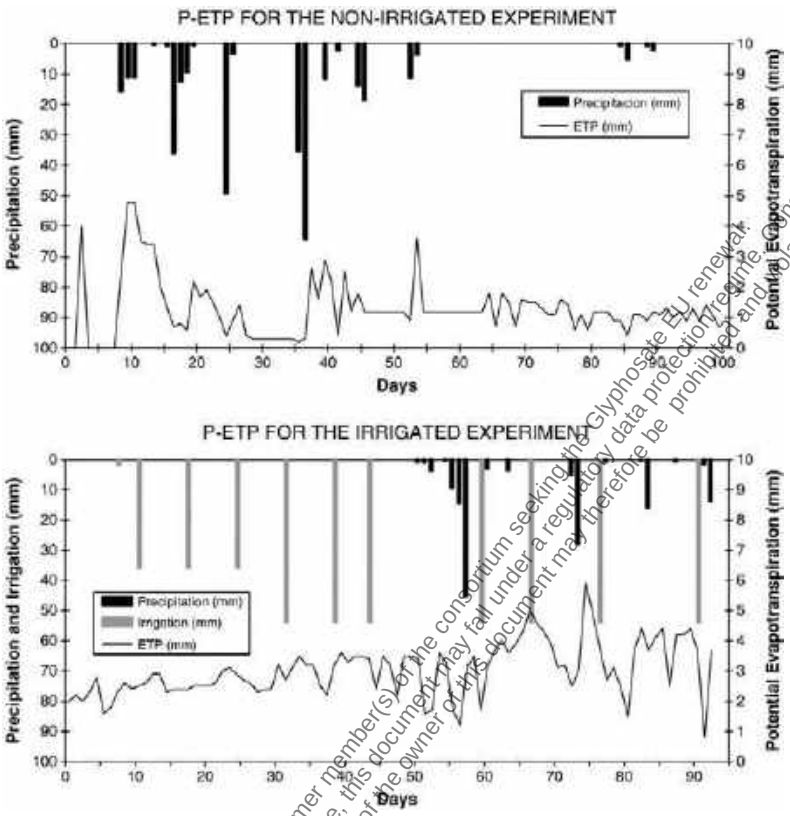
Figure 7.1.4.3-11: Study area location and non–irrigated (NI) and irrigated (I) experimental plots. The location of in situ field instrumentation and drillings is shown for the two sites; 0 denotes location of background drillings; duplicate drillings for *NI* are denoted as A and B (e.g., 1A and 1B). The vertical dashed lines (in *I*) denote the location of soak bands. The location of a groundwater well is also shown



For initial characterization of the unsaturated zone profile, before starting the experiments, two boreholes were drilled (location denoted as "0" in the non–irrigated–*NI* and irrigated–*I* plots) and undisturbed soil samples were taken down to 4.50 m. In both plots soil matrix characterization and the monitoring of pore water content was performed by destructive sampling. The amount of pesticide in the vadose zone at given times was determined in undisturbed soil sampling. Groundwater quality was monitored in an existing pumping well in plot *I* (Figure 7.1.4.3-11).

Precipitation amounts for both experimental periods were provided by the IRTA meteorological station. Irrigation was based on soak bands that were installed in the *I* plot (Figure 7.1.4.3-11) with a separation of 0.30 m in order to obtain a uniform spatial distribution of water. Two irrigation doses of 36 mm per week, were applied during two hours for the first three weeks of the study period (March I-14 to I-27). Subsequently, the amount of irrigation increased to 53 mm per week (Figure 7.1.4.3-12).

Figure 7.1.4.3-12: Precipitation and evapotranspiration during the non-irrigated (September–December) and irrigated (February–June) experiments



Application of Glyphosate and bromide
Both Glyphosate (*Roundup*®, 36 % p/v, Monsanto Europe S.A.), and bromide (NaBr; conservative tracer) were applied under non–irrigated (*NI*) and irrigated (*I*) conditions (Figure 7.1.4.3-11). The first field experiment, non–irrigated, was conducted during the rainy season (September to December, 1994) and sampling and monitoring activities extended over 92 days. During the second field experiment, irrigated, that lasted 87 days, the area was irrigated from February to May (1995).

Glyphosate, along with a solution of NaBr, was applied on the soil surface on September 13 on the *NI* plot and on March 7 on the *I* plot using an automated spray system to ensure uniformity. The pesticide and bromide solutions were prepared at the study site before application. The concentrations of BrNa solutions for the *NI* and *I* plots were of 20 g/L and 17 g/L of BrNa respectively. Glyphosate solution was prepared by mixing 400 cm^3 and 420 cm^3 of a commercial 36 % (p/v) Glyphosate EC formulation with 20 and 21 L of groundwater for the *NI* and *I* plots, respectively. This procedure of pesticide application follows standard agricultural practice in the Maresme area.

Vadose zone soil and water sampling methodology
Soil samples were obtained with a hollow–stem auger after pesticide and bromide application in both field plots. A random sampling scheme with duplicate soil cores was applied in the non–irrigated area (Figure 7.1.4.3-11; *NI*–0(A,B) to 92 (A,B)) where undisturbed soil cores were taken at 0.20 m intervals till a depth of 1 m, and at 0.50 m intervals below it (Figure 7.1.4.3-11). Due to field and experimental constraints a circular sampling pattern and single cores, where undisturbed soil samples were obtained at 0.20 m intervals, was applied in the irrigated experimental plot (Figure 7.1.4.3-11; *I*–0 to 69). To prevent possible contamination from overlying layers, two samples of the soil to be analyzed were taken from the inner part of each core, after discarding the top and the bottom portions of it. One sample was carefully wrapped in aluminum foil, and frozen until pesticide and Br laboratory analyses. The other one was used

for the determination of volumetric water content, saturated and unsaturated hydraulic conductivity, and bulk density (following ASTM 1993 standards) clay content and clay type (RX diffraction) and organic matter content. Also pH, CEC and Al and Fe oxides were determined in samples following standard techniques described in Melo (1996) and Candela *et al.* (2007).

During the field experiments (Table 7.1.4.1.1-19 and Table 7.1.4.1.1-20), groundwater samples, soil cores and soil–water potential measurements from the tensiometers were obtained after each rain or irrigation episode. Groundwater samples were obtained with a bailer from the existing open well where also the depth of the water table was monitored. Due to analytical constrains, the concentration of AMPA was monitored in the irrigated plot only.

Table 7.1.4.3-19: Sampling dates and precipitation amounts for the non–irrigated (NI) experiment conducted in 1994 (September–December)

Sampling survey	NI-0 Background	NI-14	NI-23	NI-34	NI-62	NI-92
Drilling date	Sep 6[a]	Sep 27	Oct 6	Oct 17	Nov 14	Dec14
Precipitation (mm)[b]	–	52.6	110.5	49.1	7.2	–
Days after glyphosate and NaBr application[c]	−7	14	23	34	62	92

[a] September 6 (*NI-0*), soil profile characterization.
[b] Cumulative values for the time interval between sampling. Total precipitation: 219.4 mm.
[c] Glyphosate and NaBr application on September 13.

Table 7.1.4.3-20: Sampling dates and precipitation amounts for the irrigated (I) experiment conducted in 1995 (February–June)

Sampling survey	I-0 background	I-7	I-14	I-20	I-36	I-43	I-48	I-59	I-69	I-87
Drilling date	Feb 28[a]	Mar 14	Mar 21	Mar 27	Apr 12	Apr 19	Apr 24	May 5	May 15	Jun 2
Irrigation mm/week[b]	36	36	36	53	53	53	53	53	53	53
Precipitation (mm)[b]	–	–	–	–	–	0.7	4.7	75.3	32.8	32.9
Days after glyphosate and NaBr application[c]	−7	7	14	20	36	43	48	59	69	87

[a] February 28 (*I-0*), soil profile characterization.
[b] Total irrigation: 479 mm. Total precipitation: 146.4 mm. Precipitation (January–March 15) 8.6 mm.
[c] Glyphosate and NaBr application on March 7.

The total length of the sampled soil cores in each survey was determined according to: (a) the depth of penetration of water through the unsaturated zone as predicted from in situ tensiometer readings, (b) the hydraulic conductivity of soil samples as determined in the laboratory, (c) the predicted theoretical depth reached by the center of mass of Br^-, and (d) the retardation factor, R (Ghodrati and Jury, 1992) of glyphosate as determined in batch experiments for soils and sediments of the area. However, as a safety measure, soil drillings and sampling depths were always greater than the calculated theoretical depth of penetration of Glyphosate.

Chemical analyses

Chemical analysis of glyphosate and AMPA residues in soil and water samples was performed using an HPLC method (Hewtlett Packard, HPLC ChemStation G1034A) based on reversed–phase chromatography, with flourescent detection using pre-column derivatization with FMOC (9–fluorenylmethylchloroformate) to give the flourescent derivative. The liquid chromatography coupled column (LC–LC) methodology described by Sancho *et al.* (1996) was used to confirm the presence of glyphosate and AMPA residues in positive samples. The LC–LC technique presents several advantages, such as improved sensitivity, selectivity, and sample throughput. The detection limit of glyphosate and AMPA was 6 ng/g and 4 ng/g for soil, and 0.15 µg/L and 0.1 µg/L for water samples respectively, with extraction efficiency greater than 95 % for both analytes. Bromide content was determined by ionic chromatography (VYDAC column) and the detection limit was 0.1 ng/g.

Results

Soil properties

The soil profile, a Typic Xerorthent (Soil Survey Staff, 1999), is very homogeneous and consists of medium to coarse sand size with low clay content (clay, 5 %; silt, 20 %; sand, 75 %). The clay fraction is mainly composed of smectite, illite and kaolinite. The soil had no visual structure except for the presence of a coarse sand layer at 1.50–1.90 m and granite debris at a depth of about 4.50 m. However, according to physico–chemical properties a top soil layer and a subsoil horizon may be distinguished. The soil chemical properties determined from samples at a depth of 0–0.20 m (top soil) and 0.70–1 m (subsoil horizon) respectively, are: cation exchange capacity (CEC), 5.2 and 4.6 meq.100/g; pH (1:1 in H_2O), 7.9 and 7.3; organic matter 1.1 and 0.09 (%); P 0.2 mg.100/g (top soil), total Fe_2O_3, 1.92 and 5.43 g.100/g; and total Al_2O_3 1.75 and 7.22 g.100/g. Average values of soil bulk density from field samples were 1.65 and 1.7 g/cm^3 for the top soil and subsoil, respectively. Residues of Glyphosate and Br were not detected along vadose zone profile before the experiments (*I*–0 and *NI*–0; Figure 7.1.4.3-13 and Figure 7.1.4.3-14).

Figure 7.1.4.3-13: Volumetric content of water, bromide and glyphosate in the different soil profiles for the non–irrigated plot (September–December 1994). *NI*–0: soil profile prior to pesticide and bromide application. The high water content level (0.20–0.14 cm^3/cm^3) at a depth of 1.5 m reflects the presence of a coarse sand layer (LoD: 6 ng/g Glyphosate; 4 ng/g AMPA; 0.1 ng/g Br)

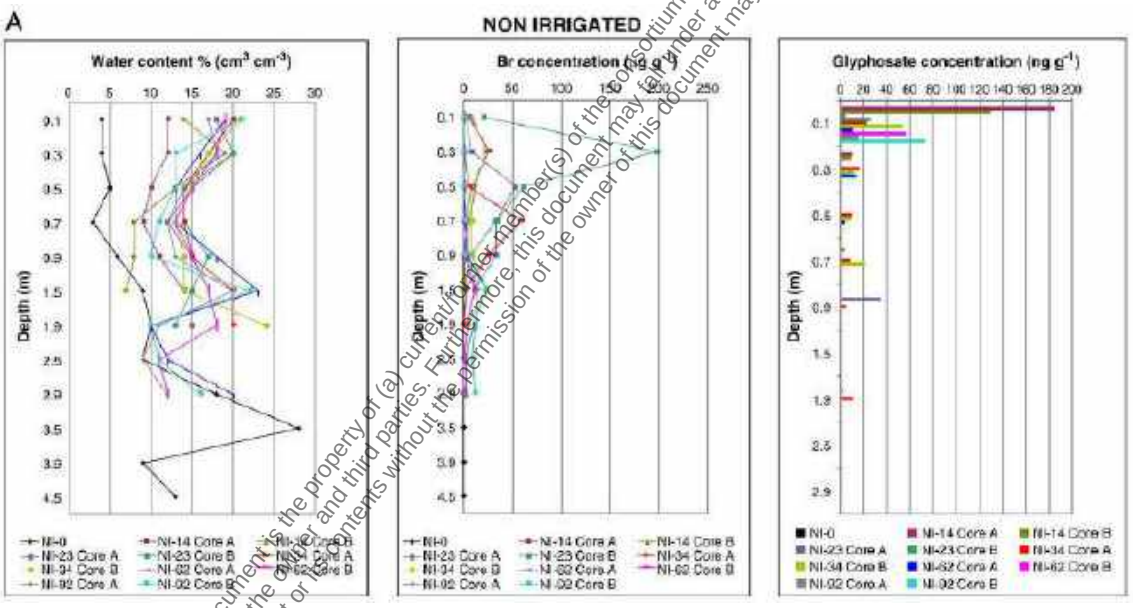
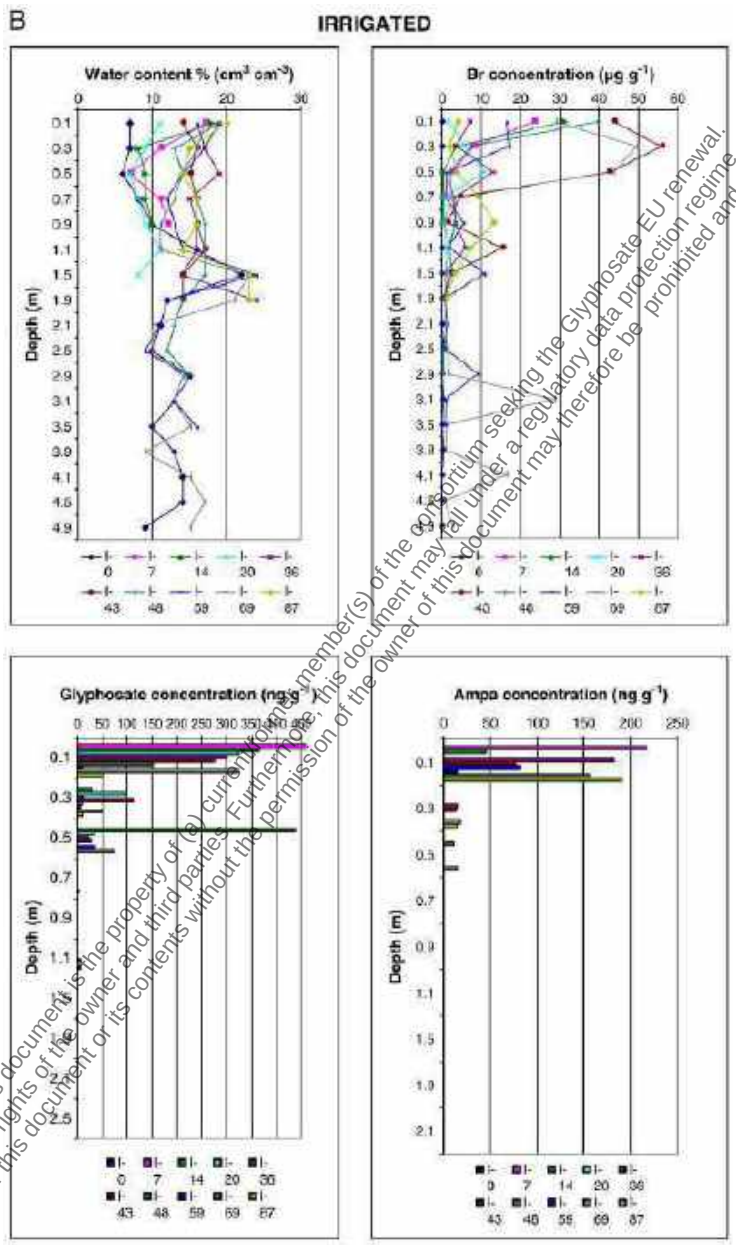


Figure 7.1.4.3-14: Volumetric content of water and bromide in the different soil profiles for the irrigated experiment (March–June 1995). *I*–0: soil profile prior to pesticide and bromide application. The high water content level (0.22–0.15 cm^3/cm^3) at a depth of 1.5 m reflects the presence of a coarse sand layer. (LoD: 6 ng/g Glyphosate; 4 ng/g AMPA; 0.1 ng/g Br)



Non-irrigated plot

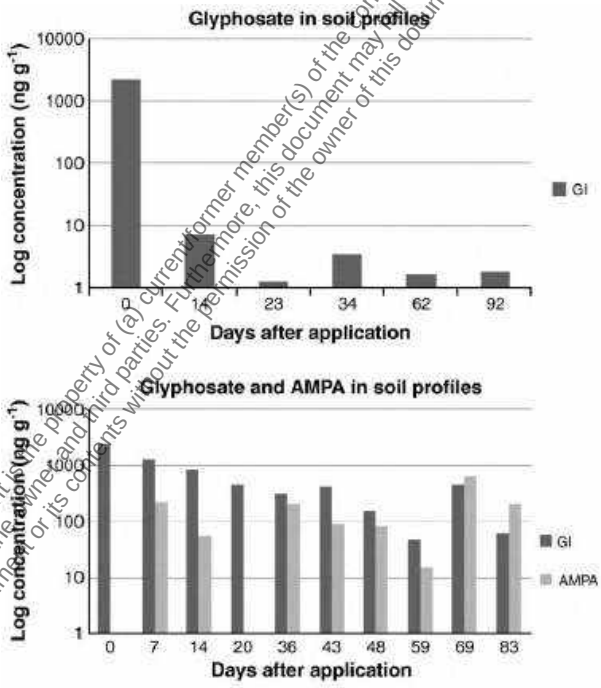
During the non–irrigated experiment the average temperature was 14.7°C, rainfall accounted for 219.4 mm, and more than 50 % of the total precipitation (163 mm) was due to three storm events in September and October. For this same time period, evapotranspiration was 118.9 mm (see Figure 7.1.4.3-12). In this plot the water content till a depth of 1.5 m was extremely low (6 % background average) due to lack of precipitation and high temperature during the summer period (*NI*, Figure 7.1.4.3-13). After the first rain event, a week after Br and pesticide application, the movement of the wetting front is clearly observed (*NI*-14). In the upper 1.50 m the water content increases up to 20 % at the end of the experimental period

and soil–water content seems to stabilize after 34 days (*NI*–34). The greatest water content along the profile was observed in the coarse sand layer at 1.5 m depth.

Maximum concentration of Glyphosate in the unsaturated zone were detected at a depth 0–0.30 m, except for *NI–23A* and *NI–34B* where residues were also detected at a depth of 0.9 and 0.7 m, respectively. The depth of penetration in individual cores varied widely. Glyphosate residues were also detected along the unsaturated zone, at concentration below the detection limit (LoD), up to a depth of 0.90 and 1.90 m after 23 and 34 days of application. After 14 days, the residual amount was 7 % of the total applied mass. After 23 days and till the end of the experiment, residual amounts account for 1 %. Glyphosate half–life (or half concentration time) calculated from in situ experimental values was 7 days, although it may be even lower considering that the first sampling campaign was undertaken after 14 days of pesticide application.

Figure 7.1.4.3-14 presents the amount of pesticide remaining in the soil profile till the end of the experiment for each core and sampled borehole. Mass estimation refers to the initial applied dose. A rapid initial dissipation phase, followed by a slower one is observed after 23 days. Degradation rate, estimated from logarithmic pesticide concentration vs. time (best fit equation) was 1.52 days. However, the small value of the correlation coefficient obtained (R^2 = 0.4) indicates the low accuracy of the calculations and the associated uncertainty.

Figure 7.1.4.3-15: Residual mass of glyphosate and AMPA remaining in soil profile as a function of time. Non–irrigated and irrigated experiment



Irrigated plot

In the irrigated plot experiment carried out during springtime, the total amount of water applied was three times higher than that of the NI plot as precipitation accounted for 146.4 mm and irrigation for 483 mm. The average temperature was of 12.3°C, and evapotranspiration (266.6 mm) was greater than in the non–irrigated experiment. The background average water content in the soil profile up to 1.5 m was 10.9 %. From *I–36* (when the irrigation dose is increased), until the end of the experiment the soil profile water content is quite constant (Figure 7.1.4.3-14, water content). The increase in water content at 1.50–1.90 m is due to the presence of a coarse sand layer.

As shown in Figure 7.1.4.3-14, maximum concentration of Glyphosate was always detected between the

first 0–0.5 m of the soil profile and concentration values were greater than those found in the non–irrigated plot. Residues of Glyphosate (below LoD) were still found at 1.50 m after 69 days of application and continued to be detected after 87 days. Residual amount of Glyphosate in soil profile after 14 days was 34 % of the applied dose, being reduced to 2 % after 59 day, and up to the end of the experiment (Figure **7.1.4.3-15**). Field half–life (or half concentration time) was around 7 days and estimated degradation rate was 0.04 days ($R^2 = 0.6$).

Glyphosate and bromide were not detected in groundwater samples obtained with a bailer along all the monitoring periods.

Discussion

For the non–irrigated experiment (NI) Br concentration along the soil profile was clearly affected by the rain episodes, and was detected up to a depth of 150 cm after 14 days of application, implying a flow velocity of 10 cm/day calculated according to Burns (1975). The observed deficit at *NI–14* profile (55 % recovery of applied dose) could be attributed to the uptake of bromide by plants (Kung, 1990a). After decomposition of plant residues, bromide may return to the soil and can be accounted for as an external input in the bulk mass balance. In the irrigated plot (Figure 7.1.4.3-14) tracer distribution over depth is fully controlled by irrigation dose, and Br^- concentration presents lower variability.

As shown in the results of both field experiments, concentrations of Glyphosate were detected, much deeper than expected according to the distribution coefficients calculated for the surface soil ($K_f = 93$), and subsoil ($K_f = 154$) in batch experiments (Melo, 1996; Candela *et al.*, 2007). The retardation factor (R, Ghodrati and Jury, 1992) of Glyphosate, as compared to that of Br for the topsoil and subsoil, is 80 and 83, respectively. Considering a worst–case scenario (R = 80) and the maximum migration depth of Br (2.90 m; and 4.90 m Figure 7.1.4.3-13, Figure 7.1.4.3-14), then, the maximum transport depth of Glyphosate should have been 0.05 m only. We hypothesize that the deep transfer of both glyphosate and AMPA can be the result of: (a) preferential transport along the unsaturated zone (Kung, 1990b; Van den Bosch *et al.*, 1999; Scorza *et al.*, 2004; Coppola *et al.*, 2009), and/or (b) colloidal–mediated transport of both components (Vereecken, 2005; Borggaard and Gimsing, 2008), a process that can be inferred from their relatively large K_f values.

The mobility of strongly adsorbing compounds as Glyphosate (Veiga *et al.*, 2001; Kjaer *et al.*, 2005; Vereecken, 2005, among others) has already been shown for pesticides such as propiconazole and fempropimorph (Krongvang *et al.*, 2004), regardless of how strongly they were found to be adsorbed under equilibrium conditions in the laboratory. For the two experiments reported here, the observed differences in soil profile distribution, and rate of degradation are probably conditioned by climatic factors prevailing during the experiments (autumn and springtime), agricultural practices (dryland–irrigated), inherent variability of soil spatial parameters, land cover and roughness of soil surface.

At the *NI* experiment the presence of glyphosate at greater depth than expected may be the consequence of rainfall events. In the *NI–34* profile, Glyphosate was detected along all the sampled profile showing a high concentration (20.3 ng/g) at 0.70 m although according to batch experiments (Candela *et al.*, 2007), after 34 days the pesticide should have been retained in the upper part of the soil. The high precipitation registered immediately after pesticide application could induce a rapid flux of water through the unsaturated zone, inhibiting adsorption onto soil particles. This process could be favored by the amount of Glyphosate available and the initial low water content in soil before rain which could promote the existence of preferential solute transport. In sandy soils with no visible structure in the top 1 m, preferential flow appears to be dependent on soil moisture and water flow tends to be channeled through low moisture zones. This effect has been observed by Kladivko *et al.* (1999) and Nolan *et al.* (2008). Previous laboratory soil column experiments carried out with the same soils and pesticide demonstrated the importance of non–equilibrium sorption under flow conditions. Mass loss is larger for longer residence times associated either to low pore–water velocity or long soil column lengths.

Mobility of AMPA is lower than Glyphosate and residues were only detected in the 0–0.30 m interval. Considering the molecular weight of both compounds, a 0.6 ratio glyphosate/AMPA concentration in soil and water samples could be expected. However, AMPA concentrations detected in soil samples only accounted for 15 % of glyphosate degradation (Figure 7.1.4.3-15). A slower glyphosate/AMPA

transformation over time, or even AMPA degradation could explain the missing amount of herbicide. The analysis on dissipation of Glyphosate and AMPA formation was not the objective of this research and the available data are not sufficient to assess the importance of biological and chemical transformation of Glyphosate. Analysis of AMPA formation (0.08 days according to best fit equation) are highly uncertain due to the low correlation coefficient obtained ($R^2 = 0.295$).

Very little is known about the nature and kinetics of this process (Grunewald *et al.*, 2001), therefore, to gain insight into it, soil microbiological activity and the fast mineralization of both Glyphosate and AMPA should be the subject of future research.

Based on the non–reacting behavior of Br and the reduced mobility of pesticide induced by adsorption, estimation of glyphosate percentage found 3 times deeper than predicted, calculated following the Ghodrati and Jury (1992) approach, would account for 18 % and 28 % for the non–irrigated and irrigated areas, respectively (Table 7.1.4.1.1-21). Note that in the non–irrigated area the transport of the pesticide is clearly influenced by the two rain events (NI–34 and NI–62; Figure 7.1.4.3-12), a phenomenon not observed in the irrigated plot where water infiltration is mainly conditioned by continuous irrigation.

Table 7.1.4.3-21: Percentage of glyphosate found three times deeper than predicted (ZG) for the different soil profiles considering achievement of equilibrium adsorption

Non irrigated (NI)			Irrigated (I)		
Profile	$3Z_G$ (cm)	PF (%)	Profile	$3Z_G$ (cm)	PF (%)
NI-14A	0.6	5	I-7	0.2	0
N1-14B	0.6	9	I-14	0.1	56
NI-23A	1.0	0	I-29	0.6	28
NI-23B	1.0	0	I-36	0.2	10
NI-34A	1.4	64	I-43	0.6	33
NI-34B	2.4	43	I-48	0.2	5
NI-62A	3.0	62	I-59	0.6	82
NI-62B	3.0	0	I-69	0.6	28
NI-92A	3.4	0	I-87	1.3	15
NI-92B	3.8	9			
Average (%)		18	Average (%)		28

Z_G : theoretical depth to the center of mass of the pesticide (Ghodrati and Jury, 1992). PF: preferential flow; $Z_G = Z_{Br}/R$ where R is the retardation factor and Z_{Br} the depth reached by the center of mass of a pulse of the conservative tracer (Br). Center of mass depth of tracer (Z_{Br}) was estimated at each sampling profile (see Fig 3).

Although in both plots detectable amounts of tracer and pesticide have been found in the soil profile, direct comparison of results is not possible as they are conditioned by climatic parameters and water application regime. The experiment under non–irrigated conditions was undertaken in autumn; the most important aspect of the precipitation pattern is its concentration in a few unevenly distributed events of heavy storms, characteristics of the Mediterranean environment. Evapotranspiration presents a decreasing trend and water content in soil profile was low at the beginning of the exercise. For the irrigated experiment, spring climatic conditions prevail and evapotranspiration is much higher than in autumn. The weekly applied irrigation dose controls water content in soil presenting a more uniform distribution along the soil profile and experimental period.

As far as the authors are aware, such deep penetration of Glyphosate has not been reported from field studies for granite soils, such as the studied ones in the Maresme area of Spain.

Conclusion

Glyhosate is commonly considered a pesticide strongly sorbed on soils, presenting a low risk for groundwater pollution due to the phosphonate functional group strong adsorption to clay minerals, Fe and Al–oxides and OM according to laboratory experiments. A problem is whether pesticide parameters measured in the laboratory are representative for predicting pesticide behaviour under field conditions. Field investigation and monitoring of pesticide leaching present the complexity of profiling pesticide concentration in soil and the difficulty of sampling pesticide migration through preferential flow paths.

As shown in the field experiments described above, Glyphosate deep leaching in a weathered granite soil profile was observed under natural field conditions regardless of the irrigated or non–irrigated conditions and climatic season. Laboratory miscible displacement experiments performed with the same soils showed that Glyhosate adsorption in soils is essentially a kinetic process and depends on the pore water velocity and residence time of soil solutions. If flow velocities are slow and enough time is given to react with the soil matrix, surface complexation and precipitation takes place. Complexation with iron and aluminum oxides, transition metals or alkaline–earth metals has been reported in literature (Sprankle *et al.*, 1975; Vereecken, 2005). Since Glyphosate adsorption is not an instantaneous process, needing time to attain equilibrium conditions, under heavy rain or irrigation just after its application on soil surface, it could leach more than predicted.

Given the typical conditions of the Maresme region vadose zone, highly permeable medium–coarse sand with low organic matter and clay content and containing Al, Fe, oxides and hydroxides, the principal mechanism affecting Glyphosate transport through the vadose zone may not be chemical equilibrium with the solid matrix alone. At field scale two possible explanations accounting for physical non–equilibrium will be of decisive importance on the transport of pesticide through the vadose zone. Since the phosphate compound of the molecule can be strongly adsorbed by Al, Fe, oxides and hydroxides, organic matter and humic acids of colloidal size, transport of colloid–bound Glyphosate and AMPA and preferential flow pathways driven by rainfall events or water application dose is likely. Presence of Glyphosate residues below detection level at depth up to 1.10 and 1.9 m in the irrigated and non–irrigated plot suggests that the pesticide may migrate into deep soil layers. This observation emphasizes the potential risk of Glyphosate transport to groundwater.

At field scale, the half–life was found to be shorter than 7 days in both experiments, much shorter than values reported in the literature (47 days average). This can be attributed to the fact that under field conditions a multitude of factors and processes contribute to herbicide disappearance, while laboratory studies are generally designed to study one of these processes. It is important to note here that these results are conditioned by the low correlation coefficient obtained (best fit equation). From the limited information obtained during the experimental study, AMPA accumulation in soil from pesticide degradation accounts for 15 % of the initial herbicide application. The parent compound transformation rate is always superior to the above–mentioned rate, leading to the conclusion that Glyphosate/AMPA transformation is a slow process or rapid AMPA degradation has occurred. Whether the transformation of the herbicide during the course of the experiment is basically controlled by chemical or biochemical processes is unknown and more research is needed regarding microbiological transformation.

3. Assessment and conclusion

Assessment and conclusion by applicant: The article describes a leaching experiment with glyphosate in an agricultural area in Spain. Leaching over a period of several months in spring and in autumn was observed under irrigated and un–irrigated conditions. Glyphosate and AMPA were found in deeper soil layers than expected from the calculations based on a tracer experiment. Two possible explanation given were colloid-facilitated transport of glyphosate adsorbed by Al, Fe, oxides, hydroxides, organic matter and humic acids on one hand and preferential flow pathways driven by rainfall events or water application dose on the other hand. Without direct comparsion of the timing and magnitude of the tracer in the actual field experiment the relevance of both processes cannot be assessed. Duration of the study was not long enough to evaluate the leaching behavior for a long–time perspective. Details regarding field sampling and sample handling practices and analysis are not sufficient to classify the study as fully reliable. The article is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

CA 7.2 Fate and Behaviour in Water and Sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

The hydrolysis of glyphosate was investigated in two studies with glyphosate which are considered valid to address the data point ([redacted] 1993, CA 7.2.1.1/004 and [redacted] 1990, CA 7.2.1.1/007). Furthermore, five studies provide supportive information ([redacted] 1995, CA 7.2.1.1/002, [redacted], 1993, CA 7.2.1.1/003, [redacted] 1992, CA 7.2.1.1/005, [redacted] 1991, CA 7.2.1.1/006 and [redacted] 1983, CA 7.2.1.1/009).

Glyphosate was found to be hydrolytically stable in sterile buffer of pH 5, 7 and 9 in the valid studies at 50°C with <10 % degradation after 29 days. Additionally, glyphosate was also hydrolytically stable in sterile buffer at pH 4, 7 and 9 at 25 °C. No degradation products were detected. The observations of the supportive studies are in line with these results.

In the scientific literature review for glyphosate (2010-2019), no article was identified to provide further information relevant to the data point.

Table 7.2.1.1-1: Hydrolytic degradation studies

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.2.1.1/001	Anonymous, 1995	Hydrolysis	Glyphosate	Invalid	Report not available
CA 7.2.1.1/002	[redacted] 1995	Hydrolysis	Glyphosate	Supportive	
CA 7.2.1.1/003	[redacted] 1993	Hydrolysis	Glyphosate	Supportive	
CA 7.2.1.1/004	[redacted] 1993	Hydrolysis	Glyphosate	Valid	
CA 7.2.1.1/005	[redacted] 1992	Hydrolysis	Glyphosate	Supportive	
CA 7.2.1.1/006	[redacted] 1991	Hydrolysis	Glyphosate	Supportive	
CA 7.2.1.1/007	[redacted] 1990	Hydrolysis	Glyphosate	Valid	
CA 7.2.1.1/008	[redacted] 1990	Hydrolysis	Glyphosate	Invalid	Report not available
CA 7.2.1.1/009	[redacted] 1983	Hydrolysis	Glyphosate-trimesium	Supportive	
CA 7.2.1.1/010	[redacted] 1978	Hydrolysis	Glyphosate	Invalid	

1. Information on the study

Data point:	CA 7.2.1.1/001
Report author	Anonymous
Report year	1995
Report title	Stability in water
Report No	R 500, WAS95-00282
Document No	
Guidelines followed in study	OECD 111
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No information available
Short description of study design and observations:	No information available
Short description of results:	Glyphosate is stable in water at 55°C.
Reasons why the study is not considered relevant/reliable or not considered as key study:	The notifier has no access to this study report and information in the monograph is limited to the results presented here. The study was not accepted in the monograph.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 7.2.1.1/002
Report author	[redacted]
Report year	1995
Report title	Determination of the hydrolysis of Glyfosaat
Report No	141784
Document No	
Guidelines followed in study	EEC A 7
Deviations from current test guideline	From OECD 111: - Basic data is missing (preparation of buffers, application procedure) - Number of test solutions is unclear - Study duration was only 5 days
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
The hydrolysis of glyphosate was determined in sterile aqueous buffer solutions at pH 4, 7 and 9 at an application rate of 130 - 248 mg/L and incubated at 50.0 ± 0.5 °C in the dark for 5 days in maximum.

Glyphosate concentrations after 5 days of incubation were 100 %, 97 % and 97 % of applied amount in buffers at pH 4, 7 and 9.

Glyphosate is hydrolytically stable under the conditions of the test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate (dutch term: Glyfosaat, non-labelled)
Lot No.:	22022
Chemical purity:	99 %

2. Buffers:
The following buffer solutions were prepared:

- sterile 0.05 M acetate buffer with pH 4: sodium acetate was combined with acetic acid in Milli-Q water
- sterile 0.05 M phosphate buffer with pH 7: potassium dihydrogenphospate was combined with sodium hydroxide in Milli-Q water
- sterile 0.05 M borate buffer with pH 9: boric acid was combined with potassium chloride and sodium hydroxide in Milli-Q water

B. STUDY DESIGN

1. Experimental conditions
Two standard solutions of glyphosate were prepared in aqueous solution at a concentration of 130 - 248 mg/L (n=2). After sonication, solutions were analysed without further pre-treatment.

An amount of approximately 20.0 mg glyphosate was added to 50.0 mL buffer solutions at pH 4, 7 and 9. After sonication, solutions were filter-sterilised through a 0.2 µm membrane filter and transferred into sterile glass vessels. To exclude oxygen, nitrogen gas was bubbled through each solution for approximately 5 minutes. Each test vessel was tightly sealed with a septum-crimcap.

In addition to test solution with glyphosate, blank buffer solutions were prepared.

Prepared test solution at pH 4, 7 and 9 were placed in a thermostatically controlled waterbath at 50.0 ± 0.5 °C in the dark.

2. Sampling
The concentration of glyphosate was determined immediately after preparation of test solutions as well as after 2.4 hours and 5 days.

pH values of test solutions were determined at study beginning and study end.

3. Analytical procedures
Immediately after samples of ≤ 5 mL were taken, they were cooled down to room temperature. Then, each test solution was analysed by HPLC without any further pre-treatment.

4. Calculations
The decrease in concentration was calculated as:

$[(C_0 - C_t) / C_0] \times 100\ \%$

Where
C_0 = concentration at time 0
C_t = concentration at time t

The relative concentration C_r was calculated as:
$Cr = [C_t / C_0] \times 100\ \%$

II. RESULTS AND DISCUSSION

A. DATA
Glyphosate concentrations are summarised in Table 7.2.1.1-2 for the respective pH values.

Table 7.2.1.1-2: Degradation of glyphosate in sterile buffer solutions at pH 4, 7 and 9

pH	Measured pH value (study start / study end)	Glyphosate concentration (mg/L) after		
		0 hours	2.4 hours	5 days
4	4.0 / 4.0	203.04	204.66 (101 % [2])	203.06 (100 % [2])
7	7.0 / 7.0	222.60	224.07 (101 % [2])	216.46 (97 % [2])
9	8.9 / 8.9	212.02	212.02 (100 % [2])	205.03 (97 % [2])

[1] Mean value of duplicate analysis
[2] Relative concentration

B. HYDROLYSIS
Glyphosate concentrations at study end (5 DAT) were 100 %, 97 % and 97 % of applied amount in buffers at pH 4, 7 and 9. The difference in test concentration of Glyphosate was determined to less than < 10 % of applied amount at study start and after 5 days of incubation in maximum.

C. KINETICS
No degradation kinetics was calculated due to stability under the test conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The hydrolysis of glyphosate was examined in sterile buffer solutions at pH 4, 7 and 9 at 50.0 ± 0.5 °C in the dark. As hydrolysis of < 10 % of applied amount was observed at all pH values at study end after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test. However, basic data is missing, e.g. on application procedure or preparation of buffers, or unclear, i.e. number of test solutions and the study duration was only five days.
The study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.1/003
Report author	[redacted]
Report year	1993
Report title	Glyphosate, ammonium salt: Determination of hydrolysis as a function of pH
Report No	93/MON033/0344
Document No	
Guidelines followed in study	OECD 111 (1981) EEC Directive 84/449/EEC (Annex V)
Deviations from current test guideline	From OECD 111: - Application rate is unclear - Basic data is missing (sterile conditions, incubation in the dark) - Number of test solutions is unclear - Study duration was only 5 days
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
The hydrolysis of glyphosate applied as its ammonium salt was investigated in aqueous buffer solutions at pH 4, 7 and 9 50.0 °C for 5 days in maximum.

Duplicate aliquots were taken for analysis after 0, 2.4, 72, 91.5, 96, 115.5 and 120 hours.

As less than 10 % of glyphosate was degraded after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: Glyphosate ammonium salt (non-labelled)
Lot No.: PSGA 1128
Chemical purity: 97.9 % glyphosate ammonium salt
Measured concentration: 88.9 % w/w glyphosate acid

2. Test Buffers:
The following aqueous buffer solutions were prepared:

- pH 4.0: Disodium hydrogen phosphate dodecahydrate (27.6 g) and citric acid (12.9 g) were dissolved in distilled water (1900 mL)
- pH 7.0: Potassium dihydrogen phosphate trihydrate (6.8 g) was dissolved in distilled water (1900 mL) and 1 M sodium hydroxide (30 mL) was added
- pH 9.0: Disodium tetraborate decahydrate (33.1 g) and potassium dihydrogen phosphate trihydrate (3.59 g) were dissolved in distilled water (1900 mL)

The volume of buffer solutions was adjusted to 2000 mL with distilled water. The pH was adjusted with 1 M hydrochloric acid or 1 M sodium hydroxide.

B. STUDY DESIGN

1. Experimental conditions

Aliquots (250 mL) of each buffer solution were measured into reagent bottles containing approximately 465 mg of glyphosate in the form of its ammonium salt to give nominal concentrations of about 1860 mg/L. The pH of the solutions was readjusted with 1 M sodium hydroxide and sealed bottles placed in a thermostatically controlled water bath at 50 °C. Following equilibration, initial samples (about 20 mL) were removed and the samples again stored in the water bath until further sampling.

Test solutions were incubated at 50 °C for 5 days in maximum.

2. Sampling

The concentration of glyphosate in test solutions was determined immediately after preparation as well as after 2.4, 72, 91.5, 96, 115.5 and 120 hours. pH values of test solutions were determined at all samplings.

3. Analytical procedures

Duplicate aliquots (1 mL) were diluted to 10 mL with the HPLC mobile phase (0.005 M aqueous potassium dihydrogen orthophosphate: methanol (97:3, v:v) adjusted to pH 2.0 with orthophosphoric acid) and then followed by analysis via HPLC.

The limit of detection was approximately 1 mg/L.

4. Calculations

The concentration of glyphosate, ammonium salt in the injection solution (C_A) was calculated from the mean response of bracketing standards:

C_A [mg/L] = sample peak area x standard concentration [mg/L] / mean peak area of bracketing standards

The concentration of glyphosate, ammonium salt in the sample solution (C_B) was then calculated as follows:

C_B [mg/L] = C_A [mg/L] x dilution factor (V_A/V_B)

Where:

V_A = volume of injection solution (10 mL)
V_B = volume of aqueous samples (1 mL)

II. RESULTS AND DISCUSSION

A. DATA

The concentration of glyphosate, ammonium salt is presented in Table 7.2.1.1-3 for buffer solutions at pH 4, 7 and 9.

Table 7.2.1.1-3: Degradation of glyphosate, ammonium salt in buffer solutions at pH 4, 7 and 9 (in mg/L)

Time (hours)	Replicate	Glyphosate, ammonium salt (mg/L)		
		pH 4	pH 7	pH 9
0	1	1794	1906	1857
	2	1793	1873	1879
	Mean	1794	1890	1868
2.4	1	1752	1905	1895
	2	1736	1900	1876
	Mean	1744	1903	1886
72	1	1646	1871	1846
	2	1639	1891	1862
	Mean	1643	1881	1854
91.5	1	1708	1883	1894
	2	1726	1941	1876
	Mean	1717	1912	1885
96	1	1717	1811	1808
	2	1729	1852	1820
	Mean	1723	1832	1814
115.5	1	1682	1860	1817
	2	1642	1910	1790
	Mean	1662	1885	1804
120	1	1744	1860	1866
	2	1735	1855	1850
	Mean	1740	1858	1858

B. TRANSFORMATION OF THE TEST ITEM

The mean concentration of glyphosate decreased from 1794 to 1740 mg/L at pH 4 and from 1890 to 1858 mg/L at pH 7, each time after 0 and 120 hours, respectively. At pH 9, mean concentrations were 1868 and 1858 mg/L after 0 and 120 hours, respectively. As less than 10 % of glyphosate was degraded after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test.
pH values in test solutions did not change significantly with time.

C. KINETICS

No degradation kinetics was calculated due to stability under the test conditions.

III. CONCLUSIONS

Results indicated that glyphosate is stable to abiotic hydrolysis in aqueous buffer at pH 4, 7 and 9 under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The hydrolysis of glyphosate (as ammonium salt) was examined in buffer solutions at pH 4, 7 and 9 at 50.0 °C. As hydrolysis of < 0 % of applied amount was observed at all pH values at study end after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test. Basic data is unclear (application rate, number of test solutions, sterile conditions, dark conditions) and the study duration was only five days. The study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.1/004
Report author	[redacted]
Report year	1993
Report title	Glyphosate isopropylamine salt. Hydrolysis in water at 3 different pH-values
Report No	PR93/009
Document No	
Guidelines followed in study	BBA-Merkblatt No. 55, part I and II (October 1980)
Deviations from current test guideline	From OECD 111: - Only single vessels prepared for each combination of pH and temperature - The test was conducted at pH 5 instead of a pH of 4
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

The hydrolysis of glyphosate isopropylammonium salt in buffer solutions at pH 5, 7 and 9 was examined at an application rate of 2 g/L at 23 °C and 50 °C, respectively, under sterile conditions and for a period of 29 days.

Samples were taken for analysis after 0, 4, 7, 14 and 29 days.

As hydrolysis of less than 10 % of applied amount was observed at study end at all pH values investigated, it was concluded that glyphosate isopropylammonium salt is hydrolytically stable under the conditions of the test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate isopropylammonium salt (non-labelled)
Lot No.:	10819
Chemical purity:	98 %

2. Buffers:

The following buffer solutions were prepared for the test:

- pH 5: 2.25 g KH_2PO_4 were dissolved in 250 mL water, 0.01 M Na_2PO_4-solution (about 100 mL) was added until pH 5 was reached
- pH 7: 0.97 gKH_2PO_4 were dissolved in 100 mL water, 150 mL 0.01 M Na_2PO_4-solution were added. pH 7 was adjusted with 0.1 M NaOH
- pH 9: 1.05 g $NaHCO_3$ were dissolved in 250 mL water, pH 9 was adjusted with 0.2 M NaOH

Buffer solutions were filtered through a sterile filter and collected into 20 mL volumetric flasks containing about 40 mg (accurately weighed) of the test substance. The flasks were topped up with buffer solution and the flasks were directly closed with glass stoppers.

All glass ware used was heated at 180 °C for 2 hours.

B. STUDY DESIGN

1. Experimental conditions

The initial concentration of the test substance was 2 g/L.

The test solutions each prepared at pH 5, 7 and 9 were incubated at 23 °C and 50.0 °C, respectively.

2. Sampling

Samples from single test vessels were taken at day 0, 4, 7, 14 and 29 under sterile conditions.

3. Analytical procedures

Samples were analysed with HPLC-UV. With the method used, only the glyphosate-anion was determined.

The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC method were not reported.

II. RESULTS AND DISCUSSION

A. DATA

Glyphosate concentrations are summarised in Table 7.2.1.1-4 for the respective pH values.

Table 7.2.1.1-4: Degradation of glyphosate in sterile buffer solutions at pH 5, 7 and 9 in g/L

Days	Sample [1]	Glyphosate (g/L)					
		pH 5		pH 7		pH 9	
		23 °C	50 °C	23 °C	50 °C	23 °C	50 °C
0	1	2.02	2.02	1.97	1.97	2.02	2.02
	2	2.04	2.04	1.89	1.89	1.96	1.96
	Mean	2.03	2.03	1.93	1.93	1.99	1.99
4	1	2.00	2.00	1.98	1.95	1.94	2.00
	2	1.99	1.99	1.96	1.94	1.93	1.94
	Mean	2.00	2.00	1.97	1.95	1.94	1.97
7	1	1.98	2.04	1.96	1.94	1.96	1.94
	2	1.95	2.04	1.90	1.89	1.90	1.97
	Mean	1.97	2.04	1.93	1.92	1.93	1.96
14	1	1.96	2.02	1.98	n.i.	1.95	1.98
	2	1.96	2.02	1.88	n.i.	1.93	1.97
	Mean	1.96	2.02	1.93	n.i.	1.94	1.98
29	1	1.88	2.08	1.93	2.00	1.91	1.97
	2	1.93	2.06	1.85	2.01	1.90	1.95
	Mean	1.91	2.07	1.89	2.01	1.91	1.96

n.i. = Not indicated
[1] Analytical replicate, true replicates are not available as per combination of pH and temperature only one vessel was prepared

B. TRANSFORMATION OF THE TEST ITEM

Glyphosate mean concentrations at pH 5 changed from 2.03 to 1.91 g/L and from 2.03 to 2.07 g/L at 23 °C and 50 °C, respectively, each from 0 DAT to 29 DAT. At pH 7, mean concentrations decreased from 1.93 (0 DAT) to 1.89 g/L (29 DAT) at 23 °C and were 1.93 at 0 DAT and 2.01 g/L at 29 DAT at 50 °C. At pH 9 mean concentrations marginally decreased from 1.99 to 1.91 g/L and from 1.99 to 1.96 g/L at 23 °C and 50 °C, respectively. As hydrolysis of < 10 % of applied amount was observed at study end after 29 days at pH 5, 7 and 9, it was concluded that glyphosate is hydrolytically stable under the conditions of the test.

C. KINETICS

No assessment of degradation kinetics was performed.

III. CONCLUSIONS

Glyphosate isopropylammonium salt was stable under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The hydrolysis of glyphosate was examined in sterile buffer solutions at pH 5, 7 and 9 at 23 and 50.0 °C. As hydrolysis of < 10 % of applied amount was observed at all pH values at study end after 29 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test. The study has some minor deviations from current guideline requirements, e.g. only single vessels were prepared per combination pH – temperature, which however do not have an impact on the results.
Therefore, the study is considered valid to adress the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.1/005
Report author	[redacted]
Report year	1992
Report title	MON-8722: Determination of hydrolysis as a function of pH
Report No	91/MON024/1207
Document No	
Guidelines followed in study	OECD 111 (1981)
Deviations from current test guideline	From OECD 111: - Not reported whether study was conducted with sterile buffers and under sterile test conditions - Study duration was only 5 days
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
The abiotic hydrolysis of the test substance glyphosate in the form of its monosodium salt was determined in aqueous buffer solutions at a test concentration of about 1.8 g/L at pH 4, 7 and 9 incubated at 50 °C in the dark.

Samples were taken after 0, 2.4 and 120 hours. Values of pH of the buffer solutions were determined at study start and at study end.

Glyphosate was considered as hydrolytically stable in aqueous buffer solutions of pH 4, 7 and 9 under the conditions of the test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: Glyphosate monosodium salt (MON-8722)
Lot No.: LLNHB210491
Chemical purity: 97.5 %

2. Buffers:
The following buffer solutions were prepared:

- pH 4.0: Disodium hydrogen phosphate (10.9 g) and citric acid monohydrate (12.9 g) were dissolved in distilled water (1900 mL)
- pH 7.0: Potassium dihydrogen phosphate (13.6 g) was dissolved in distilled water (1900 mL), 1 M sodium hydroxide (60 mL) was added
- pH 9.0: Disodium tetraborate decahydrate (33.1 g) and potassium dihydrogen phosphate (3.59 g) were dissolved in distilled water (1900 mL)

The volume of all solutions was adjusted to 2000 mL with distilled water. The pH was adjusted with 1 M hydrochloric acid or 1 M sodium hydroxide.

B. STUDY DESIGN

1. Experimental conditions

Portions of about 250 mL of each buffer solution were placed in Pyrex bottles and incubated at 50 °C in the dark. Following equilibration, a sample (100 mL) of each buffer solution was added to weighed amounts (about 180 mg) of glyphosate in separate Pyrex bottles resulting in test concentrations of about 1.8 g/L. The samples were purged with nitrogen. Storage areas were monitored for temperature.

Test solutions were incubated at 50 °C for 5 days. The pH of each test solution was measured at the beginning and the end of the test period.

2. Sampling

Samples were taken after 0, 2.4 and 120 hours. On each sampling, duplicate aliquots (1 mL) were removed from each sample.

3. Analytical procedures

Sampled aliquots were diluted to volume (10 mL) with HPLC mobile phase (0.005 M potassium dihydrogen phosphate in water/methanol (96:4; v/v) adjusted to pH 2.0 with orthophosphoric acid) and analysed for glyphosate by HPLC using a UV detector.

The limit of detection was approximately 5 mg/L.

4. Calculations

The concentration of glyphosate in the injection solution (C_A) was calculated as:

C_A [mg/L] = sample peak area x standard concentration [mg/L] / mean peak area of bracketing standards

The concentration of glyphosate in the sample solution (C_B) was calculated as:

C_B [mg/L] = C_A [mg/L] x dilution factor (V_A / V_B)

II. RESULTS AND DISCUSSION

A. DATA

The results of glyphosate concentration determinations are summarised in Table 7.2.1.1-5 for the respective pH values.

Table 7.2.1.1-5: Degradation of glyphosate in sterile buffer solutions at pH 4, 7 and 9 (in g/L)

pH	Glyphosate (g/L)					
	0 hours		2.4 hours		120 hours	
	Replicates	Mean	Replicates	Mean	Replicates	Mean
4	1.80 / 1.76	1.78	1.88 / 1.85	1.87	1.91 / 1.91	1.91
7	1.52 / 1.71	1.62	1.59 / 1.53	1.56	1.67 / 1.65	1.66
9	1.82 / 1.68	1.75	1.71 / 1.70	1.71	1.68 / 2.06	1.87

B. TRANSFORMATION OF THE TEST ITEM
Measurements of pH values showed that there was no significant change in the pH of the buffer solutions with time.

Glyphosate concentrations did not decrease until study end for pH 4, 7 and 9. Thus, glyphosate was considered as hydrolytically stable under the conditions of the test.

C. KINETICS
Kinetic assessments of the data were not conducted.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The hydrolysis of glyphosate was examined in buffer solutions at pH 4, 7 and 9 at 50 °C in the dark. As hydrolysis of < 10 % of applied amount was observed at all pH values at study end after 5 days, it was concluded that glyphosate is hydrolytically stable under the conditions of the test. It was not reported whether sterile conditions were applied and the study duration was only five days.
Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.1/006
Report author	[redacted]
Report year	1991
Report title	Behaviour of Glyphosate in water and soil. Part 1: Hydrolysis as a function of pH
Report No	PR90/002
Document No	
Guidelines followed in study	BBA-Guideline "Prüfung des Verhaltens von Pflanzenschutzmitteln in Wasser" (Merkblatt 55, part I and II)
Deviations from current test guideline	From OECD 111: - The analytical procedure is not described - The test was conducted at pH 5 instead of a pH of 4 - The test period was longer than 30 days - Not reported whether study was conducted with sterile buffers and under sterile test conditions
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary
The hydrolysis of glyphosate in buffer solutions at pH 5, 7 and 9 was examined at an application rate of 1.03 mg/L at 22 °C and for a period of 56 days.

Samples were taken for analysis at days 0, 4, 7, 28 and 56.

As hydrolysis of less than 10 % of applied amount was observed at study end at all pH values investigated, it was concluded that glyphosate is hydrolytically stable under the conditions of the test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate, free acid (non-labelled)
Lot No.:	00516
Chemical purity:	99 %

2. Buffers:

The following buffer solutions were prepared for the test:

- pH 5: 9 g/L KH_2PO_4; about 4 mL of Na_2HPO_4-solution (23.8 g/L) were added to reach pH 5
- pH 7: 9.65 g/L (0.071 M) KH_2PO_4 plus 0.01 M Disodiumhydrogenphosphate (1.42 g/L)
- pH 9: 0.05 M NaHCO3 (4.2 g/L); pH 9 was adjusted with 1 N NaOH

B. STUDY DESIGN

1. Experimental conditions

A solution containing 20.6 mg glyphosate in 100 mL water was prepared (1.03 mg/ 5 mL). 5 mL of this solution was diluted to 1 L of each buffer solution resulting in a test concentration of 1.03 mg test item/L. Eight brown glass bottles each filled with 125 mL treated buffer solution were prepared per buffer solution.

The test was performed at 22 °C.

2. Sampling

Samples were taken at days 0, 4, 7, 28 and 56. At each sampling day, two samples were investigated.

3. Analytical procedures

Procedures for determination of glyphosate resides are not detailed in the study report.

II. RESULTS AND DISCUSSION

A. DATA

Degradation of glyphosate in buffer solutions at pH 5, 7 and 9 is summarised in Table 7.2.1.1-6.

Table 7.2.1.1-6: Recovery of glyphosate at pH 5, 7 and 9 (mg/L)

Day	Replicate	Glyphosate (mg/L)		
		pH 5	pH 7	pH 9
0	1	988	1080	1064
	2	862	1193	1034
	Mean	925	1137	1049
4	1	779	909	819
	2	758	996	1171
	Mean	769	953	995
7	1	938	978	1098
	2	889	904	1068
	Mean	914	941	1083
15	1	999	1037	1014
	2	966	1145	1102
	Mean	983	1111	1058
28	1	785	1222	1099
	2	970	983	1103
	Mean	878	1103	1101
56	1	951	1066	1042
	2	937	990	1039
	Mean	944	1028	1041

B. HYDROLYSIS

Glyphosate concentrations varied slightly between start and at the end of the study: at pH 5, mean concentrations of 925 and 944 µg/L were measured, whereas at pH 7 mean concentrations of 1137 and 1028 µg/L were detected at 0 DAT and 56 DAT, respectively. At pH 9, mean values of 1049 and 1041 µg/L were determined at 0 DAT and 56 DAT, respectively.

As hydrolysis of less than 10 % of applied amount was observed at study end at all pH values investigated, it was concluded that glyphosate is hydrolytically stable under the conditions of the test.

C. KINETICS

Glyphosate was stable to abiotic hydrolysis in aqueous buffer at pH 5, 7 and 9.

III. CONCLUSIONS

For none of the three pH values there was hydrolysis to be seen within the time of investigation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The hydrolysis of glyphosate in buffer solutions at pH 5, 7 and 9 was examined at an application rate of 1.03 mg/L. The analytical procedure is not described. It is not reported whether study was conducted with sterile buffers and under sterile conditions.

Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.1/007
Report author	[redacted]
Report year	1990
Report title	Hydrolysis determination of 14C-Glyphosate (PMG) at different pH values
Report No	238500
Document No	
Guidelines followed in study	US EPA 540/9-85-013: section 161-1
Deviations from current test guideline	From OECD 111: - Test performed in aqueous buffer of pH 5 instead of pH 4 - Test performed solely at temperature of 25 °C
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The abiotic hydrolysis in sterile aqueous buffer solution was investigated at pH 4, 7 and 9 of [^{14}C]glyphosate (PMG) at a temperature of 25 °C.

The concentration of the test article was 0.32 mg/L. The test solutions were sampled at the following sampling intervals: 0, 5, 9, 15, 20, 26 and 30 days of incubation. [^{14}C]glyphosate was identified by analysing the aqueous samples directly by thin-layer chromatography (TLC) using two different solvent systems and the unlabelled parent compound for co-chromatography. After an incubation time of 30 days, no hydrolysis products were detected in the test solutions and no significant amount of volatile products were observed in the absorption traps (< 0.1 %).

The balance of radioactivity of the three test solutions resulted in a mean value of 100.2 % ± 0.9 %. Therefore, [^{14}C]glyphosate can be considered as hydrolytically stable at pH 5, 7 and 9, respectively, and 25 °C (DT_{50} > 30 days).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Radiolabelled Test Material

Identification:	[^{14}C]glyphosate (PMG), labelled in the methyl position
Lot No.:	CFA.745 C5
Specific activity:	11.2 MBq/mg (304 µCi/mg)
Radiochemical purity:	97.4 %

Unlabelled Test Material

Identification:	Glyphosate
Lot No.:	185-ff-131
Chemical purity:	99.5 %

2. Buffers
The following aqueous buffer solutions were prepared for the test:

- 0.71 g potassium hydrogen phthalate were combined with 350 mL water, pH 5 was adjusted with 0.01 M NaOH (170 ml); pH of 4.99 was determined
- 75 mL buffer pH 7 (Merck No. 9439, phosphate) were combined with 425 mL water, pH 7 was adjusted with monopotassium hydrogen phosphate; pH of 7.03 was determined
- 100 mL buffer pH 9 (Merck No. 9461, boric acid/potassium chloride – soidium hydroxide) were combined with 400 mL water; pH of 8.96 was determined

The buffer solutions were sterilised at 120 °C for 30 minutes.

B. STUDY DESIGN

1. Experimental conditions
A stock solution was prepared from 200 µL of the acetonic solution supplied (radioactive concentration: 200 µCi/mL; specific activity of the test article: 304 µCi/mg) were combined with 800 µL of water to 1.0 mL (stock solution). By liquid scintillation counting (LSC) the total content of [^{14}C]glyphosate in the aqueous stock solution was found to be 0.128 mg. For the preparation of the test solutions aliquots of 100 mL of the respective sterile buffer solution were combined each with 250 µL of the stock solution (128 mg/L of test article in water) in three neck round-bottomed flasks. Therefore, the concentration in each test solution was 0.32 mg/L. For each pH, one test solution was prepared.

The study was performed in a glass-apparatus with an open gas (nitrogen)-flow system. For the incubation procedure the flasks containing the test solutions were connected with the absorption bottles and the nitrogen flow was adjusted to about 1-2 bubbles per second. Finally, the flasks were incubated at 25 + 0.1 °C in the dark. The incubation flasks were controlled by weighing at each sampling interval to detect possible evaporation of water, these water losses were negligible.

Sterility of the test solutions was checked by adding 1 ml of each test solution on the top of agar plates, which were exposed for 24 to 48 h at 37.8 °C, afterwards the number of colonies was counted.

A high germ formation was determined after 30 days of hydrolysis at pH 7 and 9. One germ was counted in sample 9/0 (pH 9, 0 days). But, as the results of the study demonstrate, no influence on the hydrolytical behaviour of the test substance could be observed. The other samples tested proved to be sterile.

2. Sampling
Approximately 4 mL of test samples were taken each for analyses at day 0 and after 5, 9, 15, 20, 26 and 30 days. The CO_2 absorption bottles (Sodium hydroxide solutions) and volatile absorption bottles (2-methoxy-ethanol solutions) were exchanged at the same intervals.

3. Analytical procedures
The radioactivity in the test solution, as well as the solutions in the CO_2 and volatile absorption bottles, was determined on a Packard Instrument (section 2.2) equipped with OPM and luminescence options. For this purpose, 100 µL test solution were measured in 10 mL scintillation mixture. 0.5 mL of the sodium hydroxide solutions from CO_2-absorption bottles were mixed with 4.0 mL of water and 10 mL of scintillation mixture. 0.5 mL of 2-methoxy-ethanol from volatiles absorption bottles were mixed with 10 mL of scintillation mixture. The radioactivity was determined by LSC.

Samples were analysed by TLC performed on pre-coated plates (20 cm x 20 cm) of cellulose with a layer thickness of 0.50 mm. The plates were developed with chamber saturation (at least 30 min.). Two different solvent system were used, SS 2: methanol / water (50:10, v/v), and SS 4: methanol / water / trichloroacetic acid / acetic acid / 15N ammonia hydroxide (55:35:3.5:2:2.5, v/v/w/v/v). The characterisation of the radioactivity in the test solutions was performed at each sampling date. The unlabelled parent compound was used for co-chromatography and visualised by spraying with ninhydrin reagent and drying for 10 to

20 minutes at 100 to 120 °C. The radioactive zones on TLC-plates were detected by using a scanner equipped with a data processing system.

II. RESULTS AND DISCUSSION

A. DATA

The radioactivity and the balance of radioactivity is presented in the table below.

Table 7.2.1.1-7: Overall mass balance in % of applied radioactivity

Test solution		Radioactivity assigned to the test item							
Temp.(°C)	pH	Incubation time (days)							Balance of radioactivity (%)
		0 *	5	9	16	20	26	30	
25	**5**	100.0	99.9	97.7	101.9	99.1	98.3	101.3	**101.2**
25	**7**	100.0	101.3	100.3	103.1	99.4	98.6	99.2	**99.6**
25	**9**	100.0	99.0	100.0	100.4	96.1	99.3	100.6	**99.8**
Mean ± S									**100.2 ± 0.9**

S = Standard deviation
* = Initial value set at 100 %

B. MASS BALANCE

The balance of radioactivity was calculated by relating the radioactivity determined in the test solutions at the end of the incubation period to the difference of the radioactivity at the start and the total radioactivity sampled from the test solutions.

No significant amount of volatile radioactivity was determined in the absorption traps and therefore these results are not included in the calculation of the balance. With 101.2, 99.6 and 99.8 % of applied radioactivity at pH 5, 7 and 9, respectively, a mean balance for the three test solutions of 100.2 + 0.9 % was obtained and further showed that the entire amount of radioactivity was kept back in the test solutions.

C. VOLATILE RADIOACTIVITY

The results reveal that during the test period of 30 days and at a temperature of 25 °C, no significant amounts of radioactivity disappeared from the test solutions. The amount of volatile radioactivity liberated from the test solutions was < 0.1 % at each sampling interval, except for one sample (sodium hydroxide trap, pH 7, 9 days) which contained an amount of volatile radioactivity of 0.17 % (presumable due to an inaccuracy of the scintillation counter). This result demonstrates that finally no significant part of the test article was hydrolytically degraded to volatile molecules.

D. CHARACTERISATION OF RADIOACTIVITY

The results of the TLC-analysis showed that besides the parent compound no further components were detected.

III. CONCLUSIONS

In conclusion, with respect to the study design, it can be stated that [^{14}C]glyphosate was stable to abiotic hydrolysis under the conditions of the test. After 30 days of incubation at 25 °C and pH 5, 7 and 9, respectively, no hydrolysis products were observed. Furthermore, no significant amount of the test article (< 0.1 %) was degraded to volatile products.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The hydrolytic degradation of glyphosate was assessed according to pertinent guideline requirements at the time of conduct, i.e. pH range for 5 to 9 was tested at a temperature of 25 °C. While conditions required by the current guidelines slightly differ, the study adequately demonstrates that glyphosate is stable at the conditions tested. Therfore, the study is considered valid to address the data point.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.1/008
Report author	[redacted]
Report year	1990
Report title	Stability of Glyphosate to hydrolysis
Report No	WAS95-00278
Document No	
Guidelines followed in study	OECD 111 (1981)
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No information available
Short description of study design and observations:	Hydrolysis of glyphosate was investigated at three different pH values for 5 days at 50°C. The purity of the test item was 99 % (w/w)
Short description of results:	Less than 10 % of degradation was observed. Therefore, glyphosate is considered as hydrolytically stable.
Reasons why the study is not considered relevant/reliable or not considered as key study:	The report is not available. The information given in the Monograph is insufficient to assess the validity of the study.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 7.2.1.1/009
Report author	[redacted]
Report year	1983
Report title	Hydrolysis and photolysis degradation studies of SC-0224
Report No	WRC-83-85
Document No	
Guidelines followed in study	U.S. Environmental Protection Agency Report EPA 540/9-82-021, October 18, 1982, Pesticide Assessment Guidelines, Subdivision N; Chemistry: Environmental Fate, Series 161
Deviations from current test guideline	From OECD 111: - Glassware was not sterilised - Sterility checks were not reported - pH 5 instead of pH 4 and a test temperature of 25 °C
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
The abiotic hydrolysis in aqueous buffer solution was investigated at pH 4, 7 and 9 for non-labelled salt of glyphosate-trimesium (trlmethylsulfonium carboxymethylaminomethylphosponate, SC-0224) at 25 °C in the dark for 32days in maximum. Only the data for the PMG-molecule (glyphosate) are considered here.

Glyphosate was stable under the conditions of abiotic hydrolysis.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: Glyphosate as glyphosate-trimesium salt (SC-0224)
Lot No.: WRC-7746-9-1
Composition: 19.3 % glyphosate-trimesium, 75.6 % water, 0.6 % isopropanol, 1.9 % sodium, 3.0 % chloride
Measured molar ratio: Glyphosate (CMP) : trimesium (TMS) = 1.00 : 1.03

2. Buffers
Aqueous phosphate buffer solutions were prepared at pH 5, 7 and 9 as published.

B. STUDY DESIGN

1. Experimental conditions
Sterilized test solutions of 10 mg/L (ppm) and 100 mg/L (ppm) glyphosate-trimesium (SC-0224) were prepared in phosphate buffer at pH 5, 7 and 9. An adequate number of aliquots of each solution were placed in individual non-sterile, Teflon®-sealed, screw top test tubes. The tubes were placed in a 25°C thermostat-controlled water bath (±0.5°C) in the dark for 32 days in maximum.

2. Sampling
Samples were removed for analysis each for the carboxymethylaminomethylphosponate anion on days 0, 1, 4, 8, 12, 24 and 32.

3. Analytical procedures
For glyphosate and AMPA (CMP and aminomethylphosphonic acid anions) single samples were analysed, if not stated otherwise, for trimesium duplicate samples were analysed. Determinations of glyphosate and AMPA were carried out by derivatisation with 9-fluorenylmethyl chloroformate followed by HPLC analysis. The ypical recovery via the method was 93 ± 10 % for anions.

II. RESULTS AND DISCUSSION

A. DATA
The results of hydrolysis of glyphosate at the test temperature of 25 °C are summarised in Table 7.2.1.1-8 and Table 7.2.1.1-9.

Table 7.2.1.1-8: Glyphosate-trimesium concentrations (mg/L) at 25 °C, 10 mg/L test concentration, based on analysis for glyphosate anion

pH	5.0	7.0	9.0
Time (days)	Observed concentration (mg/L)		
0	8.9	9.2	10.2 / 10.3
1	10.0	9.7	10.7
4	8.2	8.6	9.8
8	8.6	9.2	11.2
12	10.8	14.8	11.2
18	9.4	9.3	9.3
24	10.2	9.7	9.3
32	9.7	8.8	9.0

Table 7.2.1.1-9: Glyphosate-trimesium concentrations (mg/L) at 25 °C, 100 mg/L test concentration, based on analysis for glyphosate anion

pH	5.0	7.0	9.0
Time (days)	Observed concentration (mg/L)		
0	78.4	105.6	94.3
1	86.8	105.6	107.5
4	82.3	70.6	97.6
8	91.7	96.4	83.5
12	133.3	147.8	130.0
18	100.0	103.0	95.0
24	91.3	102.6	94.8
32	100.0	99.0	100.0

B. TRANSFORMATION OF THE TEST SUBSTANCE
For glyphosate anion (CMP), no detectable loss was observed for any pH at either glyphosate-trimesium concentration (10 or 100 mg/L) during the 32-day test period.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The hydrolytic degradation of glyphosate was tested in line with pertinent guideline requirements at the time of conduct. While conditions required by the current guidelines slightly differ, the study provides information on the hydrolytic stability of glyphosate at the conditions tested. In view of the lacking sterilisation of glassware used, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.1/010
Report author	[redacted]
Report year	1978
Report title	Solubility, volatility, adsorption and partition coefficients, leaching and aquatic metabolism of MON 0573 and MON 0101
Report No	MSL-0207
Document No	
Guidelines followed in study	None
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Short description of study design and observations:	Study type: Hydrolysis Test item: [^{14}C] glyphosate, phosphonomethyl-label (94 % radiochemical purity) Test buffers: 0.05 M potassium biphthalate-hydrochloric acid (pH 3.0); 0.05 M potassium phosphate monobasic-sodium hydroxide buffer (pH 6.0); 0.1 M boric acid-potassium chloride-sodium hydroxide buffer (pH 9.0) Test water: Cattail Swamp, Sphagnum bog, Ballard pond pH: 6.2, 4.2, 7.3 Sterilized by Millipore filtration, 0.45 µm Experimental conditions: Hydrolysis was investigated in two buffers and three natural waters. Test labeld item was diluted with the unlabled test item and was applied at concentrations of 25 and 250 ppm (buffer tests) and 0.1 ppm (natural water tests). Buffer test vials were incubated at 5 and 35°C for 35 days. Samples were taken 0, 7, 14, 21 and 32 days after treatment. Natural water solutions were incubated at 30°C for 5 weeks. Samples were taken after filtration 0, 1, 3 and 5 weeks after treatment. Analytical procedures: Buffer samples were analysed by LSC and TLC (all samples) and by HPLC (35 DAT). Natural water samples were analysed by LSC, TLC and HPLC.

Short description of results:	Buffer tests: No indication that the test item is hydrolysed under these conditions. Natural water tests: Degradation of the test substance in two water samples is attributed to biodegradation
Reasons why the study is not considered relevant/reliable or not considered as key study:	The study was considered invalid due to the following deficiencies: Deviations from OECD Guideline 111 (April 2004): - pH 3.0 buffer solution used instead of pH 4 - Usage of natural water for hydrolysis test - Measures to avoid oxygen were not taken - Recovery >110 % in two cases Hydrolysis and biodegradation can not be separated within the experiment.
Category study in AIR 5 dossier (L docs)	Category 3b

CA 7.2.1.2 Direct photochemical degradation

The molar decadic absorption coefficient (ε) of glyphosate is << 10 L mol^{-1} cm^{-1} at wavelengths >295 nm (see Section 2.4). Therefore, it is expected that photolysis does not significantly contribute to degradation of glyphosate in aquatic systems. Thus, experimental studies on direct photolysis are formally not required. For completeness, the studies previeously evaluated are presented below.

The direct photochemical degradation of glyphosate and glyphosate-trimesium was investigated in sterile water and in aqueous buffer solution at pH 5, 7 and 9 in the course of two studies which are considered valid to address the data point ([redacted] 2005, CA 7.2.1.2/002 and [redacted] 1992, CA 7.2.1.2/006). Further two studies provide supportive information to address the data point ([redacted] 1992, CA 7.2.1.2/004 and [redacted] 1990, CA 7.2.1.2/005). In addition, experiments on indirect photolysis were conducted in two studies ([redacted] 2005, CA 7.2.1.2/002 and [redacted] 2001, CA 7.2.1.2/003). For studies performed with glyphosate-trimesium only the results for the glyphosate (PMG) anion are considered for evaluation and further assessment. A review report on four of the above studies on direct and indirect photolysis was also summarised ([redacted] 2012, CA 7.2.1.2/001), further there are own mechanistic experiments of the author included in the review. Please also refer to the monitoring chapter on water treatment regarding discussion on effects of indirect photolysis (CA 7.5).

Glyphosate was stable in an experiment on direct photodegradation in sterile distilled water with an amount of 100.3 % of applied recovered after 12 days of continuous irradiation ([redacted] 2005, CA 7.2.1.2/002). In experiments with aqueous solutions at pH 9.2, 7.3 and 5.1, it degraded slowly with half-lives of of 77, 69 and 33 days, respectively ([redacted] 1992, CA 7.2.1.2/006). AMPA was found at levels above 10 % only at pH 7.3 and 5.1 with maximum amounts of 11.6 and 16.0 %, respectively. The supportive studies on direct photochemical degradation confirm these findings showing no or only very little degradation of glyphosate under exposure to artificial or natural sunlight ([redacted] 1992, CA 7.2.1.2/004; [redacted] 1990, CA 7.2.1.2/005). In the supportive experiments on indirect photolysis glyphosate was degraded faster in sterile natural water with half-lifes of about 9 and 34 days (spring solar day irradiation. 35 °N). Besides the natural compound methanediol and the known metabolite AMPA, no degradation products were observed above 10 %.

Table 7.2.1.2-1: Direct photochemical degradation studies

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.2.1.2/001	[redacted], 2012	Review	Glyphosate	Supportive	Review on direct and indirect photolysis
CA 7.2.1.2/002	[redacted] 2005	Aqueous photolysis	Glyphosate	Valid	Direct and indirect photolysis
CA 7.2.1.2/003	[redacted] 2001	Aqueous photolysis	Glyphosate-trimesium	Supportive	Indirect photolysis
CA 7.2.1.2/004	[redacted]1992	Aqueous photolysis	Glyphosate-trimesium	Supportive	Direct photolysis
CA 7.2.1.2/005	[redacted]1990	Aqueous photolysis	Glyphosate	Supportive	Direct photolysis
CA 7.2.1.2/006	[redacted]1992	Aqueous photolysis	Glyphosate	Valid	Direct photolysis
CA 7.2.1.2/007	[redacted] 1983	Aqueous photolysis	Glyphosate-trimesium	Invalid	Direct photolysis
CA 7.2.1.2/008	[redacted] 1978	Aqueous photolysis	Glyphosate	Invalid	Indirect photolysis

1. Information on the study

Data point:	CA 7.2.1.2/001
Report author	[redacted]
Report year	2012
Report title	Review of Direct and Indirect Photolysis of Glyphosate
Report No	MSL0024051
Document No	
Guidelines followed in study	None
Deviations from current test guideline	Not applicable (review report). Only aqueous photolysis aspect of study is summarised
Previous evaluation	Not previously evaluated (submitted in AIR2 but not evaluated)
GLP/Officially recognised testing facilities	No, not applicable for this study type
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

I. INTRODUCTION

In an expert statement, results of several aqueous photolysis studies are summarised and the impact on direct and indirect photolytic process on the degradation of glyphosate are discussed.

Photodegradation of pesticides in the environment can occur either by direct or indirect absorption of light. A prerequisite for direct photolysis of pesticides is its ability to absorb light at wavelengths equal or greater than 290 nm to reach excited electronic states. The excited species then may undergo chemical transformations. In contrast, during indirect photolysis, light energy is absorbed by other substances in soil or water and the excited species can then transfer the energy to a pesticide, undergo electron transfer with the pesticide, or form highly reactive species which may enter into a series of reactions with pesticides. Glyphosate does not absorb light significantly at wavelengths longer than 230 nm. Thus, in highly purified

sterile water, in which direct photolysis is the only mechanism for photo-transformation, glyphosate is expected to be photo-stable. Indeed, aqueous photolysis studies have shown that glyphosate is relatively stable to photodegradation in distilled water; confirming that it does not absorb incident radiation directly as expected based on its UV spectrum. However, photo-induced degradation of glyphosate can occur in water under certain conditions. Studies using artificial light and solutions containing calcium ions show that glyphosate is susceptible to a slow indirect photodegradation. Similarly, under intense artificial light, glyphosate in natural river water degrades via oxidative transformation induced by photochemical excitation of humic acids as reported for other pesticides. Naturally-occurring organic and inorganic solutes such as humic acid, tryptophan, tyrosine, organic peroxides, and various metal ions are known to absorb strongly in the ultraviolet and visible region to form reactive species such as singlet molecular oxygen, hydrogen peroxide, hydroxyl radical, organic peroxyradicals, and other free radicals. These photooxidants then could react with oxidizable compounds like glyphosate in the aqueous environment.

Although indirect photodegradation of glyphosate in water can occur, under normal environmental conditions photolysis is expected to be a slow process and compared to microbial degradation is, at most, a very minor pathway for the degradation of glyphosate in the environment. Several glyphosate aqueous photolysis studies have been conducted. Two studies were conducted in distilled water utilising artificial light and two more recent studies were conducted in natural water using artificial light in one and natural sunlight in the other study. The results of these studies are discussed below.

II. DISCUSSION OF PHOTOLYSIS STUDIES

1. Direct photolysis

[redacted] (1990), please refer to CA 7.2.1.2/005 (supportive in this submission)

The rate of photodegradation and the nature and extent of formation of degradation products of glyphosate in pure sterile pH 5, 7, and 9 aqueous buffers were investigated in this study.

No ^{14}C-activity was detected in the ethylene glycol traps at levels greater than 0.5 % of the applied. The amount of ^{14}C-activity evolved as $^{14}CO_2$ from the irradiated solutions was minimal and was approximately the same as that evolved from the non-irradiated solutions. In addition to minor amounts of CO_2, no other degradation product of glyphosate was detectable in the pH 5, 7, and 9 buffer solutions after 31 days of irradiation. The fluctuation in glyphosate concentrations in both light exposed and dark control samples during various sampling intervals is not indicative of glyphosate degradation but rather is attributed to variations in mass balance data due to normal errors in radioactivity measurement by liquid scintillation counting. This study suggests that in the purified sterile water glyphosate is not susceptible to photodegradation.

[redacted] (1992), please refer to CA 7.2.1.2/006 (valid in this submission)

In this study, the rate of photolysis of [^{14}C]glyphosate in aqueous buffers at pH 5.1, 7.3, and 9.2 under the influence of simulated, artificial sunlight was investigated. TLC on cellulose and HPLC analyses of illuminated aqueous buffers at all pHs showed glyphosate as a major constituent and AMPA as a minor product. No other significant unknown degrades were detected by analyses at any sampling point.

The TLC immobile radioactive fraction was detected at all sampling points including day zero and in the [^{14}C]glyphosate dosing solution as well. The immobile radioactive fraction detected near the origin of the TLC plate (TLC Rf value in the range of 0.00-0.02) was assigned by the authors of the report as M4 unidentified fraction and those which were slightly more mobile relative to M4 (TLC Rf values in the range of 0.02-0.19), was assigned as M3 unidentified fraction we now believe that fractions identified as M3 and M4 in this study are not distinct metabolites of glyphosate but rather are chromatographic artefacts of the silica gel TLC method used in the study. It is stated that the relatively immobile radioactive fractions observed in this study are glyphosate and AMPA which are strongly and reversibly binding to the polar surface of silica gel causing the smear of the radioactivity in the TLC plates. The binding of glyphosate and

AMPA to silica gel and other minerals and organic matter has been widely reported and is consistent with the highly polar nature of these molecules.

Taking into account the lack of significant degradation of glyphosate during the 15-day photolysis period and the fact that AMPA was also detected in the non-irradiated control samples, coupled with the results from the aqueous photolysis study conducted by [redacted] it is concluded that glyphosate is stable to direct photodegradation in purified sterile water. This conclusion is consistent with the fact that glyphosate does not absorb incident radiation based on its UV spectrum.

2. Indirect photolysis

[redacted] (1978), please refer to CA 7.2.1.2/008 (invalid in this submission)

The rate of aqueous photodegradation of [^{14}C]glyphosate was determined in natural water, purified natural water, and deionized water fortified with $CaCl_2$.

Extensive photodegradation of [^{14}C]glyphosate to AMPA was found in this study. In natural water, irradiation for 14 and 21 days resulted, respectively, in 58.4-68.8 % and 78.6-86.7 % degradation of glyphosate to AMPA, compared to 5.8-9.8 % and 7.2-13.5 % degradation in the non-irradiated controls. Carbon dioxide evolution accounted for 0.5 % of the applied activity after 21 days of irradiation. Degradation of glyphosate to AMPA was 67.1 and 78.1 % after 14 days of irradiation in deionized water containing 3 and 30 ppm $CaCl_2$, respectively. In contrast, only 38.3 % degradation of glyphosate to AMPA occurred in purified natural water after irradiation for 14 days. The purified natural water contained 0.4 ppm $CaCl_2$ compared to 26.0 ppm for unpurified natural water. These results indicate that calcium sensitises the photodegradation of glyphosate to AMPA in water.

[redacted] (2005), please refer to CA 7.2.1.2/002 (valid in this submission)

In [redacted] (2005), the aqueous photolysis of glyphosate was studied using test substances labelled with 14C in either the glycine portion of the molecule [C1-^{14}C]glyphosate, or the phosphonomethylene carbon of the molecule, [C3-^{14}C]glyphosate.

Consistent with the previous studies, glyphosate was stable to photolysis in distilled water and showed low degradation throughout the 12 days of continuous irradiation. However, in natural water, glyphosate degraded rapidly when exposed to artificial light and represented an average of 19.8 % and 21.5 % of the dose in [C1-^{14}C] and [C3-^{14}C]glyphosate labelled photolysis experiments, respectively, following 12 days of continuous irradiation. In [C1-^{14}C]glyphosate photolysis experiments, the main degradate detected was $^{14}CO_2$, which represented an average of 75.4 % of dose at the end of the exposure period. In the [C3-^{14}C]glyphosate experiments, aminomethylphosphonic acid (AMPA) and methanediol were the main degradates detected and represented 19.6 % and 52.0 % of the dose, respectively, at the end of the exposure period. Glyphosate was relatively stable in dark control samples in both test systems and represented > 92 % of the dose throughout the incubation period for all sample sets. The photo-induced degradation half-life of glyphosate in natural water ranged from 33.9 to 34.4 solar days based on pseudo-first order kinetics.

Degradation of glyphosate in natural water when exposed to artificial light is proposed to proceed via oxidative transformation induced by photochemical excitation of humic acids in natural water. A mechanistic pathway as described below is based on detailed mechanistic work conducted on the oxidation reaction of glyphosate and glycine with sodium hypochlorite, conducted by the author of this report. It is proposed that photooxidation of glyphosate is induced by active oxidising species (such as peroxides or hydroxyl radicals), which are known to form from the photolysis of natural humic acids present in natural waters. Oxidative breakdown of glyphosate with hydrogen peroxide and sodium hypochlorite to form methanediol, glycine, and AMPA are described as having been previously reported. Further, reference to the degradation of glyphosate in the presence of Mn(II) and molecular oxygen is made, suspected to occur in the dark via intramolecular electron transfer mechanism.

The photoinduced oxidation of glyphosate in natural water may proceed via N-hydroxylation, followed by dehydration of the hydroxylamine, to form Imines I and II depending on which hydrogen is eliminated from glyphosate. Imines are known to hydrolyse rapidly under the reaction conditions, and hydrolysis of Imines I and II would lead to formation of glycine and AMPA, methanediol, orthophosphoric acid, and CO_2. The detection of significant amounts of AMPA in the photolysis experiment coupled with the lack of glycine detection suggests that Imine I was formed preferentially under the reaction conditions.

AMPA and glycine are expected to undergo similar oxidative transformation because of their structural similarities to glyphosate. However, since AMPA concentration gradually increased during the irradiation period, it can be concluded that its oxidation rate was slower than its rate of formation from glyphosate.

Methanediol is the hydrated form of formaldehyde in dilute aqueous solutions. Methanediol is present in the environment from both natural and non-natural sources and is derived from natural metabolic processes as well as combustion processes (automobile exhaust or burning of wood) and building materials. Large quantities of methanediol are formed in the troposphere by the oxidation of hydrocarbons. Methanediol is stated to be a metabolic intermediate involved in one-carbon metabolic processes and several publications on the presence of methanediol in plants and drinking are referenced. Reference is further made to the fact that production of methanediol under certain laboratory conditions is not unique to glyphosate and would also be expected from the oxidative fragmentation of many carbon containing small molecules, amino acids, and other natural organic compounds such as humic and fulvic acids. In the environment, formaldehyde/methanediol is rapidly metabolized in soil or water by bacteria and in the air by oxidative photolytic processes. Therefore, it is not expected that photoinduced oxidation of glyphosate will result in any additional accumulation of methanediol in the environment.

III. CONCLUSIONS

The outcome of several aqueous photolysis studies is summarised in this review report. Further, the impact on direct and indirect photolytic process on the degradation of glyphosate are discussed. Direct photolysis as assessed in sterile buffer solutions under standard conditions are considered to be negligible, whereas an impact of indirect photolysis in natural waters to the degradation of glyphosate in the environment was found.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The review plus expert statment provides a concise overview of the impact of direct and indirect photolysis on the transformation processes of glyphosate observed in the studies considered. As such the review is regarded as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.2/002
Report author	[redacted]
Report year	2005
Report title	Degradation Study: Photodegradation of [^{14}C]glyphosate in Sterilized Pure Water and Natural Water by Artificial Light
Report No	1318W-2
Document No	
Guidelines followed in study	Japan MAFF 12-Nousan-No. 8147, Part 2-6-2 Photodegradation in Water
Deviations from current test guideline	From OECD 316: - For direct photolysis test, distilled water was used instead of buffer solution
Previous evaluation	Not previously evaluated (submitted in AIR2 but not evaluated)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The aqueous photolysis of glyphosate, N-(phosphonomethyl)glycine, was studied using test substances labelled with ^{14}C in either glycine portion of the molecule [C1-^{14}C]glyphosate, or the phosphonomethylene carbon of the molecule, [C3-^{14}C]glyphosate.

Aqueous photolysis experiments were conducted on [C1-^{14}C]glyphosate in sterile distilled water and in sterile natural water collected from Lake Herman, Benicia, CA, at a nominal dose rate of 1.0 µg/mL. An additional photolysis experiment was conducted with [C3-^{14}C]glyphosate test substance using sterile natural water as the test system. The samples were irradiated at constant temperature for up to 12 days of continuous exposure to artificial light source, which was equivalent to 77.5 days of Japanese spring sunlight.

Aqueous solutions of [^{14}C]glyphosate in quartz sample tubes were irradiated with a Suntest CPS+ apparatus equipped with a xenon arc lamp with filters blocking infrared light and wavelengths below 290 nm. The average integrated intensity of the light source for the 300-400 nm range was 50.2 W/m^2. Light exposed samples were placed in a temperature controlled deionized water bath maintained at 25 ± 1 °C throughout the study periods. Dark control samples were incubated concurrently in amber borosilicate vials for each experiment. Duplicate samples were analysed periodically by Liquid Scintillation Counting (LSC) to determine accountability of radioactivity in solution (mass balance) and by high performance liquid chromatography (HPLC) with radioactivity detection for quantitation of components and co-chromatography with reference standards.

Average mass balance ranged from 97.8 ± 1.6 % to 104.9 ± 1.7 % in distilled water and natural water test systems throughout the study period. Glyphosate was stable to photolysis in distilled water and showed very little degradation throughout the 12 days of continuous irradiation. In natural water, glyphosate degraded rapidly when exposed to artificial light and represented an average of 19.8 % and 21.5 % of the dose in [C1-^{14}C] and [C3-^{14}C]glyphosate labelled photolysis experiments, respectively, following 12 days of continuous irradiation. In [C1-^{14}C]glyphosate photolysis experiments, the main degradate detected was $^{14}CO_2$, which represented an average of 75.4 % of dose at the end of the exposure period. In the [C3-^{14}C]glyphosate experiments, aminomethylphosphonic acid (AMPA) and methanediol were the main

degradates detected and represented 19.6 % and 52.0 % of the dose, respectively, at the end of the exposure period.

Glyphosate was relatively stable in dark control samples in both test systems, and represented > 92 % of the dose throughout the incubation period for all sample sets.

The artificial photodegradation half-life of glyphosate (DT_{50}) was calculated using the percent glyphosate detected in the aqueous solutions and the time of irradiation of xenon lamp source, using pseudo-first order kinetics. The solar equivalent half-life of glyphosate was then calculated by comparing the artificial light source to the light irradiance in Tokyo during spring, in the wavelength range of 300-400 nm. Very little degradation was observed during photolysis of glyphosate in distilled water and in all dark control samples. Therefore, no meaningful degradation half-life could be calculated when small changes in the concentration of glyphosate were fitted to pseudo-first order kinetics.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [C1-^{14}C]glyphosate
Lot No.: 040806
Specific activity: 55.0 mCi/mmol)
Radiochemical purity: 96.9 %

Identification: [C3-^{14}C]glyphosate
Lot No.: C-2278
Specific activity: 39.0 mCi/mmol)
Radiochemical purity: 98.6 %

2. Test systems

Glass distilled HPLC grade water with a conductivity of 7µS/cm at experimental start and natural water were used.

Water from Lake Herman, Benicia, CA (38°5'47.4" N latitude, 122°9'3.9" W longitude) was collected on October 28, 2004 and characterised as follows:

Table 7.2.1.2-2: Physico chemical characteristics of the lake water Herman

pH	8.0
Calcium	29 ppm
Magnesium	19 ppm
Sodium	43 ppm
Hardness	150 mg equiv. $CaCO_3$/L
Conductivity	61 µS/cm
Sodium absorption ratio (SAR)	1.52
Total dissolved solids	386 ppm
Turbidity	10.6 NTU
Chloride	32.3 ppm

The pH of the test systems was measured at each sampling and averaged 8.08, 7.24 and 8.29 for distilled water and natural water containing [C1-^{14}C]glyphosate and natural water containing [C3-^{14}C]glyphosate, respectively.

B. STUDY DESIGN

1. Experimental conditions

Photolysis set-up was conducted in Quartz sample tubes (10 mm i.d., 80 nm length), equipped with Teflon-lined silicon septum screw for the irradiated samples. For the dark control samples, amber borosilicate glass vials with Teflon-lined caps were used. The following tests were conducted

- Sterile distilled water treated with [C1-^{14}C]glyphosate
- Sterile natural water treated with [C1-^{14}C]glyphosate
- Sterile natural water treated with [C3-^{14}C]glyphosate

The test systems were sterilised by filtering through a 0.22 micron Falcon Bottle top filter into sterile Erlenmeyer flasks immediately prior to use. The sterility of the test systems was confirmed throughout the experiment. The pH of the sterile solutions was checked with a pH meter prior to dosing. The test substances arrived at PTRL as aqueous solutions prepared in sterile water. Concentration of these stock solutions were 0.1 mCi/mL and 39.6 µCi/mL for [C1-^{14}C]glyphosate and [C3-^{14}C]glyphosate, respectively. The dose solutions were prepared by transferring aliquots of corresponding test substance stock solution in water to sterile amber glass bottles and combining with an aliquot of the respective sterile test system.

Samples were prepared by transferring aliquots (5 mL) of the respective dose solution to sterile quartz or Pyrex sample holders using a 10 mL sterile glass pipette. Aliquots (3 x 100 µL) of the dosing solutions were taken at least before and after application of each set to determine the dose concentration and the homogeneity of the solutions during the dosing process. Stability of the dosing solutions under conditions of administration was demonstrated by HPLC analysis of the time zero samples. The nominal concentration of glyphosate in the samples was 1.0 µg/mL.

The light exposed samples for the natural water set dosed with [C3-^{14}C]glyphosate test substance were set up with continuous trapping of volatiles. Additionally, a set of tubes containing natural water dosed with [C1-^{14}C]glyphosate was equipped with a trapping system and volatiles were collected at the end of the test period. The aeration set up for continuous trapping of headspace was performed during the whole photolysis period. An air pump was used to circulate air through the sample holders. The samples were connected to the air source via Teflon tubing threaded through the septum caps and connected to manifolds. The circulating air was first pumped through a vessel containing sterile deionized water before connecting to the samples to minimize evaporation losses. Each sample was connected at the outlet, to an individual set of traps consisting of one ethylene glycol trap (20 mL) to collect organic volatiles, and two 10 % aqueous sodium hydroxide traps (20 mL each) for CO_2 collection. Trap solutions were housed in glass vials (40 mL capacity) fitted with open top caps with Teflon-lined silicon septa through which the Teflon tubing was threaded in the same fashion as the samples, except that the inlet tubing was placed under the surface of the liquid to bubble the headspace through the trap solutions.

After dosing, light exposed sample tubes were placed in a deionized water bath maintained at an average temperature of 25 ± 1°C by continuous circulation using a circulation bath. Dark control samples were placed in a Hotpack constant temperature chamber maintained at 25 ± 1 °C during the incubation period. Aliquots (2 x 0.1 mL) of the dose solutions were plated on trypticase soy agar for sterility assay at the time of application. The [C3-^{14}C]glyphosate light exposed sample set and the Day 12 samples (duplicate light exposed samples and dark control samples) with natural water, containing [C1-^{14}C]glyphosate were connected to the traps for volatiles and connected to an aeration set up for continuous trapping of headspace during the photolysis period.

The apparatus utilized for exposure of [^{14}C]glyphosate in aqueous solutions to artificial light was a Heraeus Suntest CPS+ unit, equipped with a xenon arc lamp with a filter blocking the radiation from the wavelengths below approximately 290 nm. The Suntest CPS+ was set at a light intensity of 600 W/m^2, which gave an average intensity of 457 W/ m^2 for the 300-800 nm range at the level of the photolysis sample tubes.

2. Sampling
Duplicate light exposed and dark control samples (when applicable) were removed from the water bath and Hotpack chamber and analysed on days 0, 1, 3, 5, 7, 9, and 12 after treatment (DAT). Samples were analysed by LSC and HPLC on the same day they were collected.

At each sampling, trapping solutions for the collection of volatiles were measured (volumes) and aliquots (3 x 1 mL) were radioassayed by LSC.

3. Analytical procedures
All radioassays utilised 5 mL or 15 mL of scintillation cocktail in 7 mL or 20 mL standard polyethylene counting vials and Beckman LS 6500 or LS 6000IC liquid scintillation spectrometers.

[^{14}C]glyphosate and degradates were analysed and quantitated based on HPLC analyses. For all samples the structural assignments for [^{14}C]glyphosate and degradates were based on co-chromatography with reference standards upon HPLC analysis. The limit of detection for individual degradates in the HPLC radiochromatograms were determined by the dpm injected, and the liquid scintillation counting detection limit. As a typical example a limit of 0.002 µg/mL is given for a background of 50 dpm and a sample size of 50,000 dpm injected of a matrix containing 1.0 µg/mL. Representative samples were co-spotted with reference standards and analysed by one-dimensional TLC. After elution, the reference standards were visualized by spraying with ninhydrin reagent and warming the plates to 135 °C to develop the spots. The plates were then scanned with an optical scanner and the radioactive spots matched against the UV trace of the standards.

Initial HPLC analyses of the irradiated samples were conducted by fraction collection followed by LSC counting. The reconstructed radiochromatograms for these analyses showed glyphosate as the only radioactive component. However, the HPLC column recoveries for light exposed natural water samples treated with [C1-^{14}C]glyphosate declined steadily throughout the study period, reaching an average of 20.9 % of the dose by the end of the irradiation period. This suggested the possibility of the presence of a volatile component that was not trapped in the scintillation cocktail during fraction collection and subsequent radioassay by LSC assumed to be $^{14}CO_2$. Since the pH of the aqueous samples averaged 7.24 and the samples were sealed during the exposure period, the $^{14}CO_2$ would be expected to stay in the solution predominantly as non-volatile bicarbonate (H$^{14}CO_3$). HPLC analysis required the use of acidic mobile phase (pH = 2), which would be expected to shift the solution equilibrium toward the volatile $^{14}CO_2$ during HPLC analyses, resulting in low HPLC column recovery due to loss of $^{14}CO_2$ during fraction collection and LSC counting. To confirm this hypothesis, the [C1-^{14}C]glyphosate-irradiated samples were reanalyzed by HPLC using a Beta-Ram flow through detector for ^{14}C detection. HPLC analyses showed the presence of a distinct peak eluting at approximately 6 minutes in addition to the glyphosate peak. The identity of the 6-minute eluting peak was confirmed as $^{14}CO_2$ by comparing its retention time to HPLC analysis of an authentic standard of NaH$^{14}CO_3$ solution. Further confirmation of the formation of $^{14}CO_2$ in the [C1-^{14}C]glyphosate light exposed natural water samples was obtained from the contingency samples that were set up with continuous trapping of volatiles over the entire study period, resulting in an average of 12.1 % of the applied dose recovered in the NaOH traps for the light exposed samples. The radiocarbon in these caustic traps was precipitated with $BaCl_2$ as Ba$^{14}CO_3$, confirming that the trapped radiocarbon was $^{14}CO_2$. As described above, due to the basic pH of the natural water used in the study, some carbon dioxide remained dissolved in the natural water samples. Partial precipitation of the radiocarbon in solution by treatment with $BaCl_2$ also confirmed the presence of dissolved carbonate in the natural water samples.

Confirmation of the identity of methanediol as the major degradate in the [C3-^{14}C]glyphosate irradiated samples was accomplished by derivatisation and co-chromatography with the 2,4-DNPH hydrazone derivative of an authentic standard of methanediol. The presence of methanediol in EG traps was confirmed qualitatively by bubbling air through an aliquot of a selected aqueous sample containing large amount of methanediol [C3-^{14}C]glyphosate treated Natural Water Light Day 9 and passing the headspace gases through three EG traps connected in series. Small amount of radiocarbon was detected in the three EG traps connected in series confirming that the radioactivity from the aqueous sample (e.g. methanediol) was relatively volatile.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate in the separate test systems are summarised in Table 7.2.1.2-3 to Table 7.2.1.2-8.

Table 7.2.1.2-3: Mass balance of [C1^{14}C]glyphosate in distilled water (expressed as percent of applied radioactivity)

DAT		Light exposed	Dark control
0	Replicate A	102.9	-
	Replicate B	102.8	-
1	Replicate A	102.5	103.1
	Replicate B	102.1	105.3
3	Replicate A	101.1	104.0
	Replicate B	101.2	104.3
5	Replicate A	105.6	107.2
	Replicate B	105.4	107.2
7	Replicate A	99.4	103.7
	Replicate B	98.6	103.4
9	Replicate A	97.3	103.6
	Replicate B	94.9	103.5
12	Replicate A	100.8	107.1
	Replicate B	101.2	106.8
Mean recovery	**-**	**101.1 ± 2.9**	**104.9 ± 1.7**

Table 7.2.1.2-4: Mass balance of [C1^{14}C]glyphosate in sterile natural water (expressed as percent of applied radioactivity)

DAT		Light exposed	Dark control
0	Replicate A	99.5	-
	Replicate B	99.9	-
1	Replicate A	99.8	99.9
	Replicate B	99.9	100.9
3	Replicate A	96.3	100.6
	Replicate B	95.3	101.1
5	Replicate A	98.3	101.8
	Replicate B	97.4	102.5
7	Replicate A	99.2	100.6
	Replicate B	98.1	102.0
9	Replicate A	97.5	99.8
	Replicate B	95.8	100.2
12	Replicate A	96.2	102.9
	Replicate B	96.6	101.6
Mean recovery	**-**	**97.8 ± 1.6**	**101.2 ± 1.0**

Table 7.2.1.2-5: Mass balance of [C3^{14}C]glyphosate in sterile natural water (expressed as percent of applied radioactivity)

DAT		Solution	NaOH traps	Ethylene glycol traps	Total recovery
Light exposed					
0	Replicate A	100.5	NA	NA	100.5
	Replicate B	100.5	NA	NA	100.5
1	Replicate A	98.5	0.0	0.0	98.5
	Replicate B	100.4	0.0	0.0	100.4
3	Replicate A	99.6	0.0	0.1	99.7
	Replicate B	101.6	0.0	0.0	101.6
5	Replicate A	97.7	0.0	0.1	97.8
	Replicate B	97.7	0.1	0.2	98.0
7	Replicate A	99.0	0.1	0.4	99.5
	Replicate B	98.1	0.4	0.3	98.8
9	Replicate A	93.9	0.7	1.2	95.8
	Replicate B	98.9	0.2	0.6	99.7
12	Replicate A	97.4	0.6	0.8	98.8
	Replicate B	100.0	0.2	0.7	100.9
Mean recovery					**99.3 ± 1.5**
Dark control					
1	Replicate A	100.6	NA	NA	100.6
	Replicate B	100.2	NA	NA	100.2
3	Replicate A	100.3	NA	NA	100.3
	Replicate B	98.9	NA	NA	98.9
5	Replicate A	97.6	NA	NA	97.6
	Replicate B	95.8	NA	NA	95.8
Mean recovery					**99.3 ± 1.8**

Table 7.2.1.2-6: Degradation of [C1^{14}C]glyphosate in distilled water (expressed as percent of applied radioactivity)

DAT		Total	Glyphosate	Others
Light exposed				
0	Replicate A	102.9	101.5	1.4
	Replicate B	102.8	102.7	0.1
Average		**102.9**	**102.1**	**0.8**
1	Replicate A	102.5	102.4	0.1
	Replicate B	102.1	100.1	2.0
Average		**102.3**	**101.3**	**1.1**
3	Replicate A	101.1	101.0	0.1
	Replicate B	101.2	101.2	0.0
Average		**101.2**	**101.1**	**0.1**
5	Replicate A	105.6	105.3	0.3
	Replicate B	105.4	105.3	0.1
Average		**105.5**	**105.3**	**0.2**
7	Replicate A	99.4	99.0	0.4
	Replicate B	98.6	98.6	0.0
Average		**99.0**	**98.8**	**0.2**
9	Replicate A	97.3	95.5	1.8
	Replicate B	94.9	92.9	2.0
Average		**96.1**	**94.2**	**1.9**
12	Replicate A	100.8	99.8	1.0
	Replicate B	101.2	100.8	0.4
Average		**101.0**	**100.3**	**0.7**
Dark control				
1	Replicate A	103.1	103.0	0.1
	Replicate B	105.3	105.2	0.1
Average		**104.2**	**104.1**	**0.1**
3	Replicate A	104.0	103.9	0.1
	Replicate B	104.3	103.8	0.5
Average		**104.2**	**103.9**	**0.3**
5	Replicate A	107.2	107.2	0.0
	Replicate B	107.2	107.2	0.0
Average		**107.2**	**107.2**	**0.0**
7	Replicate A	103.7	103.5	0.2
	Replicate B	103.4	103.4	0.0
Average		**103.6**	**103.5**	**0.1**
9	Replicate A	103.6	103.6	0.0
	Replicate B	103.5	103.1	0.4
Average		**103.6**	**103.4**	**0.2**
12	Replicate A	107.1	106.8	0.3
	Replicate B	106.8	104.9	1.9
Average		**107.0**	**105.9**	**1.1**

Table 7.2.1.2-7: Degradation of [C1-^{14}C]glyphosate in natural water (expressed as percent of applied radioactivity)

DAT		Glyphosate	others	CO_2	Escaped CO_2[1]	Total recovery CO_2[2]
Light exposed						
0	Replicate A	99.9	0.1	0.0	0.0	0.0
	Replicate B	99.8	0.2	0.0	0.0	0.0
Average		**99.9**	**0.2**	**0.0**	**0.0**	**0.0**
1	Replicate A	76.7	0.0	11.0	12.2	23.2
	Replicate B	77.5	0.0	10.5	11.9	22.4
Average		**77.1**	**0.0**	**10.8**	**12.1**	**22.8**
3	Replicate A	66.8	0.0	17.2	12.3	29.5
	Replicate B	52.6	0.0	19.3	23.4	42.7
Average		**59.7**	**0.0**	**18.3**	**17.9**	**36.1**
5	Replicate A	37.8	0.0	38.9	21.6	60.5
	Replicate B	34.1	0.0	36.7	26.6	63.3
Average		**36.0**	**0.0**	**37.8**	**24.1**	**61.9**
7	Replicate A	56.9	0.0	26.6	15.7	42.3
	Replicate B	35.2	0.0	25.3	37.6	62.9
Average		**46.1**	**0.0**	**26.0**	**26.7**	**52.6**
9	Replicate A	37.7	0.0	39.2	22.6	59.8
	Replicate B	14.9	0.0	53.7	27.3	81.0
Average		**26.3**	**0.0**	**45.5**	**25.0**	**70.4**
12	Replicate A	21.4	0.0	16.2	58.6	74.8
	Replicate B	18.2	2.4	0.0	76.0	76.0
Average		**19.8**	**1.2**	**8.1**	**67.3**	**75.4**
Dark control						
1	Replicate A	99.9	0.1	0.0	NA	0.0
	Replicate B	99.9	0.1	0.0	NA	0.0
Average		99.9	0.1	0.0	NA	0.0
3	Replicate A	100.0	0.0	0.0	NA	0.0
	Replicate B	99.8	0.2	0.0	NA	0.0
Average		99.9	0.1	0.0	NA	0.0
5	Replicate A	99.7	0.3	0.0	NA	0.0
	Replicate B	99.6	0.4	0.0	NA	0.0
Average		99.7	0.4	0.0	NA	0.0
7	Replicate A	95.3	4.7	0.0	NA	0.0
	Replicate B	94.6	5.4	0.0	NA	0.0
Average		95.0	5.1	0.0	NA	0.0
9	Replicate A	98.9	1.1	0.0	NA	0.0
	Replicate B	97.3	2.7	0.0	NA	0.0
Average		98.1	1.9	0.0	NA	0.0
12	Replicate A	98.3	1.7	0.0	NA	0.0
	Replicate B	97.1	2.9	0.0	NA	0.0
Average		97.7	2.3	0.0	NA	0.0

NA = Not applicable

[1] Loss of radioactivity from solution during storage of light exposed samples assumed to be due to loss of CO_2; calculated as (% of dose in aqueous immediately after sampling)-(% of dose remaining in aqueous solution after storage and prior to HPLC analysis) (see Appendix J).

[2] Calculated from % CO_2 from HPLC + % CO_2 from loss of activity attributed to CO_2 escape (Appendix J).

Table 7.2.1.2-8: Degradation of [C3^{14}C]glyphosate in natural water and the dark control (expressed as percent of applied radioactivity)

DAT		Glypho-sate (%)	AMPA (%)	D-2 2.3 min (%)	D-3 4 min (%)	Methane-diol (%)	Others	Ethylene glycol	NaOH	Total
Light exposed										
0	Replicate A	99.3	0.0	0.0	0.0	0.0	0.7	NA	NA	0.0
	Replicate B	99.3	0.0	0.0	0.0	0.0	0.7	NA	NA	0.0
Average		**99.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.7**	**NA**	NA	0.0
1	Replicate A	83.7	2.7	0.3	0.2	11.6	0.0	0.0	0.0	0.0
	Replicate B	85.0	3.5	0.6	0.3	10.9	0.0	0.0	0.0	0.0
Average		**84.4**	**3.1**	**0.5**	**0.3**	**11.3**	**0.0**	**0.0**	0.0	0.0
3	Replicate A	50.0	11.5	1.0	1.4	35.8	0.0	0.1	0.0	0.1
	Replicate B	55.5	10.6	1.1	1.3	32.9	0.2	0.0	0.0	0.0
Average		**52.8**	**11.1**	**1.1**	**1.4**	**34.4**	**0.1**	**0.1**	0.0	**0.1**
5	Replicate A	56.4	10.1	2.8	1.5	26.8	0.0	0.1	0.0	0.1
	Replicate B	31.9	14.9	3.6	2.3	44.9	0.0	02	0.1	0.3
Average		**44.2**	**12.5**	**3.2**	**1.9**	**35.9**	**0.0**	**0.2**	**0.1**	**0.2**
7	Replicate A	25.7	17.3	1.4	3.5	51.1	0.0	0.4	0.1	0.5
	Replicate B	25.3	18.8	1.5	3.3	49.2	0.0	0.3	0.4	0.7
Average		**25.5**	**18.1**	**1.5**	**3.4**	**50.2**	**0.0**	**0.4**	**0.3**	**0.6**
9	Replicate A	27.5	16.9	2.2	2.8	46.3	0.0	1.2	0.7	1.9
	Replicate B	25.5	17.2	3.4	2.5	50.3	0.0	0.6	0.2	0.8
Average		**26.5**	**17.1**	**2.8**	**2.7**	**48.3**	**0.0**	**0.9**	**0.5**	**1.4**
12	Replicate A	21.5	19.6	1.5	2.9	52.0	0.0	0.8	0.6	1.4
	Replicate B[1]	45.6	13.6	2.6	3.8	34.4	0.0	0.7	0.2	0.9
Average		**21.5**	**19.6**	**1.5**	**2.9**	**52.0**	**0.0**	**0.8**	**0.6**	**1.4**
Dark control										
3	Replicate A	98.2	0.0	0.0	0.0	0.0	1.8	NA	NA	0.0
	Replicate B	97.8	0.0	0.0	0.0	0.0	2.2	NA	NA	0.0
Average		**98.0**	**0.0**	**0.0**	**0.0**	**0.0**	**2.0**	**NA**	**NA**	**0.0**
7	Replicate A	92.3	5.2	0.6	0.0	1.7	0.2	NA	NA	0.0
	Replicate B	99.4	0.1	0.0	0.5	0.0	0.0	NA	NA	0.0
Average		**95.9**	**2.7**	**0.3**	**0.3**	**0.9**	**0.1**	**NA**	**NA**	**0.0**
12	Replicate A	95.7	0.6	0.3	0.0	0.9	0.1	NA	NA	0.0
	Replicate B	94.7	0.1	0.2	0.0	0.5	0.3	NA	NA	0.0
Average		**95.2**	**0.4**	**0.3**	**0.0**	**0.7**	**0.2**	**NA**	**NA**	**0.0**

NA = Not applicable
[1] Outlier Not used for product distribution / half-life calculations.

B. MASS BALANCE

The material balance for the study was determined as the radiocarbon recovered in the aqueous samples, and as the sum of radiocarbon in the aqueous samples and volatile traps for those samples with continuous trapping of volatiles and is expressed as percent of applied radiocarbon based on aliquots of the dosing solution. Mass balance for irradiated samples averaged from 101.1 ± 2.9 % and 97.8 ± 1.6 % of the dose in distilled water and natural water containing [C1-^{14}C]glyphosate samples, respectively. In the [C3-^{14}C]glyphosate photolysis experiment, the average radiocarbon recovered in the light exposed samples following 12 days of continuous irradiation was 99.3 ± 1.5 % of the dose.

C. VOLATILE RADIOACTIVITY

The radioactivity trapped in the ethylene glycol (EG) trap (maximum of 1.2 % of the dose) was characterised as methanediol. Additionally, from the irradiation of [C3-^{14}C]glyphosate treated natural water, a maximum of 0.6 % of the applied dose was recovered in the NaOH traps. Treatment of the caustic traps with $BaCl_2$ resulted in very little precipitation of radioactivity as $Ba^{14}CO_3$, demonstrating that majority

of the radioactivity collected in the caustic traps was not due to $^{14}CO_2$ but may have originated as a result of methanediol volatilization.

D. TRANSFORMATION OF THE TEST SUBSTANCE

Glyphosate is relatively stable to photodegradation in distilled water as expected based on its UV spectrum and represented >92 % of the applied dose throughout the irradiation period. Significant degradation is observed in natural water when exposed to artificial light with mean values of 19.8 % AR and 21.5 % AR for C1- and C3 labelled glyphosate, respectively, and the end of the irradiation period. $^{14}CO_2$ was the major degradate observed in the [C1-^{14}C]glyphosate treated light exposed natural water samples and represented an average of 75.4 % of the dose following 12 days of continuous irradiation.

In the [C3-^{14}C]glyphosate experiments, AMPA and methanediol were observed at levels above 10 %AR with maximum amounts of 19.6 % AR and 52.0 % AR, respectively, after 12 days of irradiation in test water treated with [C3-^{14}C]glyphosate.

The proposed pathway in the natural water experiments is indirect photodegradation of glyphosate, induced by active oxidising species (such as peroxides or hydroxyl radicals), which are known to form from the photolysis of natural humic acids present in natural waters. The photoinduced oxidation of glyphosate in natural water may proceed via N-hydroxylation, followed by dehydration of the hydroxylamine, hydrolysis and decarboxylation to obtain methanediol, AMPA and CO_2. AMPA is expected to undergo similar oxidative transformation because of its structural similarity to glyphosate. However, since the AMPA concentration gradually increased during the irradiation period, it can be concluded that its oxidation rate was slower than its rate of formation from glyphosate. Two minor degrades were also observed represented an average of 1.5 % and 2.9 % of dose, respectively, by the end of the photolysis period.

E. HALF-LIFE OF [^{14}C]GLYPHOSATE

The half-life of glyphosate was calculated using pseudo-first order kinetics based on hours of continuous irradiation (light exposed) or days of incubation (dark controls). Very little degradation was observed during photolysis of glyphosate in distilled water and in all dark control samples. Therefore, no meaningful degradation half-life could be calculated when small changes in the concentration of glyphosate were fitted to pseudo-first order kinetics.

The artificial photolysis half-life of glyphosate in natural water was determined as 128 and 126 hours (5.33 and 5.25 days) for [C1-^{14}C] and [C3-^{14}C]glyphosate treated samples, respectively, please refer to Table 7.2.1.2-9. This is equivalent to 34.4 and 33.9 solar days in [C1-^{14}C] and [C3-^{14}C]glyphosate treated samples, respectively, based on Tokyo spring solar day irradiation.

Table 7.2.1.2-9: Determined DT_{50} and DT_{90} of glyphosate

Sample Set	Artificial Light (days)		Solar Days (Tokyo)		R^2
	DT_{50}	DT_{90}	DT_{50}	DT_{90}	
Natural Water [C1-^{14}C]glyphosate Light Exposed	5.33	17.8	34.4	115	0.9247
Natural Water [C3-^{14}C]glyphosate Light Exposed	5.25	17.5	33.9	113	0.9208

III. CONCLUSIONS

Aqueous photolysis of glyphosate was studied using sterile distilled water (pure water) and natural water from Lake Herman, Benicia, CA and exposing a 1.0 µg/mL solution of [^{14}C]glyphosate to an artificial light source for up to 12 days of continuous irradiation. Glyphosate was relatively stable to photolysis in distilled water and represented > 92 % of the applied dose throughout the irradiation period. In contrast, glyphosate degraded rapidly in natural water when exposed to artificial light and represented 19.8 % and 21.5 % of the dose in [C1-^{14}C] and [C3-^{14}C]glyphosate samples, respectively, at the end of the exposure period. The major

degradates detected during photolysis in natural water were CO_2 from the [C1-^{14}C]glyphosate (up to 75.4 % of dose), and AMPA and methanediol (up to 19.6 % and 52.0 %, respectively) from the [C3-^{14}C]glyphosate. The photo induced degradation half-life of glyphosate in natural water ranged from 33.9 to 34.4 solar days (Tokyo, spring) based on pseudo-first order kinetics.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The use of distilled water instead of buffer solution for the direct photolysis test, does not have a significant impact on the outcome of the study as pH was measured.
Therefore, the experiment on direct and indirect photolysis is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.2/003
Report author	[redacted]
Report year	2001
Report title	Glyphosate trimesium Determination of the rate of photolytic degradation in natural water under laboratory conditions
Report No	ZCA/069
Document No	
Guidelines followed in study	Requirements of Japanese Ministry of Agriculture, Forestry and Fisheries Guideline: Photolysis of a Pesticide in Water
Deviations from current test guideline	From OECD 316: - Only the test substance was quantified in test solutions, transformation products were not assessed
Previous evaluation	Not previously evaluated (submitted in AIR2 but not evaluated)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
Filtered and sterilised River water was treated with glyphosate trimesium, ^{14}C-labelled in either the (phosphonomethyl anion, PMG) or trimesium (trimethyl silyl cation, TMS) moiety at a nominal concentration of 2 mg/L. The solutions were continuously irradiated with light from a xenon arc lamp that was filtered to give a spectral distribution close to that of natural sunlight. Samples were maintained at 25 ± 2 °C and irradiated for defined periods up to 144 hours (equivalent to approximately 34 days of Tokyo spring sunlight). Sterility was maintained throughout the course of the study. The pH values ranged from 7.8 - 8.3 throughout the study duration.

Samples of incubated solutions treated with the [^{14}C]trimesium were analysed at zero-time and at approximately 1, 2, 3, 4, 5 and 6 days after application. Samples treated with the ^{14}C-phosphonomethyl anion were analysed at zero-time and at approximately 0.5, 1, 1.5, 2, 2.5 and 3 days after application. Dark

control samples were also prepared and maintained at 25 ± 2 °C in the dark. Single samples were analysed at the same times as the irradiated test solutions.

Total recovery of the applied radioactivity following [^{14}C]trimesium treatment was in the range 96.5 - 99.7 % applied radioactivity (% AR) for irradiated samples and 96.7 – 100 % AR for the dark control samples. At the final analysis time, unchanged [^{14}C]trimesium accounted for a mean of 96.9 % AR and 97.9 % AR in irradiated and dark control solutions respectively. Therefore the photolytic half-life of [^{14}C]trimesium in natural water could not be determined as no significant degradation of the test substance was observed during the test period.

Total recovery of the applied radioactivity following [^{14}C]glyphosate trimesium treatment of natural water was in the range 97.6 – 100 % AR for all irradiated samples and 99.3 – 101 % AR for all dark control samples.

[^{14}C]glyphosate was degraded rapidly in irradiated samples from a mean of 93.7 % AR at zero-time to a mean of 42.5 % AR after 1.5 days (8.1 days sunlight equivalents) and 25.1 % AR after 3 days (16.1 days sunlight equivalents). No degradation was observed in the dark controls and after 3 days the ^{14}C-phosphonomethyl anion accounted for 92.6 % AR. The [^{14}C]glyphosate degraded in irradiated sterile natural river water with a DT_{50} equivalent to 8.8 days of natural spring sunlight in Tokyo (latitude 35°N).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]trimesium
Batch.:	99-42
Specific activity:	2.07 GBq/mmol
Radiochemical purity:	96.8 %
Chemical purity:	not indicated

Identification:	[^{14}C]glyphosate
Batch.:	00-J12
Specific activity:	2.04 GBq/mmol
Radiochemical purity:	94.5 %
Chemical purity:	not indicated

2. Test system:

The natural water used to prepare the samples was from the Great River Ouse, Huntingdon, and Cambridgeshire, UK and was stored at +4 °C until required. The temperature, pH and oxygen saturation of the water was measured at the time collection. The pH was in a range of 7.8 and 8.3 through the study.

Table 7.2.1.2-10: Characterisation of the natural river water

pH[a]	7.75
pH[b]	7.84
Oxygen saturation (%)[a]	70.1
Oxygen saturation (%)[c]	87.8
Oxygen saturation (%)[d]	85.4
Temperature (°C)[a]	11.7
Electrical conductivity (µS/cm)[b]	60 ± 117
Suspended solids (g/L)[b,e]	0.0036 ± 0.0021
Total residue on evaporation (g/L)[b,d]	0.50 ± 0.032

[a] Measured at the time of collection
[b] Measured in the laboratory following filtering and sterilisation
[c] Measured prior to addition of [^{14}C]trimesium
[d] Measured prior to addition of [^{14}C]glyphosate
[e] Mean of three replicates determinations

B. STUDY DESIGN

1. Experimental conditions

The following tests were conducted:

- Sterile natural water treated with [^{14}C]trimesium
- Sterile natural water treated with [^{14}C]glyphosate

First the natural water was filtered through a 212 µm filter to remove large particulate matter prior to filtration through Whatman Grade 5 filter paper to remove further particulate matter. The filtered water was stored at +4 °C in the dark when not in use. Water was stored for no longer than two months. Sterile water was aseptically dispensed into a sterile plastic bottle. An aliquot of the prepared stock solution of [^{14}C]trimesium was added and the solution mixed by inversion to obtain a nominal concentration of 2 mg/L. Same procedure was also done for the stock solution of [^{14}C]glyphosate.

Aliquots of the test solutions (20 mL) were transferred into each of the 22 pre-weighed, sterile photolysis and dark control vessels. The photolysis and control vessels were then capped and re-weighed to determine the exact weight of test solution dispensed. Aliquots (1 mL) of the test solution were taken and radioassayed. The actual application rates were determined as 1.95 µg/mL for [^{14}C]trimesium and 2.00 µg/mL for [^{14}C]glyphosate.

The samples to be irradiated were placed in the Suntest apparatus and irradiation started. The study was conducted using a Suntest Accelerated Exposure Unit (Heraeus Equipment Ltd, Brentwood, Essex, UK) fitted with a xenon arc light source. A system of mirrors and filters prevented ultra-violet radiation with a wavelength of less than 290 nm from reaching the test solutions. Light intensity (irradiance) measurements were made at five representative positions in the Suntest apparatus at the beginning and end of the irradiation period over the wavelength range 250 – 800 nm. The measurements were integrated to provide the total light intensity over the wavelength range 300 – 400 nm. A mean value was and then used to calculate the equivalent time of irradiation of natural Tokyo spring sunlight (latitude 35 °N) received by each test solution.

Irradiated test solutions were maintained within the range 25 ± 2 °C and were stirred continuously. Control vessels were maintained in darkness in a temperature controlled growth room within the range 25 ± 2 °C, and were oscillated continuously.

2. Sampling

Duplicate test solutions were taken for analysis immediately after test substance application and provided a zero-time analysis for both irradiated and dark control experiments. Duplicate irradiated and single non-irradiated treated solutions were taken for analysis at approximately 1, 2, 3, 4, 5 and 6 days for [^{14}C]trimesium after application and 0.5, 1, 1.5, 2, 2.5 and 3 days after application for [^{14}C]glyphosate. Three further treated samples were taken, one at zero-time and one irradiated and one dark control at the final sample time for sterility testing.

3. Analytical procedures

Measurement of Radioactivity was conducted by liquid scintillation counting, using liquid scintillation counters with automatic quench correction. Twice the background was considered as the limit of accurate determination.

High Performance Liquid Chromatography with radiodetection was carried out for analysis of [^{14}C]glyphosate samples. For quantitative analysis, following sample injection, 1 minute fractions of column eluate were collected and radioassayed. The proportion of the total net eluted radioactivity in each fraction was calculated, as well as the recovery of radioactivity from the column. The proportion of [^{14}C]glyphosate in test solutions was derived from this data using only those fractions greater than or equal to twice the background value. Normal phase TLC was carried out to provide confirmatory quantitative data for representative [^{14}C]glyphosate samples.

Reverse phase thin layer chromatography was used for analysis of [^{14}C]trimesium samples. Each radiochromatogram was divided into regions, corresponding to the separated radioactive components and areas in between. The average radioactivity level in the background regions was calculated and subtracted from all other regions to determine total net radioactivity used as to determine the percentage of total net radioactivity in each region.

II. RESULTS AND DISCUSSION

A. DATA

Recoveries of radioactivity of trimesium or glyphosate, respectively in irradiated and dark control samples are summarised in Table 7.2.1.2-11 to Table 7.2.1.2-12.

Table 7.2.1.2-11: Total radioactivity and concentration of [^{14}C]trimesium (TMS) in irradiated and dark control test solutions (expressed as % AR)

DAT	Equivalent duration[1] (days)	Irradiated		Dark control	
		Total radioactivity	TMS	Total radioactivity	TMS
0	0	98.6	99.4	-	-
		99.5	99.1		
1	5.6	99.5	97.8	98.8	98.9
		99.2	98.1		
2	10.4	99.1	97.4	98.6	98.3
		98.9	98.1		
3	16.0	96.5	96.7	96.7	98.9
		97.2	98.1		
4	21.8	99.7	97.3	100	97.3
		99.6	96.4		
5	27.7	99.5	96.5	99.4	97.3
		98.9	96.1		
6	33.8	99.5	96.8	98.3	97.9
		99.5	96.9		

[1] For Tokyo at latitude 35 C spring sunlight

Table 7.2.1.2-12: Total radioactivity and concentration of [^{14}C]glyphosate (PMG) in irradiated and dark control test solutions (expressed as % AR)

DAT	Equivalent duration[1] (days)	Irradiated		Dark control	
		Total radioactivity	PMG	Total radioactivity	PMG
0	0	98.1	93.3	-	-
		97.6	94.1		
0.5	2.6	99.7	68.3	100	92.9
		99.8	67.2		
1	5.5	100	59.2	101	93.8
		100	54.7		
1.5	8.1	99.4	42.5	99.8	90.9
		99.3	42.5		
2	10.9	100	42.0	101	93.4
		100	35.3		
2.5	13.8	100	30.9	101	93.0
		99.9	28.4		
3	16.1	99.3	26.0	99.3	92.6
		98.6	24.2		

[1] For Tokyo at latitude 35 C spring sunlight

Table 7.2.1.2-13: Photodegradation Half-Life of [^{14}C]glyphosate and [^{14}C]trimesium in Natural Water

Sample Set	Artificial Light (days)	Solar Days (Tokyo)	R^2
	DT_{50}	DT_{50}	
Natural Water [^{14}C]glyphosate	1.6 (35.5 hours)	8.8	0.98
Natural Water [a] [^{14}C]trimesium	-	-	-

[a] No significant degradation was observed after irradiation to > 30 days

B. MASS BALANCE

[^{14}C]trimesium material balances ranged from 96.5 to 99.7 % of applied radioactivity (% AR) and [^{14}C]glyphosate material balances ranged from 97.6 to 100 % of applied radioactivity.

C. TRANSFORMATION OF THE TEST SUBSTANCE

At zero-time [^{14}C]trimesium accounted for a mean 99.3 % AR. In all other test solutions, both irradiated and dark control [^{14}C]trimesium accounted for ≥ 96.1 % AR. There was no significant decline in the concentration of [^{14}C]trimesium after approximately 34 days equivalent of Tokyo spring sunlight at latitude 35 °N.

At zero-time, [^{14}C]glyphosate accounted for a mean of 93.7 % AR. After 5.5 days equivalent of Tokyo spring sunlight at latitude 35 °N [^{14}C]glyphosate accounted for a mean of 57.0 % AR. This declined further to a mean of 25.1 % AR after 16.1 days sunlight equivalents. This compared to a 92.6 % AR in the terminal dark control sample treated with this radiolabel.

D. KINETICS

Assuming first order kinetics, the estimated DT_{50} value for [^{14}C]glyphosate in natural river water was reported as 38.5 hours equivalent to 8.8 days of natural spring sunlight in Tokyo (latitude 35 °C). The correlation coefficient (r^2) of the data was 0.98. There was no significant degradation of [^{14}C]glyphosate in dark control solutions.

III. CONCLUSIONS

The phosphonomethyl anion of glyphosate trimesium is photolytically labile and degraded in natural river water under sterile conditions with a DT_{50} of approximately 8.8 days of natural spring sunlight in Tokyo (latitude 35°N). However, the trimethyl silyl cation is photolytically stable in sterile natural water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes the indirect photodegradation rate of glyphosate trimesium in sterilised natural water. Samples were analysed for glyphosate only. The study considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.2/004
Report author	[redacted]
Report year	1992
Report title	Glyphosate-Trimesium – aqueous photolysis
Report No	RR91-065B
Document No	
Guidelines followed in study	U.S. EPA 161-2
Deviations from current test guideline	From OECD 316: - LOQ not specified - Test systems were sterilised but sterility was not confirmed
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The aqueous photolysis of trimesium glyphosate (phosphonomethylglycine, trimethylsulfonium salt) in sterile buffers at pH 7 was investigated under simulated sunlight at a temperature of 25 °C for 14 days. Xenon arc lamp light was filtered to give a spectral distribution approximating that of sunlight. The photostability of both glyphosate and trimesium was investigated with anion and cation ^{14}C radiolabeled test substance in separate tests.

Light intensity emitted by the xenon arc lamp was calculated to be 446, 469 and 437 watt/m^2 after 0, 4 and 13.6 days of continuous artificial irradiation for [^{14}C]glyphosate and 434 and 454 watt/m^2 after 0 and 15.8 days for [^{14}C]trimesium.

Duplicate tubes of photolysed and dark control samples were withdrawn on days 0, 1.7, 4.0 6.7, 8.7, 11.8 and 13.6 for all [^{14}C]trimesium samples and [^{14}C]glyphosate irradiated samples. Dark control samples for [^{14}C]glyphosate were sampled on days 0, 2.0, 5.9, 8.1, 10.0, 11.9 and 13.9.

Dark control tests established both the anion and cation were stable to hydrolysis over the 2 weeks required for the photolysis tests. At an initial total [^{14}C]glyphosate concentration of 186 mg/L, the photolytic DT_{50} at 25 °C was determined to be 81 days of solar exposure for glyphosate in aqueous solution at pH 7. At an initial [^{14}C]trimesium glyphosate concentration of 7.1 mg/L, the trimesium was stable within experimental error, with a DT_{50} greater than 1 year.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate trimesium, glyphosate (phosphono[^{14}C]methyl)labeled, [^{14}C]PMG
Lot No.:	WRC 13269-04
Specific activity:	56 Ci/mol
Radiochemical purity:	95 %

Identification:	Glyphosate trimesium, trimesium (tri[^{14}C]methylsulfonium)labeled, [^{14}C]TMS
Lot No.:	WRC 13453-21
Specific activity:	25 Ci/mol
Radiochemical purity:	>96 %

2. Buffers:
Phospate buffer solutions (25 mM, pH 7) were prepared from potassium dihydrogen phosphate and sodium hydroxide using distilled water. The pH was adjusted to 7.00 ± 0.05 with sodium hydroxide. Buffer solutions were then autoclaved at 121 °C for 1 hour.

B. STUDY DESIGN

1. Experimental conditions
Photolysis tubes (1 cm x 10 cm) consisted of cylindrical quartz tubes. Each tube had a tapered ground-glass joint fitted with a polytetrafluoroethylene (PFTE) stopper. Irradiated and dark control samples were placed in a separate tank covered with aluminium foil to exclude light. Distilled water was then poured into the photolysis chamber covering all test samples. The photoreactor was a stainless steel chamber closed with a quartz window at the top and equipped with a cooling system keeping test solutions at approximately 25 ± 1 °C during irradiation. The temperature of samples was monitored with a thermocouple inserted into a photolysis tube filled with distilled water and a recirculating water bath was used to control the temperature of test solutions. Sterility of the test systems throughout the experiments was not confirmed.

For each test, the glyphosate-trimesium stock solution was prepared by adding radiolabelled glyphosate-trimesium in water to a sterilized volumetric flask (100 or 200 mL) and filled up with pH 7 buffer. The resulting solution was filtered through a sterile 0.2 µm filter in a laminar flow hood. A 3 to 7 mL aliquot of the filtered glyphosate-trimesium stock solution was added to each photolysis and dark control photolysis tube. The concentration of glyphosate-labelled test substance was 186 mg/L (as glyphosate-trimesium), which consisted of 182.3 mg/L of nonlabelled and 3.3 mg/L of labelled glyphosate-trimesium. The radioactivity concentration was 1690 and 1677 dpm/µL in irradiated and dark control samples, respectively. The concentration of trimesium-labelled test substance was 7.1 mg/L glyphosate-trimesium corresponding to 1599 dpm/µL.

The photolysis chamber was irradiated continuously under a Heraeus Suntest xenon arc lamp. The lamp output was collimated with aluminum parabolic reflectors. UV filters were used to remove wavelengths below 290 nm. A spectroradiometer (spectral range 300 – 850 nm) was used to measure the light intensity and emission spectrum of the xenon arc lamp inside the chamber. The integrated xenon light intensity over

the wavelength range 300 – 800 nm was measured at least at beginning and end of the study. The averaged intensity was used to calculate the sunlight equivalent received by samples. The local solar spectrum at Richmond, CA (latitude 37° 56´N) was similarly measured for comparison of solar and xenon lamp emission spectra. Three and two measurements of light intensity were averaged for anion and cation labelled glyphosate trimesium. The integrated light intensities for the samples treated with glyphosate labelled test substance over 13.6 days of continuous artificial light were equivalent to 29.3 days of natural sunlight. For the trimesium labelled test, the integrated light intensities from 300 to 800 nm over 15.8 days of continuous artificial irradiation amounted to an equivalent of 33.5 days of natural sunlight.

2. Sampling
Duplicate tubes of photolysed and dark control samples were withdrawn on days 0, 1.7, 4.0 6.7, 8.7, 11.8 and 13.6 for all [^{14}C]trimesium samples and [^{14}C]glyphosate irradiated samples. Dark control samples for [^{14}C]glyphosate were sampled on days 0, 2.0, 5.9, 8.1, 10.0, 11.9 and 13.9.

3. Analytical procedures
Photolysis and dark control samples were analysed by directly injecting the solutions into HPLC. HPLC fractions were collected in scintillation vials after the eluent was mixed with scintillation cocktail. Later the radioactivity in each fraction was measured by LSC. The radioactivity in the solutions was measured by counting 20 or 25 mL aliquots of each sample solution. All components were analysed relative to the radioactivity in solution at the start of the test, which served as a check on losses by volatilisation, sorption, or precipitation of test substance or products. Analysis by TLC was performed as confirmatory method.

The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC/radiodetection method were not reported.

4. Calculations
The pseudo first-order rate constant was determined from the slope of a line generated by a linear least-squares fit of the natural logarithm of the relevant glyphosate-trimesium ion concentration (glyphosate or trimesium) versus time. The fraction of the initial total radioactivity present as [^{14}C]glyphosate or [^{14}C]trimesium was used to measure the concentration of the relevant glyphosate-trimesium ion.

The net pseudo first-order photolytic DT_{50} was calculated as:
$t_{1/2} = \ln 2 / (k_i - k_d)$

Where
$t_{1/2}$ = net photolysis DT_{50}
k_i = pseudo first-order rate constant for the irradiated samples
k_d = pseudo first-order rate constant for the dark control samples

As both glyphosate trimesium ions were stable in the dark controls, the above equation can be simplified to
$t_{1/2} = \ln 2 / k_i$

The integrated light intensities (300 – 800 nm) were calculated as follows:

$I_{ave} = (I_0 + \ldots I_f) / n$

Where
I_0 = integrated intensity (watt/m^2) measured at the beginning of irradiation period
I_f = integrated intensity (watt/m^2) measured at the end of irradiation period
n = number of intensity measurements
I_{ave} = average intensity during the test

The amount of radiation received from the continuous exposure for time t (days) can be converted to natural summer equivalent days (SED) with the following equation:

$SED = (I_{ave} \times 24 \times t) / 5030$

Where
5030 = the averaged daily sunlight irradiation measured for three consecutive days (June 21-23, 1988) at Richmond, CA
I_{ave} = average intensity during the test

II. RESULTS AND DISCUSSION

A. DATA

Radioactivity measurements for trimesium or glyphosate labelled test substance in irradiated and dark control samples are summarised in Table 7.2.1.2-14 to Table 7.2.1.2-17.

Table 7.2.1.2-14: Degradation of glyphosate labelled glyphosate trimesium (^{14}C-PMG) and metabolites in irradiated test solutions (expressed as % of applied radioactivity)

Sample point	Irradiation time (days)	Solar sunlight equivalent (days)	% AR			
			^{14}C-PMG	AMPA	Unassigned radioactivity	Mass balance[1]
0	0	0	94.0	4.0	1.6	100
			97.3	4.1	2.1	104
1	1.7	3.8	95.5	6.9	2.4	105
			97.3	6.6	1.5	105
2	4.0	8.6	93.5	7.2	2.5	103
			90.3	9.4	3.2	103
3	6.7	14.4	80.8	12.0	3.7	96
			87.7	12.7	4.5	105
4	8.7	18.6	83.2	11.6	3.6	98
			86.3	9.0	4.1	99
5	11.8	25.3	82.9	12.6	4.7	100
			80.0	10.7	4.7	95
6	13.6	29.3	75.8	18.1	6.1	100
			71.2	18.9	6.9	97
Avarage						**100.8**
Standard deviation						**3.4**

[1] Total radioactivity collected after each HPLC injection expressed as a percent of the initial radioactivity per unit volume multiplied by the sample loop volume.

Table 7.2.1.2-15: Degradation of glyphosate labelled glyphosate trimesium (^{14}C-PMG) and metabolites in dark control test solutions (expressed as % of applied radioactivity)

Sample point	Sampling dates (days)	% AR			
		^{14}C-PMG	AMPA	Unassigned radioactivity	Mass balance[1]
0	0	93.0	3.9	2.1	99
		93.7	4.0	2.0	100
1	2.0	93.0	3.2	1.6	98
		94.7	3.4	1.5	100
2	5.9	94.5	3.6	1.2	99
		97.7	4.3	3.6	106
3	8.1	89.5	3.4	1.3	94
		94.5	3.8	1.7	100
4	10.0	92.4	3.3	0.9	97
		94.7	3.6	1.6	100
5	11.9	95.9	3.6	1.6	101
		95.5	4.2	2.3	102
6	13.9	94.9	3.4	1.2	99
		95.0	4.0	2.3	101
Avarage					**99.7**
Standard deviation					**2.6**

[1] Total radioactivity collected after each HPLC injection expressed as a percent of the initial radioactivity per unit volume multiplied by the sample loop volume.

Table 7.2.1.2-16: Degradation of trimesium labelled glyphosate trimesium (^{14}C-TMS) and metabolites in irradiated test solutions (expressed as % of applied radioactivity)

Sample point	Irradiation time (days)	Solar sunlight equivalent (days)	% AR			
			^{14}C-TMS	Unknown	Unassigned radioactivity	Mass balance[1]
0	0	0	92.7	0.9	1.0	95
			94.6	1.0	1.2	97
1	1.7	1.6	96.3	0.9	1.1	98
			97.2	1.0	1.2	99
2	4.0	4.7	96.5	1.0	1.3	99
			97.1	1.1	1.2	100
3	6.7	7.6	94.8	0.8	0.7	96
			97.3	0.9	0.6	99
4	8.7	10.6	96.0	3.3	0.6	100
			93.3	1.0	0.7	95
5	11.8	27.6	84.7	1.1	0.6	86
			95.9	1.0	0.5	98
6	13.6	33.5	97.9	0.9	0.4	99
			96.3	0.9	0.5	98
Avarage						**97.0**
Standard deviation						**3.5**

[1] Total radioactivity collected after each HPLC injection expressed as a percent of the initial radioactivity per unit volume multiplied by the sample loop volume.

Table 7.2.1.2-17: Degradation of trimesium labelled glyphosate trimesium (^{14}C-TMS) and metabolites in dark control test solutions (expressed as % of applied radioactivity)

Sample point	Sampling dates (days)	% AR			
		^{14}C-TMS	Unknown	Unassigned radioactivity	Mass balance[1]
0	0	92.7	0.9	1.0	95
		94.6	1.0	1.2	97
1	1.7	96.4	1.0	1.1	98
		96.2	1.0	1.2	98
2	4.0	95.8	1.0	1.3	98
		96.0	1.0	1.3	98
3	6.7	96.2	0.8	0.7	98
		95.5	0.8	0.5	97
4	8.7	95.0	0.8	0.6	96
		94.7	0.9	0.8	96
5	11.8	96.2	1.2	0.6	98
		96.4	0.8	0.6	98
6	13.6	97.3	0.8	0.4	98
		97.1	0.8	0.5	98
Avarage					**97.5**
Standard deviation					**1.1**

[1] Total radioactivity collected after each HPLC injection expressed as a percent of the initial radioactivity per unit volume multiplied by the sample loop volume.

B. MASS BALANCE

Mass balances were determined by relating the ratio of radioactivity collected during an HPLC run to the initial total radioactivity. [^{14}C]trimesium material balances ranged from 95 to 100 % AR and [^{14}C]glyphosate material balances ranged from 95 to 100 % AR.

C. TRANSFORMATION OF THE TEST SUBSTANCE

At zero-time [^{14}C]trimesium accounted for 93.7 % AR (mean of two duplicates). With the exception of a single sample accounting for 84.7 % AR, values were > 93 AR in all other test solutions, both irradiated and dark control. There was no significant decline in the concentration of [^{14}C]trimesium after approximately 34 days equivalent of natural sunlight at latitude 38 °N.

At 0 DAT, [^{14}C]glyphosate accounted for 95.7 % AR (mean of two duplicates). After 33.5 days equivalent of natural sunlight at latitude 38 °N [^{14}C]glyphosate was decreased to 73.5 % AR(mean of two duplicates). In the dark control samples treated with this radiolabel 95 % AR were encountered at the end of the test period. At the beginning of the irradiation period, AMPA accounted for 4.05 % AR (mean of two duplicates). After 33.5 days equivalent of natural sunlight at latitude 38 °N AMPA had increased to 18.5 % AR (mean of two duplicates). No other degradation products were identified in the study. In the dark control, AMPA remained at a level of ca. 4 % AR throughout the study period.

D. KINETICS

The photolysis DT_{50} reported was 81 sunlight equivalent days for glyphosate at pH 7 and 25 °C.

III. CONCLUSIONS

After irradiation by light from a xenon arc lamp, [^{14}C]glyphosate photolysed in solution at pH 7 and 25 °C, to yield aminomethylphosphonic acid. The pseudo first-order DT_{50} was 81 days of clear weather summer sunlight at 38° N.

[^{14}C]trimesium was stable to the equivalent of one month of exposure to clear weather summer sunlight at 38° N. The calculated pseudo first-order DT_{50} was 5.1 years of clear weather summer sunlight at 38° N.

The overall rate of photolysis of [^{14}C]trimesium should be considered the rate of photolysis of the anion, [^{14}C]glyphosate, with a half-life of 81 days of clear weather summer sunlight at 38° N.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted mainly in agreement with the current guideline. The test items were sterilised but sterility was not checked throughout the experiment. Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.2/005
Report author	[redacted]
Report year	1990
Report title	Degradation Study: Photodegradation of [^{14}C]glyphosate in Buffered Aqueous Solution at pH 5, 7 and 9 by Natural Sunlight
Report No	233W-1
Document No	
Guidelines followed in study	U.S. EPA 161-2
Deviations from current test guideline	From OECD 316: - test systems were exposed to natural sunlight instead of artificial light
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
[^{14}C]glyphosate [N-(phosphonomethyl)glycine] was exposed to natural sunlight in sterile pH 5, 7, and 9 aqueous buffers, concurrendy with dark control samples.. All samples were maintained in a water bath at 24.5±0.7°C. The nominal test substance concentration was 0.9, 0.9, and 0.8 ppm for the pH 5, pH 7 and pH 9 buffer solutions, respectively.

The pH 7 test was conducted with volatile trapping and consisted of a zero time and five additional samples taken over a 31 day period. The extrapolated half-life of degradation of glyphosate was 413 days in light exposed and 555 days in dark control samples. The correlation coefficients (r^2) for the linear regression calculations were poor (0.15 and 0.09 for the light and dark respectively) reflecting the minimal degradation which occured during the 31 day study period. No unknown products were observed by HPLC analysis, organic volatiles represented less than 0.6 % of applied radioactivity (AR) and 0.4 % was trapped as CO_2. Material balance was averaged 97.1±4.5 % and 95.7±4.7 % for the light and dark samples, respectively.

The comparative studies, conducted under the same conditions in pH 5 and 9 buffer solutions, consisted of 0 DAT and 29 DAT samples in sealed containers without volatile trapping. Material balance averaged 101.0+1.5 % and 100.5+0.5 % for the light and dark samples in pH 5 buffer, and 100.8±0.3 % and 98.8±0.6 % in pH 9 buffer, respectively. Results of the HPLC analysis of these samples were consistent with the pH 7 study. Minimal photodegradation of [^{14}C]glyphosate was observed at pH 5, 7, or 9.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate
Lot No.:	C927-51B
Specific activity:	8.08 mCi/mmole
Radiochemical purity:	100 %
Chemical purity:	-

2. Test systems:

All water used in the preparation of buffer solutions was filtered using a Barnstead NANO-Pure II system which produces Type I Reagent Grade water per ASTM-D1193 (conductivity: 70fiS, dissolved solids: 30 ppm as NaCl, 27 ppm as $CaC0_3$).

- pH 5: Acetic Acid-Sodium Acetate: 146 mL of 0.1 M acetic acid added to 100 mL of 0.1 M NaOH and then deionized water added to a final volume of one liter.
- pH 7: Potassium Dihydrogen Phosphate-Disodium Hydrogen Phosphate: 22.4 mL of 0.1 M KH_2PO_4 was added to 25.8 mL of 0.1 M Na_2HP0_4 and then deionized water added to a final volume of one liter.
- pH 9: Sodium Borate-Hydrochloric Acid: 11.5 mL of 0.04 M HCl added to 125 mL of 0.01 M $Na_2B_4O_7$ and deionized water then added to a final volume of 250 mL.

All solutions were adjusted to the precise pH by addition of NaOH or HCl as indicated. The nominal ionic strength of each buffer solution was 0.01 M. The solutions were sterilised by filtering through a 0.2 micron Falcon filter and the pH was rechecked. The pH was also measured in the test samples and found to be stable throughout each of the study periods. Sterility of the test systems throughout the experiments was confirmed.

B. STUDY DESIGN

1. Experimental conditions

Sample tubes used for exposure of [^{14}C]glyphosate in pH 7 buffer to natural sunlight were made of quartz for the irradiated samples, those used for the dark control samples were made of pyrex. Individual pyrex tubes (8 mL) with teflon lined caps sealed with Parafilm were used for the pH 5 and 9 buffer solutions. All dark control samples were covered with aluminum foil to prevent irradiation. The sample tubes were placed in a distilled water bath at a 60 degree vertical angle to maximize irradiation during periods of strong sunlight intensity. The temperature in the water bath was maintained at approximately 25° C by continuous circulation. The temperature was continuously monitored and recorded at 20 minute intervals throughout the study.

Ethylene glycol and 10 % NaOH were used to trap volatile organic compounds and CO_2, respectively in the pH 7 test. Air was drawn through sterilised bacterial filters into both the light and dark sample tubes and then into separate sets (light and dark) of three traps (1 EG, 2 10 % NaOH). Gas dispersion tubes were used to maximize the trapping efficiency. Trapping efficiency for $^{14}CO_2$ (100.9 %) was determined, using the identical system, by introducing a measured amount of ^{14}C-sodium bicarbonate (Sigma) as an aqueous

solution into a sample holder and adding an excess (3 mL) of glacial acetic acid while air was being drawn through the system. $^{14}CO_2$ was trapped by two sodium hydroxide traps in series over a 2 day period. Volatiles were not trapped in the pH 5 and 9 samples.

Mean light intensity and daily total light energy ranged from 9953 µW/cm² to 16789 µW/cm² and 8.19 to 11.08 W min/cm² for the pH 7 test period. For pH 5 and pH 9 ranges of 7684 µW/cm² to 13897 µW/cm² and 6.5 to 11.08 W min/cm² were determined.

Application solutions for each pH were prepared by adding aliquots of [^{14}C]glyphosate to the sterilised buffer solutions. For pH 7, 144 µL was added to 300 mL buffer. For pH 5 and 9, 24 µl was added to 50 mL of each buffer solution. The resulting solutions were stirred. Aliquots (10 mL) of the pH 7 test solution were transferred into each sample holder using aseptic technique. Aliquots taken from the time 0 samples were averaged to determine the applied radiocarbon. Similarly, aliquots (5 mL) of the pH 5 and 9 test solutions were transferred to pyrex tubes. Aliquots from the stock solutions were used to determine the applied radiocarbon. The measured concentration of glyphosate in the pH 5, 7 and 9 solutions was 0.9, 0.9 and 0.8 ppm respectively.

2. Sampling

For pH 7, duplicate light exposed and dark control samples were removed from the water bath at 0, 5, 11, 17, 26 and 31 DAT. Volumes and pH were measured and the samples were analysed promptly. Aliquots of the samples were subjected to LSC in triplicate (3 x 50 µl). A separate rinse of the sample holders with approximately 2 mL of ammonium bicarbonate was analysed by LSC to determine if any radiocarbon had deposited on the walls. Total volumes in each gas dispersion trap were measured and aliquoted (3 x 0.5 mL) for radioassay (LSC) at each sampling time. Recovered radiocarbon from each trap was divided equally among the contributing samples.

Duplicate light exposed and dark control samples for the pH 5 and 9 samples were taken from the water bath at 0 DAT and 29 DAT. The volumes and pH were measured and the samples were analysed first by LSC then by HPLC.

3. Analytical procedures

Samples were analysed by LSC immediately following their removal from the water bath. All radioassays utilised 5 mL of scintillation cocktail in 7 mL standard polyethylene counting vials and Beckman LS 5000 CE liquid scintillation spectrometers.

Test and control samples were analysed by HPLC by direct injection of 100 µl of the aqueous samples typically within 48 hours of sampling. Chromatographic methods (HPLC) were validated with authentic standards achieving the necessary resolution and sensitivity. Both the UV and radiocarbon peak of glyphosate using the initial HPLC method were characteristically broad peaks. Selected samples were re-analysed with a second HPLC method that provided better resolution and peak shape. LOQ and LOD are described as 0.6 % AR 0.1 % AR, respectively.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate in pH 7 buffer under irradiation and respective dark controls and mass balance for pH 5 and pH 9 buffers are summarised in Table 7.2.1.2-18 to Table 7.2.1.2-22 for the respective pH values.

Table 7.2.1.2-18: Material balance of [^{14}C]glyphosate in pH 7 buffer under irradiation with natural sunlight (expressed as percent of applied radioactivity)

Sample/Replicate	Buffer solution pH 7	Volatiles		Total Recovery
		CO_2	Ethylene glycol	
Hour 0				
Irradiated 1	100.8			100.8
Irradiated 2	100.6			100.6
Day 5				
Irradiated 1	92.2	0.2	0	92.4
Irradiated 2	92.3	0.2	0	92.5
Day 11				
Irradiated 1	100.2	0.2	0	100.5
Irradiated 2	100	0.2	0	100.2
Day 17				
Irradiated 1	102	0.3	0.1	102.4
Irradiated 2	97.6	0.3	0.1	98
Day 26				
Irradiated 1	96.5	0.3	0.3	97.2
Irradiated 2	92.6	0.3	0.3	93.3
Day 31				
Irradiated 1	86.9	0.4	0.5	87.8
Irradiated 2	98	0.4	0.5	98.9

Table 7.2.1.2-19: Material balance of [^{14}C]glyphosate in pH 7 buffer dark controls (expressed as percent of applied radioactivity)

Sample/Replicate	Buffer solution pH 7	Volatiles		Total Recovery
		CO_2	Ethylene glycol	
Hour 0				
Dark Control (I)	100.2	-	-	100.2
Dark Control (2)	98.4	-	-	98.4
Day 5				
Dark Control (1)	91.5	0.2	0	91.7
Dark Control (2)	90.1	0.2	0	90.3
Day 11				
Dark Control (1)	100.7	0.2	0	101
Dark Control (2)	93.8	0.2	0	94
Day 17				
Dark Control (1)	104.2	0.3	0.1	104.5
Dark Control (2)	98.1	0.3	0.1	98.5
Day 26				
Dark Control (1)	91.1	0.3	0.1	91.6
Dark Control (2)	90.7	0.3	0.1	91.1
Day 31				
Dark Control (1)	94.1	0.4	0.1	94.5
Dark Control (2)	93.1	0.4	0.1	93.6

Table 7.2.1.2-20: Degradation of [-^{14}C]glyphosate in pH 7 buffer solution irradiated with natural sunlight irradiation and dark controls(expressed as percent of applied radioactivity)

Compound	Replicate	DAT					
		0	5	11	17	26	31
Glyphosate	Irradiated 1	100.8	92.2	100.2	102.0	96.5	86.9
	Irradiated 2	100.6	92.3	100.0	97.6	92.6	98.0
	Dark Control (1)	100.2	91.5	100.7	104.2	91.1	94.1
	Dark Control (2)	98.4	90.1	93.8	98.1	90.7	93.1
CO2	Irradiated 1	-	0.2	0.2	0.3	0.3	0.4
	Irradiated 2	-	0.2	0.2	0.3	0.3	0.4
	Dark Control (1)	-	0.2	0.2	0.3	0.3	0.4
	Dark Control (2)	-	0.2	0.2	0.3	0.3	0.4
Unknowns	Irradiated 1	0.0	0.0	0.0	0.1	0.3	0.5
	Irradiated 2	0.0	0.0	0.0	0.1	0.3	0.5
	Dark Control (1)	0.0	0.0	0.0	0.0	0.1	0.1
	Dark Control (2)	0.0	0.0	0.0	0.0	0.1	0.1
Total recovery	Irradiated 1	100.8	92.4	100.4	102.4	97.1	87.8
	Irradiated 2	100.6	92.5	100.2	98.0	93.2	98.9
	Dark Control (1)	100.2	91.7	100.9	104.5	91.5	94.6
	Dark Control (2)	98.4	90.3	94.0	98.4	91.1	93.6

DAT: Days after treatment

Table 7.2.1.2-21: Degradation of [-14C]glyphosate in pH 5 buffer solution under natural sunlight irradiation and dark controls (expressed as percent of applied radioactivity)

Compound	Replicate	DAT	
		0	29
Glyphosate	Irradiated 1	100.1	103.2
	Irradiated 2	99.8	100.9
	Dark Control (1)	100.3	100.9
	Dark Control (2)	99.8	100.9
Total recovery	Irradiated 1	100.1	103.2
	Irradiated 2	99.8	100.9
	Dark Control (1)	100.3	100.9
	Dark Control (2)	99.8	100.9

DAT: Days after treatment

Table 7.2.1.2-22: Degradation of [-14C]glyphosate in pH 9 buffer solution under natural sunlight irradiation and dark controls (expressed as percent of applied radioactivity)

Compound	Replicate	DAT	
		0	29
Glyphosate	Irradiated 1	100.4	100.7
	Irradiated 2	101.1	100.9
	Dark Control (1)	99.2	99.7
	Dark Control (2)	99.3	98.3
Total recovery	Irradiated 1	100.4	100.7
	Irradiated 2	101.1	100.9
	Dark Control (1)	99.2	99.7
	Dark Control (2)	99.3	98.3

B. MASS BALANCE
Mass balance for pH 7 averaged 97.1 ± 4.5 % AR and 95.7 ± 4.7 % AR in light and dark samples, respectively. Radiocarbon recoveries based on solute measurements only for pH 5 averaged 101.0 ± 1.5 % and 100.5 ± 0.5 % for the light and dark samples, respectively. For pH 9, light and dark samples averaged 100.8 ± 0.3 % and 98.8 ± 0.6 % of the applied radiocarbon, respectively.

D. VOLATILE RADIOACTIVITY
Carbon dioxide at study end amounted to 0.4 % AR in buffer pH 7 for both, irradiated and dark control samples. Organic volatiles determined were ≤ 0.5 % AR for pH 7 buffer at the end of the study (31 DAT).

E. TRANSFORMATION OF THE TEST ITEM
Glyphosate degraded only minimally over the study period in any of the pH buffer test solutions. No difference in degradation was observed for the separate buffer solutions tested over the study period.

F. KINETICS
The extrapolated half-life (regression analysis assuming first-order kinetics) of light exposed and dark control samples in pH 7 buffer was determined in the report as 413 days ($R^2 = 0.15$) and 555 days ($R^2 = 0.09$), respectively.

III. CONCLUSIONS

[^{14}C]glyphosate degrades very slowly in pH 5, 7 or 9 buffer solutions when exposed to natural sunlight for up to 31 days. The extrapolated half-life of light exposed and dark control samples in pH 7 buffer is 413 days ($R^2 = 0.15$) and 555 days ($R^2 = 0.09$) respectively. The poor correlation coefficients reflect the minimal amount of degradation observed during the study period with respect to the long half-life. No significant difference in material balance or degradation is detected among pH 5, 7 or 9 samples. These results indicate that photodecomposition is a minor process for degradation of glyphosate in the environment.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The test systems were exposed to natural sunlight instead of artificial light. Nevertheless, the study indicates that photodegradation is a minor process in aquatic degradation process.
Therefore, the study is considered as supportive information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.2/006
Report author	[redacted]
Report year	1992
Report title	Photodegradation study of [^{14}C]glyphosate in water at pH 5, 7 and 9
Report No	250751
Document No	
Guidelines followed in study	U.S. EPA 540/9-82-021 Section 161-2 Photodegradation Studies in Water
Deviations from current test guideline	From OECD 316: - Impurities of up to 7 % were present at day 0
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The photolytic degradation behaviour of [^{14}C]glyphosate (N-(Phosphonomethyl)-glycine), was investigated in aqueous buffer solutions at pH 7.3, 5.1 and 9.2. [^{14}C]glyphosate was applied to the aqueous solution at a dose of 94 – 102 µg/mL and continuously exposed for 15 days, i.e. the equivalent of 30 days natural sunlight (12 hours of light/day).

Total recoveries of radioactivity at all time intervals amounted, on average, to 99.2 + 2.7 % (pH 7.3), 99.5 + 3.2 % (pH 5.1) and 99.5 ± 2.3 % (pH 9.2). Only small amounts of volatile radioactivity (0.1 - 0.3 %) could be trapped by NaOH at every pH. No radioactivity was trapped by 2-methoxy-ethanol, even when the trap was additionally acidified with acetic acid. In the dark, total recovery at every pH ranged, on average, from 99.4 + 1.3 % to 101.7 + 2.9 %. At pH 7.3 and 9.2, small amounts of volatiles (0.4 - ~0.5 %) were trapped by NaOH and none by 2-methoxy-ethanol/acetic acid. At pH 5.1, no volatiles were trapped. The amounts of radioactivity in the aqueous solutions at every pH mainly reflected the amounts of radioactivity totally recovered at all time intervals and ranged from 95.8 - 102.2 % (pH 7.3), from 94.0 - 102.9 % (pH 5.1) and from 96.1 - 102.6 % (pH 9.2).

At pH 7.3, the amount of parent compound decreased from 94.0 % at day 0 to 83.9 % and 82.3 % at days 7 and 15, respectively. Besides the parent compound, within the first 7 days, one to three minor radioactive fractions (M2 - M4) were detected, accounting for 0.4 - 6.7 %. At day 15, only two radioactive fractions (M2 and M4) were found. The major radioactive fraction M2 (characterised as aminomethylphosphonic acid/AMPA) accounted for 11.6 % and the radioactive fraction M4 was detected at a minor amount of 1.9 %. At pH 5.1, the amount of parent compound decreased from 95.5 % at day 0 to 80.8 % and 70.7 % at days 7 and 15, respectively. At all time intervals, besides the parent compound, two radioactive fractions were detected. The major radioactive fraction was characterised as AMPA, increasing, to 10.1 % and 16.0 % at days 7 and 15, respectively. Radioactive fraction M3 occurred in minor amounts and ranged from 2.7 - 8.4 % during the incubation period. At pH 9.2, the amount of parent compound decreased from 95.0 % (day 0) to 89.0 % (day 7) and to 83.1 % (day 15). Besides the parent compound, at all time intervals, two minor radioactive fractions AMPA and M3 occurred, ranging from 1.8 % to 6.5 %.

In the dark, at all three pH-values, besides minor amounts (1.6 - 5.3 %) of radioactive fractions AMPA and M3, merely parent compound was found after 0, 7 and 15 days, ranging from 90.7 - 96.7 %. At every pH, the parent compound was not significantly degraded in the dark, i.e. the amount of parent compound from

day 0 to day 15 did not decrease more than 3.5 %. Taking into account the occurrence of the radioactive fractions AMPA and M3 in comparable amounts already in the stock solution, no hydrolytic products were detected in the aqueous solutions at various pH-values. Furthermore, the occurrence of small but significant amounts of volatile radio-activity (0.1 - 0.5 %, mainly $^{14}CO_2$) at every pH indicated that significant amounts of parent compound were completely degraded.

In conclusion, the results showed that photolytic half-lives of [^{14}C]glyphosate in aqueous solutions at pH 9.2, 7.3 and 5.1 were 77, 69 and 33 days, respectively, the major degradation product being AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate (N-(Phosphonomethyl)-glycine)
Lot No.:	185-ff-131
Specific activity:	0.9 mCi
Radiochemical purity:	95.8 %.
Chemical purity:	99.5 %

2. Test systems:
Since the test article may reversibly ionize within the pH range 5-9, photolysis was performed at three different pH's:

- 67.8 mL sodium acetate (0.1 mol/1) was combined with 32.2 mL acetic acid (0.1 mol/1) for a pH 5.1 buffer solution
- 29.6 mL 0.1 N NaOH (0.1 mol/1) was combined with 50.0 mL monopotassium phosphate (0.1 mol/1) for a pH 7.3 buffer solution.
- 21.3 mL 0.1 N NaOH (0.1 mol/1) was combined with 50.0 mL boric acid (0.1 mol/1) for a pH 9.2 buffer solution.

To minimize buffer effects during incubation, the buffer solution was used at a final concentration of 0.01 mol/L. In a pre-test, based on the total amount needed, 130 mg glyphosate were dissolved in buffer solutions pH 5.1 and pH 9.2 at a concentration of 2.6 mg/mL. No significant pH-changes were observed, indicating that the addition of the test article did not affect the pH of the buffer solutions. Total plate counts (bacteria) were determined after 48 hours of exposure for 0, 7 and 15 days at every pH. The results indicated that microbial degradation did not play a significant role in the present study.

B. STUDY DESIGN

1. Experimental conditions
The incubation vessel with an aliquot of 200 mL buffer solution containing the test article (diameter: 9.5 cm; height: 6 cm) consisted of pyrex glass covered with a quartz glass-plate and was equipped with a septum to take samples by means of a Hamilton syringe. The system was continuously ventilated through a sterile filter with air (about 30 mL per minute) and pre-moistened by bubbling through a flask with sterile bidistilled water. The outcoming air was passed through a CO_2-trapping system (2N NaOH) and through 2-methoxy-ethanol at room temperature for absorption of volatiles. Since the occurrence of methylamine was assumed, the methoxy-ethanol trap was acidified with glacial acetic acid (2 %, v/v) from day l on. At the beginning of the incubation period, the depth of the buffer solution in the reaction vessel was about 2.82 cm.

For each buffer solution, in addition to the illuminated reaction vessel, a reaction vessel (diameter: 8.5 cm; height: 7 cm) with an aliquot of 150 mL sterile buffer solution containing the test article was incubated under identical conditions in the dark. The outcoming air was trapped through NaOH and 2-methoxy-ethanol/acetic acid at room temperature. At the beginning of the incubation period, the depth of

each buffer solution in the corresponding reaction vessel was about 2.64 cm. Sterility of the test systems throughout the experiments was confirmed.

The study was performed in the Hanau Suntest apparatus which is equipped with a xenon burner (1.1 kW) and an UV-filter (290 to 800 nm) with controllable irradiance between 400 W/m^2 and 765 W/m^2 to a preset value. Specimen Area was about 50 cm^2 per reaction vessel. Light Intensity was measured by means of a Lux-meter and ranged from 80-94 KLux which was comparable to the light intensity of natural daylight in the summer with vertical incidence of the sun on a clear, cloudless day (about 90-100 KLux).

The photolytis apparatus was set at a target temperature of 25 °C and cooled by means of a waterjacket connected to a waterbath. The actual temperature in the main test was monitored at regular time intervals. In the illuminated incubation vessels, the temperature during the first 7 days ranged from 24.5 - 24.8 °C. Due to disfunctioning of the cooling system at day 11 and an increase to at least 40 °C, the last illumination sampling interval (day 16) had to be repeated. Except for about 2 hours at day 11 (33.3 °C), the temperature for the repeated illumination during 15 days ranged from 24.5 - 24.7 °C. The temperature of the controls during 16 days of incubation ranged from 24.3 - 25.1 °C.

Based on a target specific radioactivity of 6 µCi/mg and an amount of 38.5 mg (including an excess of 10 %) three stock solutions were prepared for each pH. An amount of 1.2 mL (0.8 mg [^{14}C]glyphosate) was diluted with 39.6 mg (for pH 5.1), 39.4 mg (for pH 7.3) and 39.6 mg (for pH 9.2) unlabelled glyphosate, respectively. Each aliquot was made up to 20.0 mL with the respective buffer solution and determined by liquid scintillation counting (LSC).

2. Sampling

Aliquotes of 10 mL for irradiated samples and 5 mL for dark controls were taken at 0, 1, 2, 4, 7 and 16 DAT. The repeated illumination was incubated for 15 days. Appropriate aliquots (50 µl) were used to determine the amount of radioactivity. Remaining samples were stored at -20 °C until further analyses, performed within 11 weeks or 17 weeks for irradiated and dark control, respectively.

Except for day 0, at each time interval samples for $^{14}C-CO_2$ and volatiles were taken for both test and control solution.

At the end of the incubation period, the incubation vessel was washed with acetone to dissolve possible precipitates and to exclude possible glass adsorption during incubation. Further, the remaining volume was noted. The difference as compared to the theoretical volume represented the amount of evaporation during incubation.

3. Analytical procedures

The radioactivity was determined on a Packard liquid scintillation. All values were corrected for instrumental background. Measurements were performed at least in duplicate. Additional characterisation of the radioactivity in the NaOH-absorption solutions of day 15 was obtained by precipitation of $^{14}C-CO_2$, to barium carbonate in a subsample (5.0 mL) after addition of 15 mL bidistilled water and 20 mL saturated barium hydroxide solution. After centrifugation, the supernatant was counted and the amount of precipitated radioactivity was obtained by means of subtraction

The aqueous samples of illuminated solutions were directly analysed by one-dimensional TLC. Aqueous samples of control solutions of days 0, 7 and 16 were analysed accordingly. TLC was performed on precoated plates of silica gel with a layer thickness of 0.25 mm or on precoated cellulose plates with a layer thickness of 0.50 mm. All non-labelled reference compounds were visualized on TLC-plates after moistening with ninhydrin-spray solution followed by heating at about 50 – 100 °C for about 10 minutes. The radioactive zones on TLC-plates were detected by using a Berthold Automatic TLC-Linear Analyser equipped with an Epson PC AX Processing System. Additionally, selected aqueous samples were submitted to high performance liquid chromatography (HPLC) after appropriate dilution in 0.05 M potassium dihydrogen phosphate, pH 3.4.

II. RESULTS AND DISCUSSION

A. DATA

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in illuminated and dark control samples are summarised in the tables below for the test systems at pH 7.3, 5.1 and 9.2. In the illuminated solutions, the time interval at day 16 represented the results after transient elevated temperature and were therefore not further discussed.

Table 7.2.1.2-23: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 5.1 after exposure to artificial sunlight at various time intervals (values in % AR)

	Sampling Interval (Days)						
	0	**1**	**2**	**4**	**7**	**15** [1]	**16**
Aqueous solution (pH 5.1)	100.0	100.0	102.9	100.4	99.3	94.0	97.6
^{14}C-CO_2-(NaOH-trapped)	n.d.	0.1	0.1	0.1	0.1	0.3	0.3
2-methoxy-ethanol-trapped [2]	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	100.1	103.0	100.5	99.4	94.3	97.9
TOTAL MEAN (except day 16)		99.5 ± 3.2					

n.d. : Not determined.
[1] Repeated incubation, clear solution; somewhat less optimal total recovery assumed to be due to a CO_2-saturated NaOH trap.
[2] With additionally 2 % acetic acid from day 1 on

Table 7.2.1.2-24: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 5.1 after in the dark at various time intervals (values in percentage % AR)

	Sampling Interval (Days)					
	0	**1**	**2**	**4**	**7**	**16**
Aqueous solution (pH 5.1)	100.0	99.8	101.4	99.3	98.7	97.9
Cumulative volatiles						
^{14}C-CO_2 (NaOH-trapped)	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
2-methoxy-ethanol-trapped [1]	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	99.8	101.4	99.3	98.7	97.9
TOTAL MEAN		99.4 ± 1.3				

n.d.: Not determined.
[1] With additionally 2 % acetic acid from day 1 on

Table 7.2.1.2-25: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 7.3 after exposure to artificial sunlight at various time intervals (values in % AR)

	Sampling Interval (Days)						
	0	**1**	**2**	**4**	**7**	**15** [1]	**16**
Aqueous solution (pH 7.3)	100.0	101.2	102.2	99.3	97.1	95.8	97.2
^{14}C-CO_2-(NaOH-trapped)	n.d.	0.1	0.1	0.1	0.2	0.1	1.0
2-methoxy-ethanol-trapped [2]	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	101.3	102.3	99.4	97.3	95.9	98.2
TOTAL MEAN (except day 16)		99.2 ± 2.7					

n.d.: determined.
[1] Repeated incubation
[2] With additionally 2 % acetic acid from day 1 on

Table 7.2.1.2-26: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 7.3 after in the dark at various time intervals (values in % AR)

	Sampling Interval (Days)					
	0	**1**	**2**	**4**	**7**	**16**
Aqueous solution (pH 7.3)	100.0	101.2	102.8	100.8	100.4	97.9
Cumulative volatiles						
^{14}C-CO$_2$ (NaOH-trapped)	n.d.	<0.05	<0.05	<0.05	<0.05	0.5
2-methoxy-ethanol-trapped [1]	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	101.2	102.8	100.8	100.4	98.4
TOTAL MEAN		100.7 ± 1.6				

n.d.: Not determined
[1] With additionally 2 % acetic acid from day 1 on

Table 7.2.1.2-27: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 9.2 after exposure to artificial sunlight at various time intervals (values in %AR)

	Sampling Interval (Days)						
	0	**1**	**2**	**4**	**7**	**15 [1]**	**16**
Aqueous solution (pH 9.2)	100.0	99.4	102.6	99.8	99.5	96.1	98.7
^{14}C-CO$_2$ (NaOH-trapped)	n.d.	<0.05	<0.05	<0.05	<0.05	0.1	0.2
2-methoxy-ethanol-trapped [2]	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	99.4	102.6	99.8	99.5	96.2	98.9
TOTAL MEAN (except day 16)		99.5 ± 2.3					

n.d.: Not determined.
[1] Repeated incubation
[2] With additionally 2 % acetic acid from day 1 on

Table 7.2.1.2-28: Balance of radioactivity of [^{14}C]glyphosate in aqueous samples at pH 9.2 after in the dark at various time intervals (values in % AR)

	Sampling Interval (Days)					
	0	**1**	**2**	**4**	**7**	**16**
Aqueous solution (pH 9.2)	100.0	100.8	104.3	103.6	102.7	96.7
Cumulative volatiles						
^{14}C-CO$_2$ (NaOH-trapped)	n.d.	<0.05	<0.05	<0.05	0.2	0.4
2-methoxy-ethanol-trapped [1]	n.d.	<0.05	<0.05	<0.05	<0.05	<0.05
TOTAL	100.0	100.8	104.3	103.6	102.9	97.1
TOTAL MEAN		101.7± 2.9				

n.d.: Not determined.
[1] With additionally 2 % acetic acid from day 1 on

Table 7.2.1.2-29: Degradation patterns of [^{14}C]glyphosate in aqueous samples at pH 5.1 after exposure to artificial sunlight and in dark control samples at various time intervals (values in % AR)

DAA/Identity	Irradiated/ dark	0	1	2	4	7	15
Parent	Irradiated	95.5	93.0	94.3	86.9	80.8	70.7
	Dark	93.0	-	-	-	92.6	90.7
AMPA	Irradiated	1.8	3.2	4.1	5.8	10.1	16.0
	Dark	2.0	-	-	-	3.3	1.9
Unknown M3	Irradiated	2.7	3.8	4.5	7.7	8.4	7.3
	Dark	5.0	-	-	-	2.8	5.3
Total	Irradiated	100.0	100.0	102.9	100.4	99.3	94.0
	Dark	100.0	-	-	-	98.7	97.9

n.d.: Not detected; -: Not determined

Table 7.2.1.2-30: Degradation patterns of [^{14}C]glyphosate in aqueous samples at pH 7.3 after exposure to artificial sunlight and in dark control samples at various time intervals (values in % AR)

DAA/Identity	Irradiated/ dark	0	1	2	4	7	15
Parent	Irradiated	94.0	94.2	93.5	90.2	83.9	82.3
	Dark	92.9	-	-	-	93.7	92.4
AMPA	Irradiated	2.2	3.1	3.9	5.0	6.7	11.6
	Dark	3.0	-	-	-	3.5	1.6
Unknown M3	Irradiated	3.8	3.9	4.8	4.1	0.4	n.d.
	Dark	4.1	-	-	-	n.d.	4.4
Unknown M4	Irradiated	n.d.	n.d.	n.d.	n.d.	6.1	1.9
	Dark	n.d.	-	-	-	n.d.	n.d.
Total	Irradiated	100.0	101.2	102.2	99.3	97.1	95.8
	Dark	100.0	-	-	-	100.4	98.4

n.d.: Not detected; -: Not determined

Table 7.2.1.2-31: Degradation patterns of [^{14}C]glyphosate in aqueous samples at pH 9.2 after exposure to artificial sunlight and in dark control samples at various time intervals (values in % AR)

DAA/Identity	Irradiated/ dark	0	1	2	4	7	15
Parent	Irradiated	95.0	93.9	--	93.8	89.0	83.1
	Dark	94.5	-	-	-	96.7	91.0
AMPA	Irradiated	2.2	2.3	--	1.8	4.0	6.5
	Dark	2.7	-	-	-	3.5	2.2
Unknown M3	Irradiated	2.8	3.2	--	4.2	6.5	6.5
	Dark	2.8	-	-	-	2.7	3.9
Total	Irradiated	100.0	99.4	--	99.8	99.5	96.1
	Dark	100.0	-	-	-	102.9	97.1

n.d.: Not detected; -: Not determined; --: Not analysed due to sample loss

B. MASS BALANCE

During illumination, radioactivity was almost completely recovered at all time intervals and amounted, on average, to 99.2 + 2.7 % for pH 7.3. At pH 5.1, during illumination, recovery of radioactivity was virtually complete and amounted, on average, to 99.5 ± 3.2 %. During illumination at pH 9.2, radioactivity was

almost completely recovered at all time intervals and amounted, on average, to 99.5 + 2.3 %. In dark controls, the mean total recovery ranged between 99.4±1.3 % AR and 101.7±2.9 % AR for all pH values.

C. VOLATILE RADIOACTIVITY

At pH 5.1, volatiles (0.3 %) were only trapped by NaOH from the illuminated solution. At pH 7.3 and 9.2, low amounts of radioactivity (0.1 - 0.5 %) were trapped by NaOH from both illuminated and control solutions.

No volatiles (<0.05 %) were trapped by 2-methoxy-ethanol/acetic acid in irradiated and dark control test system.

D. TRANSFORMATION OF THE TEST SUBSTANCE

At pH 7.3 and pH 9.2, the parent compound was degraded to a similar extend. At pH 5.1, degradation was somewhat more pronounced. At all time intervals, the major radioactive fraction was the parent compound.

The parent compound accounted for 93.0 % after 1 day at pH 5.1, thereafter, the amount of parent compound steadily decreased to 86.9 %, 80.8 % and 70.7 % after 4, 7 and 15 days of illumination, respectively. Accordingly, radioactive fraction AMPA steadily increased from 3.2 % at day 1 to 5.8 % at day 4, 10.1 % at day 7 and 16.0 % at day 15. Radioactive fraction M3 increased from 3.8 % at day 1 to 7.7 % at day 4. After 0, 7 and 16 days of incubation in the dark, besides the parent compound (90.7 - 93.0 % of the radioactivity applied), minor amounts of radioactive fractions AMPA and M3 were found, ranging from 1.9 - 5.0 %.

The amount of parent compound amounted to 94.2 % after 1 day of illumination at pH 7.3. Radioactive fraction AMPA increased from 3.1 % (day 1) to 5.0 % (day 4). Radioactive fraction M3 remained constant and ranged from 3.9 - 4.8 %. In dark control, after 0, 7 and 16 days of incubation in the dark, besides the parent compound (92.4 - 93.7 % of the radioactivity applied), minor amounts of radioactive fractions AMPA and M3 were found at all time intervals, ranging from 1.6 - 4.4 %.

The parent compound remained constant up to 4 days (93.8 %) in the pH 9.2 irradiated buffer solution. Thereafter, it decreased to 89.0 % and 83.1 % at days 7 and 15, respectively. Accordingly, radioactive fraction AMPA increased from 1.8 % at day 4 to 4.0 % at day 7 and 6.5 % at day 15. Radioactive fraction M3 increased from 3.2 % at day 1 to 6.5 % at days 7 and 15. After 0, 7 and 16 days of incubation in the dark, besides the parent compound (91.0 - 96.7 as), minor amounts of radioactive fractions AMPA and M3 were found at all time intervals, ranging from 2.2 - 3.9 %.

At pH 7.3 and 5.1, the amount of AMPA accounted for more than 10 % of the radioactivity applied at the end of the illumination period (day 15). Radioactive fraction M3 occurred at minor amounts (below 9 %) at each time interval and every pH. Furthermore, only at pH 7.3 radioactive fraction M4 occurred in minor amounts (below 7 %) after 7 and 15 days of illumination.

After incubation in the dark at every pH, the parent compound was not degraded, i.e. the amount of parent compound from day 0 to day 15 did not decrease more than 3.5 %. Additionally, radioactive fractions AMPA and M3 were found in minor amounts (below 6 %) at every pH and at all time intervals.

E. KINETICS

After continuous illumination half-lives of 77, 69 and 33 days were obtained (regression analysis assuming first order kinetics) for the photolysis rate of [^{14}C]glyphosate at pH 9.2, 7.3 and 5.1, respectively.

III. CONCLUSIONS

The data demonstrated that after 15 days of continuous illumination (the equivalent of 30 days natural sunlight, 12 hours of light per day), the photolytic degradation of [^{14}C]glyphosate in aqueous solutions at pH 9.2, 7.3 and 5.1 proceeded with decreasing half-lives of 77, 69 and 33 days, respectively.

In the dark, at every pH the parent compound was not significantly degraded.

Low but significant amounts of radioactivity (0.1 - 0.5 %) were trapped by NaOH. At pH 5.1, volatiles were only detected in the illuminated solution, indicating that significant amounts of parent compound could be completely degraded at acidic pH 5.1 due to the process of photolysis. At pH 7.3 and pH 9.2, volatiles were also found in the corresponding dark controls, assuming an additional breakdown process at more alkalic pH-values (7.3 and 9.2). Volatile radioactivity mainly represented ^{14}C-$C0_2$, although at more acidic pH 5.1, additional volatile compounds may occur.

At all three pH-values, radioactivity was almost completely recovered (on average above 98 %) during illumination and in the dark controls.

After analyses of the illuminated aqueous solutions, at every pH, mainly parent compound was found at all time intervals. Radioactive fractions AMPA and M3 were common at every pH. Radioactive fraction M4 was exclusively found at pH 7.3.

The major radioactive fraction M2, characterised as AMPA, accounted at pH 7.3 and pH 5.1 for more than 10 % of the radioactivity applied at the end of the illumination period. All other degradation products (M3 and M4) occurred in minor amounts (below 9 %) at any time interval during illumination.

During incubation in the dark, radioactive fractions AMPA and M3 were detected in minor amounts (below 6 %) at every pH at all time intervals. Taking into account the occurrence of radioactive fractions AMPA and M3 in similar minor amounts already in the stock solution, no significant amounts of hydrolytic products of [^{14}C]glyphosate occurred in the aqueous solutions at various pH-values.

Finally, taking into account the sterility of the aqueous solutions and the elimination of the process of hydrolysis by means of the control values in the dark, the present data reflect merely the process of photolysis of [^{14}C]glyphosate in aqueous solution, mainly resulting in aminomethylphosphonic acid.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted mainly in line with the current guideline. At day 0 impurities of up to 7 % were present in the test systems but this does not have a serious impact on the results.
Therefore, the study it is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.2/007
Report author	[redacted]
Report year	1983
Report title	Hydrolysis and photolysis degradation studies of SC-0224
Report No	WRC-83-85
Document No	
Guidelines followed in study	U.S. EPA 161
Deviations from current test guideline	From OECD 316: - Test was conducted at 40 °C - No duplicate samples were used - UV light was used - Test sytsems were sterilized but sterility was not confirmed - Exact application rate was not reported
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The aqueous photolysis of glyphosate as glyphosate-trimesium (SC-0224) was investigated as a function of pH in buffer solutions at pH 5, 7 and 9 with an application rate of 50 – 60 mg/L. Test solutions were incubated at 40 ±0.5 °C for up to 30 days under sterile conditions.

UV black-light lamps were used as an artificial light source. Light intensity emitted by UV lamps was calculated to be 2.097 x 10^4 erg/sec/cm^2, or approximately 2100 µWatt/cm^2.

Reactor tubes of irradiated samples were removed for sampling on days 0, 4, 7, 11, 15, 19, 22 and 29. Dark controls were sampled after 30 days. Single samples were analysed by HPLC.

Photolytic breakdown of the glyphopsate (CMP) occurred at pH 5, 7 and 9. AMPA and phosphoric acid were identified as the degradation products. Photolytic breakdown of the trimesium (TMS) occurred at pH 9. No photolysis products at pH 9 were observed for the trimesium.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: Glyphosate as glyphosate-trimesium (SC-0224)
Lot No.: WRC-8146-27-1
Composition: 90.9 % glyphosate-trimesium, 4.2 % water
Measured molar ratio: Glyphosate (CMP) : trimesium (TMS) = 1.00 : 1.09

2. Buffers:

Buffer solutions were prepared in buffer systems of pH 5.0 (biphthalate), 7.0 (phosphate) and 9.0 (borate).

B. STUDY DESIGN

1. Experimental conditions

Test solutions between 50 mg/L and 60 mg/L were prepared in buffer systems of pH 5.0 (biphthalate), 7.0 (phosphate) and 9.0 (borate). The water used was free of bacteria, having passed through a 0.2 µm filter. Flasks and photoreactor tubes were sterilised prior to use. Sterility of the test systems throughout the experiments was not confirmed.

Each of the three reactor tubes was filled to the 1300 mL level with one of the test solutions. Reactor tubes were placed into a 40 °C thermostated bath and UV lamps were turned on. Baths of dark control samples were covered. Irradiated and dark control samples were incubated for 29 and 30 days, respectively.

UV black-light lamps (GE Lamp No. F40 BL) were used as artificial light source. Each lamp was mounted vertically inside a double-walled, cylindrical Pyrex glass photoreactor. Comparative measurements with natural sunlight have shown that distribution for the GE F40 BL lamp is similar to that of the sunlight at the high energy (low wavelength) end of the spectrum. Light intensity emitted by UV lamps was measured chemically at the beginning and the end of the study period. The light intensity was calculated to be 2.097×10^4 erg/sec/cm^2, or approximately 2100 µWatt/cm^2.

The temperature of each test solution remained constant at 40 ±0.5 °C throughout the study period.

2. Sampling

Reactor tubes of irradiated samples were removed for sampling on days 0, 4, 7, 11, 15, 19, 22 and 29. Dark controls were sampled after 30 days. Single samples were analysed.

3. Analytical procedures

At each sampling, 2 to 3 mL aliquots were removed from the bottom sampling port of the reactor and submitted for analysis.

Determinations of glyphosate and AMPA (CMP and aminomethylphosphonic acid anions) were carried out by derivatisation with 9-fluorenylmethyl chloroformate followed by HPLC analysis. The trimesium cation (TMS) was dealkylated to dimethylsulfide prior to analysis by gas chromatography. Typical recoveries via these methods are 93 ± 10 % for anions and 94 ± 7 % for TMS.

In the pH 5 solution an unknown response was observed, as well as discoloration. To identify the unknown compound, the extract was directly analysed by GC/MS but no response was detected. In a separate study, about 1000 mL of the final (aqueous) pH 5 solution was evaporated to dryness using a rotary evaporator at 38 °C. About 2.5 mL of D_2O and 0.5 mL of 50 % NaOH were added to the residue. The resulting deuterium oxide solution was analysed by phosphorous NMR.

II. RESULTS AND DISCUSSION

A. DATA

Glyphosate and AMPA concentrations are summarised in Table 7.2.1.2-32. Concentrations of trimesium are presented in Table 7.2.1.2-33.

Table 7.2.1.2-32: Concentrations (mg/L) of glyphosate and AMPA at 40 °C

pH	5.0		7.0		9.0	
Time (days)	Observed concentration (mg/L)					
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
0	45.1	0.3	45.0	0.3	49.2	0.3
4	34.5	4.4	45.0	1.5	44.1	1.8
7	29.4	8.5	43.4	2.1	43.0	2.9
11	22.9	10.0	40.0	2.9	40.0	3.3
15	21.6	8.5	40.7	2.7	37.0	3.8
19	16.6	8.1	n.a.	4.2	35.8	6.0
22	16.1	8.5	38.1	3.7	34.1	5.5
29	10.5	7.0	34.9	3.9	29.4	6.7
Dark Control (30 days)	46.3	n.a.	49.7	n.a.	45.7	n.a.

n.a. = Not available

Table 7.2.1.2-33: Trimesium concentrations (mg/L) at 40 °C

pH	5.0	7.0	9.0
Time (days)	Observed concentration (mg/L)		
0	18.7	21.1	20.0
4	23.6	21.3	20.4
7	20.7	23.5	18.2
11	18.3	22.3	20.1
15	23.5	22.1	14.8
19	18.8	21.6	11.9
22	18.8	23.5	11.3
29	20.8	20.9	13.1
Dark Control (30 days)	20.8	20.6	19.9

B. TRANSFORMATION OF THE TEST ITEM

Glyphosate concentrations decreased from 45.1 at study start to 10.5 mg/L after 29 days at pH 5.0 and from 45.0 to 34.9 mg/L at pH 7.0. At pH 9.0, glyphosate concentrations decreased from 49.2 to 29.4 mg/L from study start to study end.

The only photolytic decomposition products identified for the glyphosate anion (CMP) were AMPA and phosphoric acid. Maximum concentrations of AMPA were 10.0 %, 4.2 % and 6.7 % at pH 5.0, pH 7.0 and pH 9.0, respectively, with concentrations qualifying AMPA as major metabolite at pH 5 and pH 9.

No other responses were observed in the analytical chromatographic scans except for pH 5 solutions, where an unknown compound represented <4 % of the original compound. Since this response was present and the pH 5 solution was discolored, the solution was further analysed by phosphorous NMR. The NMR spectrum indicated that the solution contained CMP, AMPA and phosphoric acid in a molar ratio of 2.5 : 1.0 : 1.9.

No decomposition of trimesium occurred at pH 5 or pH 7.

C. KINETICS

For glyphosate, DT_{50} values of 14.6, 77.9 and 41.6 days were calculated based on pseudo first-order.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In view of the test conditions using UV light and of lacking information e.g. on mass balance or the exact application rate the study is considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.1.2/008
Report author	[redacted]
Report year	1978
Report title	Photodegradation and anaerobic aquatic metabolism of Glyphosate, N-Phosphono-Methylglycine
Report No	MSL-0598
Document No	
Guidelines followed in study	None
Deviations from current test guideline	From OECD 316: - The study was conducted with artificial light with wavelengths of 350-450 nm, but no details of light source are reported. - No details on characteristics of test sytsems are reported - Test temperature is not reported - Test systems were sterilised by microfiltration but sterility was not confirmed - Only four sampling dates within experimental period - AMPA was present as impurity of test solution with 7.4 % - No analysis results for glyphosate are reported
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary
The aqueous photodegradation of glyphosate, N-(phosphono-merhyl)glycine, was assessed in natural water, deionised natural water and deionised natural water amended with $CaCl_2$. To that end, the rate of formation of photodegradation products of glyphosate in sterile aqueous solutions treated with [^{14}C]glyphosate was investigated in a reactor exposed to artificial light at 350-450 nm and aerated for 1, 7, 14, and/or 21 days.

In a first test with natural water formation of 78.5 % AR of AMPA (aminonethylphospohnic acid) was encountered after 21 days of continuous irradiation. The half-life of glyphosate was determined to be 19 days. In the second test formation of AMPA amounted to 86.7 %, 38.3 %, 67.1 % and 78.1 % of applied radioactivity after 14 days of irradiation in natural water, deionised natural water, deionised natural water

containing 3 mg/L $CaCl_2$ and deionised natural water containing 30 mg/L $CaCl_2$, respectively. Thus, $CaCl_2$ was shown to act as agent in the photodegradation of glyphosate.

In the same study, anaerobic aquatic metabolism and photodegradation of glyphosate on soil surfaces. The results related to soil photolysis of the study are summarised in section CA 7.1.1.3.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate (N-(phosphono-methyl-^{14}C-glycine), PMG)
Lot No.:	not indicated
Specific activity:	10.12 mC/mM
Radiochemical purity:	98 – 99 % (TLC)

2. Test system:
The natural lake water used was sampled at lake number 34 at the Busch Wildlife Area, Weldon Springs, Missouri, USA. A pH of 6.6 was determined. For test no. 1, the test water was purified by AG 50-X8 resin. Further, for test no. 2, a second natural water sample was obtained to assess the photosensitising impact of $CaCl_2$ on degradation of glyphosate natural water. The test water for the second test was deionised and $CaCl_2$.was added at a level of 3 or 30 mg/L, respectively. Further, the test water was analysed for metal ions before and after clean up.

B. STUDY DESIGN

1. Experimental conditions
The photolysis reactors were sterilised at 20 psi and 120 °C for 20 minutes. After dosing, the test waters were sterilised by Millipore filtration (0.20 µm). The test solutions were fortified with 1.0 mg/L glyphosate (0.1 mg/L [^{14}C]glyphosate mixed with unlabelled glyphosate at a ratio 1:10). Prior to use, the radiolabeled test material applied in the second test was purified by D-50 column chromatography to remove AMPA present in the stock solution. Ascarite towers were placed on the reactors to monitor the formation of $^{14}CO_2$ over the study duration. Sterility of the test systems throughout the experiments was not confirmed.

The test solutions were exposed to artificial light emitting wave lengths between 350-450 nm for two to three weeks. An exposure period of 14 days to this source of artificial light corresponds to 112 eight-hour days of exposure to sunlight at Davis, California, USA. Simultaneously, dark control test solutions were maintained.

2. Sampling
In the first test using purified natural water, aliquots were removed at 0, 1, 7, 14 and 21 days. Test no. 2 solutions were sampled after 0, 1, 7 and 14 days.

3. Analytical procedures
The samples were analysed via HPLC for glyphosate and AMPA (aminonethylphospohnic acid) by collecting eluant at 0.5 min intervals for LSC. The respective retention times were determined using radiolabelled standards. TLC was further used as confirmatory method for aliquots taken at the last sampling event. The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC/TLC//LSC were not reported.

II. RESULTS AND DISCUSSION

A. DATA

Mass balances or recoveries of glyphosate are not given in the study report. The degradation of glyphosate in irradiated and dark control test solutions is reflected by results for AMPA as indicated in the table below.

Table 7.2.1.2-34: Recovery of AMPA in irradiated and dark control samples after treatment with [^{14}C]glyphosate (expressed as percent of applied radioactivity)

		DAT				
Variant	**Incubation**	**0**	**1**	**7**	**14**	**21**
Water no. 1	Irradiated	7.4	25.9	39.5	58.4	78.6
	Dark control	7.4	11.3	8.8.	9.8	14.6
Water no. 2	Irradiated	-	18.4	68.8	86.7	-
	Dark control	-	2.0	5.8	13.5	-
Water no. 2 deionised	Irradiated	-	6.0	23.7	38.3	-
	Dark control	-	2.1	1.0	3.2	-
Water no. 2 deionised with 3 mg/L $CaCl_2$	Irradiated	-	7.5	57.5	67.1	-
	Dark control	-	1.0	1.0	1.2	-
Water no. 2 deionised with 30 mg/L $CaCl_2$	Irradiated	-	5.3	38.4	78.1	-
	Dark control	-	0	0	2.5	-

- Not determined

B. MASS BALANCE

The total recovery of the irradiated and dark control samples was reported to be 96.4 % and 106.3 % AR, respectively.

C. VOLATILISATION

CO_2 was reported to amount to 0.5 % after 21 days of irradiation.

D. TRANSFORMATION OF THE TEST SUBSTANCE

In the first test, 78.6 % AR formation of AMPA was encountered after 21 days of continuous irradiaton. There was no evidence of any photodegration product other than AMPA. Initially, the test substance contained 7.4 % AMPA and corrected for this initial value, 7.2 % photodegradation in the dark control sample was encountered after 21 days. This degradation was suspected to be due to enzymes present in the water samples after Millipore filtration rather than microbial contamination.

In the second test, formation of AMPA amounted to 86.7 %, 38.3 %, 67.1 % and 78.1 % of applied radioactivity after 14 days of irradiation in natural water, deionised natural water, deinonised natural water with 3 mg/L $CaCl_2$ and deinonised natural water with 30 mg/L $CaCl_2$, respectively. In the dark controls 13.5 % AMPA was found in natural water, but only 3.2 % in deionised natural water. Degradation seems markedly reduced in the natural water after deionisation, while $CaCl_2$ enhances photodegradation of glyphosate. However, sodium, silica, and calcium ions were still present in the water after deionisation. All in all, the results are considered to indicate that while $CaCl_2$ has a sensitising effect photodegradation may also be influenced by other unknown factors.

E. KINETICS

The half-life of glyphosate designated in the abstract of the report was 19 days, however it's not further elaborated how the half-life was derived from the given data.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In view of the limited information on test conditions, test systems and analytical results and due to the wavelength spectrum, the study is considered invalid.

Assessment and conclusion by RMS:

CA 7.2.1.3 Indirect photochemical degradation

The molar decadic absorption coefficient (ε) of glyphosate is << 10 L mol^{-1} cm^{-1} at wavelengths >295 nm (see Section 2.4). Therefore, it is expected that photolysis does not significantly contribute to degradation of glyphosate in aquatic systems. Thus, experimental studies on indirect photolysis are formally not required. For completeness, the available studies are submitted as supportive information. The results are presented above together with the studies on direct photodegradation (see CA 7.2.1.2).

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

Three studies are available which are considered valid to address the ready biodegradability of glyphosate (Feil, 2009, CA 7.2.2.1/001; Carrick, 1991 CA 7.2.2.1/002; Wüthrich, 1990, CA 7.2.2.1/003).

Glyphosate is classified as not readily biodegradable.

Table 7.2.2.1-1: Studies on ready biodegradability with glyphosate

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.2.2.1/001	[redacted] 2009	Ready biodegradability	Glyphosate	Valid	
CA 7.2.2.1/002	[redacted]1991	Ready biodegradability	Glyphosate	Valid	
CA 7.2.2.1/003	[redacted] 1990	Ready biodegradability	Glyphosate	Valid	

1. Information on the study

Data point:	CA 7.2.2.1/001
Report author	[redacted]
Report year	2009
Report title	Ready biodegradability of Glyphosate in a manometric respirometry test
Report No	53981163
Document No	
Guidelines followed in study	OECD 301 F Commission Regulation 440/2008/EC, Method C.4-D
Deviations from current test guideline	From OECD 301 F - The concentration of activated sludge slightly exceeded the concentration of suspended solids given in OECD 301 F
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test item glyphosate was investigated for its ready biodegradability in a manometric respirometry test over a period of 28 days at 21 – 22°C in the dark. The biodegradation has been assessed by the oxygen uptake of activated sludge which was received from a domestic wastewater treatment plant.

The test system comprised five treatment groups containing all inoculated mineral salt medium except the abiotic control, which was made up of mineral salts medium plus test substance at 103 mg/L, poisoned with $HgCL_2$ The inoculum control group remained without any further additions. The procedure control group and the glyphosate group received either sodium benzoate or glyphosate at levels of 104 mg/L and 103 mg/L, respectively, whereas the toxicity control group comprised both (104 mg/L and 103 mg/L of sodium benzoate and glyphosate, respectively). Incubations were conducted for 28 days at a temperature of 21°C in the dark.

The test item glyphosate contains nitrogen, therefore the evaluation of biodegradation was based on formation of ammonium salts ($ThOD_{NH4}$) and of nitrate ($ThOD_{N03}$). However, the biodegradation of glyphosate did not reach 60 % after 28 days and therefore no determination of nitrification was made. The mean biodegradation of glyphosate was 26 % ($ThOD_{NH4}$) and 16 % ($ThOD_{N03}$). Therefore, glyphosate is considered not to be readily biodegradable.

The reference item sodium benzoate was sufficiently degraded to 90 % after 14 days and to 98 % after 28 days of incubation, thus confirming the suitability of the aerobic activated sludge inoculum used.

In the toxicity control containing both the test item and reference 65 % or 55 % biodegradation was noted within 14 days based on $ThOD_{NH4}$ and $ThOD_{N03}$, respectively. After 28 days of incubation biodegradation of the toxicity control was 69 % or 59 %, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate
Lot No.:	07-b-151
Chemical purity:	97.7 % (w/w)
Molecular formula:	$C_3H_8NO_5P$
Molecular weigth:	169.01 g/mol (calculated)

Reference substance:

Identification:	Sodium benzoate
Lot No.:	098K0700
Chemical purity:	100 % (w/w)
Molecular formula:	$C_7H_5O_2Na$
Molecular weigth:	144.1 g/mol

2. Inoculum and test medium:

Inoculum

A sample of activated sludge was supplied from a domestic waste water treatment plant by the sewage plant Darmstadt, Germany. Activated sludge was used as inoculum with a concentration corresponding to 31 mg dry solids per litre. Dry solid of the activated sludge was 1.5 g/L by weight measurements. The activated sludge was washed three times by centrifugation of the sludge, decanting the supernatant and re-suspending the sludge in tap water. After the last washing step, the pellet was re-suspended in test water and aerated overnight.

Test medium

Analytical grade salts were added to deionised water to prepare the following stock solutions:

(A) 8.5 g KH_2PO_4, 21.75 g K_2HP0_4, 33.4 g Na_2HP0_4 x 2 H_20, 0.5 g NH_4Cl filled up with deionised water to 1000 mL volume
(B) 22.5 g $MgS0_4$ x $7H_20$ filled up with deionised water to 1000 mL volume
(C) 36.4 g $CaCl_2$ x $2H_20$ filled up with deionised water to 1000 mL volume
(D) 0.25 g $FeC1_3$ x $6H_20$ filled up with deionised water to 1000 mL volume

In order to avoid precipitation of iron hydroxide in the stock solution (D) after storage and before use, one drop of concentrated HCl per litre was added.

10 mL of stock solution (A) and 1 mL of the stock solutions (B) to (D) were combined and filled up to a final volume of 1000 mL with deionised water. The pH-value was 7.5, thus no adjustment had to be done. 5 mL activated sludge was filled up to 244 mL with 239 mL mineral medium corresponding to 31 mg/L dry solids.

B. STUDY DESIGN AND METHODS

1. Experimental conditions

Five treatment groups were established:

- Inoculum Control: inoculated mineral salts medium
- Procedure Control: inoculated mineral salts medium plus sodium benzoate at 104 mg/L organic carbon
- Glyphosate: inoculated mineral salts medium plus test substance at 103 mg/L, corresponding to an oxygen demand of about 59 mg/L ($ThOD_{NH4}$) and 97 mg/L ($ThOD_{NO3}$)
- Toxicity Control: inoculated mineral salts medium plus the test substance at 103 mg/L and the reference substance at 104 mg/L
- Abiotic Control: not inoculated mineral salts medium plus test substance at 103 mg/L, poisoned with $HgCL_2$ (5 mL of stock solution with 48.72 mg/mL was made up to a final volume of 244 mL)

The purpose of the toxicity control was to assess the biodegradation of the reference substance in the presence of the test substance. Duplicate vessels were established for the glyphosate treatment and the inoculum control. Single vessels were established for the procedure, the abiotic and the toxicity control.

The amounts of test item and reference item were directly weighed into the test flasks of approximately 500 mL volume. No emulsifiers or solvents were used, but the solutions were dispersed by stirring to stirring to achieve a homogeneous solution of the test item.

2. Analytical procedures

The closed test flasks were incubated in a climatised room under continuous stirring in the dark. The consumption of oxygen was determined daily by measuring the change of pressure in the flasks by means of a manometric method (BSB/BOD-Sensor-System). The temperature was measured each working day in the climatised room and was 22 ± 1 °C throughout the whole study.

The pH-values were measured in control, procedure control and a separately prepared test flask with test item at test start (to prevent loss of test item in the test flasks) and in all flasks at the end of the test using a pH-electrode WTW pH 340i.

Evolved carbon dioxide was absorbed in an aqueous absorbed in an aqueous solution (45 %) of potassium hydroxide.

The pH value was 7.5 and 6.8 – 7.6 measured at start and at the end of the test, respectively.

3. Calculations

Biodegradation related to oxygen demand

The biodegradability (% BOD = mg O_2 per mg test item) exerted after each period was calculated as:

BOD = (mg O_2 uptake of test item – mg O_2 uptake of inoculum control) / mg test item in flask

The percentage biodegradation of the test item and of the reference item sodium benzoate was calculated as:

% degradation = (BOD (mg O_2 / mg test item or reference item)) / ($ThDO_{NH4}$ (mg O_2 / mg test item or reference item)) x 100

or in case of nitrification of the test item:

% degradation = (BOD (mg O_2 / mg test item or reference item)) / ($ThOD_{NO3}$ (mg O_2 / mg test item or reference item)) x 100

II. RESULTS AND DISCUSSION

A. DATA

Biodegradation of glyphosate, sodium benzoate and the toxicity control based on $ThODN_{H4}$ and $ThOD_{NO3}$ are summarised in the table below.

Table 7.2.2.1-2: Percentage biodegradation of glyphosate, sodium benzoate and the toxicity control based on $ThOD_{NH4}$ and $ThOD_{NO3}$

Time (days)	Glyphosate				Sodium benzoate	Toxicity control	
	$ThOD_{NH4}$ [1]		$ThOD_{NO3}$ [3]		$ThOD_{NH4}$ [2]	$ThOD_{NH4}$ [1,2]	$ThOD_{NO3}$ [3,4]
	Flask 1	Flask 2	Flask 1	Flask 2	Flask 5	Flask 7	Flask 7
1	0	0	0	0	29	22	18
2	0	0	0	0	43	28	24
3	-4	-4	-3	-3	65	46	40
4	-9	-9	-5	-5	67	52	44
5	-9	-9	-5	-5	72	56	48
6	0	0	0	0	75	58	50
7	0	0	0	0	78	60	52
8	-4	-4	-3	-3	80	61	53
9	-9	-9	-5	-5	81	62	53
10	-9	0	-5	0	81	62	53
11	0	0	0	0	84	65	55
12	0	0	0	0	87	65	55
13	0	0	0	0	87	65	55
14	0	0	0	0	90	65	55
15	0	9	0	5	90	65	55
16	9	9	5	5	93	67	57
17	4	4	3	3	94	66	56
18	0	0	0	0	94	65	55
19	9	9	5	5	96	65	55
20	9	9	5	5	96	65	55
21	9	9	5	5	96	65	55
22	9	9	5	5	96	65	55
23	9	9	5	5	96	65	57
24	9	17	5	10	98	67	57
25	17	17	10	10	98	67	57
26	17	17	10	10	98	67	57
27	26	26	15	16	98	69	59
28	26	26	15	16	98	69	59

[1] $ThOD_{NH4}$ of glyphosate: 0.568 mg/mg
[2] $ThOD_{NH4}$ of sodium benzoate: 1.666 mg/mg
[3] $ThOD_{NO3}$ of glyphosate: 0.946 mg/mg

B. BIODEGRADATION

The relevant pass levels for ready biodegradability of glyphosate are 60 % of ThOD for respirometric methods. The mean percentage biodegradation at the end of the 28-day exposure period was 26 % ($ThOD_{NH4}$). The occurrence of nitrification was considered but not experimentally confirmed. Based on $ThOD_{NH3}$, the mean percentage biodegradation at the end of the exposure period at 28 DAT was 16 %. The degradation rate of glyphosate did not reach 60 % within 28 days of incubation. Therefore, glyphosate is considered not to be readily biodegradable.

The reference item sodium benzoate was sufficiently degraded to 90 % after 14 days and to 98 % after 28 days of incubation. The percentage biodegradation of the reference item confirms the suitability of the used aerobic activated sludge inoculum.

In the toxicity control containing both the test item and reference 65 % or 55 % biodegradation was noted within 14 days based on $ThOD_{NH4}$ and $ThOD_{N03}$, respectively. After 28 days of incubation biodegradation of the toxicity control was 69 % or 59 %, respectively. According to the test guidelines the test item can be assumed to be not inhibitory on the aerobic activated sludge micro-organisms because degradation was >25 % within 14 days.

The oxygen demand in the abiotic control was zero. No correction of the test item degradation rates had to be done.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted in line with the relevant guideline OECD 301 F. It is therefore considered valid to describe the ready biodegradability of glyphosate. Glyphosate is considered not to be readily biodegradable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.2.1/002
Report author	[redacted]
Report year	1991
Report title	A study to evaluate Ready Biodegradability of Glyphosate Technical
Report No	RB-09
Document No	
Guidelines followed in study	OECD 302 B (1981) EEC Directive 87/302 Biodegradation, Zahn Wellens Test
Deviations from current test guideline	From OECD 302 B: - Information on inoculum is limited
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The objective of this test was to measure the biodegradability of glyphosate in an aqueous medium by measuring the DOC (dissolved organic carbon) loss at 50 mg/L DOC over a test period of 28 days. The medium was inoculated by activated sludge from a sewage treatment plant.

Prior to the biodegradation test a microbial toxicity test was carried out to check that microbial inhibition was not greater than 50 % at the test substance DOC concentration of 50 mg/L as required in the biodegradation test. Four glyphosate concentrations (100, 50, 10 and 1 mg/L) were investigated.

The test system comprised four treatment groups containing all inoculated mineral salt medium. The blank solution group remained without any further additions. The control and the glyphosate group received either sodium acetate or glyphosate, both at levels of 50 mg/L. The adsorption check solutions were made up like the glyphosate solutions in order to check that there was no adsorption of glyphosate to the flask walls. The mixtures were stirred and aerated at 22 ± 3 °C in virtual darkness.

In flasks with glyphosate, 2 % (mean of three replicates) biodegradation was calculated at 28 DAT. Therefore, glyphosate is considered not to be ready or inherent biodegradable.

The reference item sodium acetate was degraded to 100 % within 2 days of incubation, thus confirming the viability of the aerobic activated sludge inoculum used.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate Technical
Lot No.:	0206-JAK-25-1
Chemical purity:	97.7 %

2. Inoculum and test solutions:

Inoculum
Activated sludge from Kendal sewage treatment plant was used as inoculum in an amount corresponding to approximately 0.2 g dry material/L.

Test medium
The test medium (2 L per flask) was prepared according to OECD 302 B:
38.5 g NH_4Cl, 33.4 g NaH_2PO_4 x $2H_2O$, 8.5 g KH_2PO_4 and 21.75 g K_2HPO_4 were dissolved in 1000 mL bidistilled water.

2.5 mL of this stock solution were added to 1000 mL test water (1:1 drinking water/bidistilled water).

On the basis of the suspended solids determination, the medium of all treatment groups was inoculated with activated sludge in an amount corresponding to approximately 0.2 g dry material/L.

The total volume used per flask was 2 litres. Per flask, 2.5 mL nutrient solution/L was added.

B. STUDY DESIGN AND METHODS

1. Experimental conditions
Prior to the biodegradation test a microbial toxicity test was carried out to check that microbial inhibition was not greater than 50 % at the test substance DOC concentration of 50 mg/L as required in the biodegradation test. In this test, a range of glyphosate concentrations (100, 50, 10 and 1 mg/L) with a fixed concentration of biodegradable standard (glucose/glutamic acid solution) were dissolved in BOD dilution water. The mixtures were saturated with air, seeded and then measured volumes stirred in partially filled bottles connected to closed-end mercury manometers. Oxygen consumption was measured by observing the change in level of the mercury columns, with any carbon dioxide evolved into the bottles absorbed by alkali held in small cups within the bottle caps. The test as carried out at 20 ± 1 °C for 5 days, and the amount of oxygen taken up was determined and compared to that from the standard solution.

For the biodegradation test four treatment groups were established:

- Blank solution: inoculated mineral salt medium
- Control solution: inoculated mineral salt medium plus sodium acetate at 50 mg/L DOC
- Glyphosate solution: inoculated mineral salt medium plus test substance at 50 mg/L DOC
- Adsorption check: inoculated mineral salt medium plus the test substance at 50 mg/L DOC

Three vessels were established for the glyphosate treatment. Single vessels were established for the blank, the standard control and the adsorption control.

The test was conducted over a period of 28 days. Flasks were placed in a tank through which water at 22 ± 3°C was circulated from a temperature controlled unit. The test flasks were stirred with continuous aeration to ensure that the sludge did not settle or the oxygen concentration fall below 2 mg/L. Sides and top of the tank was covered but 15 cm holes under each of the tests flasks at the bottom of the tank allowed a small amount of diffuse daylight into the system.

Evaporation losses from the flasks were made up with deionised water just prior to sampling by marking the liquid levels in the flasks before starting the test, and after each sampling. Samples were taken 3 hours after the start of the test in order to allow for any adsorption of glyphosate by the activated sludge.

2. Analytical procedures
Daily samples (weekdays) were removed for DOC analysis. A 20 mL sample was removed from each flask and filtered through a washed filter paper with the first 5 mL filtrate returned to the test flask.

The pH of the glyphosate and blank test solutions was checked at regular intervals and adjusted to pH 7-8 by addition of M NaOH.

3. Calculations
Inhibition of microbial activity was calculated as:

$$((BOD_{std} - BOD_{test}) / BOD_{std}) \times 100$$

The degradation rate was calculated as:

$$D\ (\%) = [(1 - (C_T - C_B)) / (C_A - C_{BA})] \times 100$$

Where

D_T = biodegradation (%) at time T
C_T = DOC value at time of sampling (mg/L)
C_B = DOC value of the blank (mg/L)
C_A = initial DOC value in the test solutions (mg/L) measured three hours after the beginning of the test
C_{BA} = DOC value of the blank (mg/L) measured three hours after the beginning of the test

II. RESULTS AND DISCUSSION

A. DATA
Results of the microbial inhibition test are summarised in Table 7.2.2.1-3, whereas biodegradation of glyphosate, sodium benzoate and results on adsorption are summarised in Table 7.2.2.1-4.

Table 7.2.2.1-3: Pre-test: microbial inhibition as BOD value and percent inhibition

Concentration of test substance in BOD solution (mg TOC/L)	3 day BOD value (mg O_2/L)		Inhibition (%)	
	Flask 1	Flask 2	Flask 1	Flask 2
100	138	142	8.6	8.4
50	162	153	-7.3	1.3
10	142	148	6.0	4.5
1	133	141	11.9	9.0
Control Standard	151	155	-	-
Blank	0	0	-	-

Table 7.2.2.1-4: Percentage biodegradation of glyphosate and sodium acetate as well as DOC values for adsorption check

Day	% Biodegradation				Arithmetic mean	Adsorption check DOC
	Control (Sodium acetate)	Glyphosate				
		Flask 1	Flask 2	Flask 3		
2	100	0	-3	-1	-1	57.8 [1]
7	-	-1	0	-4	-2	60.0
14	-	3	5	1	3	56.7
21	-	1	2	4	3	59.3
28	-	2	2	1	2	55.6

[1] Initial adsorption check value

B. BIODEGRADATION

In flasks with glyphosate, 2 % (mean of three replicates) biodegradation was calculated at 28 DAT. Therefore, glyphosate is considered not to be readily biodegradable.

The test is considered valid if the procedural control shows the removal of the reference compound by at least 70 % within 14 days. The validity of this study was ratified by the 100 % biodegradation of the sodium acetate control within 2 days confirming the viability of the inoculum.

The temperature of the flasks was maintained between 22.15 – 22.55 °C (hourly logged values) and the lowest measured oxygen concentration in the flasks was 8.3 mg O_2.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was conducted in line with the relevant guideline OECD 302 B. It is therefore considered valid to describe the ready biodegradability of glyphosate. Glyphosate is considered not to be inherently biodegradable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.2.1/003
Report author	[redacted]
Report year	1990
Report title	Glyphosate technical: inherent biodegradability: "modified Zahn-Wellens Test"
Report No	271653
Document No	
Guidelines followed in study	OECD 302 B (1981)
Deviations from current test guideline	From OECD 302 B : - Ratio between inoculum and test compound as TOC was lower than recommended (1.5-1.6:1) - The pH was slightly higher in some tests than recommended – 28 DAT samples were stored at 4 °C for more than 48 hours due to defect of TOC analyser

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The degradation of glyphosate technical was investigated in an aqueous medium by measuring the removal of TOC over a test period of 28 days.

The medium was inoculated by activated sludge originating from two treatment plants, with not adapted and adapted microorganisms in sets 1 and 2, respectively. Glyphosate technical was applied at a test concentration of 620 mg/L corresponding to 121.5 mg TOC/L in Test set 1 and 131 mg TOC/L in Test set 2.

The test system comprised three treatment groups containing all inoculated mineral salt medium. The inoculum control group remained without any further additions. The functional control and the glyphosate group received aniline at a level 100 mg/L corresponding to a theoretical amount of 77.4 mg TOC/L. The glyphosate solution flasks received test substance at 1240 mg/2 L (620 mg/L) corresponding to 121.5 mg TOC/L in Test set 1 and 131 mg TOC/L in Test set 2.

The study was run at 20 – 23 °C protected from light. The flasks were aerated with a flow rate of about 0.5 – 0.7 L/min, resulting in an oxygen concentration of 7.7 – 9.0 mg O_2 per litre. The pH was adjusted to 7.0 and 8.2.

No removal of glyphosate was detected in flasks treated with sludge sets 1 and 2 demonstrated by unchanged TOC. Glyphosate was neither eliminated nor degraded within 28 days of incubation. Therefore, glyphosate is considered not to be readily biodegradable.

The reference compound aniline was biodegraded within 7 days of exposure by 88 % and 89 %, respectively, with microorganisms sets 1 and 2, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: Glyphosate technical (N-(Phosphonomethyl)-glycine)
Lot No.: 229-Jak-5-1
Chemical purity: 98.9 %

2. Inoculum and test solutions:

Inoculum
Two sets of microorganisms were used, both originating from the secondary effluent of a domestic waste water sewage plant, with not adapted and adapted microorganisms in sets 1 and 2, respectively. Microorganisms from set 1 were provided by ARA Sissach/Switzerland, whereas those from set 2 were supplied by the sponsor.

Test medium
The test medium (2 L per flask) was prepared according to OECD 302 B:
38.5 g NH_4Cl, 33.4 g NaH_2PO_4 x $2H_2O$, 8.5 g KH_2PO_4 and 21.75 g K_2HPO_4 were dissolved in 1000 mL bidistilled water.

2.5 mL of this stock solution were added to 1000 mL test water (1:1 drinking water/bidistilled water).

An amount of sludge from a domestic waste-water sewage plant corresponding to 0.2 g dry material was added per litre final test medium.

B. STUDY DESIGN

1. Experimental conditions
Three treatment groups were established:

- Inoculum Control: inoculated mineral salt medium
- Functional solution: inoculated mineral salt medium plus aniline at 100 mg/L corresponding to a theoretical amount of 77.4 mg TOC/L
- Glyphosate solution: inoculated mineral salt medium plus test substance at 1240 mg/2 L (620 mg/L) corresponding to 121.5 mg TOC/L in Test set 1 and 131 mg TOC/L in Test set 2

Three vessels were established for the glyphosate treatment. Single vessels were established for the inoculum control and the functional control.

A pre-test was conducted investigating potential inhibitory effects of different glyphosate concentrations on sludge.
The study was run at 20 – 23 °C protected from light. The flasks were aerated with a flow rate of about 0.5 – 0.7 L/minute, resulting in an oxygen concentration of 7.7 – 9.0 mg O_2 per litre. The pH was adjusted to 7.0 and 8.2.

2. Analytical procedures
Per sampling interval, two subsamples of 30 mL were taken per flask and analysed for TOC in duplicate. Samples were taken at day 0 (0 and 3 hours after treatment), 7, 14, 21 and 28 of the incubation period. Water evaporation losses were compensated by adding bidistilled water.

Samples were filtered through a washed fluted filter paper. The first 5 mL of the filtrate were replaced into the reactor. The remaining 25 mL were used for TOC analysis. Samples were analysed on day of sampling, except on 28 DAT where the samples were stored at 4 °C for four days due to a defect of the TOC-Analyser.

TOC analyses were performed with the various filtrates using a total carbon analyser.

3. Calculations
The degradation rate was calculated as:

$$Dt\ (\%) = (1 - (Ct - Cbl) / (C0 - Cbl)) \times 100$$

Where
Dt = degradation in percent TOC-removal at time t
C0 = starting TOC-concentration of the culture medium (mg TOC/L)
Ct = TOC-concentration of the culture medium at time t (mg TOC/L)
Cbl(0) = starting TOC-concentration of the blank (mg TOC/L)
Cbl(t) = TOC-concentration of the blank at time t (mg TOC/L)

Degradation is stated as the percentage TOC-removal within 28 days with respect to the test article (% TOC-removal).

II. RESULTS AND DISCUSSION

A. DATA

Biodegradation of glyphosate technical and reference compound aniline expressed as percent TOC removal is summarised in Table 7.2.2.1-5 and Table 7.2.2.1-6 for microorganism sets 1 and 2, respectively.

Table 7.2.2.1-5: Degradation of glyphosate technical by activated sludge (microorganisms set 1 and set 2, respectively) expressed as percent TOC-removal

Replicate	% TOC-removal after				
	3 hours	7 d	14 d	21 d	28 d
Microorganisms test set 1 (supplied by ARA Sissach, Switzerland)					
1	-12	-17	-2	-25	-2
2	-10	-14	-3	-21	-6
Microorganisms test set 2 (supplied by Sponsor)					
1	-15	0	-17	-19	-11
2	-8	-13	-15	-23	-3

Table 7.2.2.1-6: Degradation of aniline by activated sludge (microorganisms set 1 and set 2, respectively) expressed as percent TOC-removal

Replicate	% TOC-removal after				
	3 hours	7 d	14 d	21 d	28 d
Microorganisms test set 1 (supplied by ARA Sissach, Switzerland)					
1	-27	88	91	94	100
Microorganisms test set 2 (supplied by Sponsor)					
1	-12	89	92	96	99

B. BIODEGRADATION

No removal of glyphosate was detected in flasks treated with sludge sets 1 and 2 demonstrated by unchanged TOC. Glyphosate was neither eliminated nor degraded within 28 days of incubation. Therefore, glyphosate is considered not to be readily biodegradable.

The test is considered valid as the reference compound aniline was biodegraded within 14 days by 91 % and 92 %, respectively, with microorganisms sets 1 and 2, respectively.

III. CONCLUSIONS

The test article glyphosate technical appeared to be non-degradable (unchanged TOC). The reference compound aniline was degraded within 14 days by 91 and 92 % by microorganisms of two different sources, demonstrating the viability of the microorganisms.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted in line with the relevant guideline OECD 302 B with minor deviations that did not have an impact on the outcome. It is therefore considered valid to describe the ready biodegradability of glyphosate. Glyphosate is considered not to be ready biodegradable.

Assessment and conclusion by RMS:

CA 7.2.2.2 Aerobic mineralisation in surface water

The aerobic mineralisation of glyphosate in surface water was investigated in a new study with glyphosate which is considered valid to address the data point ([redacted] 2020, CA 7.2.2.2/001).

Glyphosate was found to be well degraded in natural surface water under aerobic conditions at 20°C in the dark with half-lives of 12.3 and 21.8 days, for low and high dose, respectively. Maximum mineralisation of glyphosate was 26.5 and 23.1 % AR, while NER accounted for 14.0 and 8.8 % AR at the end of the study, in the low and high dose, respectively. AMPA was the only major metabolite identified and was almost exclusively detected in the water phase. The maximum amounts of AMPA, detected in the water phase, were 42.7 and 39.8 % AR, in the low and high dose, respectively.

Analysis by a secondary chromatographic method showed the presence of an unidentified peak with >5 % AR. Several attempts to identify this peak were not successful. Analyses by a tertiary chromatographic method showed that this peak was comprised of three individual peaks. Further attempts to characterize this radioactivity are currently made and will be reported in an amendment to this study report.

In the scientific literature review for glyphosate (2010-2019), no article was identified to provide further information relevant to the data point.

Table 7.2.2.2-1: Aerobic mineralisation in surface water studies

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.2.2.2/001	[redacted] 2020	Aerobic mineralisation in surface water	Glyphosate	Valid	

Table 7.2.2.2-2: Summary of degradation endpoints in surface water for glyphosate

Study	Water system	Concentration	pH water phase	pH sed[1]	T (°C)	DT_{50} (d)	DT_{90} (d)	St. (χ^2err) (%)	Kinetic model
[redacted] (2020) CA 7.2.2.2/001	Calwich Abbey	10 µg/L	8.2	7.6	20	12.3	41.0	8.4	SFO
	Calwich Abbey	95 µg/L	8.2	7.6	20	21.8	72.4	5.2	SFO

[1] In water

1. Information on the study

Data point:	CA 7.2.2.2/001
Report author	[redacted]
Report year	2020
Report title	Glyphosate – Aerobic Mineralisation of [^{14}C]Glyphosate in Surface Water
Report No	815731
Document No	
Guidelines followed in study	OECD Guideline 309
Deviations from current test guideline	From OECD 309: - Material balance below 90 % for some samples, losses explained by incomplete trapping of carbon dioxide - Procedural recovery for HPLC analysis was <90 % for some samples - Single replicates for sterile samples
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The mineralisation of [phosphonomethyl-^{14}C]glyphosate was studied in surface water under conditions of a suspended sediment test under aerobic conditions in the laboratory at 20 ± 2°C in the dark for 62 days in maximum.

The study was performed at test concentrations of 10 µg/L (low dose) and 95 µg/L (high dose).

The test systems consisted of glass Erlenmeyer flasks filled with with 100 mL natural water and its associated suspended sediment. The sediment concentration was 0.54 g/L. During incubation, the test vessels were connected to traps for collection of $^{14}CO_2$ and other volatiles.

Duplicate samples were removed for analysis immediately following test item application (0 DAT) and 3, 7, 14, 30, 44 and 62 days after treatment (DAT). For both test concentrations, single replicates of sterile samples were sampled at zero DAT and 62 DAT.

Mean material balances ranged from 87.7 to 94.5 % AR for the low dose and from 88.2 to 94.7 % AR for the high dose. Material balances for the sterile test system were between 86.6 and 91.9 % AR.

Total mineralisation to ^{14}C-carbon dioxide was 26.5 % and 23.1 % AR, for the low and high dose, respectively. Formation of other volatiles was not significant as demonstrated by values <LOQ in all samples. The amount of carbon dioxide determined in sterile samples after 62 days was 2.0 and 0.5 % AR for the low and high dose, respectively. Formation of other volatiles was not significant as demonstrated by values <LOQ in all samples.

The amount of non-extractable residues (NER) increased from 0 DAT to 62 DAT from 0.2 to 14.0 % AR for the low dose and from 0.3 to 8.8 % AR for the high dose.

At the low (10 µg/L) dose level, glyphosate in the water phase declined from 72.6 % AR on 0 DAT to 2.0 % AR on 62 DAT. In the sediment extracts, glyphosate increased from 14.9 % AR on 0 DAT to

23.0 % AR on 7 DAT and declined to 3.0 % AR on 30 DAT and was not detectable afterwards. In the total system, glyphosate decreased from 87.5 % AR at 0 DAT to 2.0 % AR at 62 DAT. The only degradation product observed was aminomethylphosphonic acid (AMPA). AMPA was mainly detected in the water phase, increasing from 3.5 % AR on 0 DAT to 42.7 % AR on 44 DAT, slightly decreasing to 42.0 % AR at 62 DAT. In the sediment extracts, AMPA increased from 0.6 % AR on 0 DAT to 3.1 % AR on 62 DAT. In the total system, AMPA increased from 4.1 % AR on 0 DAT to 42.7 % AR on 44 DAT and then slightly decreased to 42.0 % AR at 62 DAT. Minor metabolites accounted for a maximum of 1.8 % AR.

At the high (95 µg/L) dose level, glyphosate in the water phase declined from 57.6 % AR on 0 DAT to 6.1 % AR on 62 DAT. In the sediment extracts, glyphosate increased from 30.6 % AR on day zero to 36.2 % AR on 7 DAT and declined to 3.8 % AR at the end of the study (62 DAT). In the total system, glyphosate decreased from 88.2 % AR on 0 DAT to 9.8 % AR on 62 DAT. The only degradation product observed was aminomethylphosphonic acid (AMPA). AMPA was mainly detected in the water phase, increasing from 2.8 % AR on 0 DAT to 39.8 % AR on 62 DAT. In the sediment extracts, AMPA increased from 1.2 % AR on 0 DAT to 5.2 % AR on 62 DAT. In the total system, AMPA increased from 4.0 % AR on 0 DAT to 45.0 % AR on 62 DAT. Minor metabolites accounted for a maximum of 1.1 % AR.

In sterile samples, degradation of glyphosate was negligible. Glyphosate decreased from 0 DAT to 62 DAT from 93.7 to 89.6 % AR for the low dose and from 92.7 to 89.9 % AR for the high dose. AMPA was determined in low dose samples with 3.8 % AR at 0 DAT and 3.2 % AR at 62 DAT and in high dose samples with 3.1 % AR at 0 DAT and 3.2 % AR at 62 DAT.

Analysis by a secondary chromatographic method showed the presence of an unidentified peak at >5 % AR. Attempts to identify the peak were not successful in a first approach. However, analyses by a tertiary chromatographic method finally demonstrate that the peak comprised of at least three individual components.

SFO, DFOP and FOMC models were applied to calculated degradation rates using CAKE version 3.3. The SFO model was selected as the best fit kinetic in all cases. The samples treated at 10 µg/L yielded a DT_{50} of 12.3 days and a DT_{90} of 41.0 days. The samples treated at 95 µg/L yielded a DT_{50} of 21.8 days and a DT_{90} of 72.4 days.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Identification: [phosphonomethyl-^{14}C]glyphosate
Batch ID: 6848SXD008-2
Specific activity: 12.18 MBq/mg
Radiochemical purity: 98.3 % (HPLC-radiodetection)
Chemical purity: Not reported

2. Test Surface Water and Sediment

Freshly collected natural sediment and water from a lake (Calwich Abbey Lake, Staffordshire, UK) was used. Upon collection, sediment was passed through a 2-mm sieve and water through a 0.2-mm sieve. Sediment and water were stored under aerobic conditions at *ca* 4°C until use for 7 days until acclimatization of test systems. Characteristics of test water and sediment are summarised in the table below.

Table 7.2.2.2-3: Characteristics of test surface water and sediment

Parameter	Results
Test system	Calwich Abbey
Country	UK
Sediment:	

Table 7.2.2.2-3: Characteristics of test surface water and sediment

Parameter	Results
Textural Class (USDA)	Silt Loam
Sand [50 µm – 2 mm] (%)	10
Silt [2 µm – 50 µm] (%)	73
Clay [< 2 µm] (%)	17
pH (in water)	7.6
pH (in 0.01 M $CaCl_2$)	7.5
Organic matter (%)	7.94
Organic carbon (%)	4.60
Maximum water holding capacity – pF0 disturbed sediment (% w/w)	100.5
Cation exchange capacity [meq/100 g]	18.4
Nitrogen, Total (% w/w)	0.35
Phosphorus, Total (mg/kg)	1312
Carbonate Content as CO_3 (%)(w:w)	30.3
Water:	
Organic Carbon (mg/L)	5.50
Dissolved Organic (mg/L)	5.64
Nitate (mg/L) (-N)	12.51 (2.83)
Nitrite (mg/L) (-N)	<0.66 (<0.20)
Total Nitrogen (mg/L)	3.55
Ammonium (mg/L) (-N)	<0.26 (<0.20)
Phosphate (mg/L) (-P)	<0.06 (<0.20)
Total phosphorous (mg/L)	<0.02
Total Suspended Solids (mg/L)	53.6
Electrical Conductivity (µS/cm)	544
CBOD (mg/L)	<1.0
pH	8.2
Total Hardness (EDTA, mg/L as calcium carbonate)	261
Alkalinity (mg $CaCO_3$/L)	149
Carbonate (mg/L)	2.4
Bicarbonate (mg/L)	177

DAT = Days after treatment; USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental Conditions

First, Calwich Abbey sediment and Calwich Abbey surface water to a 10-L glass duran bottle and thoroughly shaken. After settling for 130 minutes, the supernatant was removed and the sediment concentration was determined. Afterwards, sediment was added to the supernatant and left to settle again. This process was repeated until a sediment concentration of 0.535 g/L was reached. The test system was then stored aerobically for four days at +4 ºC prior to being weighed into test vessels.

The study was performed in 250 mL Erlenmeyer flasks filled with approximately 100 g of the test water containing sediment (0.535 g/L). Test vessels were contained on an orbital shaker and the water was gently agitated through the study. Each flask was connected to a series of four liquid traps, the first being a safety trap, the second containing ethanediol to trap organic volatiles and the final two containing 2 M NaOH to trap CO_2. Moist air was drawn through the test apparatus (via a dip tube just below the bulk inlet water

surface) and the air leaving each test vessel was drawn through the series of traps. The air had at a rate of flow such that only one air bubble was observed in the trapping solutions at any time.

Flasks containing the test water were each treated with the corresponding treatment solution, prepared in ultrapure water to receive final nominal concentrations of 10 µg/mL (low concentration) and 95 µg/mL (high concentration). For both test concentrations sterile samples were prepared.Volumes and dosing technique were the same as for non-sterile flasks. Measured concentration were 9.8 and 96.2 µg/L.

Additionally, two further vessels were treated with sodium [ring-U-^{14}C]benzoate at a concentration of 10 µg/L as a reference control to prove biological viability of the test systems.

Samples were maintained under aerobic conditions for 62 days at 20 ± 2◦C in the laboratory in the dark.

2. Sampling

Seven sampling intervals were distributed over the entire incubation period of 62 days. For each of the two test concentrations (10 and 95 µg/L) duplicate flasks removed 0, 3, 7, 14, 30, 44 and 62 days after treatment (DAT). The sterile controls were sampled after 0 and 62 days. Reference controls were sampled with other terminal samples at day 62. Traps were collected and replenished at 7, 14, 30, 44 and 62 days. The sodium benzoate reference controls had an additional trap change on Day 3.

3. Analytical Procedures

At each sampling interval, the dissolved oxygen content (mg/L) and pH of the water was measured in control vessels.

Sediment and water were separated by filtration using 0.45-µm filter membrane and Büchner apparatus. The test vessels were rinsed with ultrapure water which was passed through the filter membrane and combined with the sample filtrate. Duplicate aliquots of the water were taken for analysis by liquid scintillation counting (LSC).

The amount of $^{14}CO_2$ trapped in the water samples was determined by LSC after acidification of subsamples of the filtered surface water to *ca* pH 2-3 and shaking on an orbital shaker overnight. An additional test for entrained $^{14}CO_2$ was performed analogously for two contingency samples.
The loss on filtration was tested with one contingency sample of the 95 µg/L group. Recovery before and after filtration through a 0.45 µm filter membrane was compared to determine if the filtering process led to a loss.

The filter membrane containing the sediment after separation of the surface water and sediment was extracted in three steps. First, the membrane was extracted in a centrifuge tube using 30 mL 0.5 M aqueous NH_4OH solution by shaking for 1 hour. Afterwards, the filter membrane was placed on the Büchner apparatus and the extractant passed through it. Final volume was made to 30 mL with extractant. The second extract was created as above, with the exception that day 3, 7 and 14 were not passed through their respective filter membrane. Final volume was made to 30 mL with extractant. The third extraction was performed as above on samples from the Day 14 timepoint onwards. For the day 30 samples onwards, the centrifuge tube was rinsed with 10 mL extractant, passed through the membrane and combined with extract 3. Final volume was made to 40 mL with extractant. Duplicate aliquots of all three extracts were analysed by LSC.

Non-extractable residues (NER) were determined by combustion of the filter membrane followed by LSC measurement of the evolved $^{14}CO_2$. As the extract 2 samples from the Day 3, 7 and 14 timepoints were not passed through their respective filter membranes after shaking, the samples were re-filtered using a fresh filter membrane and these membrane were also combusted.

Trap solutions were removed for analysis at each sampling time and duplicate aliquots were analysed by LSC.

Surface water samples of the first sampling (0 DAT) were analysed directly by HPLC. All other time points were admixed (50:50, v:v) with mobile phase A of the respective HPLC method and analysed by HPLC without further processing.

Extract 1 was analysed by HPLC for all samples while extract 2 was only analysed by HPLC when containing >5 % AR and extract 3 was not analysed by HPLC. An aliquot (5 mL) of the sediment extract was concentrated to dryness under a stream of nitrogen. The samples was reconstituted with mobile phase A, sonicated for 10 min and analysed by HPLC.

HPLC involving a porous graphitic carbon (PGC) 'Hypercarb' column was used as primary analytical method for radiochemical purity determination of the test item, stock and application solutions of the test item and for determining the initial patterns of degradation in surface water and sediment extract samples. The limit of quantification (LOQ) for HPLC was deemed to be 200 dpm in a single peak for online radiodetector analysis. The limit of quantification for low dose samples (10 µg/L) was 1.5 % AR for surface water and 0.22 % AR for sediment extracts. The limit of quantification for high dose samples (95 µg/L) was 0.15 % AR for surface water and 0.02 % AR for sediment extracts. All values reported are in excess of the limit of detection, unless stated otherwise.

A secondary method based on HPLC involving a strong cation exchange column to differ from the primary method was used in addition to confirm the initial profiles for selected water and sediment extracts.

Following the observation of an unknown component from use of the confirmatory secondary method, additional attempts were made for characterisation. Tests to characterise the unknown as AMPA and/or glyphosate associated to metal ions failed. The unknown was thus isolated by fraction collection using the secondary analytical method for a representative sample of 62 DAT (high dose). The isolated unknown was subject to investigation by a tertiary chromatographic method, i.e. HPLCinvolving a strong anion exchange (SAX) column to result in separation of the unknown peak into three components. The result has to be confirmed for the whole range of samples of the low and high dose being subject of ongoing work and to an amendment to report.

LC-MS experiments were performed on selected surface waters, sediment extracts and the isolated unidentified peak observed using the secondary method, to confirm assignments made by HPLC through co-chromatography with reference standards.
Control samples treated with sodium benzoate were analysed by reversed phase HPLC.

The identity of carbon dioxide was confirmed by precipitation with barium chloride.

II. RESULTS AND DISCUSSION

The pH value of the water remained relatively constant during the study between 7.32 and 8.61. The dissolved oxygen decreased from 0 DAT to 62 DAT from ≥8.69 to ≤6.34 mg/L. At each sampling interval of sterile samples, sterility was proven.

Recovery from the sodium [^{14}C]benzoate reference controls had a mean value of 97 % AR at Day 62. Radioactivity accounted for a mean of 0.7 % AR in surface water only and 0.8 % AR in the sediment/filter membrane extracts. The sediment/filter membrane combustions accounted for a mean of 8.0 % AR. The majority of the recovery was in the NaOH traps, which accounted for a mean of 87.3 % AR by Day 62, showing that the test system was viable.

Radioactive mass balance and distribution of glyphosate and metabolites in surface water and suspended sediment are summarised in Table 7.2.2.2-4 to Table 7.2.2.2-9. Comparison of analyses by primary and secondary method are presented in Table 7.2.2.2-10 and Table 7.2.2.2-11.

A. DATA

Table 7.2.2.2-4: Material balance of radioactivity from [^{14}C]glyphosate at an application rate of 10 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT						
		0	3	7	14	30	44	62
Surface Water	1	77.0	59.8	45.9	49.0	48.7	44.2	45.4
	2	78.7	56.1	48.1	52.3	50.3	47.8	44.8
	Mean	**77.9**	**58.0**	**47.0**	**50.7**	**49.5**	**46.0**	**45.1**
Sediment Extract 1	1	15.0	15.4	23.2	14.2	6.1	3.6	3.0
	2	15.8	15.9	24.9	16.1	6.0	3.9	3.9
	Mean	**15.4**	**15.7**	**24.1**	**15.2**	**6.1**	**3.8**	**3.5**
Sediment Extract 2	1	1.0	2.0	2.7	2.8	1.5	1.4	0.6
	2	1.1	2.0	1.3	2.9	1.3	0.9	0.5
	Mean	**1.1**	**2.0**	**2.0**	**2.9**	**1.4**	**1.2**	**0.6**
Sediment Extract 3	1	NA	NA	NA	2.7	0.6	0.8	0.3
	2	NA	NA	NA	2.7	0.7	0.8	0.3
	Mean	**NA**	**NA**	**NA**	**2.7**	**0.7**	**0.8**	**0.3**
NER [1]	1	0.1	10.6	14.8	7.0	12.1	11.5	14.7
	2	0.2	13.8	9.3	8.9	11.9	13.8	13.2
	Mean	0.2	12.2	12.1	8.0	12.0	12.7	14.0
Volatiles	1	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	2	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Mean	**NA**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**
$^{14}CO_2$ [2]	1	NA	0.5	<LOQ	10.3	19.0	23.8	27.9
	2	NA	0.6	3.8	11.0	20.0	23.2	25.1
	Mean	**NA**	**0.6**	**1.9**	**10.7**	**19.5**	**23.5**	**26.5**
Apparatus Wash	1	<LOQ	<LOQ	<LOQ	0.8	<LOQ	0.6	<LOQ
	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.2	0.8
	Mean	**<LOQ**	**<LOQ**	**<LOQ**	**0.4**	**<LOQ**	**0.9**	**0.4**
Centrifuge Tube Wash	1	NA	0.4	1.4	0.6	0.9	0.7	NA
	2	NA	1.4	NA	NA	NA	NA	0.5
	Mean	**NA**	**0.9**	**1.4**	**0.6**	**0.9**	**0.7**	**0.5**
Total	1	93.1	88.7	88.0	87.4	88.9	86.6	91.9
	2	95.8	89.8	87.4	93.9	90.2	91.6	89.1
	Mean	**94.5**	**89.3**	**87.7**	**90.7**	**89.6**	**89.1**	**90.5**

DAT: Days after treatment
<LOQ = Below the limit of quantification
NA = Not Applicable
[1] Combined sediment and filter membrane
[2] Combined total recoveries from both NaOH traps

Table 7.2.2.2-5: Material balance of radioactivity from [^{14}C]glyphosate at an application rate of 95 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT						
		0	3	7	14	30	44	62
Surface Water	1	63.7	46.8	47.2	41.0	48.1	43.7	48.6
	2	57.0	46.2	24.3	49.6	41.0	48.2	45.0
	Mean	**60.4**	**46.5**	**35.8**	**45.3**	**44.6**	**46.0**	**46.8**
Sediment Extract 1	1	28.4	33.9	27.4	24.8	15.9	11.8	7.8
	2	35.1	31.5	42.9	23.8	17.8	11.6	10.0
	Mean	**31.8**	**32.7**	**35.2**	**24.3**	**16.9**	**11.7**	**8.9**
Sediment Extract 2	1	2.1	3.3	3.2	3.3	3.6	3.4	1.5
	2	2.5	4.0	7.9	3.1	3.4	4.0	2.3
	Mean	**2.3**	**3.7**	**5.6**	**3.2**	**3.5**	**3.7**	**1.9**
Sediment Extract 3	1	NA	NA	NA	1.6	1.3	1.8	0.5
	2	NA	NA	NA	2.8	1.4	1.6	1.0
	Mean	**NA**	**NA**	**NA**	**2.2**	**1.4**	**1.7**	**0.8**
NER [1]	1	0.3	5.7	6.9	2.4	7.4	8.7	7.5
	2	0.2	6.8	5.5	4.7	8.7	9.4	10.1
	Mean	**0.3**	**6.3**	**6.2**	**3.6**	**8.1**	**9.1**	**8.8**
Volatiles	1	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	2	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Mean	**NA**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**
$^{14}CO_2$ [2]	1	NA	0.4	2.4	11.0	15.2	20.6	20.9
	2	NA	0.4	6.2	8.3	17.1	19.1	25.3
	Mean	**NA**	**0.4**	**4.3**	**9.7**	**16.2**	**19.9**	**23.1**
Apparatus Wash	1	<LOQ	0.1	0.3	0.4	0.4	0.1	0.5
	2	<LOQ	0.1	0.1	0.2	0.3	0.1	0.2
	Mean	**<LOQ**	**0.1**	**0.2**	**0.3**	**0.4**	**0.1**	**0.4**
Centrifuge Tube Wash	1	NA	0.4	1.3	0.4 [3]	NA	NA	0.3
	2	NA	0.5	0.7	NA	0.3	NA	NA
	Mean	**NA**	**0.5**	**1.0**	**0.4**	**0.3**	**NA**	**0.3**
Total	1	94.5	90.6	88.7	84.9	91.9	90.1	87.6
	2	94.8	89.5	87.6	92.5	90.0	94.0	93.9
	Mean	**94.7**	**90.1**	**88.2**	**88.7**	**91.0**	**92.1**	**90.8**

DAT: Days after treatment
<LOQ = Below the limit of quantification
NA = Not Applicable
[1] Combined sediment and filter membrane
[2] Combined total recoveries from both NaOH traps
[3] Combined total recovery from centrifuge tube wash containing filter membranes for extractions 1 and 2

Table 7.2.2.2-6: Material balance of radioactivity from [^{14}C]glyphosate at two test concentrations in sterilised surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Test concentration [µg/L]	DAT	
		0	62
Surface Water	10	97.5	92.8
	95	96.0	93.1
Sediment Extract 1	10	0.4	3.6
	95	2.0	4.0
Sediment Extract 2	10	<LOQ	0.4
	95	0.1	0.3
Sediment Extract 3	10	NA	0.3
	95	NA	0.1
NER [1]	10	<LOQ	2.0
	95	<LOQ	0.5
Volatiles	10	NA	<LOQ
	95	NA	<LOQ
$^{14}CO_2$ [2]	10	NA	1.2
	95	NA	1.2
Apparatus Wash	10	<LOQ	<LOQ
	95	0.1	0.1
Total	10	97.9	100.3
	95	98.2	99.3

DAT: Days after treatment
<LOQ = Below the limit of quantification
NA = Not Applicable
[1] Combined sediment and filter membrane
[2] Combined total recoveries from both NaOH traps

Table 7.2.2.2-7: Degradation of [^{14}C]glyphosate at an application rate of 10 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Phase	Replicate	DAT						
			0	3	7	14	30	44	62
Glyphosate	Surface Water	1	71.5	55.2	39.6	31.8	9.7	2.6	2.6
		2	73.7	52.3	40.9	35.9	9.4	4.0	1.4
		Mean	**72.6**	**53.8**	**40.3**	**33.9**	**9.6**	**3.3**	**2.0**
	Sediment extracts	1	14.2	15.1	22.2	12.5	3.2	NS	NS
		2	15.5	15.6	23.7	14.5	2.7	NS	NS
		Mean	**14.9**	**15.4**	**23.0**	**13.5**	**3.0**	**NS**	**NS**
	Total	1	85.7	70.3	61.8	44.3	12.9	2.6	2.6
		2	89.2	67.9	64.6	50.4	12.1	4.0	1.4
		Mean	**87.5**	**69.1**	**63.2**	**47.4**	**12.5**	**3.3**	**2.0**
AMPA	Surface Water	1	3.9	4.6	6.3	17.2	39.0	41.6	41.7
		2	3.1	3.8	7.2	16.4	40.9	43.8	42.3
		Mean	**3.5**	**4.2**	**6.8**	**16.8**	**40.0**	**42.7**	**42.0**
	Sediment extracts	1	0.8	0.3	1.0	1.7	2.9	NS	NS
		2	0.3	0.3	1.2	1.6	3.3	NS	NS
		Mean	**0.6**	**0.3**	**1.1**	**1.7**	**3.1**	**NS**	**NS**
	Total	1	4.7	4.9	7.3	18.9	41.9	41.6	41.7
		2	3.4	4.1	8.4	18.0	44.2	43.8	42.3
		Mean	**4.1**	**4.5**	**7.9**	**18.5**	**43.1**	**42.7**	**42.0**
Total Minor Unidentified Degradation Products [1]	Surface Water	1	1.6	ND	ND	ND	ND	ND	1.1
		2	1.9	ND	ND	ND	ND	ND	1.0

Table 7.2.2.2-7: Degradation of [^{14}C]glyphosate at an application rate of 10 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

		Mean	**1.8**	**ND**	**ND**	**ND**	**ND**	**ND**	**1.1**
	Sediment extracts	1	ND	ND	ND	ND	ND	NS	NS
		2	ND	ND	ND	ND	ND	NS	NS
		Mean	**ND**	**ND**	**ND**	**ND**	**ND**	**NS**	**NS**
	Total	1	1.6	ND	ND	ND	ND	ND	1.1
		2	1.9	ND	ND	ND	ND	ND	1.0
		Mean	**1.8**	**ND**	**ND**	**ND**	**ND**	**ND**	**1.1**

DAT: Days after treatment
NS = Sample not analysed as insufficient radioactivity in sample
ND = Not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

Table 7.2.2.2-8: Degradation of [^{14}C]glyphosate at an application rate of 95 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions (expressed as percent of applied radioactivity)

Compound	**Phase**	**Replicate**	**DAT**						
			0	**3**	**7**	**14**	**30**	**44**	**62**
Glyphosate	Surface Water	1	61.1	42.8	42.6	31.5	25.8	12.5	8.5
		2	54.1	41.6	18.8	39.4	18.3	14.5	3.6
		Mean	**57.6**	**42.2**	**30.7**	**35.5**	**22.1**	**13.4**	**6.1**
	Sediment extracts	1	27.6	31.9	26.0	22.9	13.4	7.8	3.9
		2	33.5	30.2	46.4	22.2	14.1	7.5	3.6
		Mean	**30.6**	**31.1**	**36.2**	**22.6**	**13.8**	**7.7**	**3.8**
	Total	1	88.7	74.7	68.6	54.4	39.2	20.3	12.4
		2	87.6	71.9	65.2	61.6	32.4	22.0	7.2
		Mean	**88.2**	**73.3**	**66.9**	**58.0**	**35.8**	**21.2**	**9.8**
AMPA	Surface Water	1	2.6	4.0	4.6	9.5	21.3	30.1	39.1
		2	2.9	4.5	5.5	9.3	21.6	33.2	40.5
		Mean	**2.8**	**4.3**	**5.1**	**9.4**	**21.5**	**31.7**	**39.8**
	Sediment extracts	1	0.8	2.0	1.4	1.9	2.5	4.0	3.9
		2	1.6	1.2	4.4	1.6	3.7	4.1	6.4
		Mean	**1.2**	**1.6**	**2.9**	**1.8**	**3.1**	**4.1**	**5.2**
	Total	1	3.4	6.0	6.0	11.4	23.8	34.1	43.0
		2	4.5	5.7	9.9	10.9	25.3	37.3	46.9
		Mean	**4.0**	**5.9**	**8.0**	**11.2**	**24.6**	**35.7**	**45.0**
Total Minor Unidentified Degradation Products [1]	Surface Water	1	ND	ND	ND	ND	1.0	1.2	1.0
		2	ND	0.2	ND	0.9	1.1	0.6	0.9
		Mean	**ND**	**0.1**	**ND**	**0.5**	**1.1**	**0.9**	**1.0**
	Sediment extracts	1	ND	ND	ND	ND	ND	ND	ND
		2	ND	ND	ND	ND	ND	ND	ND
		Mean	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
	Total	1	ND	ND	ND	ND	1.0	1.2	1.0
		2	ND	0.2	ND	0.9	1.1	0.6	0.9
		Mean	**ND**	**0.1**	**ND**	**0.5**	**1.1**	**0.9**	**1.0**

DAT: Days after treatment
NS = Sample not analysed as insufficient radioactivity in sample
ND = Not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

Table 7.2.2.2-9: Degradation of [^{14}C]glyphosate at two test concentrations in sterilised surface water containing suspended sediment (0.54 g/L) under aerobic conditions (total system; expressed as percent of applied radioactivity)

Compound	Test concentration [µg/L]	DAT	
		0	62
Glyphosate	10	93.7	89.6
	95	92.7	89.9
AMPA	10	3.8	3.2
	95	3.1	3.2
Total Minor Unidentified Degradation Products [1]	10	ND	ND
	95	0.2	ND

DAT: Days after treatment
ND = Not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

Table 7.2.2.2-10: Comparison of degradation of [^{14}C]glyphosate at an application rate of 10 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions through analysis by the primary and secondary chromatographic methods (expressed as percent of applied radioactivity)

Compound	Chromatographic method	DAT									
		0	3	7		14	30		44		62	
		Rep 1	Rep 2	Rep 1	Rep 2	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Surface water												
Glyphosate	Primary	71.5	52.3	39.6	40.9	35.9	9.7	9.4	2.6	4.0	2.6	1.4
	Secondary	75.7	51.3	20.1	23	34.5	6.9	3.9	ND	ND	ND	ND
	Difference	**4.2**	**1.0**	**19.5**	**17.9**	**1.4**	**2.8**	**5.5**	**2.6**	**4.0**	**2.6**	**1.4**
AMPA	Primary	3.9	3.8	6.3	7.2	16.4	39.0	40.9	41.6	43.8	41.7	42.3
	Secondary	1.3	3.1	2.7	2.7	14.8	35.5	39.3	38	40.3	38.0	37.0
	Difference	**2.6**	**0.7**	**3.6**	**4.5**	**1.6**	**3.5**	**1.6**	**3.6**	**3.5**	**3.7**	**5.3**
Unidentified Peak	Primary	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Secondary	ND	1.7	23.2	22.5	3.0	6.3	7.1	6.2	7.5	7.4	7.8
	Difference	**ND**	**1.7**	**23.2**	**22.5**	**3.0**	**6.3**	**7.1**	**6.2**	**7.5**	**7.4**	**7.8**
Total Minor Unidentified Degradation Products [1]	Primary	1.6	ND	ND	ND	ND	ND	ND	ND	ND	1.1	1.0
	Secondary	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Difference	**1.6**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**1.1**	**1.0**
Sediment extracts												
Glyphosate	Primary	14.2	NS	NS	NS	14.5	NS	NS	NS	NS	NS	NS
	Secondary	15.0	NS	NS	NS	14.9	NS	NS	NS	NS	NS	NS
	Difference	**0.8**	**NS**	**NS**	**NS**	**0.4**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
AMPA	Primary	0.8	NS	NS	NS	1.6	NS	NS	NS	NS	NS	NS
	Secondary	ND	NS	NS	NS	0.8	NS	NS	NS	NS	NS	NS
	Difference	**0.8**	**NS**	**NS**	**NS**	**0.8**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Unidentified Peak	Primary	ND	NS	NS	NS	ND	NS	NS	NS	NS	NS	NS
	Secondary	ND	NS	NS	NS	ND	NS	NS	NS	NS	NS	NS
	Difference	**ND**	**NS**	**NS**	**NS**	**ND**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Total Minor Unidentified Degradation Products [1]	Primary	ND	NS	NS	NS	ND	NS	NS	NS	NS	NS	NS
	Secondary	ND	NS	NS	NS	0.4	NS	NS	NS	NS	NS	NS
	Difference	**ND**	**NS**	**NS**	**NS**	**0.4**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

DAT: Days after treatment
NS = Sample not analysed as insufficient radioactivity in sample
ND = Not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

Table 7.2.2.2-11: Comparison of degradation of [^{14}C]glyphosate at an application rate of 95 µg/L in surface water containing suspended sediment (0.54 g/L) under aerobic conditions through analysis by the primary and secondary chromatographic methods (expressed as percent of applied radioactivity)

Compound	Chromatographic method	DAT										
		0	3	7		14	30		44		62	
		Rep 2	Rep 2	Rep 1	Rep 2	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Surface water												
Glyphosate	Primary	54.1	42.8	42.6	18.8	39.4	25.8	18.3	12.5	14.5	8.5	3.6
	Secondary	53.8	42	33.7	9.0	39.7	25.2	17.3	10.6	13.9	6.3	2.6
	Difference	**0.3**	**0.8**	**8.9**	**9.8**	**0.3**	**0.6**	**1.0**	**1.9**	**0.6**	**2.2**	**1.0**
AMPA	Primary	2.9	4.0	4.6	5.5	9.3	21.3	21.6	30.1	33.2	39.1	40.5
	Secondary	2.4	3.4	3.4	2.7	8.5	19.4	19.8	27.2	29.3	36.9	35.6
	Difference	**0.5**	**0.6**	**1.2**	**2.8**	**0.8**	**1.9**	**1.8**	**2.9**	**3.9**	**2.2**	**4.9**
Unidentified Peak	Primary	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Secondary	0.7	1.4	10.0	12.6	1.3	3.5	4.0	5.0	5.0	5.4	6.8
	Difference	**0.7**	**1.4**	**10.0**	**12.6**	**1.3**	**3.5**	**4.0**	**5.0**	**5.0**	**5.4**	**6.8**
Total Minor Unidentified Degradation Products [1]	Primary	ND	ND	ND	ND	0.9	1.0	1.1	1.2	0.6	1.0	0.9
	Secondary	ND	ND	ND	ND	ND	ND	ND	0.9	ND	ND	ND
	Difference	**ND**	**ND**	**ND**	**ND**	**0.9**	**1.0**	**1.1**	**0.3**	**0.6**	**1.0**	**0.9**
Sediment extracts												
Glyphosate	Primary	33.5	NS	NS	NS	22.2	NS	NS	NS	7.5	3.9	NS
	Secondary	34.0	NS	NS	NS	22.0	NS	NS	NS	7.3	3.4	NS
	Difference	**0.5**	**NS**	**NS**	**NS**	**0.2**	**NS**	**NS**	**NS**	**0.2**	**0.5**	**NS**
AMPA	Primary	1.6	NS	NS	NS	1.6	NS	NS	NS	4.1	3.9	NS
	Secondary	1.1	NS	NS	NS	1.5	NS	NS	NS	3.5	3.7	NS
	Difference	**0.5**	**NS**	**NS**	**NS**	**0.1**	**NS**	**NS**	**NS**	**0.6**	**0.2**	**NS**
Unidentified Peak	Primary	ND	NS	NS	NS	ND	NS	NS	NS	ND	ND	NS
	Secondary	ND	NS	NS	NS	0.2	NS	NS	NS	0.9	0.7	NS
	Difference	**ND**	**NS**	**NS**	**NS**	**0.2**	**NS**	**NS**	**NS**	**0.9**	**0.7**	**NS**
Total Minor Unidentified Degradation Products [1]	Primary	ND	NS	NS	NS	ND	NS	NS	NS	ND	ND	NS
	Secondary	ND	NS	NS	NS	ND	NS	NS	NS	ND	ND	NS
	Difference	**ND**	**NS**	**NS**	**NS**	**ND**	**NS**	**NS**	**NS**	**ND**	**ND**	**NS**

DAT: Days after treatment
NS = Sample not analysed as insufficient radioactivity in sample
ND = Not detected
[1] Maximum combined unknown minor degradation products, with no individual components accounting for ≥5 % AR

B. MATERIAL BALANCE

Mean material balances ranged from 87.7 to 94.5 % AR for the low dose and from 88.2 to 94.7 % AR for the high dose. Material balances for the sterile test system were between 86.6 and 91.9 % AR.

The decreased material balances obtained from 3 DAT onward were likely due to one of two factors, or a combination of both. One factor involved the challenge in accounting for relatively low levels of radioactivity across multiple compartments. The other factor is that $^{14}CO_2$ generated during the course of the study may not have been fully accounted for because of low amounts entrained in surface waters, which were lost during sample processing. Results from testing for entrained $^{14}CO_2$ support this possibility. The relatively low amounts that were lost ultimately have no impact on the data for the calculation of biotransformation and kinetic data.

C. VOLATILES

Total mineralisation of the samples accounted for 26.5 and 23.1 % AR, for the low and high dose, respectively. Formation of other volatiles was not significant as demonstrated by values <LOQ in all

samples. The amount of carbon dioxide determined in sterile samples after 62 days was 2.0 and 0.5 % AR for the low and high dose, respectively. Formation of other volatiles was not significant as demonstrated by values <LOQ in all samples.

The results from the acidified surface water sub-samples showed some entrained $^{14}CO_2$ to be present in the surface water. Samples in the 10 µg/L and 95 µg/L groups lost between 0.9 % AR and 5.2 % AR upon acidification. In the sterile samples, a mean of 5.8 % AR $^{14}CO_2$ was evolved in the 0 DAT samples. However, these results are considered anomalous as the rest of the sampling data does not support such a rapid degradation to $^{14}CO_2$ as the cumulative total in the $^{14}CO_2$ traps at 62 DAT for the sterile samples was only 1.2 % AR.

D. NON-EXTRACTABLE RESIDUES

The amount of non-extractable residues (NER) increased from 0 DAT to 62 DAT from 0.2 to 14.0 % AR for the low dose and from 0.3 to 8.8 % AR for the high dose.

E. DEGRADATION OF PARENT COMPOUND

At the low (10 µg/L) dose level, glyphosate in the water phase declined from 72.6 % AR on 0 DAT to 2.0 % AR on 62 DAT. In the sediment extracts, glyphosate increased from 14.9 % AR on 0 DAT to 23.0 % AR on 7 DAT and declined to 3.0 % AR on 30 DAT and was not detectable afterwards. In the total system, glyphosate decreased from 87.5 % AR at 0 DAT to 2.0 % AR at 62 DAT. The only degradation product observed was aminomethylphosphonic acid (AMPA). AMPA was mainly detected in the water phase, increasing from 3.5 % AR on 0 DAT to 42.7 % AR on 44 DAT, slightly decreasing to 42.0 % AR at 62 DAT. In the sediment extracts, AMPA increased from 0.6 % AR on 0 DAT to 3.1 % AR on 62 DAT. In the total system, AMPA increased from 4.1 % AR on 0 DAT to 42.7 % AR on 44 DAT and then slightly decreased to 42.0 % AR at 62 DAT. Minor metabolites accounted for a maximum of 1.8 % AR.

At the high (95 µg/L) dose level, glyphosate in the water phase declined from 57.6 % AR on 0 DAT to 6.1 % AR on 62 DAT. In the sediment extracts, glyphosate increased from 30.6 % AR on day zero to 36.2 % AR on 7 DAT and declined to 3.8 % AR at the end of the study (62 DAT). In the total system, glyphosate decreased from 88.2 % AR on 0 DAT to 9.8 % AR on 62 DAT. The only degradation product observed was AMPA. It was mainly detected in the water phase, increasing from 2.8 % AR on 0 DAT to 39.8 % AR on 62 DAT. In the sediment extracts, AMPA increased from 1.2 % AR on 0 DAT to 5.2 % AR on 62 DAT. In the total system, AMPA increased from 4.0 % AR on 0 DAT to 45.0 % AR on 62 DAT. Minor metabolites accounted for a maximum of 1.1 % AR.

In sterile samples, degradation of glyphosate was negligible. Glyphosate decreased from 0 DAT to 62 DAT from 93.7 to 89.6 % AR for the low dose and from 92.7 to 89.9 % AR for the high dose. AMPA was determined in low dose samples with 3.8 % AR at 0 DAT and 3.2 % AR at 62 DAT and in high dose samples with 3.1 % AR at 0 DAT and 3.2 % AR at 62 DAT.

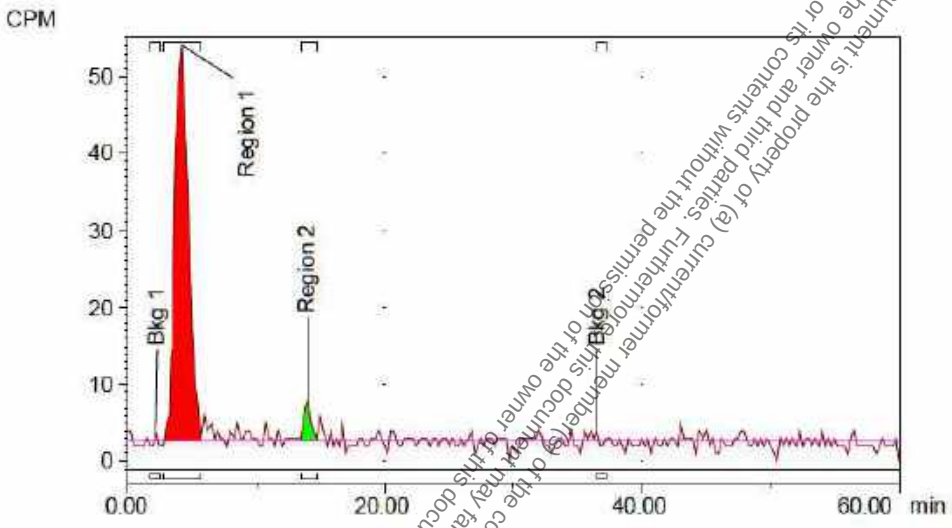
The results of the secondary HPLC analysis for glyphosate were mostly comparable to those obtained from the primary analysis. Absolute differences between primary and secondary analysis (excluding values of 7 DAT) were <5 % (mean values, if applicable) for surface water and sediment extracts of both concentrations. On 7 DAT, absolute differences for glyphosate determined by both methods were 18.7 and 9.4 % AR for the low and high dose samples, respectively. In the secondary analysis an unidentified peak with a retention time between 3 and 4 minutes was observed in surface water accounting for a maximum of 22.9 and 11.3 % AR at 7 DAT for the low and high dose samples, respectively. The maximum of the unknown peak in the secondary analysis coincides with the drop of the glyphosate associated radioactivity compared to the primary method. At the following sampling days the amount of the unidentified peak was <8 % AR and the amounts of glyphosate determined by both methods differed by maximum 5.5 % AR for individual samples (see Table 7.2.2.2-10 and Table 7.2.2.2-11).

An LC-MS/MS analysis was performed using reference items to confirm the identity of glyphosate and AMPA and to investigate whether the isolated unknown peak could be assigned to the known water/sediment metabolite hydroxymethylphosphonic acid (HMPA). Analyses confirmed the identity of glyphosate and AMPA but did not support confirmation of the unknown peak as HMPA.

Further attempts were made to characterise the unidentified peak using the secondary method after addition of EDTA solution to surface water to test whether the peak was comprised of AMPA and/or glyphosate coordinated to metal ions. Since chromatograms prior to and after complexation of metal with EDTA were comparable these attempts were proven not successful.

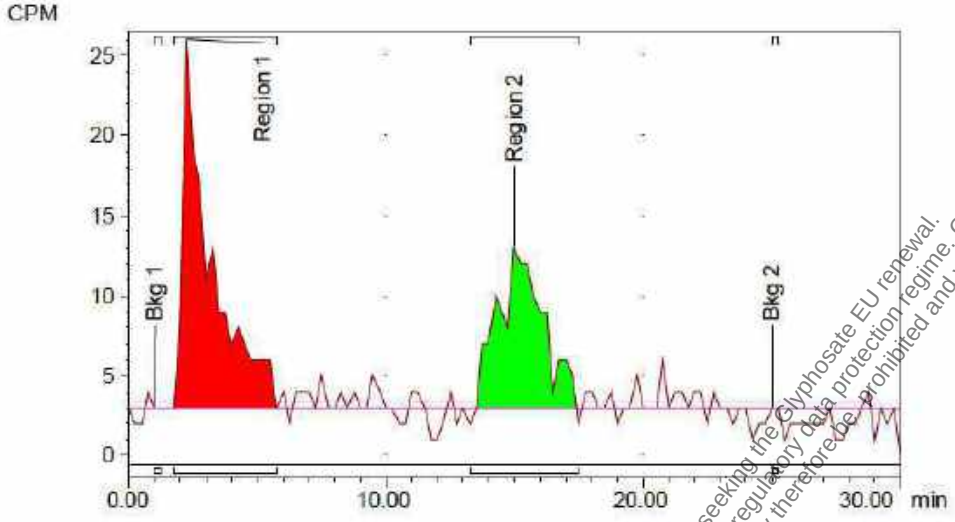
The unidentified peak was isolated by the secondary chromatographic method and the isolated fraction was analysed by the primary method. By the primary method, a peak was present at the correct retention time for AMPA, providing evidence that the unidentified peak co-eluted with AMPA by the primary method. The second region observed in this chromatogram has a retention time that does not relate to anything else seen in the second method analysis and is considered to be contamination rather than a metabolite (see Figures below).

Figure 7.2.2.2-1: Representative Secondary Method HPLC Chromatography of Isolated Sample Treated with [^{14}C]glyphosate at an Application Rate of 95 µg/L at Day 62



Name	Retention (min)	%ROI (%)
Bkg 1	2.25	
Region 1	4.25	94.9
Region 2	14.00	5.1
Bkg 2	36.50	
2 Peaks		100.0

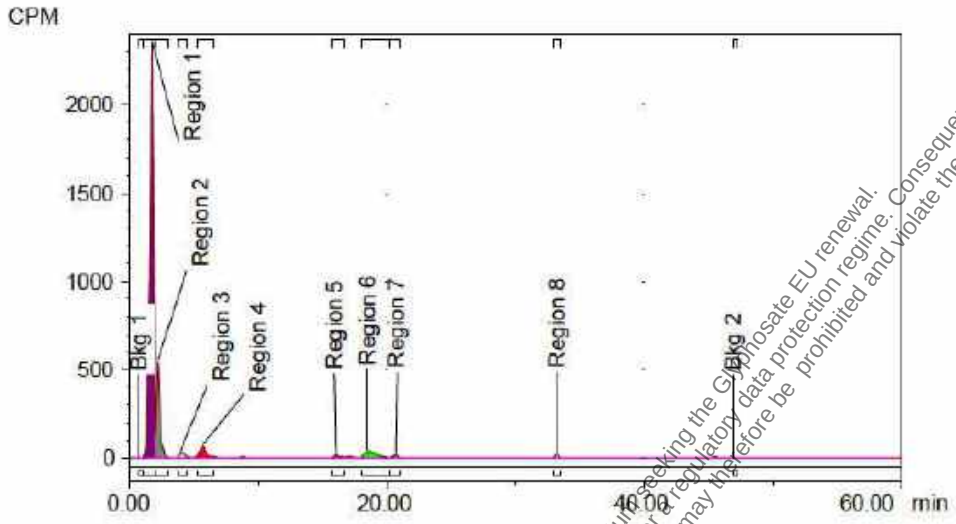
Figure 7.2.2.2-2: Representative Primary Method HPLC Chromatography of Isolated Sample Treated with [^{14}C]glyphosate at an Application Rate of 95 µg/L at Day 62



Name	Retention (min)
Bkg 1	1.00
Region 1	7.25
Region 2	15.00
Bkg 2	25.00
2 Peaks	

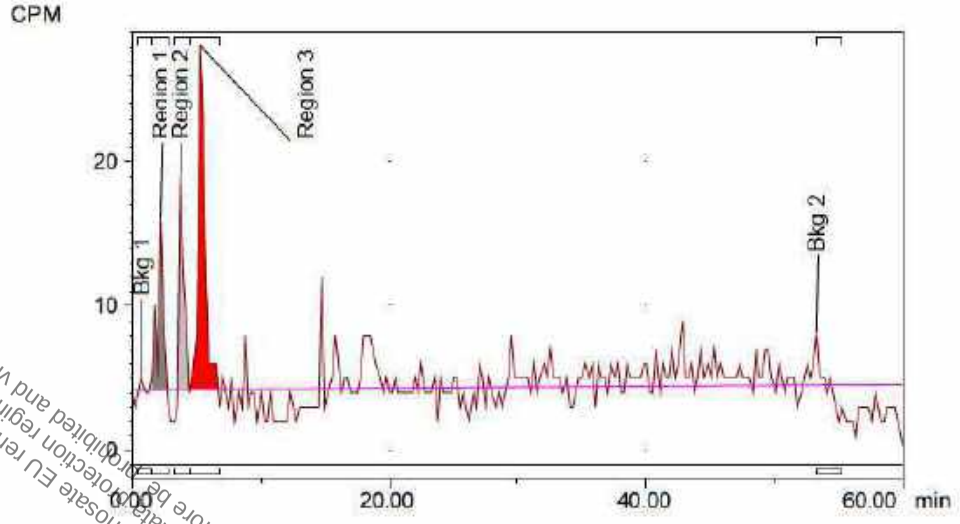
Afterwards, the isolated unidentified peak as well as a surface water samples (high dose, 62 DAT, replicate 1) were analysed by the tertiary method utilising a strong anion exchange (SAX) column. In the chromatograms of the tertiary method no region was observed that corresponds to the unidentified peak. Instead, there were several smaller regions, suggesting that the peak is comprised of multiple components (see Figures below).

Figure 7.2.2.2-3: Representative Tertiary Method HPLC Chromatography of Surface water Treated with [^{14}C]glyphosate at an Application Rate of 95 µg/L at Day 62 (Rep 1)



Name	Retention (min)	%RCF (%)	%TRR (%)
Bkg 1	0.75		
Region 1	1.75	73.8	35.9
Region 2	3.25	16.9	8.2
Region 3	4.00	1.4	0.7
Region 4	5.75	3.1	1.5
Region 5	16.00	0.6	0.3
Region 6	18.50	3.1	1.5
Region 7	20.75	0.6	0.3
Region 8	33.25	0.5	0.2
Bkg 2	47.00		
8 Peaks		100.0	48.6

Figure 7.2.2.2-4: Representative Tertiary Method HPLC Chromatography of Isolated Sample Treated with [^{14}C]glyphosate at an Application Rate of 95 µg/L at Day 62



Name	Retention (min)	%ROI (%)
Bkg 1	0.75	
Region 1	2.25	22.0
Region 2	3.75	21.9
Region 3	5.25	56.1
Bkg 2	53.25	
3 Peaks		100.0

F. KINETIC EVALUATION

SFO, DFOP and FOMC models were applied to calculated degradation rates using CAKE version 3.3. The SFO model was selected as the best fit kinetic in all cases. The samples treated at 10 µg/L yielded a DT_{50} of 12.3 days and a DT_{90} of 41.0 days. The samples treated at 95 µg/L yielded a DT_{50} of 21.8 days and a DT_{90} of 72.4 days. The results of the kinetic evaluation are summarised in the tables below.

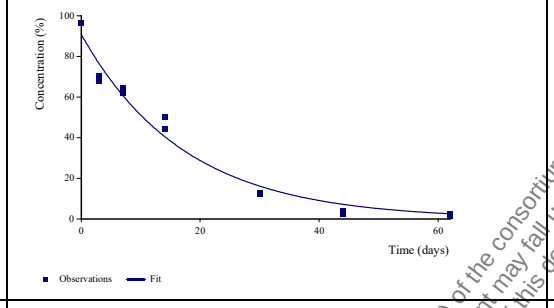
Table 7.2.2.2-12: Kinetic models and goodness-of-fit statistics of parent-only fits in the total system following application of 10 µg glyphosate/L

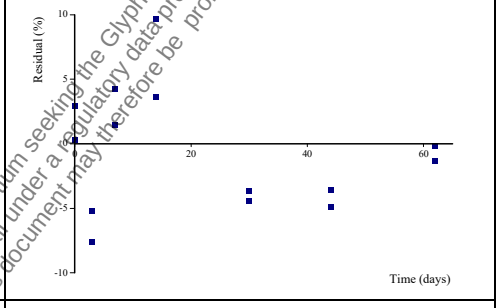
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	r^2	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Good	89.4	k: 0.0562	8.4	0.982	k: <0.001	k: 0.047	k: 0.066	12.3	41.0
FOMC	Good	93.7	α: 5150 β: 73500	9.0	0.982	-[a]	β: nd	β: nd	9.89	32.9
DFOP	Good	89.4	k_1: 0.0562 k_2: 0.0562 g: 0.971	10.0	0.982	k_1: 0.5 k_2: 0.5	k_1: -1631 k_2: -53840	k_1: 1630 k_2: 53800	12.3	41.0

The visual and statistical fits from the SFO model are good and describe the best fit.

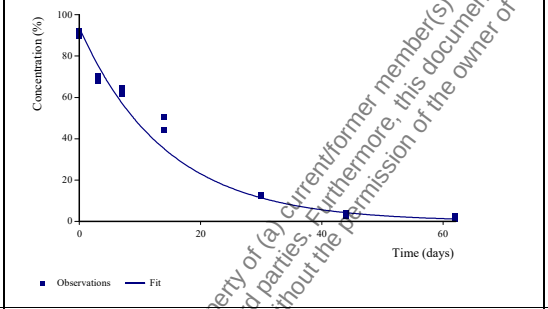
Conclusion: SFO will be used for determination of trigger endpoints.

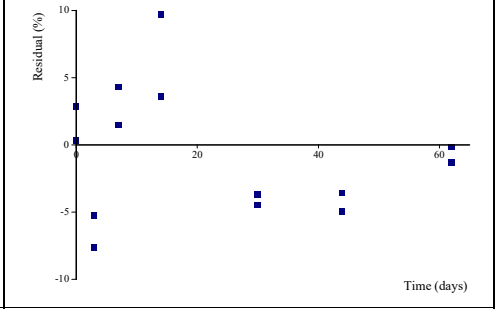
SFO



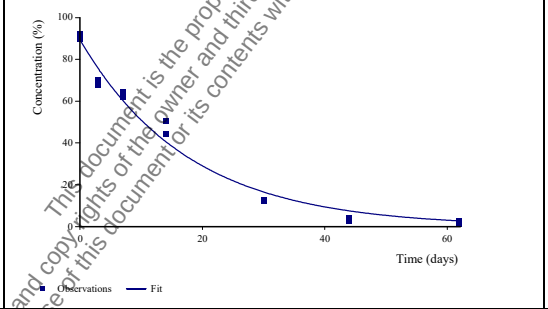


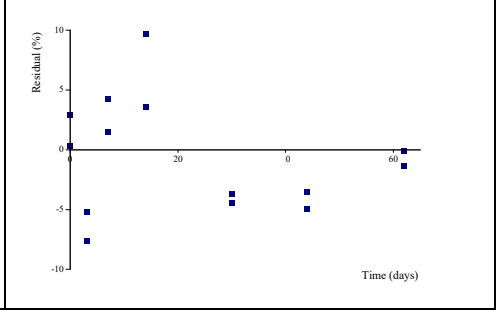
FOMC





DFOP





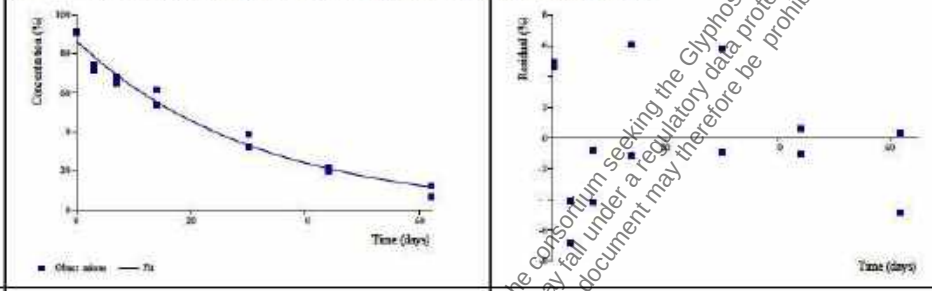
[a] t-test not relevant for kinetic parameter β

Table 7.2.2.2-13: Kinetic models and goodness-of-fit statistics of parent-only fits in the total system following application of 95 µg glyphosate/L

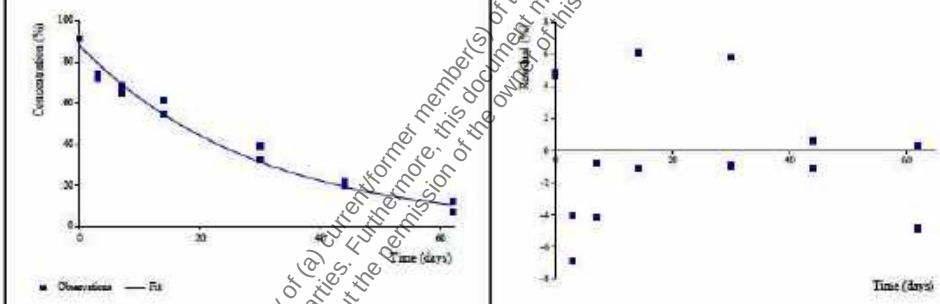
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	r^2	Prob > t (5 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	86.7	k: 0.0318	5.2	0.979	k: <0.001	k: 0.027	k: 0.036	21.8	72.4
FOMC	Acceptable	88.1	α: 587.9 β: 17100	5.6	0.979	-[a]	β: 12700	β: 21500	20.2	67.1
DFOP	Acceptable	86.7	k_1: 0.0382 k_2: 0.0382 g: 0.114	6.1	0.979	k_1: 0.5 k_2: 0.5	k_1: -448.3 k_2: -57.5	k_1: 448.4 k_2: 57.6	21.8	72.4

The visual and statistical fits from the SFO model are good and describe the best fit.
Conclusion: SFO will be used for determination of trigger endpoints.

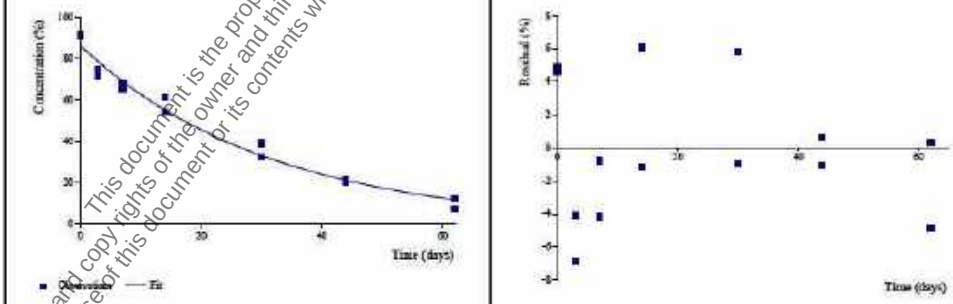
SFO



FOMC



DFOP



[a] t-test not relevant for kinetic parameter β

III. CONCLUSIONS

The aerobic mineralisation of glyphosate in a surface water system containing suspended sediment was studied at two concentrations, 10 µg/L and 95 µg/L. Dissipation of glyphosate in the surface water system occurred through a combination of microbial degradation and formation of non-extractable residues in the suspended sediment.

The major degradation product observed in the water phase was AMPA reaching a maximum mean level of 42.7 % AR. Analysis by a secondary chromatographic method showed the presence of an unidentified peak with >5 % AR. Several attempts to identify this peak were not successful. Analyses by a tertiary chromatographic method finally showed that this peak was comprised of three individual peaks. Mineralisation to $^{14}CO_2$ was significant in both test systems reaching mean values of 26.5 and 23.1 % AR by the end of the study in the 10 µg/L and 95 µg/L systems, respectively. Formation of non-extractable residues also contributed to the dissipation of glyphosate residues reaching a mean maximum level of 14.0 % AR in the 10 µg/L system by the end of the study and a maximum level of 9.1 % AR on Day 44 in the 95 µg/L system before declining slightly to 8.8 % AR at the end of the study.

The dissipation rate of glyphosate in the total system (water + sediment) was evaluated using CAKE v. 3.3 software. The best-fit kinetics were obtained using an SFO kinetic model giving DT_{50} values of 12.3 and 21.8 days and DT_{90} values of 41.0 and 72.4 days for the 10 µg/L and 95 µg/L concentrations, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline, showing only minor deviations. The mass balance shows values below 90 % for several sampling points, this might be caused by the formation and loss of carbon dioxide during processing. In conclusion, the deviations do not influence the overall results and general outcome of the study.

Analysis by a secondary chromatographic method showed the presence of an unidentified peak with >5 % AR. Several attempts to identify this peak were not successful. Analyses by a tertiary chromatographic method showed that this peak was comprised of three individual peaks. Further attempts to characterize these radioactivity will be reported in an amendment to this study report.

The study is considered valid to cover this data point and is in full compliance with the current guidances including the presented kinetic evaluation.

Assessment and conclusion by RMS:

Expert statement (Dougan & Raykova, 2020)

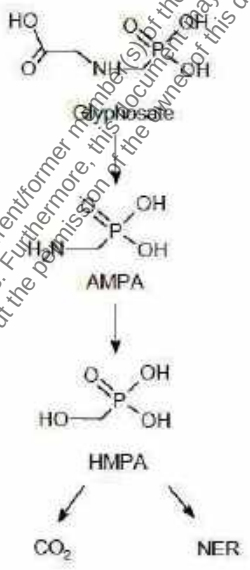
The kinetic evaluation in the report was conducted according to FOCUS (2014). According to FOCUS, material balance values are applied as input data on 0 DAT, however due to the high impurity of the test item, the material balance values at 0 DAT (Table 7.2.2.2-4) were corrected for purity of test item of 96.3 %, resulting in corrected mass balance values of 89.7 %AR and 92.3 %AR at 0 DAT for the 10 µg/L test concentration. Similarly, the material balance values for the 95 µg/L test concentration (Table 7.2.2.2-5) were corrected for the purity of 96.5 %, resulting in input data at 0 DAT.

CA 7.2.2.3 Water/sediment studies

The fate of glyphosate was investigated in four different water/sediment systems in the course of two studies with glyphosate or glyphosate-trimesium which are considered valid to address the data point ([redacted] 1999, CA 7.2.2.3/002 and [redacted] 1993, CA 7.2.2.3/005). For studies performed with glyphosate-trimesium only the results for the glyphosate (PMG) anion are considered for evaluation and further assessment. Furthermore, four acceptable studies are available where the metabolite aminomethylphosphonic acid (AMPA) was applied to eight water/sediment systems ([redacted] 2004, CA 7.2.2.3/018; [redacted] 2003, CA 7.2.2.3/019; [redacted] 2002, CA 7.2.2.3/020 and [redacted] 1999, CA 7.2.2.3/021). Finally, one study is available that proves the storage stability of glyphosate and AMPA in natural water for at least 24 months ([redacted] 1989, CA 7.2.2.3/022).

Glyphosate degraded in the water phase and also partitioned to the sediment where it was further degraded. The extent of mineralisation was high with a maximum amount of 48 % AR after 100 days indicating the potential for complete mineralisation of glyphosate. The formation of non-extractable residues was moderate with a maximum amount of 22.0 % AR after 100 days. The major degradation products observed in water/sediment systems were aminomethylphosphonic acid (AMPA) and hydroxymethylphosphonic acid (HMPA). AMPA was determined in water, sediment and total system with maximum occurrences of 15.7 % AR (14 DAT), 18.7 % AR (58 DAT) and 27.1 % AR (30 DAT), respectively. HMPA was not observed in sediment extracts but in the water phase with a maximum occurrence of 10.0 % AR at 61 DAT. The proposed degradation pathway for glyphosate in water/sediment system is presented below.

Figure 7.2.2.3-1: Proposed degradation pathway of glyphosate in water/sediment systems



The results of the two water/sediment studies with glyphosate were evaluated according to the current FOCUS kinetic guidances ([redacted] 2020, CA 7.2.2.3/001). The persistence DT_{50} and DT_{90} of glyphosate for the total system range from 8.4 to 196 days and from 45.6 to >1000 days, respectively. In addition, the persistence DT_{50} and DT_{90} for the water phase range from 1.1 to 7.9 days and from 22.2 to 78.2 days. For modelling purpose the geometric mean $DegT_{50}$ in the total system is 143 days (n = 4). An evaluation on the Level P-II ($DegT_{50}$ for water and sediment phase) was not possible on the basis on the study results.

In addition to the route and rate of glyphosate in water/sediment systems, the studies with the metabolite AMPA applied provide further information on the behaviour of AMPA in aquatic systems. The results of these studies showed rapid dissipation of AMPA from the water phase by adsorption to the sediment followed by microbial degradation to CO_2. The results demonstrated the degradation of AMPA to carbon dioxide and non-extractable residues. The extent of mineralisation was moderate to high with a maximum

amount of 40.1 % AR after 104 days. The formation of non-extractable residues was moderate with a maximum amount of 40.7 % AR after 29 days.

The degradation of AMPA was evaluated according to the current FOCUS kinetic guidances based on the data of the four water/sediment studies with AMPA and the two water/sediment studies with glyphosate ([redacted] 2020, CA 7.2.2.3/001). The persistence DT_{50} and DT_{90} of AMPA for the total system ranged from 2.4 to 43.5 days and from 197 to >1000 days, respectively. In addition, the persistence DT_{50} and DT_{90} for the water phase range from 0.6 to 6.6 days and from 5.5 to 50.7 days. For modelling purpose the geometric mean $DegT_{50}$ in the total system, derived from evaluation at Level P-I and Level M-I dissipation is 102.5 days (n = 7).

In the studies where AMPA was applied unidentified radioactivity was reported in water phase and/or sediment extracts (P1a, P3, unknown 1 and unknown 2, M3.3 and M7, peak at Rf-value zero and peak at Rf-value 0.9**)**. As these potential minor transient degradation products they were only observed in AMPA water/sediment studies but were never detected in any of the available studies where glyphosate was applied, they are not considered relevant for further evaluation or risk assessment.

In the scientific literature review for glyphosate (2010-2019), one article was identified to provide further information relevant to the data point. In this article, degradation of stable isotope co-labeled [$^{13}C_3{}^{15}N$]glyphosate was investigated in one water/sediment system over a period of 80 days. During the experiment, glyphosate dissipated rapidly from the water and was mineralised to a high extend. It was shown that sediment plays a key role in glyphosate degradation since it was incorporated into amino acids, indicating no risk bearing biogenic residue formation. The reliability of the article was assessed as "reliable with restrictions". Thus, no new endpoints were derived, and the article is considered as supportive information.

Table 7.2.2.3-1: Water/Sediment studies

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.2.2.3/001	[redacted]u, I., 2020	Kinetic evaluation	Glyphosate, AMPA	Valid	
CA 7.2.2.3/002	[redacted] 1999	Water/sediment	Glyphosate-trimesium	Valid	Updated kinetic evaluation in [redacted] 2020
CA 7.2.2.3/003	[redacted] [redacted] 1997	Water/sediment	Glyphosate-trimesium	Invalid	
CA 7.2.2.3/004	[redacted], 1996	Water/sediment	Glyphosate	Invalid	
CA 7.2.2.3/005	[redacted] [redacted] 1993	Water/sediment	Glyphosate	Valid	Addendum: [redacted] [redacted] 1995; Updated kinetic evaluation in [redacted] 2020
CA 7.2.2.3/006	[redacted] [redacted] 1995	Water/sediment	Glyphosate	Valid	Addendum to [redacted] [redacted] 1993
CA 7.2.2.3/007	[redacted] 1993	Water/sediment	Glyphosate	Invalid	
CA 7.2.2.3/008	[redacted], 1991	Water/sediment	Glyphosate-trimesium	Invalid	
CA 7.2.2.3/009	[redacted] [redacted] 1990	Water/sediment	Glyphosate	Invalid	Addendum: [redacted] 1992, CA 7.2.2.3/010 & CA 7.2.2.3/013
CA 7.2.2.3/010	[redacted] 1992	Water/sediment	Glyphosate	Invalid	Addendum to [redacted] [redacted] 1990, CA 7.2.2.3/009

Table 7.2.2.3-1: Water/Sediment studies

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.2.2.3/011	[redacted] 1990	Water/sediment	Glyphosate	Invalid	Addendum: [redacted] 1992, CA 7.2.2.3/012 & CA 7.2.2.3/013
CA 7.2.2.3/012	[redacted] 1992	Water/sediment	Glyphosate	Invalid	Addendum to [redacted] 1990, CA 7.2.2.3/011
CA 7.2.2.3/013	[redacted] 1992	Water/sediment	Glyphosate	Invalid	Addendum to [redacted] 1990, CA 7.2.2.3/009 and [redacted] 1990, CA 7.2.2.3/011
CA 7.2.2.3/014	[redacted] 1988	Water/sediment	Glyphosate	Invalid	
CA 7.2.2.3/015	[redacted] 1979	Water/sediment	Glyphosate	Invalid	
CA 7.2.2.3/016	[redacted] 1978	Water/sediment	Glyphosate	Invalid	
CA 7.2.2.3/017	[redacted] 1972	Water/sediment	Glyphosate	Invalid	
CA 7.2.2.3/018	[redacted] 2004	Water/sediment	AMPA	Valid	Updated kinetic evaluation in [redacted] 2020
CA 7.2.2.3/019	[redacted] 2003	Water/sediment	AMPA	Valid	Updated kinetic evaluation in [redacted] 2020
CA 7.2.2.3/020	[redacted] 2002	Water/sediment	AMPA	Valid	Updated kinetic evaluation in [redacted] 2020
CA 7.2.2.3/021	[redacted] 1991	Water/sediment	AMPA	Valid	Updated kinetic evaluation in [redacted] 2020
CA 7.2.2.3/022	[redacted] 1989	Storage stability	Glyphosate, AMPA	Supportive	

Table 7.2.2.3-2: Water/Sediment – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.2.2.3/023	Wang *et al.*, 2016	Water/sediment	Glyphosate	Reliable with restrictions	

Table 7.2.2.3-3: Summary of degradation endpoints in water/sediment for glyphosate: trigger endpoints Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	DT_{50} (d)[1]	DT_{90} (d)[1]	St. (χ^2err) (%)	Kinetic model
Total system								
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	8.4	45.6	2.7	FOMC
	Putah	8.4	7.5	20	195.8	902.3	4.4	DFOP
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	15.8	329.4	2.2	HS
	Unter Widdersheim	8.6	7.68	20	121.6	>1000	4.8	DFOP
Water phase								
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	5.0	22.7	2.3	DFOP
	Putah	8.4	7.5	20	7.9	78.2	10.0	FOMC
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	2.0	22.2	5.2	DFOP
	Unter Widdersheim	8.6	7.68	20	1.1	28.7	2.6	DFOP
Sediment phase								
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	33.9	112.6	8.4	SFO
	Putah	8.4	7.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	158.7	965.3	3.6	DFOP
	Unter Widdersheim	8.6	7.68	20	-[3]	-[3]	-[3]	-[3]

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[3] No acceptable fit obtained and no endpoints could be derived

Table 7.2.2.3-4: Summary of degradation endpoints in water/sediment for glyphosate: modelling endpoints Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	Model	SFO DT_{50} (d)[1]	St. (χ^2err) (%)
Total system							
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	SFO	9.7	5.3
	Putah	8.4	7.5	20	DFOP	301.4[2]	4.4
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	HS	144.4[2]	2.2
	Unter Widdersheim	8.6	7.68	20	DFOP	1000[3]	4.8
Water phase							
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	SFO	5.9	8.5
	Putah	8.4	7.5	20	FOMC	23.6[4]	10.0
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	DFOP	6.7[4]	5.2
	Unter Widdersheim	8.6	7.68	20	DFOP	8.6[4]	2.6
Sediment phase							
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	SFO	33.9	8.4
	Putah	8.4	7.5	20	-[5]	-[5]	-[5]

Table 7.2.2.3-4: Summary of degradation endpoints in water/sediment for glyphosate: modelling endpoints Level P-I

[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	DFOP	346.6[2]	3.6
	Unter Widdersheim	8.6	7.68	20	-[6]	-[6]	-[6]
Geometric mean (total system) (n = 4)						143.3	
Geometric mean (water phase) (n = 4)						9.5	
Geometric mean (sediment phase) (n = 2)						108.4	

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] Calculated from slow phase degradation rate (k_2) as 10 % of the initial amount was not reached within experimental period
[3] The estimated degradation rate is not significantly different from zero, default $DegT_{50}$ of 1000 d to be used
[4] Back-calculated from DT_{90} of bi-phasic model (DT_{90}/3.32) as 10 % of the initial amount was reached within experimental period
[5] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[6] No acceptable fits obtained and no endpoints could be derived

Table 7.2.2.3-5: Summary of degradation endpoints in water/sediment for AMPA: trigger endpoints from evaluation at Level P-I (AMPA applied) and Level M-I dissipation (glyphosate applied)

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	DT_{50} (d)[1]	DT_{90} (d)[1]	St. (χ^2err) (%)	Kinetic model
Total system, Level P-I								
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	12.6	>1000	1.6	FOMC
	Schäphysen	8.0	7.34	20	2.4	>1000	6.2	DFOP
[redacted] (2003) CA 7.2.2.3/019	Bickenbach	8.5	8.5	20	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.5	8.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	43.5	196.8	3.5	DFOP
	Unter Widdersheim	8.2	7.5	20	17.7	579.8	3.4	HS
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	-[3]	-[3]	-[3]	-[3]
	Manningtree B	7.1	6.3	20	-[4]	-[4]	-[4]	-[4]
Water phase, Level P-I								
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	2.2	22.1	2.1	FOMC
	Schäphysen	8.0	7.34	20	1.1	6.6	3.2	FOMC
[redacted] (2003) CA 7.2.2.3/019	Bickenbach	8.5	8.5	20	2.4	37.1	5.3	FOMC
	Unter Widdersheim	8.5	8.5	20	2.1	25.9	8.0	FOMC
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	6.6	50.7	4.5	DFOP
	Unter Widdersheim	8.2	7.5	20	2	17.3	8.2	DFOP
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	0.6	8.1	1.8	FOMC
	Manningtree B	7.1	6.3	20	1.1	5.5	1.0	HS
Sediment phase, Level P-I								
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	168.1	558.3	1.9	SFO
	Schäphysen	8.0	7.34	20	-[3]	-[3]	-[3]	-[3]
[redacted] (2003)	Bickenbach	8.5	8.5	20	-[2]	-[2]	-[2]	-[2]

Table 7.2.2.3-5: Summary of degradation endpoints in water/sediment for AMPA: trigger endpoints from evaluation at Level P-I (AMPA applied) and Level M-I dissipation (glyphosate applied)

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	DT$_{50}$ (d)[1]	DT$_{90}$ (d)[1]	St. (χ^2err) (%)	Kinetic model
CA 7.2.2.3/019	Unter Widdersheim	8.5	8.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	-[5]	-[5]	-[5]	-[5]
	Unter Widdersheim	8.2	7.5	20	-[3]	-[3]	-[3]	-[3]
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	-[5]	-[5]	-[5]	-[5]
	Manningtree B	7.1	6.3	20	-[4]	-[4]	-[4]	-[4]
Total system, Level M-I dissipation								
[redacted] (1999) CA 7.2.2.3/002	Cache[5]	8.2	8.1	20	224.6	746.2	3.2	SFO
	Putah	8.4	7.5	20	-[6]	-[6]	-[6]	-[6]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	26.8[7]	88.9[7]	7.9	SFO
	Unter Widdersheim	8.6	7.68	20	15.1[7]	50.0[7]	5.8	SFO
Water phase, Level M-I dissipation								
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	53.8	178.8	6.1	SFO
	Putah	8.4	7.5	20	-[6]	-[6]	-[6]	-[6]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	26.8	88.9	7.9	SFO
	Unter Widdersheim	8.6	7.68	20	15.1	50.0	5.8	SFO

[1] DT$_{50}$ = DegT$_{50}$ for total system but DisT$_{50}$ for water and sediment phase

[2] The data of the sediment phase and the total system were not considered in the kinetic evaluation

[3] No acceptable fits obtained and no endpoints could be derived

[4] Due to experimental problems, the total system and the sediment phase were not considered in the kinetic evaluation

[5] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration

[6] No evaluations could be conducted for any compartment at Level M-I dissipation due to the limited number of data points available after the peak concentration

[7] Since AMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for total system

Table 7.2.2.3-6: Summary of degradation endpoints in water/sediment for AMPA: modelling endpoints from evaluation at Level P-I (AMPA applied) and Level M-I dissipation (glyphosate applied)

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	Model	SFO DT_{50} (d)[1]	χ^2 err (%)
Total system, Level P-I							
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	DFOP	95.0[2]	3.8
	Schäphysen	8.0	7.34	20	DFOP	1000[3]	6.2
[redacted] (2003) CA 7.2.2.3/019	Bickenbach	8.5	8.5	20	-[4]	-[4]	-[4]
	Unter Widdersheim	8.5	8.5	20	-[4]	-[4]	-[4]
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	SFO	47.7	5.9
	Unter Widdersheim	8.2	7.5	20	HS	288.8[2]	3.4
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	-[5]	-[5]	-[5]
	Manningtree B	7.1	6.3	20	-[6]	-[6]	-[6]
Water phase, Level P-I							
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	FOMC	6.7[7]	2.1
	Schäphysen	8.0	7.34	20	SFO	1.5	10.7
[redacted] (2003) CA 7.2.2.3/019	Bickenbach	8.5	8.5	20	FOMC	11.2[7]	5.3
	Unter Widdersheim	8.5	8.5	20	FOMC	7.8[7]	8
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	DFOP	15.3[7]	4.5
	Unter Widdersheim	8.2	7.5	20	DFOP	5.2[7]	8.2
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	FOMC	2.4[7]	1.8
	Manningtree B	7.1	6.3	20	HS	1.7[7]	1
Sediment phase, Level P-I							
[redacted] (2002) CA 7.2.2.3/020	Rückhaltebecken	8.7	7.64	20	SFO	168.1	1.9
	Schäphysen	8.0	7.34	20	-[5]	-[5]	-[5]
[redacted] (2003) CA 7.2.2.3/019	Bickenbach	8.5	8.5	20	-[4]	-[4]	-[4]
	Unter Widdersheim	8.5	8.5	20	-[4]	-[4]	-[4]
[redacted] (1999) CA 7.2.2.3/021	Bickenbach	8.3	7.4	20	-[8]	-[8]	-[8]
	Unter Widdersheim	8.2	7.5	20	-[5]	-[5]	-[5]
[redacted] (2004) CA 7.2.2.3/018	Manningtree A	7.2	7.6	20	-[8]	-[8]	-[8]
	Manningtree B	7.1	6.3	20	-[6]	-[6]	-[6]
Total system, Level M-I dissipation							
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	SFO	224.6	3.2
	Putah	8.4	7.5	20	-[9]	-[9]	-[9]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	SFO	26.8[10]	7.9
	Unter Widdersheim	8.6	7.68	20	SFO	15.1[10]	5.8

Table 7.2.2.3-6: Summary of degradation endpoints in water/sediment for AMPA: modelling endpoints from evaluation at Level P-I (AMPA applied) and Level M-I dissipation (glyphosate applied)

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	Model	SFO DT_{50} (d)[1]	St. (χ^2err) (%)
Water phase, Level M-I dissipation							
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	SFO	53.8	6.1
	Putah	8.4	7.5	20	-[9]	-[9]	-[9]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	SFO	26.8	7.9
	Unter Widdersheim	8.6	7.68	20	SFO	15.1	5.8
Geometric mean (total system) (n = 7, derived from Level P-I and M-I dissipation)						**102.5**	
Geometric mean (water phase) (n = 11, derived from Level P1 and M-I dissipation)						**7.8**	
Geometric mean (sediment phase) (n = 1, derived from Level M-I dissipation)						**168.1**	

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] Calculated from slow phase degradation rate (k_2) as 10 % of the initial amount was not reached within experimental period
[3] The estimated degradation rate is not significantly different from zero, default $DegT_{50}$ of 1000 d to be used
[4] The data of the sediment phase and the total system were not considered in the kinetic evaluation
[5] No acceptable fits obtained and no endpoints could be derived
[6] Due to experimental problems, the total system and the sediment phase were not considered in the kinetic evaluation
[7] Back-calculated from DT_{90} of bi-phasic model (DT_{90}/3.32) as 10 % of the initial amount was reached within experimental period
[8] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[9] No evaluations could be conducted for any compartment at Level M-I dissipation due to the limited number of data points available after the peak concentration
[10] Since AMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for total system

Table 7.2.2.3-7: Summary of degradation endpoints in total system for AMPA: modelling and trigger endpoints Level M-I degradation (pathway fit)

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Formation fraction (-)	St. (χ^2err) (%)	Model
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	20	172.8	573.9	0.339 (from parent)	7.0	FOMC-SFO
	Putah	8.4	7.5	20	-[1]	-[1]	-[1]	-[1]	-[1]
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	15.7	52.3	0.488 (from parent)	9.4	HS-SFO
	Unter Widdersheim	8.6	7.68	20	8.8	29.2	0.321 (from parent)	22.4	DFOP-SFO

[1] No acceptable fits obtained and no endpoints could be derived

Table 7.2.2.3-8: Summary of degradation endpoints in total system for HMPA: modelling and trigger endpoints Level M-I degradation (pathway fit)

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Formation fraction (-)	St. (χ^2err) (%)	Model
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	128.8	427.8	0.366 (from AMPA)	20.5	HS-SFO
	Unter Widdersheim	8.6	7.68	20	10	33.4	0.359 (from AMPA)	39.3	DFOP-SFO

Table 7.2.2.3-9: Summary of dissipation endpoints in water/sediment for HMPA: modelling and trigger endpoints Level M-I dissipation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DisT_{50}$ (d)	$DisT_{90}$ (d)	St. (χ^2err) (%)	Model
Total system and water phase[1]								
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	20	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.6	7.68	20	8.9	29.5	7.1	SFO

[1] Since HMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system.
[2] No evaluations could be conducted at Level M-I dissipation due to the limited number of data points available after the peak concentration

Table 7.2.2.3-10: Summary of maximum occurrence of parent in sediment, mineralisation and non-extractable residues (from glyphosate dosed experiments)

Study	Water / sediment system	pH water phase	pH sed	Parent max x % in sediment after n d.	Mineralisation x % after n d. (end of the study).	Non-extractable residues in sed. max x % after n d
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	15.9 (3 d)	**48.0** (100 d)	13.5 (58 d)
	Putah	8.4	7.5	58.2 (100 d)	5.9 (100 d)	20.3 (58 d)
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	53.1 (7 d)	20.2 (61 d)	**22.0** (100 d)
	Unter Widdersheim	8.6	7.68	**61.4** (7 d)	19.4 (61 d)	13.6 (100 d)

Table 7.2.2.3-11: Summary of maximum occurrence of major metabolites of glyphosate in different comparments of water/sediment systems (from glyphosate dosed experiments)

Study	Water / sediment system	pH water phase	pH sed	AMPA			HMPA		
				Water	Sediment	Total system	Water	Sediment	Total system
[redacted] (1999) CA 7.2.2.3/002	Cache	8.2	8.1	10.3 (30 d)	**18.7** (58 d)	**27.1** (30 d)	-	-	-
	Putah	8.4	7.5	1.5 (58 d)	3.8 (58 d)	5.3 (58 d)	-	-	-
[redacted] (1993) CA 7.2.2.3/005	Bickenbach	8.6	7.8	**15.7** (14 d)	-	15.7 (14 d)	**10.0** (61 d)	-	**10.0** (61 d)
	Unter Widdersheim	8.6	7.68	5.8 (14 d)	-	5.8 (14 d)	1.9 (30 d)	-	1.9 (30 d)

AMPA: Aminomethylphosphonic acid; HMPA: Hydroxymethylphosphonic acid; -: Not detected

Updated kinetic evaluation of water/sediment studies with glyphosate and AMPA as test item

1. Information on the study

Data point:	CA 7.2.2.3/001
Report author	[redacted]
Report year	2020
Report title	Estimation of kinetic endpoints for glyphosate and its metabolites AMPA and HMPA from laboratory water-sediment studies
Report No	112148-002
Document No	
Guidelines followed in study	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS, EC Document Reference SANCO/10058/2005 version 2.0, June 2006. FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.
Deviations from current test guideline	From FOCUE kinetics guidance: none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A kinetic re-evaluation of six aquatic water-sediment studies was performed in order to derive trigger (persistence) and modelling endpoints for glyphosate and its aquatic metabolites AMPA and HMPA. The kinetic endpoints may be used in calculating predicted environmental concentrations of glyphosate, AMPA

and HMPA in surface water and sediment (modelling endpoints) or as trigger values for aquatic ecotoxicology studies (trigger endpoints).

Kinetic analyses at different evaluation levels were conducted for the water-sediment systems according to FOCUS kinetics guidance (2006, 2014) using the fitting software KinGUI v2.1.

I. MATERIALS AND METHODS

The FOrum for the Coordination of Pesticide Fate-Models and their USe (FOCUS) developed recommendations on the kinetic evaluation of water-sediment degradation studies conducted in the laboratory (FOCUS; 2006, 2014). These recommendations intend to harmonise the derivation of degradation parameters and to provide more certainty for these important parameters in increasingly complex risk assessments.

The purpose of this assessment was to conduct a kinetic modelling re-evaluation for glyphosate and its aquatic metabolites AMPA and HMPA using results from six laboratory water-sediment degradation studies ([redacted] 1999, CA 7.2.2.3/002; [redacted], 1993, CA 7.2.2.3/005; [redacted] 2002, CA 7.2.2.3/020; [redacted] 2003, CA 7.2.2.3/019; [redacted], 1999, CA 7.2.2.3/021 and [redacted], 2004, CA 7.2.2.3/018). The aim of the evaluation was to derive the following endpoints:

- Trigger endpoints for glyphosate and its aquatic metabolites AMPA and HMPA to be used as triggers for aquatic ecotoxicology studies.
- Modelling endpoints for use in calculating predicted environmental concentrations of glyphosate and its aquatic metabolites AMPA and HMPA in surface water and sediment.

The parent compound glyphosate was applied as test substance in two of the studies ([redacted] 1999, CA 7.2.2.3/002 and [redacted] 1993, CA 7.2.2.3/005, amended by [redacted] 1995, CA 7.2.2.3/006) while AMPA was applied as test substance in the remaining studies ([redacted] 2002, CA 7.2.2.3/020; [redacted] 2003, CA 7.2.2.3/019; [redacted] 1999, CA 7.2.2.3/021 and [redacted] 2004, CA 7.2.2.3/018).

Besides the major metabolite AMPA, the metabolite HMPA was detected with up to 10 % of applied radioactivity (AR) in the water phase of both test systems in the study of [redacted] (1993, , CA 7.2.2.3/005, amended by [redacted] 1995, CA 7.2.2.3/006). Both metabolites were considered for kinetic evaluation assuming degradation of glyphosate → AMPA → HMPA.

1. Data pre-processing

Datasets were prepared for the kinetic analysis at different evaluation levels. According to the FOCUS kinetic guidance (2006, 2014), the kinetic analyses for water-sediment study were conducted at Level P-I and Level P-II for the parent and Level M-I for the metabolites. At Level P-I, the kinetic analyses were conducted using the dataset in a single compartment to determine the degradation $DegT_{50}$ in the total system and dissipation DT_{50} in water and sediment. At Level P-II, kinetic analyses were conducted as a two-compartmental approach to estimate degradation in the water column and sediment compartments. At Level M-I, pathway and decline fits were conducted to determine the degradation $DegT_{50}$ in total system and dissipation DT_{50} in total system, water and sediment, where applicable.

The standard procedures recommended by FOCUS (2006, 2014) were followed for all residues to adjust the experimental data for the kinetic modelling. Replicate samples were available for all studies except [redacted] (2004, CA 7.2.2.3/018).

The initial amount of the test substance in total system and water was set to the value of the material balance at day 0. Accordingly, the initial amount was set to zero for the test substance in sediment for evaluation at Level P-II and for the metabolites AMPA and HMPA for evaluation at Level M-I degradation. The assessment of dissipation in sediment at Level P-I and Level M-I dissipation (total system, water and

sediment) only requires kinetics to be fitted to the corresponding decline data, starting from the maximum observed concentration in the compartment. The dissipation of the respective compound was thus evaluated starting at the day of maximum occurrence that was defined as 0 days after maximum concentration. All later time points were adjusted accordingly as days after maximum concentrations.

It is recommended that values below the LOD should be replaced by half the LOD (FOCUS; 2006, 2014). Where LOD values were not available, the values were set to half the lowest measured value.

Further details of the data pre-processing are given further below. Processed residue data are presented in the following.

[redacted] *(1999, CA 7.2.2.3/002)*

The sediment residue values were not reported in the study report. Therefore, the sediment values were obtained by subtracting the water phase residues values from the total system residues values.

At Level P-I for glyphosate, no evaluation could be conducted for the sediment phase for system Putah due to the limited number of data points available after the peak concentration. For the same reason, no evaluation could be conducted at Level M-I dissipation for AMPA in sediment in system Cache as well as in water and sediment in system Putah.

Table 7.2.2.3-12: Experimental data for system Cache of study [redacted] (1999, CA 7.2.2.3/002) used for kinetic evaluation

Sampling day (d)	Glyphosate residues (% AR)			AMPA residues (% AR)		
	Total system	Water	Sediment[1]	Total system	Water	Sediment[2]
0	99.0[3]	99.0[3]	0.00[4]	0.00[5]	0.24	0.00
0	101.7[3]	101.7[3]	0.00[4]	0.00[5]	0.66	0.00
0.25	94.09	87.38	6.71	0.91	0.33	0.58
0.25	94.65	87.17	7.48	1.08	0.46	0.62
1	84.94	74.26	10.68	2.02	1.30	0.72
1	85.92	71.54	14.38	2.28	1.30	0.98
2	79.04	66.24	12.80	3.47	1.62	1.85
2	82.44	67.13	15.31	4.03	2.17	1.86
3	76.88	59.90	**16.98**	5.18	2.19	2.99
3	76.04	61.27	**14.77**	5.12	2.45	2.67
7	53.61	39.51	14.10	12.32	5.24	7.08
7	54.18	39.82	14.36	11.56	5.30	6.26
14	33.25	21.98	11.27	17.93	8.07	9.86
14	34.57	22.22	12.35	18.92	8.93	9.99
30	16.79	7.34	9.45	**26.97**	**10.52**	16.45
30	18.60	8.30	10.30	**27.18**	**10.10**	17.08
58	4.56	1.53	3.03	27.26	8.07	**19.19**
58	5.37	1.61	3.76	26.28	8.08	**18.20**
100	4.85	0.79	4.06	20.71	3.69	17.02
100	4.17	0.87	3.30	22.89	3.97	18.92

Numbers in **bold** represent peak concentrations considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly

[1] Since the sediment phase residues were not reported in the study report, they were obtained by subtracting the water phase residues values from the total system residues values

[2] No evaluation was conducted at Level M-I dissipation since no decline was observed in the sediment phase

[3] Values at day 0 were set to material balance according to FOCUS (2014)

[4] Set to zero for evaluation at Level P-II

[5] Set to zero for evaluation at Level M-I degradation

Table 7.2.2.3-13: Experimental data for system Putah of study [redacted] (1999) used for kinetic evaluation

Sampling day (d)	Glyphosate residues (% AR)			AMPA residues (% AR)		
	Total system	Water	Sediment[1]	Total system[2]	Water[2]	Sediment[2]
0	103.9[3]	103.9[3]	0.0[4]	0.0[5]	0.41	0.00
0	101.6[3]	101.6[3]	0.0[4]	0.0[5]	0.37	0.00
0.25	96.11	90.68	5.43	0.80	0.80	0.00
0.25	97.67	91.77	5.90	0.69	0.69	0.00
1	86.13	74.05	12.08	1.71	0.96	0.75
1	79.04	65.68	13.36	1.79	0.89	0.90
2	89.22	76.63	12.59	0.81	0.81	0.00
2	89.17	75.39	13.78	1.39	0.90	0.49
3	82.40	63.52	18.88	1.53	0.64	0.89
3	83.45	63.28	20.17	1.84	0.86	0.98
7	81.42	60.24	21.18	2.01	1.10	0.91
7	81.43	60.74	20.69	1.43	0.82	0.61
14	70.31	34.02	36.29	2.68	1.08	1.60
14	67.03	32.47	34.56	2.15	1.11	1.04
30	70.82	18.64	52.18	5.33	1.32	4.01
30	79.88	22.11	57.77	3.02	0.58	2.44
58	65.73	11.45	54.28	**6.15**	**1.78**	**4.37**
58	69.19	9.04	60.15	**4.37**	**1.12**	**3.25**
100	61.77	5.26	**56.51**	3.67	0.54	3.13
100	64.90	4.97	**59.93**	3.46	0.50	2.96

Numbers in **bold** represent peak concentrations considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly

[1] Since no decline was observed in the sediment phase, no evaluation could be conducted at Level P-I for the sediment phase

[2] No evaluation was conducted at Level M-I dissipation due to the limited number of data points available after the peak concentration

[3] Values at day 0 were set to material balance according to FOCUS (2014)

[4] Set to zero for evaluation at Level P-II

[5] Set to zero for evaluation at Level M-I degradation

[redacted] (1993, CA 7.2.2.3/005)

Since the metabolites AMPA and HMPA were not detected in the sediment phase, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system. However, no evaluation could be conducted at Level M-I dissipation for HMPA for system Bickenbach due to the limited number of data points available after the peak concentration.

The metabolite AMPA was not detected in the system Bickenbach on days 0 and 0.25. Therefore, the residue values on day 0.25 were set to half of the lowest reported value across the experimental study (0.2 % AR for glyphosate on day 100) as no LOD/LOQ values were available.

The same was done for the metabolite HMPA in the system Unter Widdersheim where HMPA was first detected on day 14 of the study period. The residual values on day 7 were set to half of the lowest measured value as described above.

Table 7.2.2.3-14: Experimental data for system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005) used for kinetic evaluation

Sampling day (d)	Glyphosate residues (% AR)			AMPA residues (% TAR)[1]		HMPA residues (% AR)[1]	
	Total system	Water	Sediment	Total system	Water	Total system	Water[2]
0	96.93[3]	96.93[3]	0.0[4]	0.0[5]	-[6]	0.00[5]	-[6]
0	98.76[3]	98.76[3]	0.0[4]	0.0[5]	-[6]	0.00[5]	-[6]
0.25	95.79	81.04	14.75	0.10[g]	-[6]	NaN[8]	-[6]
0.25	96.33	80.29	16.04	0.10[g]	-[6]	NaN[8]	-[6]
1	96.41	63.18	33.23	2.86	2.86	NaN[8]	-[6]
1	94.78	64.22	30.56	2.08	2.08	NaN[8]	-[6]
2	86.84	47.68	39.16	4.21	4.21	NaN[8]	-[6]
2	91.30	51.68	39.62	5.65	5.65	NaN[8]	-[6]
7	74.35	21.52	**52.83**	12.45	12.45	0.10[7]	0.10[7]
7	77.69	24.37	**53.32**	8.98	8.98	0.10[7]	0.10[7]
14	53.50	14.92	38.60	**15.39**	**15.39**	3.75	3.75
14	48.24	12.10	36.14	**16.10**	**16.10**	3.01	3.01
30	40.32	5.86	34.46	11.41	11.41	2.67	2.67
30	42.25	9.39	32.86	11.61	11.61	4.78	4.78
61	36.75	1.10	35.65	4.83	4.83	11.37	**11.37**
61	34.67	0.62	34.05	5.23	5.23	8.58	**8.58**
100	29.93	0.20	29.73	0.39	0.39	7.63	7.63
100	29.07	0.33	28.74	0.56	0.56	7.41	7.41

Numbers in **bold** represent peak concentrations considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly

[1] Since the metabolites were not detected in sediment, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system

[2] No evaluation was conducted at Level M-I dissipation due to the limited number of data points available after the peak concentration

[3] Values at day 0 were set to material balance according to FOCUS (2014)

[4] Set to zero for evaluation at Level P-II

[5] Set to zero for evaluation at Level M-I degradation

[6] The metabolites were not detected at the beginning of the experiment

[7] Since no LOD/LOQ values are available in the study report, the value was set to half of the lowest measured value in the study (lowest measured value: 0.2 % AR, system Bickenbach, glyphosate on day 100, water phase)

[8] HMPA not detected; values omitted according to FOCUS (2014), NaN (= not a number) was used as input for KinGUI

Table 7.2.2.3-15: Experimental data for system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005) used for kinetic evaluation

Sampling day (d)	Glyphosate residues (% AR)			AMPA residues (% AR)[1]		HMPA residues (% AR)[1]	
	Total system	Water	Sediment	Total system	Water	Total system	Water
0	93.48[2]	93.48[2]	0.0[3]	0.0[4]	4.37	0.0[4]	-[5]
0	95.92[2]	95.92[2]	0.0[3]	0.0[4]	3.65	0.0[4]	-[5]
0.25	99.32	78.03	21.29	2.35	2.35	NaN[6]	-[5]
0.25	96.32	73.24	23.08	1.64	1.64	NaN[6]	-[5]
1	86.69	47.17	39.52	2.95	2.95	0.10[7]	-[5]
1	95.02	50.74	44.28	1.18	1.18	0.10[7]	-[5]
2	82.86	34.41	52.83	2.77	2.77	0.24	0.24
2	82.05	31.06	57.31	2.11	2.11	0.15	0.15
7	82.86	16.77	**66.09**	3.91	3.91	0.63	0.63
7	76.30	25.43	**56.62**	4.88	4.88	0.51	0.51
14	61.16	14.78	46.38	5.41	**5.41**	0.81	0.81
14	59.91	17.07	42.84	6.14	**6.14**	0.77	0.77
30	51.67	8.30	43.37	3.22	3.22	1.76	**1.76**
30	52.47	8.25	44.22	2.45	2.45	2.09	**2.09**
61	58.07	3.31	54.76	0.47	0.47	0.11	0.11
61	58.68	3.66	55.02	0.51	0.51	0.21	0.21
100	45.97	1.83	44.14	0.39	0.39	0.12	0.12
100	47.17	3.02	44.15	0.39	0.39	0.10	0.10

Numbers in **bold** represent peak concentrations considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly

[1] Since the metabolites were not detected in sediment, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system

[2] Values at day 0 were set to material balance according to FOCUS (2014)

[3] Set to zero for evaluation at Level P-II

[4] Set to zero for evaluation at Level M-I degradation

[5] The metabolite HMPA was not detected at the beginning of the experiment

[6] HMPA not detected; values omitted according to FOCUS (2014), NaN (= not a number) was used as input for KinGUI

[7] Since no LOD/LOQ values are available in the study report, the value was set to half of the lowest measured value in the study (lowest measured value: 0.2 %, system Sandy Sediment, glyphosate on day 100, water phase)

[redacted] (2002, CA 7.2.2.3/020)

In the water phase of the system Schäphysen, AMPA was not detected in one of the replicates on day 60 while in one replicate each on days 90 and 119 amounts of AMPA were below the LOQ. The residue values were set to half of the individual reported LOQ values on the respective sampling dates (0.48 % AR on day 60, 0.42 % AR on day 90 and 0.39 % AR on day 119).

Table 7.2.2.3-16: Experimental data of AMPA for system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment
0	100.2[1]	100.2[1]	0.0[2]
0	100.4[1]	100.4[1]	0.0[2]
3	68.08	39.27	28.82
3	72.94	46.05	26.90
7	59.42	24.96	34.46
7	59.59	25.39	34.19
14	51.90	19.17	**32.73**
14	48.41	11.94	**36.47**
31	39.40	7.36	32.04
31	37.43	8.37	29.05
60	30.67	4.04	26.62
60	31.21	3.27	27.94
89	28.78	0.88	27.91
89	24.38	3.18	21.21
119	19.86	2.06	17.80
119	27.86	0.75	27.10

Numbers in **bold** represent peak concentrations considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly

[1] Values at day 0 were set to material balance according to FOCUS (2014)

[2] Set to zero for evaluation at Level P-II

Table 7.2.2.3-17: Experimental data of AMPA for system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment
0	97.4[1]	97.4[1]	0.0[2]
0	99.7[1]	99.7[1]	0.0[2]
3	38.49	18.91	19.58
3	44.67	24.94	19.73
7	35.28	15.80	19.48
7	33.38	13.95	19.43
14	25.16	3.69	21.47
14	26.95	3.46	23.49
31	19.87	0.82	19.04
31	18.65	0.30	18.35
60	27.52	0.24[3]	**27.28**
60	24.36	0.88	**23.48**
89	20.75	0.21[4]	20.54
89	19.54	0.36	19.18
119	23.18	0.24	22.94
119	23.75	0.20[5]	23.55

Table 7.2.2.3-17: Experimental data of AMPA for system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020) used for kinetic evaluation

Numbers in **bold** represent peak concentrations considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly
[1] Values at day 0 were set to material balance according to FOCUS (2014)
[2] Set to zero for evaluation at Level P-II
[3] Value was set to ½ LOQ (LOQ at sampling day 60: 0.48 %)
[4] Value was set to ½ LOQ (LOQ at sampling day 89: 0.42 %)
[5] Value was set to ½ LOQ (LOQ at sampling day 119: 0.39 %)

[redacted] (2003, CA 7.2.2.3/019)

In the report document available for the evaluation, the individual results of HPLC analysis for water and sediment phase were missing. Thus, the evaluation could only be based on results of TLC analysis. The missing data led to inconsistencies in the reporting of the amounts of AMPA in sediment extracts in the text of the study report compared to tabulated results from TLC analysis. Therefore, no kinetic evaluation was performed for the sediment phase as well as the total system of both systems and only a kinetic evaluation for the water phase is included in the current assessment.

A complete report document including the results of HPLC analysis was received after completion of the kinetic evaluation. The complete data may be used to update the evaluation at a later time point.

Table 7.2.2.3-18: Experimental data of AMPA for system Bickenbach of study [redacted] (2003, CA 7.2.2.3/019) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)[1]
	Water
0	97.3[2]
0	101.7[2]
0.25	87.8
0.25	87.4
1	63.9
1	62.5
2	58.5
2	59.5
7	27.9
7	28.6
14	16.7
14	15.4
30	9.7
30	13.8
62	7.6
62	7.6
104	4.7
104	5.8

[1] The data of the sediment phase and the total system were not considered in the kinetic evaluation
[2] Values at day 0 were set to material balance according to FOCUS (2014)

Table 7.2.2.3-19: Experimental data of AMPA for system Unter Widdersheim of study [redacted] (2003, CA 7.2.2.3/019) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)[1]
	Water
0	100.7[2]
0	102.4[2]
0.25	80.6
0.25	82.1
1	60.2
1	63.1
2	52.7
2	54.4
7	28.9
7	32.0
14	12.2
14	14.4
30	7.2
30	6.8
62	1.3
62	1.5
104	1.8
104	0.8

[1] Due to inconsistencies of the reported residues in sediment phase in the study report, the data of the sediment phase and the total system were not considered in the kinetic evaluation
[2] Values at day 0 were set to material balance according to FOCUS (2014)

[redacted] (1999, CA 7.2.2.3/021)

For each water-sediment system, the extracts were analysed at each sampling time, with two different TLC systems (SS 1 and SS 2). The values resulting from the TLC systems were considered to be analytical replicates and were therefore averaged prior to kinetic evaluation for each sampling time.

For the system Unter Widdersheim some water samples with overall less than 5 % AR were not analysed by TLC on days 30, 59 (one of two replicates) and 100 (both replicates). These data points were not considered in the kinetic evaluation.

At Level P-I, no evaluations could be conducted for the sediment phase for system Bickenbach due to the limited number of data points available after the peak concentration.

Table 7.2.2.3-20: Experimental data of AMPA for system Bickenbach of study [redacted] (1999, CA 7.2.2.3/021) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment[1]
0	103.6[2]	103.6[2]	0.0[3]
0	103.2[2]	103.2[2]	0.0[3]
0.25	99.9	91.6	8.3
0.25	98.6	90.2	8.4
1	100.2	84.6	15.7
1	98.8	79.4	19.4
2	90.5	68.2	22.3
2	91.8	73.6	18.2
7	84.0	52.8	31.2
7	83.4	52.0	31.5
14	66.5	31.6	34.9
14	73.9	35.0	38.9
30	51.0	17.7	33.3
30	61.4	26.0	35.5
59	48.4	7.5	**41.0**
59	50.0	9.4	**40.6**
100	27.0	4.9	22.2
100	22.0	3.3	18.7

Numbers in **bold** represent peak concentrations considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly

[1] No evaluation was conducted at Level P-I for the sediment phase due to the limited number of data points available after the peak concentration

[2] Values at day 0 were set to material balance according to FOCUS (2014)

[3] Set to zero for evaluation at Level P-II

Table 7.2.2.3-21: Experimental data of AMPA for system Unter Widdersheim of study [redacted] (1999, CA 7.2.2.3/021) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment
0	102.2[1]	102.2[1]	0.0[2]
0	102.1[1]	102.1[1]	0.0[2]
0.25	98.2	84.0	14.2
0.25	97.5	81.3	16.2
1	92.9	53.4	39.5
1	96.5	48.4	48.1
2	85.6	58.5	27.2
2	85.1	58.2	27.0
7	73.3	34.1	39.3
7	71.3	25.8	45.5
14	58.5	20.4	**38.2**
14	60.2	6.1	**54.1**
30	39.1	-[3]	39.1

Table 7.2.2.3-21: Experimental data of AMPA for system Unter Widdersheim of study [redacted] (1999, CA 7.2.2.3/021) used for kinetic evaluation

30	33.6	-[3]	33.6
59	33.9	-[3]	33.9
59	32.0	2.3	29.7
100	26.6	-[3]	26.6
100	35.0	-[3]	35.0

Numbers in **bold** represent peak concentrations considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly
[1] Values at day 0 were set to material balance according to FOCUS (2014)
[2] Set to zero for evaluation at Level P-II
[3] Sediment extracts containing less than 5 % AR were not analysed further. These data points were not considered in the kinetic evaluation

[redacted] *(2004, CA 7.2.2.3/018)*

At Level P-I, no evaluation could be conducted for the sediment phase for system Manningtree A due to the limited number of data points available after the peak concentration.

Due to problems analysing extracts obtained from system Manningtree B, explained to be caused by endogenous co-extracted material disrupting the ion-exchange chromatography, the total system and sediment phase of the system Manningtree B were not considered in the kinetic evaluation. Therefore, Level P-II evaluation could not be conducted for the system Manningtree B.

Table 7.2.2.3-22: Experimental data of AMPA for system Manningtree A of study [redacted] (2004, CA 7.2.2.3/018) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)		
	Total system	Water	Sediment[1]
0	96.6[2]	96.6[2]	0.0[3]
1	53.1	37.7	15.4
7	27.1	10.7	16.4
14	9.4	5.9	3.5
29	34.3	4.7	29.6
61	32.5	2.3	**30.2**
103	13.1	0.8	12.3

Number in **bold** represent peak concentration considered for single-compartment evaluation; previous values were omitted and sampling dates were adjusted accordingly
[1] No evaluation was conducted at Level P-I for the sediment phase due to the limited number of data points available after the peak concentration
[2] Values at day 0 were set to material balance according to FOCUS (2014)
[3] Set to zero for evaluation at Level P-II

Table 7.2.2.3-23: Experimental data of AMPA for system Manningtree B of study [redacted] (2004, CA 7.2.2.3/018) used for kinetic evaluation

Sampling day (d)	AMPA residues (% AR)[1]
	Water
0	97.2[2]
1	52.7
7	8.60
14	6.20
29	1.90
61	0.10
103	0.20

[1] Due to experimental problems, the total system and the sediment phase were not considered in the kinetic evaluation
[2] Values at day 0 were set to material balance according to FOCUS (2014)

3. Kinetic models and analysis

Kinetic models

Four kinetic degradation models were considered to describe the degradation behaviour of the compounds in the water-sediment systems: single first-order (SFO), first-order multi-compartment (FOMC = Gustafson and Holden model), double-first-order-in-parallel (DFOP) and Hockey-stick (HS) (FOCUS; 2006, 2014). At Level P-I and M-I dissipation, all of the four models were considered, where applicable, based on the recommended procedure to derive endpoints in FOCUS (2014) and the number of available data points. At Level P-II, the SFO model was applied for both water and sediment compartments. At Level M-I degradation, the best-fit model derived from Level P-I was applied for parent, and the SFO model was used for the metabolites.

The best-fit model was accepted for deriving trigger endpoints, while the DT_{50} calculated from SFO model was preferably selected as modelling endpoint.

Optimisation

The kinetic analyses were conducted using the software KinGUI v2.1. The data were directly fitted with the complete dataset and unconstrained initial concentration (M_0) for glyphosate and AMPA (when applied as test substance). Iteratively Reweighted Least Square (IRLS) was used as the solver, as implemented in KinGUI. Optimisations were carried out for the initial residue (M_0), degradation model parameters k, α, β, g or t_b, depending on the respective kinetic model. The initial estimates for the parameters were specified manually, based on the observed degradation pattern and preliminary model runs. By default, the initial amount of the parent substance in sediment and the metabolite in total system were fixed to 0 at Level P-II and Level M-I degradation, respectively. The parameters were optimised by minimising the sum of squared differences between measured and calculated data. The error tolerance and the number of iterations were set to the default values of 1×10^{-5} and 100, respectively.

Criteria for selection of the appropriate kinetic model

Evaluation of model fit

The goodness of fit of the estimated to the measured residue data was evaluated visually (concentration vs. time plots and residual plots) and statistically (Chi-square (χ^2) test). The visual inspection focused on the residuals which should not be distributed systematically around the zero line, but randomly. However in the case of systematic but sufficiently small deviations, a fit was considered to be visually acceptable. Specifically, the visual acceptance of a model fit has been judged according to the following classification:

- Good: excellent conformity of measured residues and fitted decline curve; low residual levels; randomly scattered
- Acceptable: acceptable conformity of measured residues and fitted decline curve; medium residual levels; residuals more or less randomly scattered

- Poor: significant deviation between measured residues and fitted decline curve; the calculated curve does not match the observed pattern; high residual levels; residuals clearly not randomly scattered around the zero line

A statistical measure of the quality of a fit is given by the χ^2-test which considers the deviations between observed and calculated values relative to the uncertainty of the measurements. The model with the smallest error percentage was defined as the most appropriate, because it described the measured data in the most robust way.

In general, it is recommended that if the χ^2 error is <15 %, then the model has adequately reflected the measured data. However, this value should only be considered as guidance and not an absolute cut-off criterion. Depending on the complexity of the curve fitting for multiple components and the scattering of the experimental data, also fits with higher χ^2 error values may be acceptable if overall the measured data are well described by the fitted curve.

Significance of parameters
A single-sided t-test was performed to evaluate whether the optimised degradation rate constants (k) of the SFO, DFOP and HS kinetic models were significantly different from zero at a chosen significance level of 10 % for water-sediment kinetics. For the FOMC kinetic model, only the confidence interval of parameter β was considered in the assessment.

The t-test was required to be passed for derivation of modelling endpoints. In case of trigger endpoints, the non-significance of parameters was not seen as a cut-off criterion but the t-test was used as supporting information for the decision making process. The KinGUI software also reports a 95 % confidence interval on the estimated parameters. It should be relatively tight and not contain 0 to be considered statistically robust.

At level P-II, no further evaluation was conducted if the visual fits are poor or the degradation rates of the water or sediment phase failed the t-test.

II. RESULTS AND DISCUSSION

[redacted] (1999, CA 7.2.2.3/002)

Table 7.2.2.3-24: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

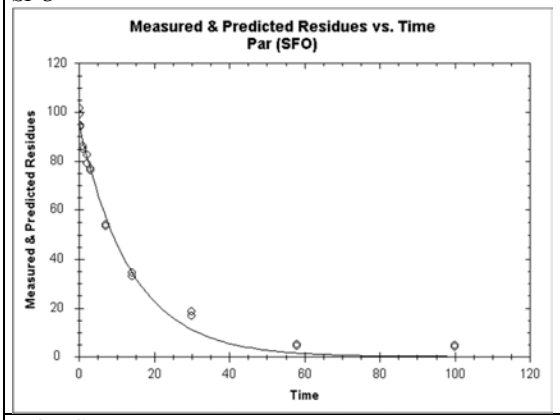
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	95.1	k: 0.0716	5.3	k: <0.001	k: 0.0634	k: 0.0800	9.7	32.1
FOMC	Good	97.8	α: 1.8669 β: 18.7550	2.7	-[1]	β: 11.2434	β: 26.2670	8.4	45.6
DFOP	Good	97.5	k_1: 0.1386 k_2: 0.0298 g: 0.5982	3.1	k_1: <0.001 k_2: <0.001	k_1: 0.0845 k_2: 0.0148	k_1: 0.1930 k_2: 0.0450	8.4	47.0
HS	Good	97.0	k_1: 0.0853 k_2: 0.0394 tb: 11	3.2	k_1: <0.001 k_2: <0.001	k_1: 0.0762 k_2: 0.0301	k_1: 0.0940 k_2: 0.0490	8.1	45.6

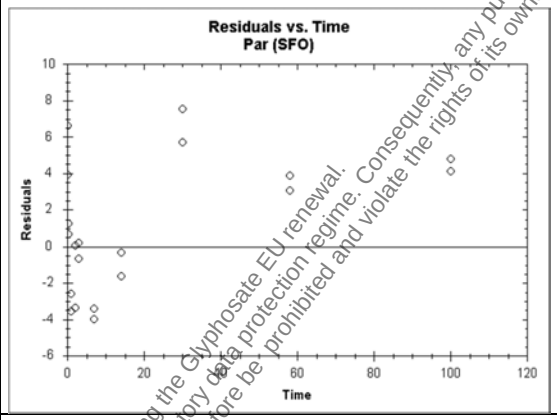
Although the visual and statistical fits of the SFO model are acceptable, the degradation of glyphosate is best described by bi-phasic models. All bi-phasic models provide equally reliable and visually good results but the least χ^2 error is provided by the FOMC model.

Conclusion: FOMC to be used for trigger endpoints and for further evaluation at Level M-I degradation
SFO to be used for modelling endpoints

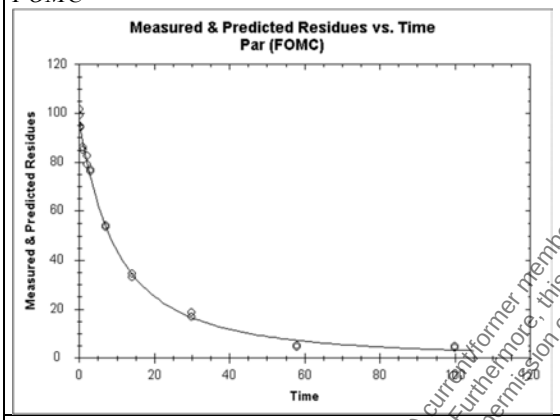
Table 7.2.2.3-24: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

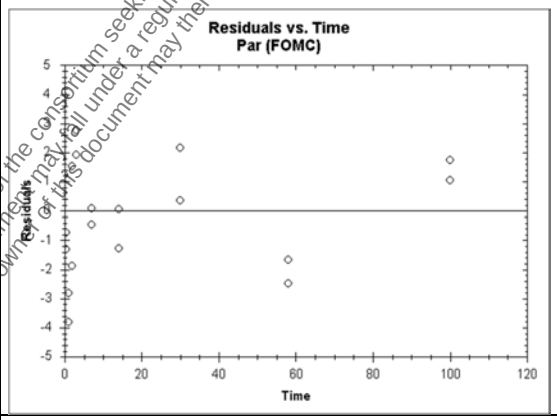
SFO



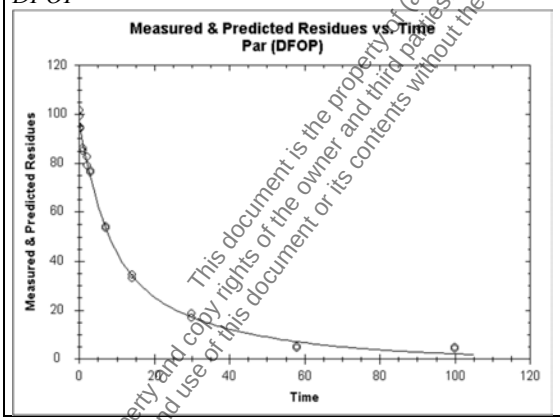


FOMC





DFOP



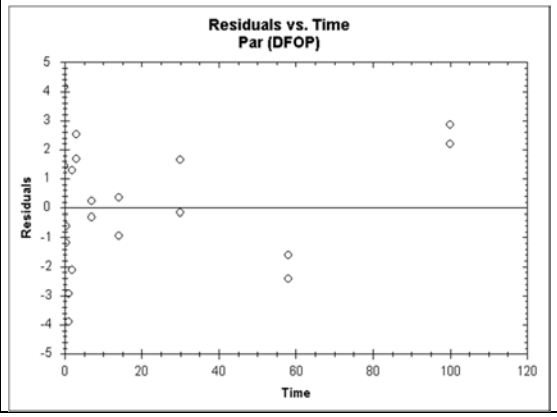
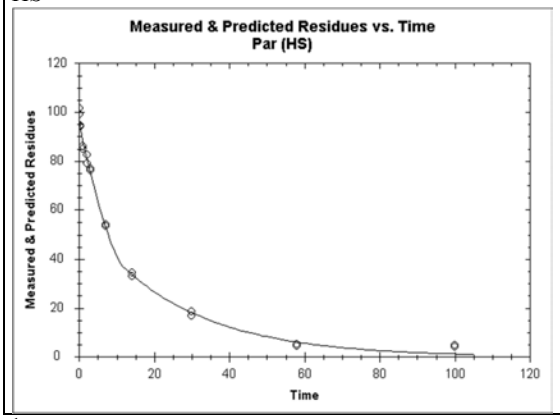
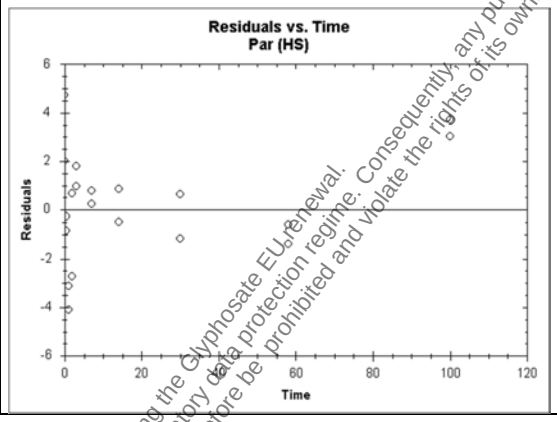


Table 7.2.2.3-24: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

HS





[1] t-test not relevant for kinetic parameter β

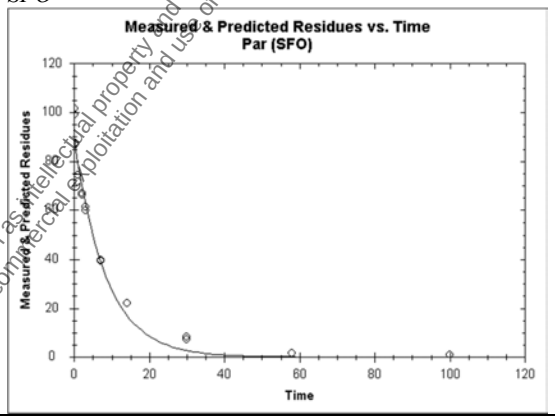
Table 7.2.2.3-25: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	90.0	k: 0.1181	8.5	k: <0.001	k: 0.0989	k: 0.1370	5.9	19.5
FOMC	Acceptable	94.2	α: 1.4851 β: 7.9603	6.0	-[1]	β: 3.2128	β: 12.7080	4.7	29.6
DFOP	Good	100.4	k_1: 3.0100 k_2: 0.0908 g: 0.2141	2.3	k_1: <0.001. k_2: <0.001	k_1: 1.9370 k_2: 0.0845	k_1: 4.0830 k_2: 0.0970	5.0	22.7
HS	Good	94.0	k_1: 0.1682 k_2: 0.0752 tb: 3.7	6.4	k_1: <0.001 k_2: <0.001.	k_1: 0.1381 k_2: 0.0498	k_1: 0.1980 k_2: 0.1010	4.6	26.0

Although the visual and statistical fits of the SFO model are acceptable, the dissipation of glyphosate in the water phase is best described by bi-phasic models. The FOMC model provides acceptable fits while the DFOP and HS models provide equally reliable and visually good results. However, the least χ^2 error is provided by the DFOP model.

Conclusion: DFOP to be used for trigger endpoints
SFO to be used for modelling endpoints

SFO



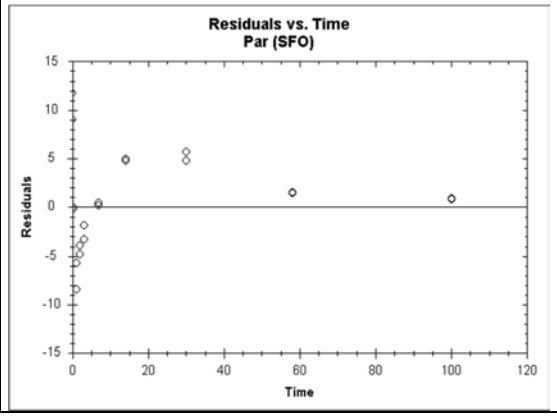
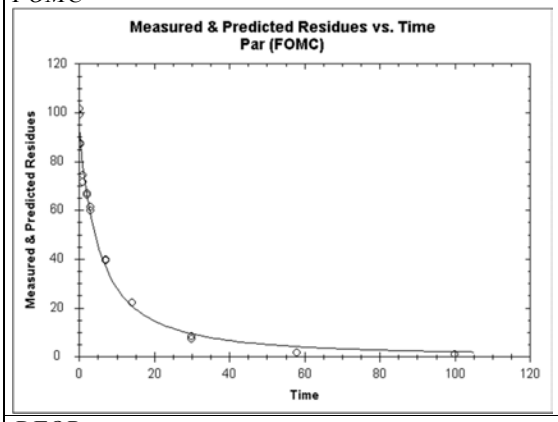
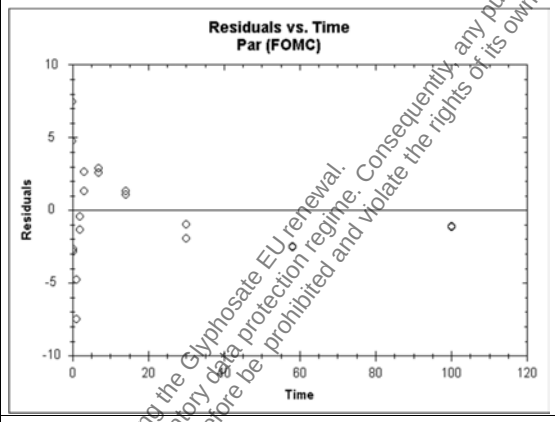


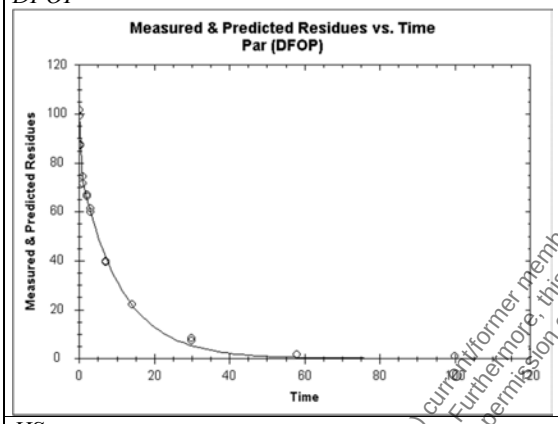
Table 7.2.2.3-25: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

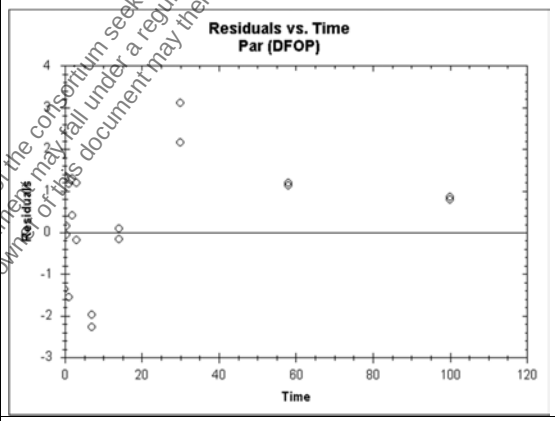
FOMC



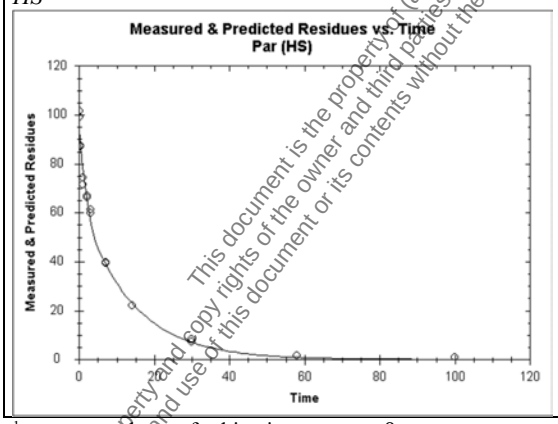


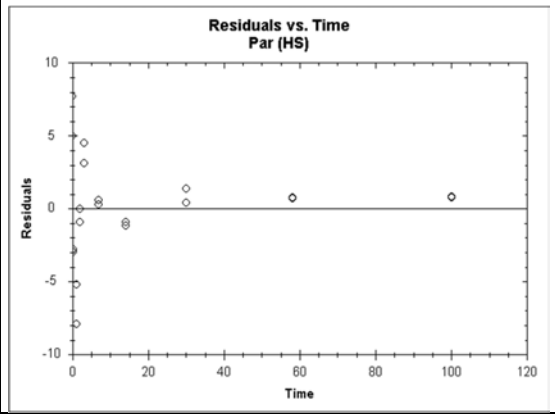
DFOP





HS





[1] t-test not relevant for kinetic parameter β

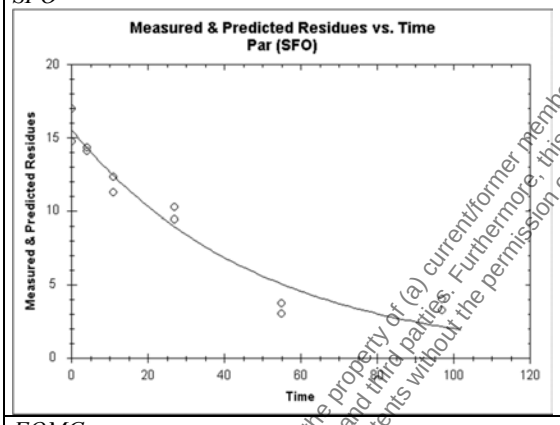
Table 7.2.2.3-26: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, sediment phase

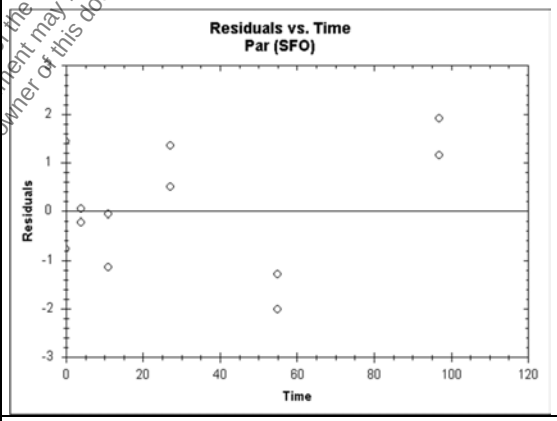
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	15.5	k: 0.0204	8.4	k: <0.001	k: 0.0156	k: 0.0250	33.9	112.6
FOMC	Acceptable	15.8	α: 2.6471 β: 103.695	8.7	-[1]	β: -191.77	β: 399.16	31.0	143.8
DFOP	Acceptable	15.9	k_1: 0.0287 k_2: 2.34×10^{-14} g: 0.8631	9.4	k_1: 0.220 k_2: >0.500	k_1: -0.0406 k_2: -0.1295	k_1: 0.0980 k_2: 0.1300	30.2	>1000
HS	Acceptable	15.9	k_1: 0.0467 k_2: 0.0197 tb: 1.6	10.4	k_1: 0.21 k_2: <0.001	k_1: -0.0609 k_2: 0.0138	k_1: 0.1540 k_2: 0.0260	33.0	114.6

Dissipation of glyphosate in sediment is best described by the SFO model. Thus, the SFO model is selected as the best-fit model as well as for deriving modelling endpoints.

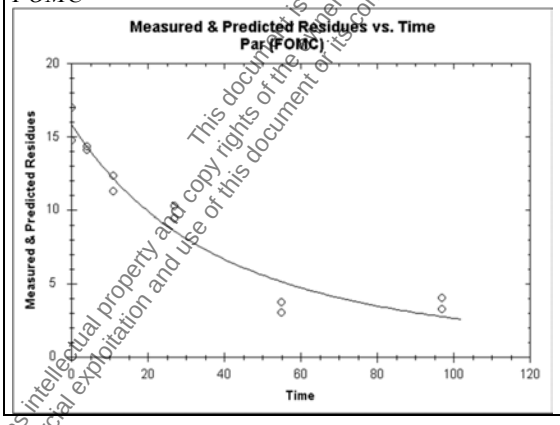
Conclusion: SFO to be used for trigger endpoints
SFO to be used for modelling endpoints

SFO





FOMC



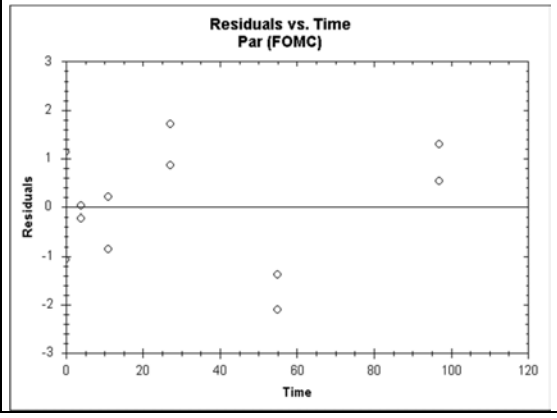
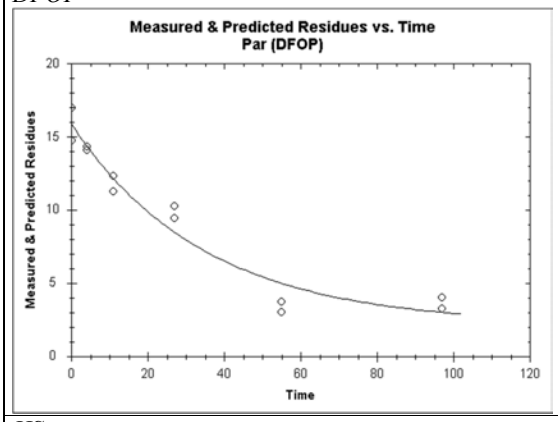
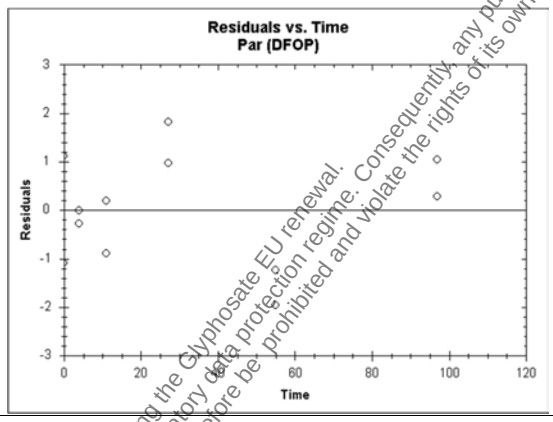


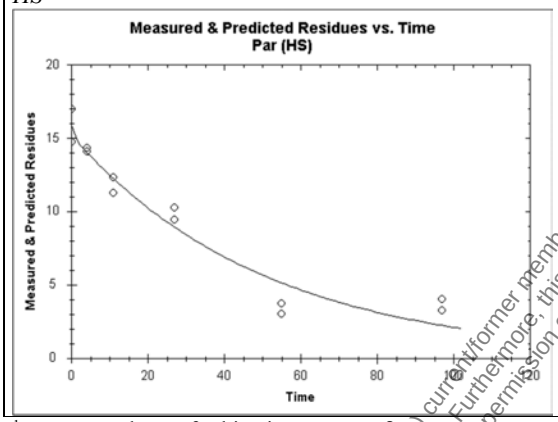
Table 7.2.2.3-26: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, sediment phase

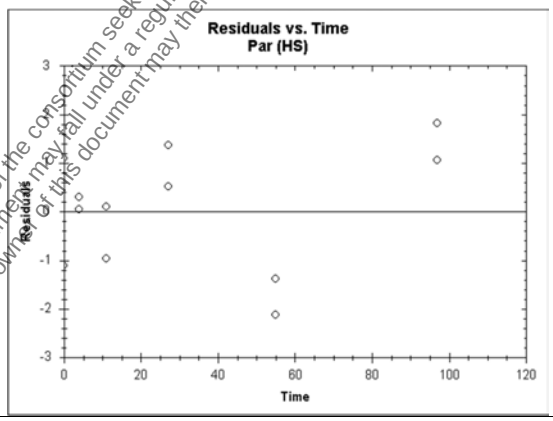
DFOP





HS





[1] t-test not relevant for kinetic parameter β

Table 7.2.2.3-27: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-II

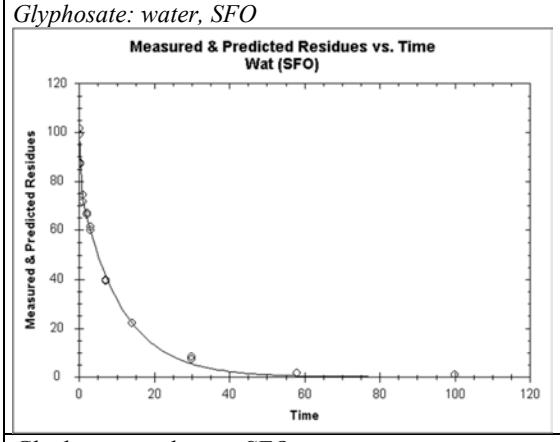
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Good	99.7	k_{wat}: 0.1129 k_{wat_sed}: 0.5175	2.3	k_{wat}: 0.0442 k_{wat_sed}: <0.001	k_{wat}: -0.0133 k_{wat_sed}: 0.3302	k_{wat}: 0.239 k_{wat_sed}: 0.705	6.1	20.4
Sediment: SFO	Poor	0.0	k_{sed}: 2.34×10^{-14} k_{sed_wat}: 2.082	34.6	k_{sed}: 0.5 k_{sed_wat}: <0.001	k_{sed}: -0.5082 k_{sed_wat}: -1.177	k_{sed}: 0.508 k_{sed_wat}: 2.987	>1000	>1000

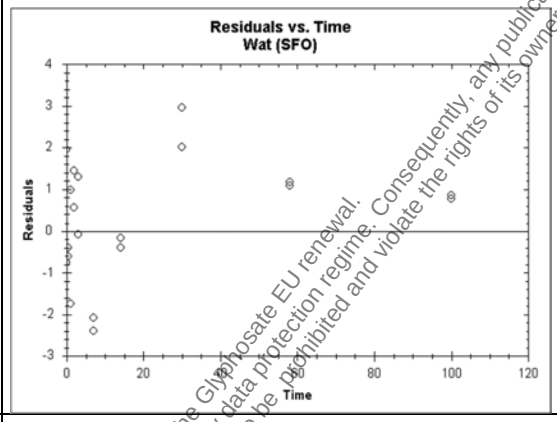
The visual and statistical fits obtained for the water phase are reliable but the visual fit obtained for the sediment phase is poor.

Conclusion: No further evaluation was conducted. No reliable endpoints could be derived

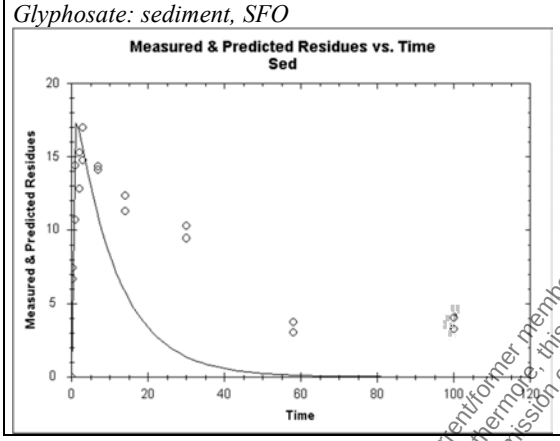
Table 7.2.2.3-27: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level P-II

Glyphosate: water, SFO





Glyphosate: sediment, SFO



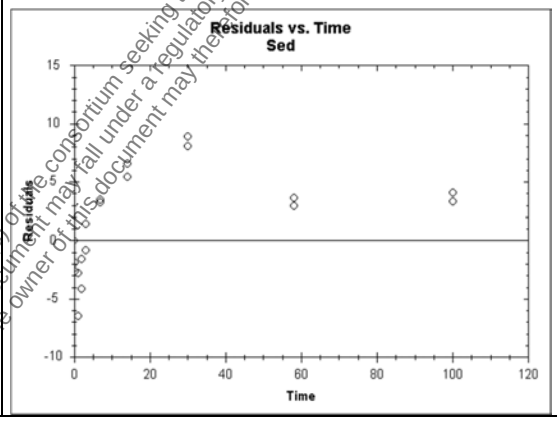


Table 7.2.2.3-28: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolite AMPA in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I degradation

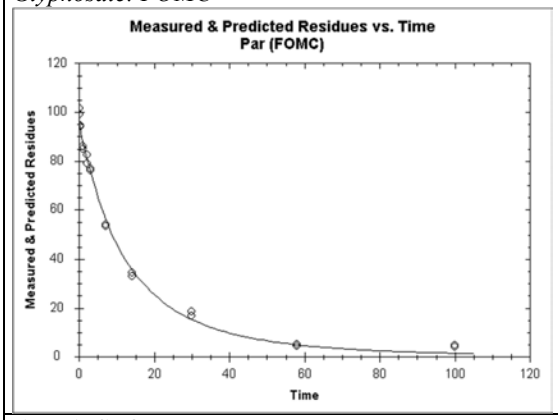
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: FOMC	Good	95.7	α: 3.544 β: 44.02	3.7	-[1]	β: 13.510	β: 74.518	9.5	40.3	-
AMPA: SFO	Accep-table	0.0	k: 0.004	7.0	k: <0.001	k: 0.0026	k: 0.0050	172.8	573.9	0.339 (±0.014)

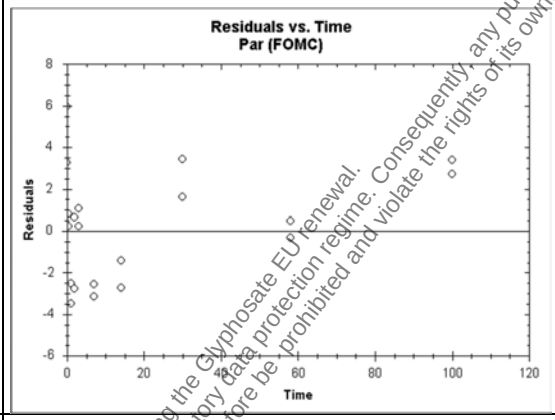
The fit of glyphosate at Level M-I degradation is comparable to that at Level P-I total system degradation. For AMPA, both the visual fit and the statistical parameters from the SFO model are acceptable.

Conclusion: FOMC-SFO to be used for trigger endpoints for AMPA
FOMC-SFO to be used for modelling endpoints for AMPA

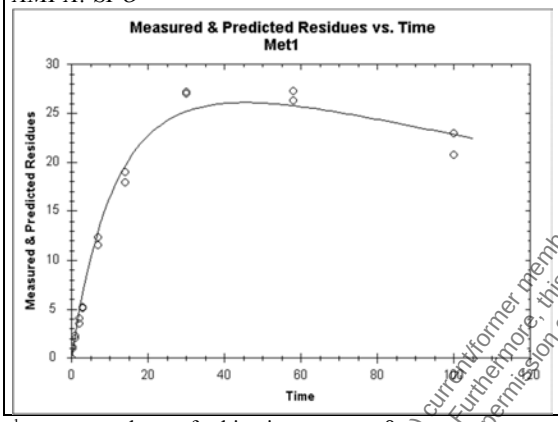
Table 7.2.2.3-28: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolite AMPA in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I degradation

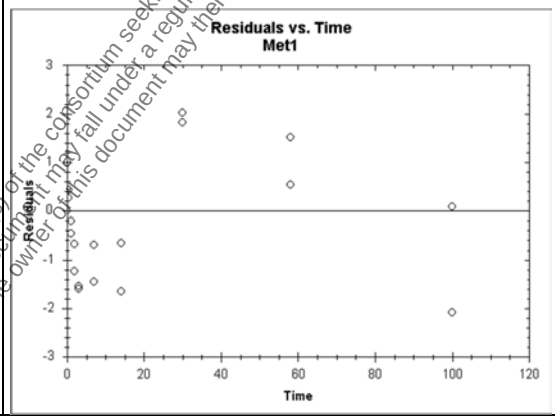
Glyphosate: FOMC





AMPA: SFO





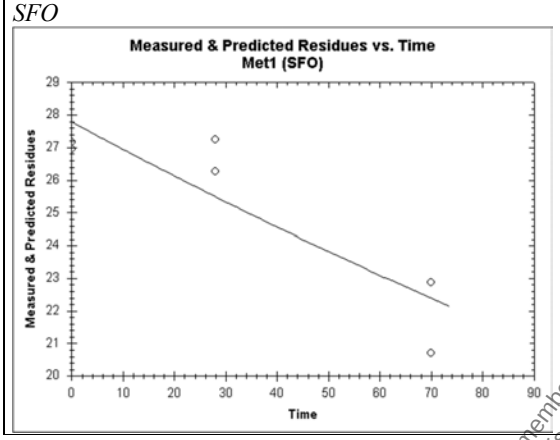
[1] t-test not relevant for kinetic parameter β

Table 7.2.2.3-29: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I dissipation, total system

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	27.8	k: 0.0031	3.2	k: 0.010	k: 0.0015	k: 0.0050	224.6	746.2

Only the SFO model was used for evaluation due to the limited number of data points.
The visual and statistical fit from the SFO model is acceptable.
Conclusion: SFO to be used for trigger endpoints
SFO to be used for modelling endpoints

SFO



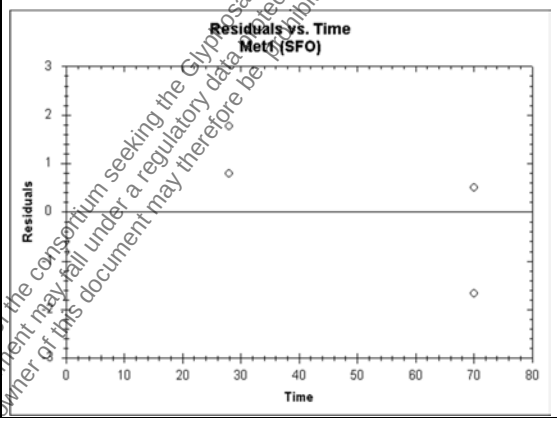
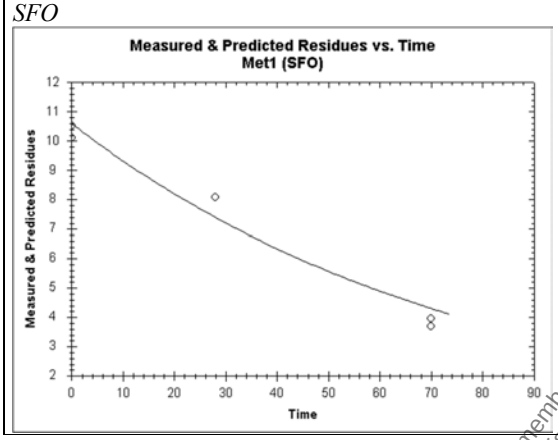


Table 7.2.2.3-30: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Cache of study [redacted] (1999, CA 7.2.2.3/002), Level M-I dissipation, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	10.6	k: 0.0129	6.1	k: <0.001	k: 0.0099	k: 0.0160	53.8	178.8
Only the SFO model was used for evaluation due to the limited number of data points. The visual and statistical fits from the SFO model are acceptable. **Conclusion:** SFO to be used for trigger endpoints SFO to be used for modelling endpoints									

SFO



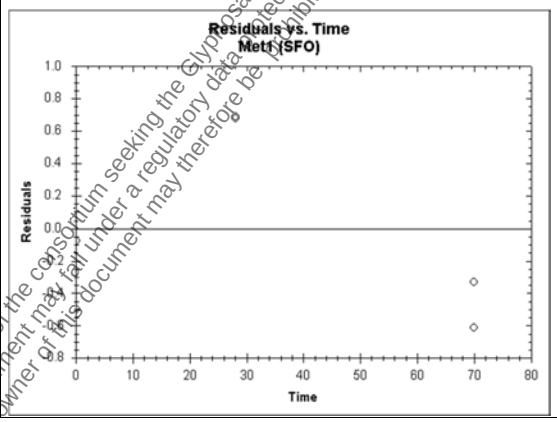
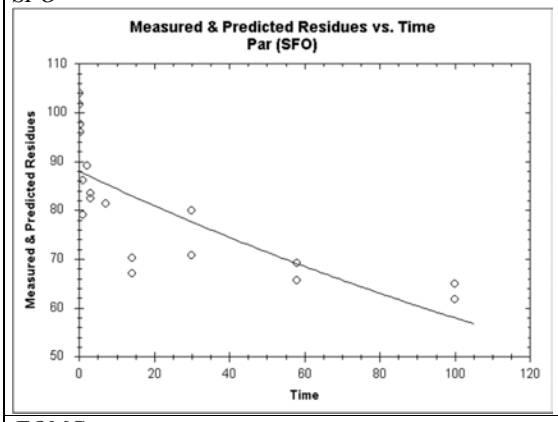


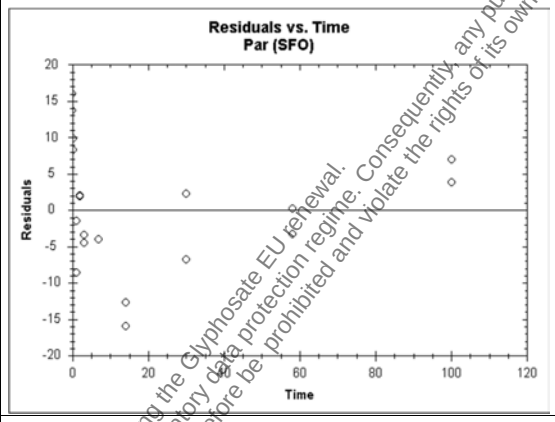
Table 7.2.2.3-31: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	87.9	k: 0.0042	7.7	k: <0.001	k: 0.0024	k: 0.0060	166.0	551.5
FOMC	Acceptable	102.8	α: 0.0686 β: 0.1287	3.7	-[1]	β: -0.0829	β: 0.3400	>1000	>1000
DFOP	Acceptable	100.4	k_1: 0.6409 k_2: 0.0023 g: 0.2189	4.4	k_1: 0.016 k_2: 0.003	k_1: 0.1071 k_2: 0.0009	k_1: 1.1750 k_2: 0.0040	195.8	902.3
HS	Acceptable	98.0	k_1: 0.0623 k_2: 0.0021 tb: 3.9	4.9	k_1: 0.001 k_2: 0.006	k_1: 0.0275 k_2: 0.0007	k_1: 0.0970 k_2: 0.0040	215.3	966.2
Degradation of glyphosate is best described by bi-phasic models. The statistical fit of the FOMC model is not reliable as the confidence interval of parameter β includes zero. Both DFOP and HS models provide equally reliable and visually acceptable results but the DFOP model provides a smaller χ^2 error and is selected as the best-fit model as well as for deriving modelling endpoints. **Conclusion:** DFOP to be used for trigger endpoints DFOP to be used for modelling endpoints									

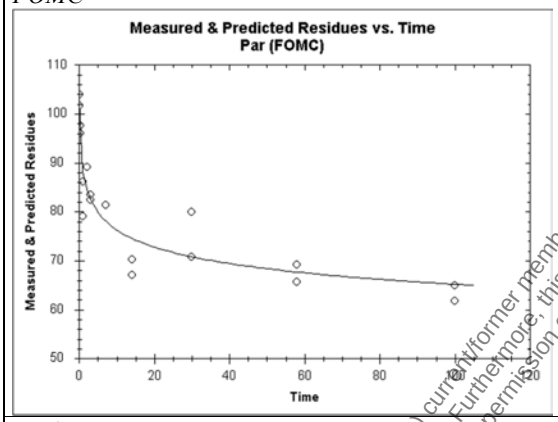
Table 7.2.2.3-31: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

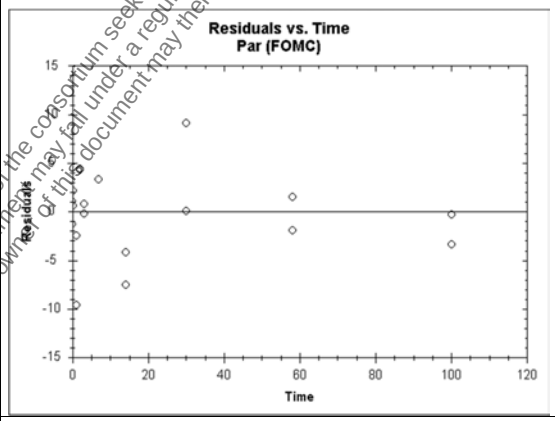
SFO



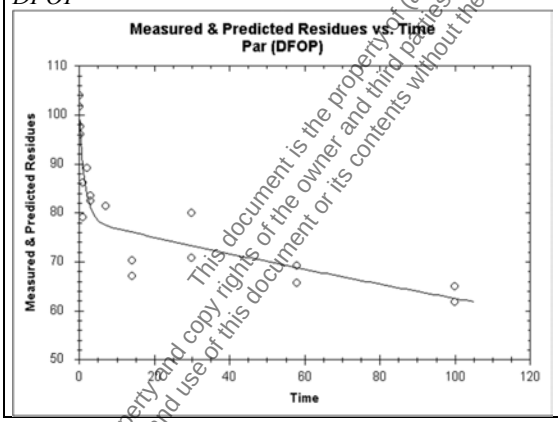


FOMC





DFOP



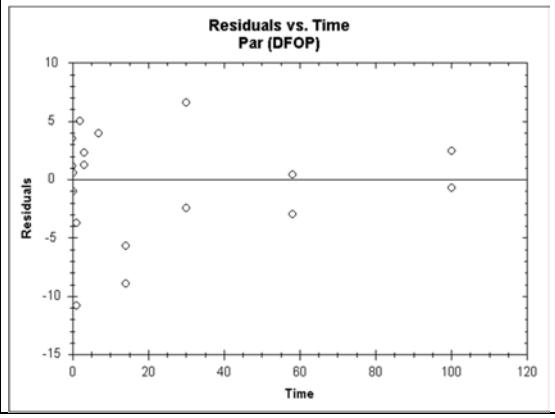
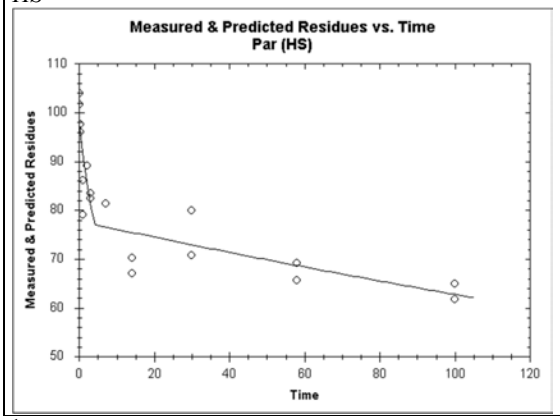
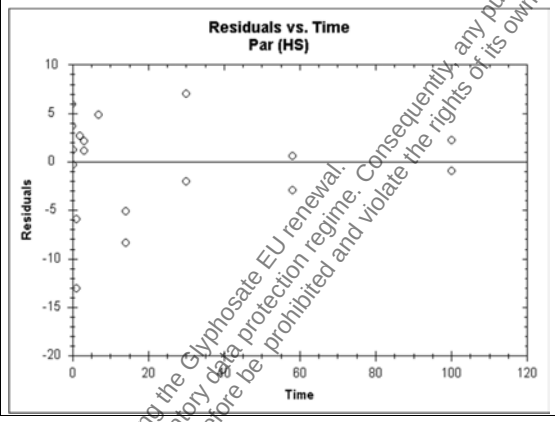


Table 7.2.2.3-31: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, total system

HS





[1] t-test not relevant for kinetic parameter β

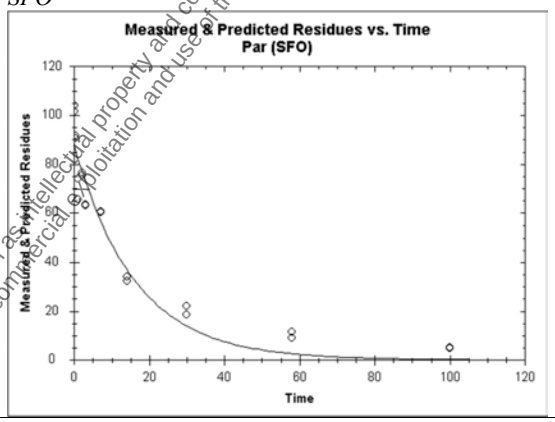
Table 7.2.2.3-32: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	87.9	k: 0.0617	12.2	k: <0.001	k: 0.0448	k: 0.0780	11.2	37.4
FOMC	Good	93.9	α: 0.9292 β: 7.158	10.0	-[1]	β: 0.0281	β: 14.288	7.9	78.2
DFOP	Acceptable	103.5	k_1: 2.724 k_2: 0.0451 g: 0.2639	7.4	k_1: 0.023 k_2: <0.001	k_1: 0.2677 k_2: 0.0354	k_1: 5.1800 k_2: 0.0550	8.6	44.3
HS	Acceptable	96.6	k_1: 0.1696 k_2: 0.0428 tb: 2.2	9.9	k_1: <0.001 k_2: <0.001	k_1: 0.0915 k_2: 0.0289	k_1: 0.2480 k_2: 0.0570	9.8	47.4

Dissipation of glyphosate in the water phase is best described by bi-phasic models. The FOMC model provides the best visual fit and is statistically reliable. Since 10 % of the initially measured substance concentration was reached within the experimental period, FOMC is selected also for deriving modelling endpoints.

Conclusion: FOMC to be used for trigger endpoints
FOMC to be used for modelling endpoints

SFO



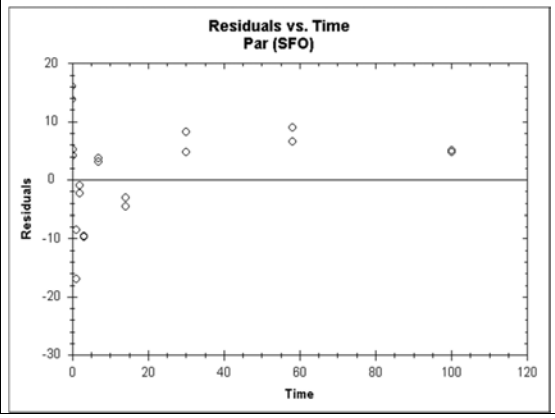
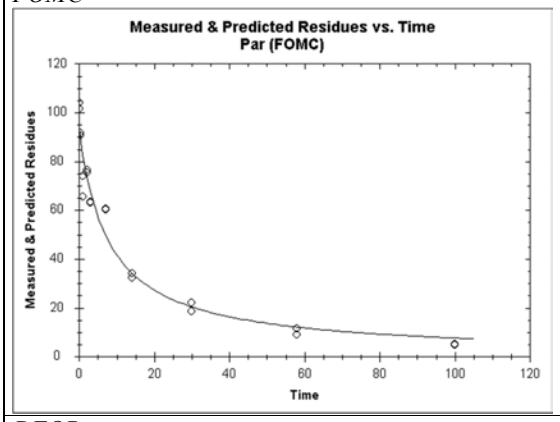
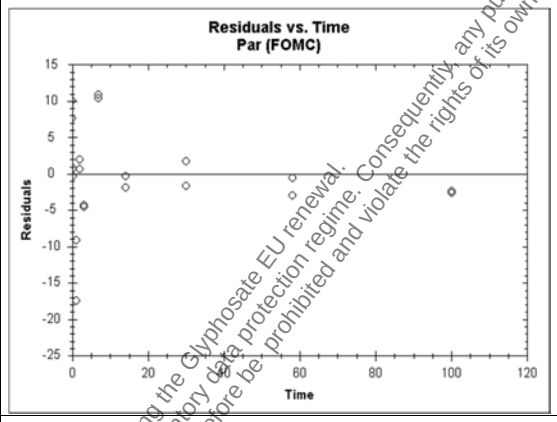


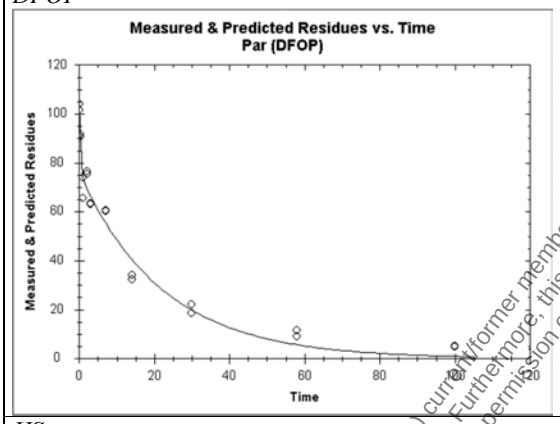
Table 7.2.2.3-32: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-I, water phase

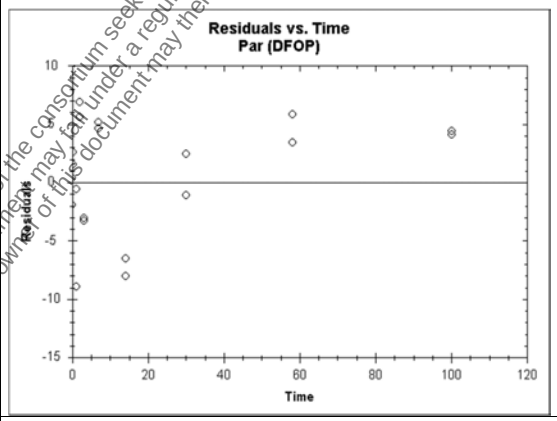
FOMC



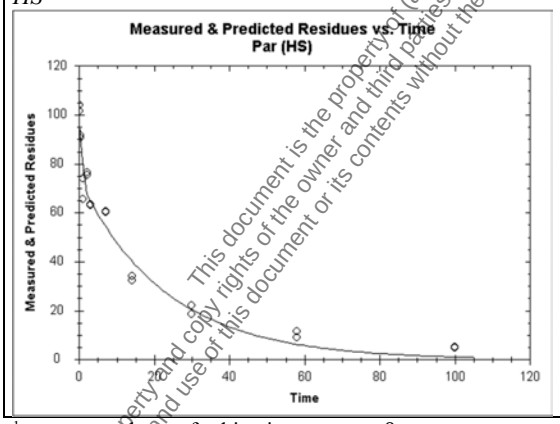


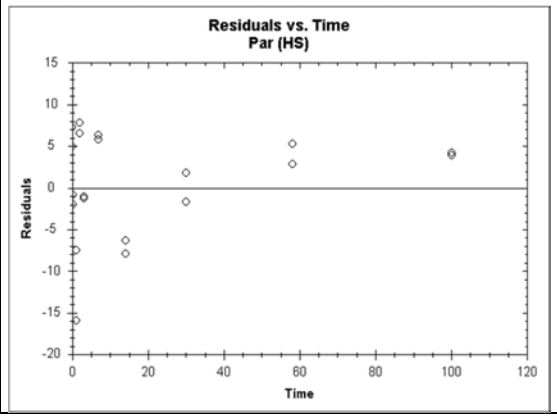
DFOP





HS





[1] t-test not relevant for kinetic parameter β

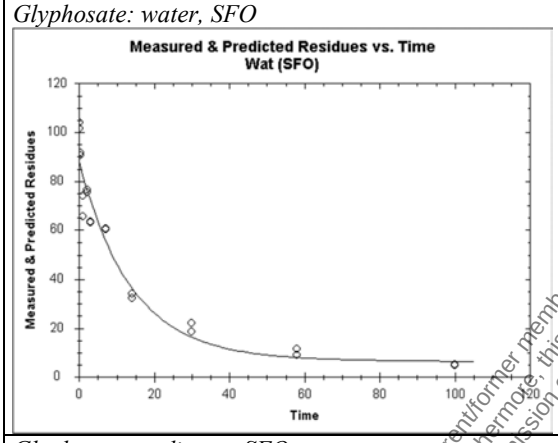
Table 7.2.2.3-33: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level P-II

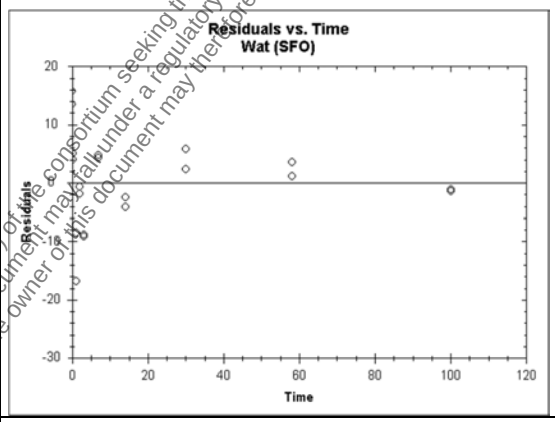
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Good	88.1	k_{wat}: 0.0144 k_{wat_sed}: 0.0521	11.5	k_{wat}: 0.0226 k_{wat_sed}: <0.001	k_{wat}: 0.0008 k_{wat_sed}: 0.0434	k_{wat}: 0.028 k_{wat_sed}: 0.061	48.3	160.4
Sediment: SFO	Good	0.0	k_{sed}: 2.34×10^{-14} k_{sed_wat}: 0.0073	12.4	k_{sed}: 0.5 k_{sed_wat}: 0.0676	k_{sed}: -0.0052 k_{sed_wat}: -0.0021	k_{sed}: 0.005 k_{sed_wat}: 0.017	>1000	>1000

Although the visual fits obtained for the water and sediment phases are good, the degradation rate in sediment is not significantly different from zero. Therefore, the statistical fits obtained for the sediment phase are not reliable.

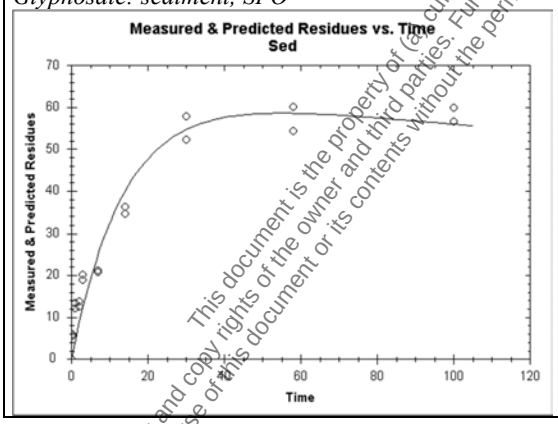
Conclusion: No further evaluation was conducted. No reliable endpoints could be derived

Glyphosate: water, SFO





Glyphosate: sediment, SFO



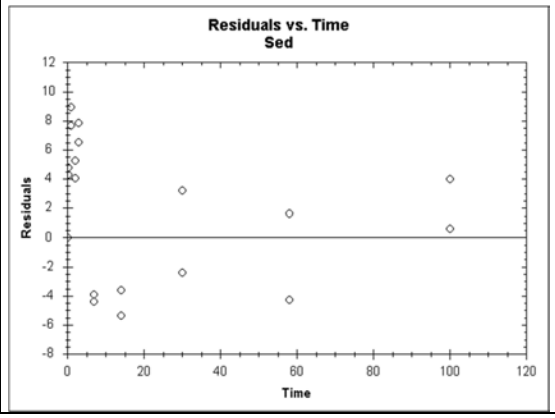
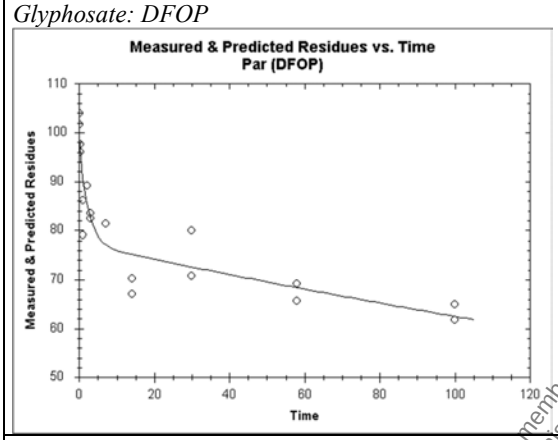


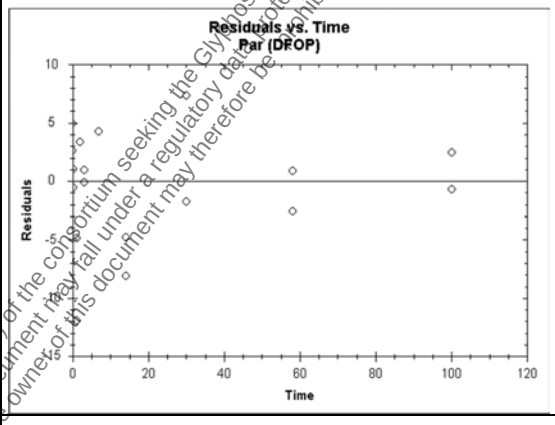
Table 7.2.2.3-34: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolite AMPA in system Putah of study [redacted] (1999, CA 7.2.2.3/002), Level M-I, degradation

Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: DFOP	Accep-table	99.0	k_1: 0.4485 k_2: 0.0021 g: 0.2183	4.5	k_1: 0.003 k_2: 0.003 g: <0.001	k_1: 0.1427 k_2: 0.0007	k_1: 0.754 k_2: 0.004	208.8	960.7	-
AMPA: SFO	Poor	0.0	k: 2.34×10^{-14}	26.9	k: >0.1	k: -0.0069	k: 0.007	>1000	>1000	0.123 (±0.027)

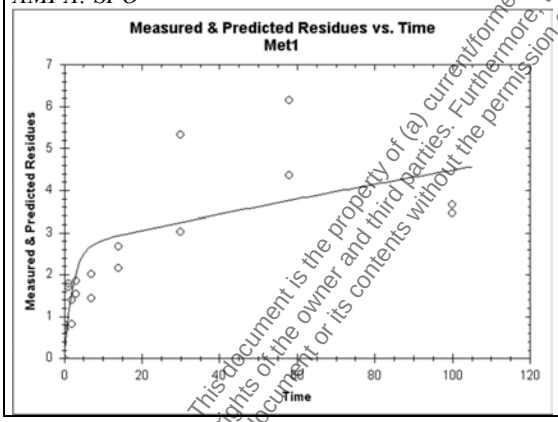
Conclusion: No reliable endpoints could be derived for AMPA

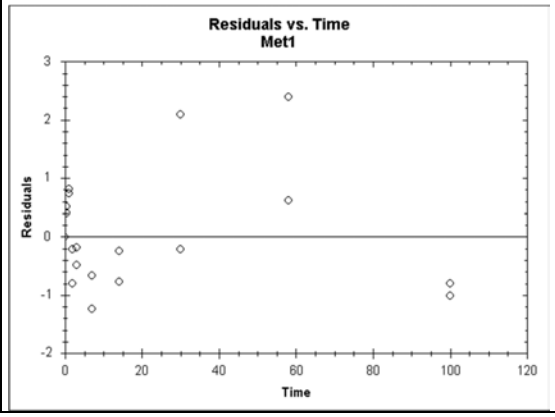
Glyphosate: DFOP





AMPA: SFO





[redacted] (1993, CA 7.2.2.3/005)

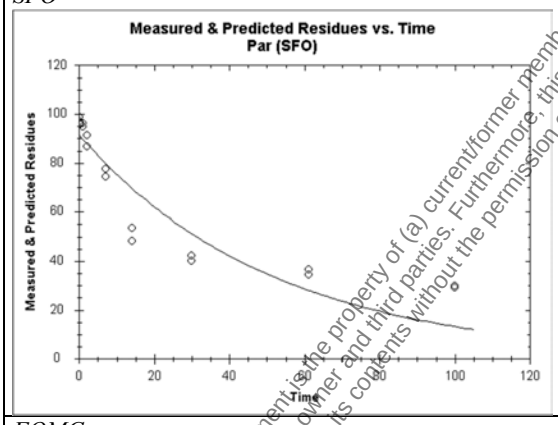
Table 7.2.2.3-35: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

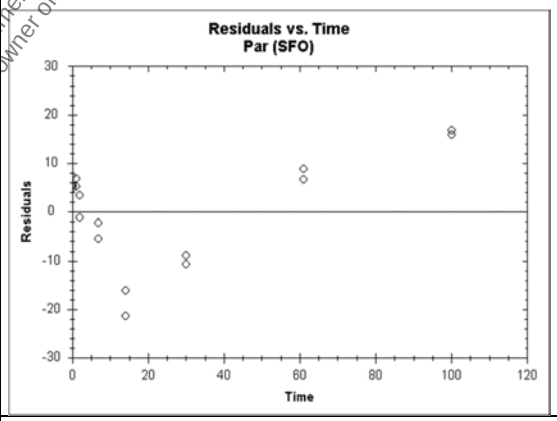
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	91.4	k: 0.0194	11.7	k: <0.001	k: 0.0136	k: 0.0250	35.8	118.8
FOMC	Good	99.7	α: 0.4556 β: 6.1123	4.5	-[1]	β: 1.9556	β: 10.2690	21.9	951.8
DFOP	Good	98.9	k_1: 0.0863 k_2: 0.0026 g: 0.6014	3.4	k_1: <0.001 k_2: 0.12	k_1: 0.0556 k_2: -0.0016	k_1: 0.1170 k_2: 0.0070	18.7	531.2
HS	Good	98.4	k_1: 0.0439 k_2: 0.0048 tb: 18.5	2.2	k_1: <0.001 k_2: <0.001	k_1: 0.0395 k_2: 0.0028	k_1: 0.0480 k_2: 0.0070	15.8	329.4

Degradation of glyphosate is best described by bi-phasic models. All bi-phasic models provide visually good fits. The HS model provided the least χ^2 error and its degradation parameters are significantly different from zero. Thus, the HS model is selected as the best-fit model as well as for deriving modelling endpoints.

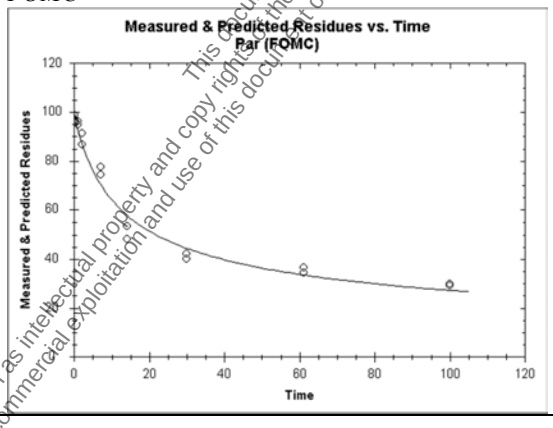
Conclusion: HS to be used for trigger endpoints
HS to be used for modelling endpoints

SFO





FOMC



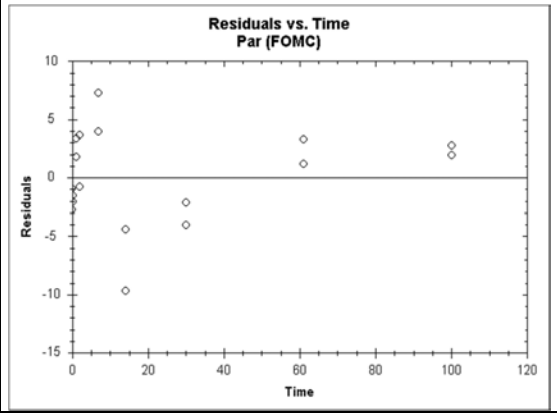
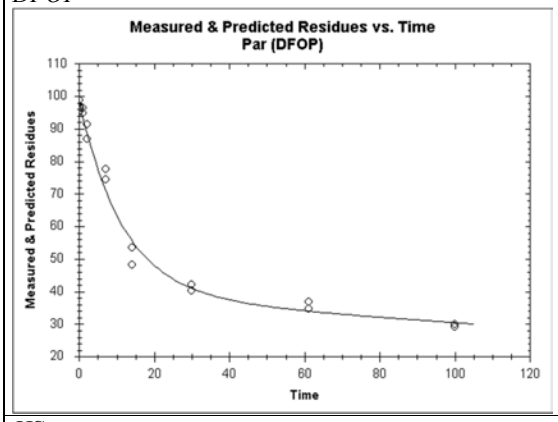
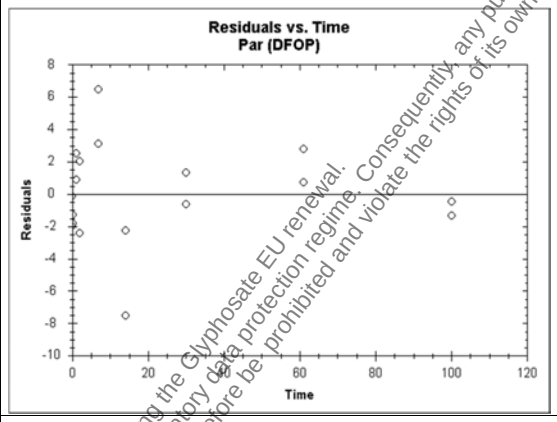


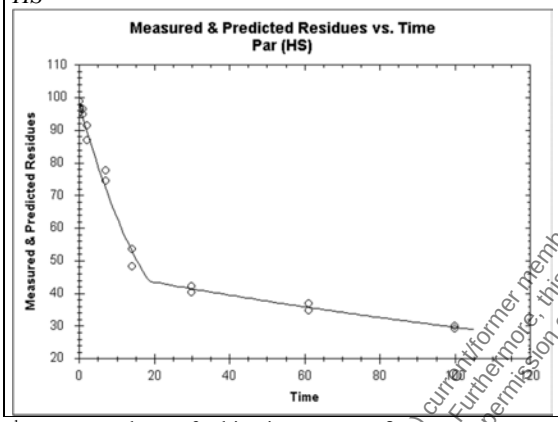
Table 7.2.2.3-35: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

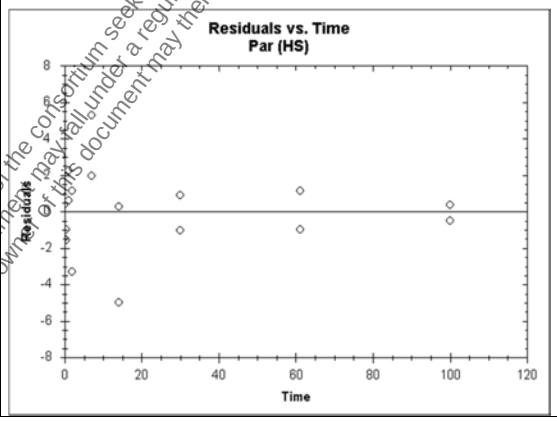
DFOP





HS





[1] t-test not relevant for kinetic parameter β

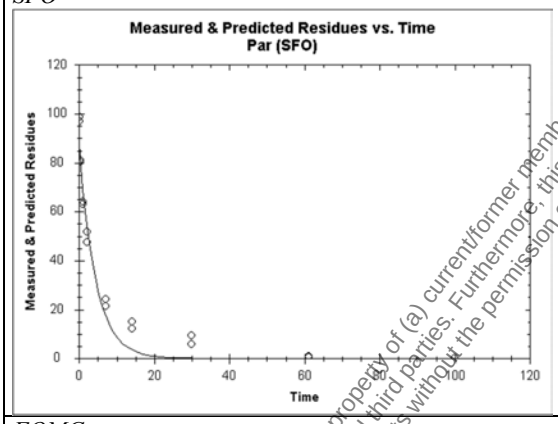
Table 7.2.2.3-36: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

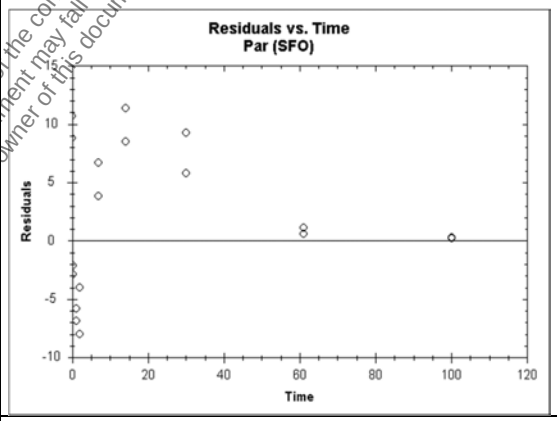
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	88.1	k: 0.2293	13.5	k: <0.001	k: 0.1699	k: 0.2980	3.0	10.0
FOMC	Good	95.1	α: 0.9278 β: 1.8600	4.6	-[1]	β: 1.1856	β: 2.5340	2.1	20.4
DFOP	Good	94.3	k_1: 0.6169 k_2: 0.0565 g: 0.6488	5.2	k_1: <0.001 k_2: <0.001	k_1: 0.4463 k_2: 0.0306	k_1: 0.7880 k_2: 0.0820	2.0	22.2
HS	Good	93.1	k_1: 0.34 k_2: 0.0546 tb: 3.6	6.4	k_1: <0.001 k_2: <0.001	k_1: 0.2938 k_2: 0.0271	k_1: 0.3920 k_2: 0.0820	2.0	22.9

Dissipation of glyphosate is best described by bi-phasic models. All bi-phasic models provide visually good fits. However, the DFOP model provides the best visual fit and is selected as the best-fit model as well as for deriving modelling endpoints.

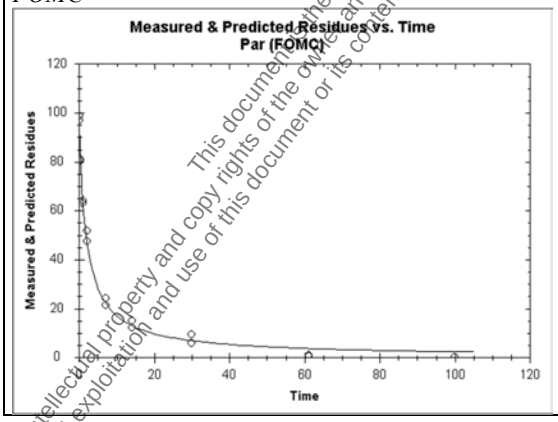
Conclusion: DFOP to be used for trigger endpoints
DFOP to be used for modelling endpoints

SFO





FOMC



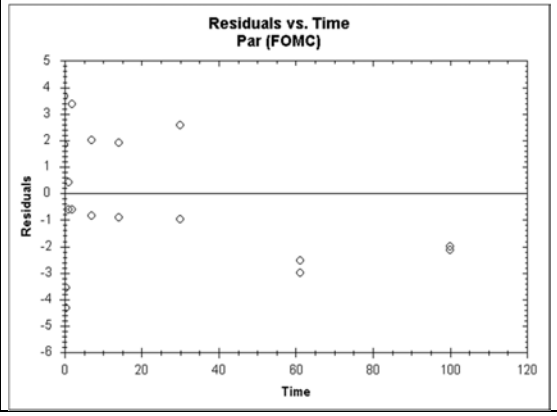
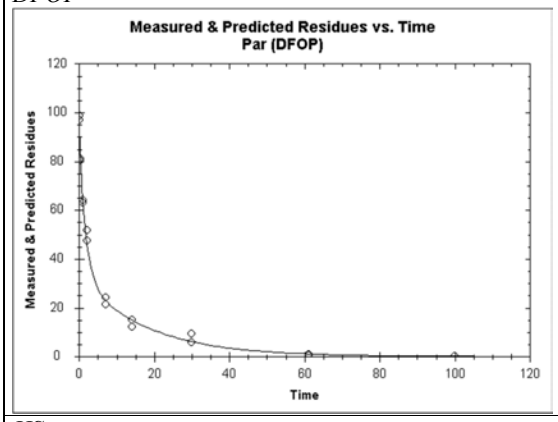
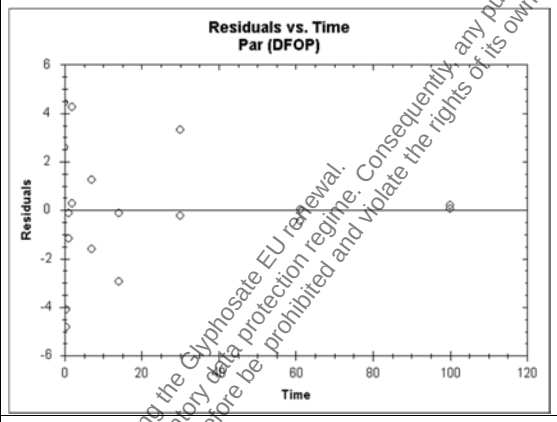


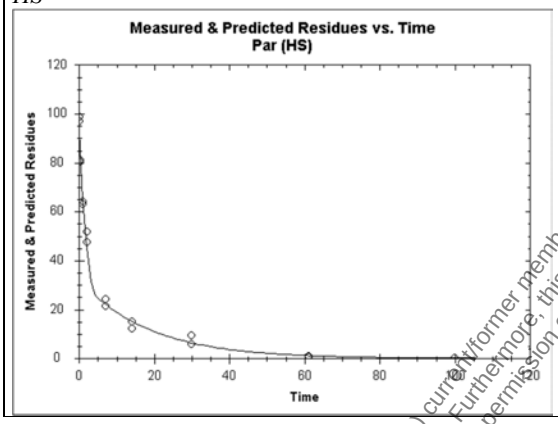
Table 7.2.2.3-36: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

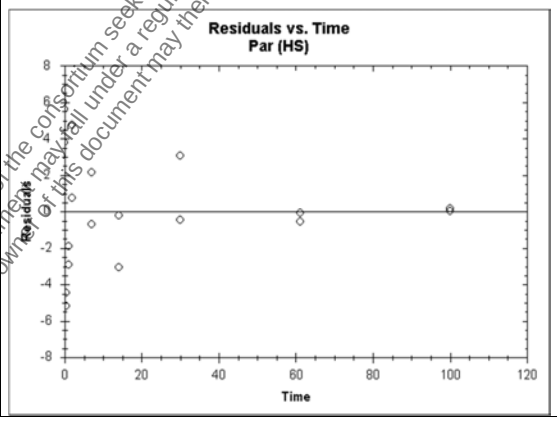
DFOP





HS





[1] t-test not relevant for kinetic parameter β

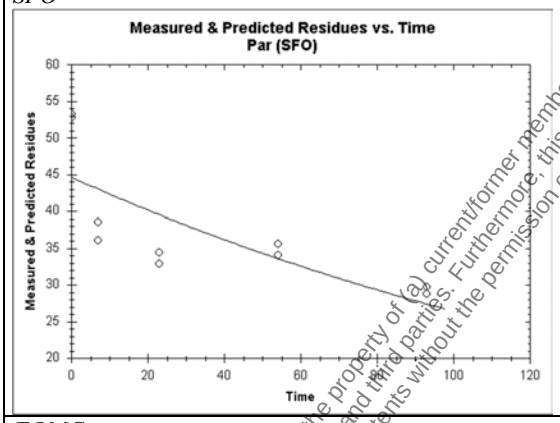
Table 7.2.2.3-37: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

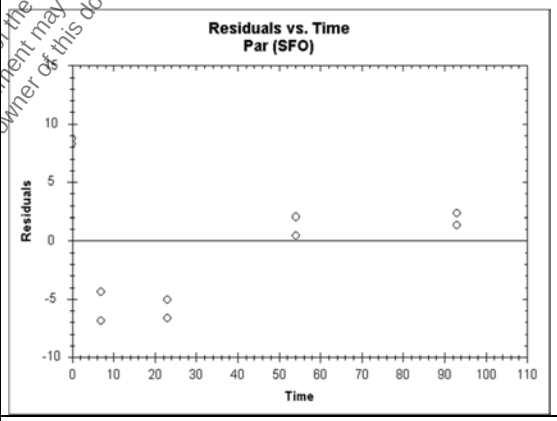
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	44.6	k: 0.0052	11.4	k: 0.008	k: 0.0019	k: 0.0090	132.3	439.4
FOMC	Acceptable	53.1	α: 0.0738 β: 0.0637	3.5	-[1]	β: -0.1582	β: 0.2860	764.6	>1000
DFOP	Good	53.1	k_1: 0.3503 k_2: 0.0020 g: 0.3137	3.6	k_1: 0.061 k_2: 0.025	k_1: -0.0310 k_2: 0.0004	k_1: 0.7320 k_2: 0.0040	158.7	965.3
HS	Good	53.1	k_1: 0.1242 k_2: 0.0024 tb: 2.9	3.8	k_1: <0.001 k_2: 0.005	k_1: 0.0973 k_2: 0.0011	k_1: 0.1510 k_2: 0.0040	145.0	825.5

Dissipation of glyphosate in sediment is best described by bi-phasic models. All bi-phasic models provide visually acceptable or good fits. The statistical fit of the FOMC model is not reliable as the confidence interval of parameter β includes zero. The DFOP model provides the best visual fit and is selected as the best-fit model as well as for deriving modelling endpoints.

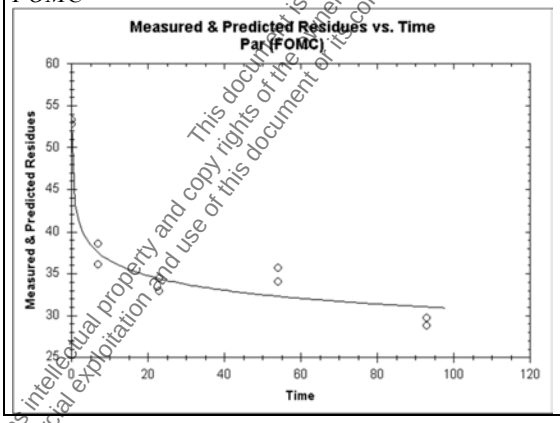
Conclusion: DFOP to be used for trigger endpoints
DFOP to be used for modelling endpoints

SFO





FOMC



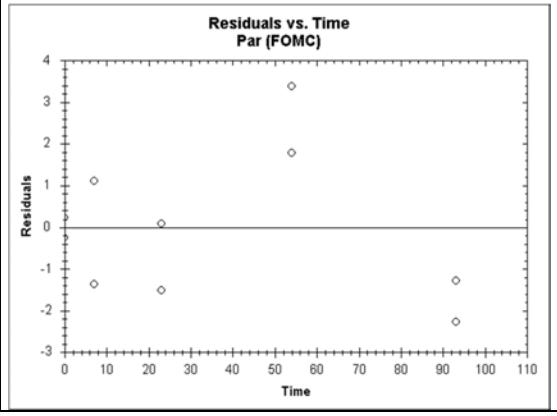
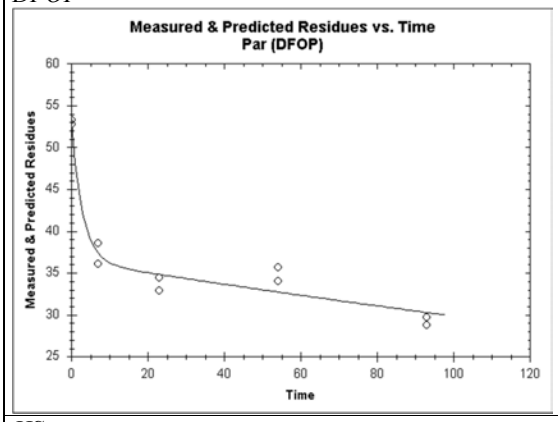
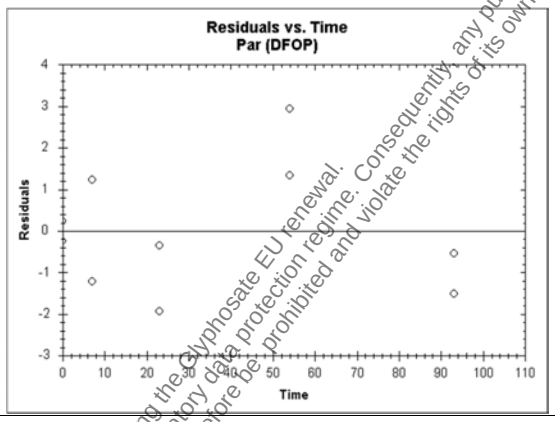


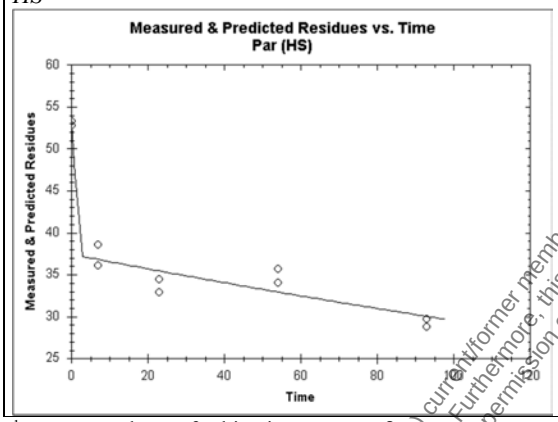
Table 7.2.2.3-37: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

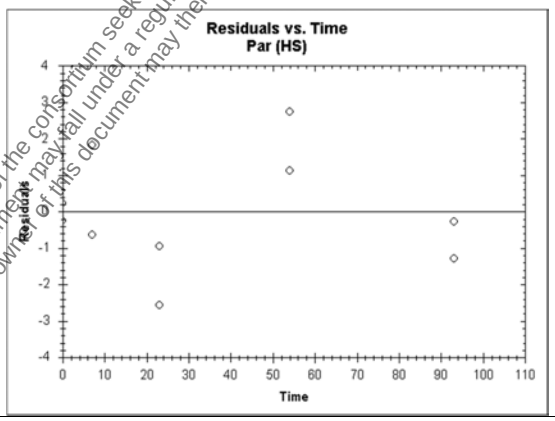
DFOP





HS





[1] t-test not relevant for kinetic parameter β

Table 7.2.2.3-38: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-II

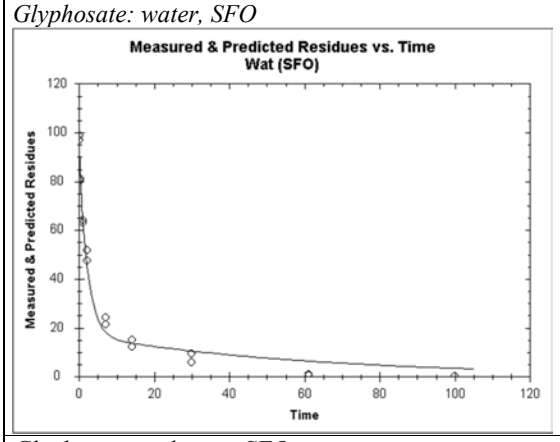
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Accep-table	93.7	k_{wat}: 0.0768 k_{wat_sed}: 0.3007	8.2	k_{wat}: 0.0144 k_{wat_sed}: <0.001	k_{wat}: 0.0111 k_{wat_sed}:0.2301	k_{wat}: 0.143 k_{wat_sed}: 0.371	9.0	30.0
Sediment: SFO	Poor	0.0	k_{sed}: 2.34×10^{-14} k_{sed_wat}: 0.0958	23.0	k_{sed}: 0.5 k_{sed_wat}: <0.001	k_{sed}: -0.0243 k_{sed_wat}:0.0551	k_{sed}: 0.024 k_{sed_wat}: 0.136	>1000	>1000

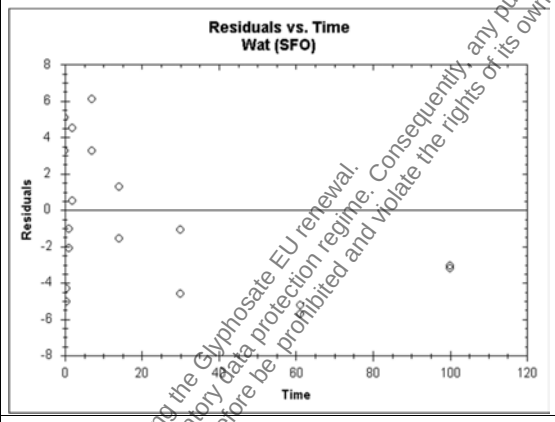
The visual and statistical fits obtained for the water phase are reliable but the visual fit obtained for the sediment phase is poor.

Conclusion: No further evaluation was conducted. No reliable endpoints could be derived

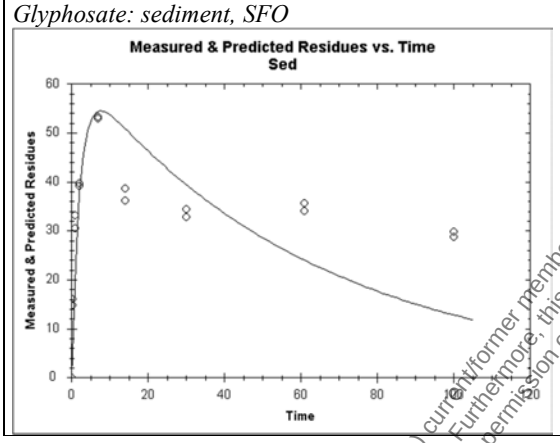
Table 7.2.2.3-38: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level P-II

Glyphosate: water, SFO





Glyphosate: sediment, SFO



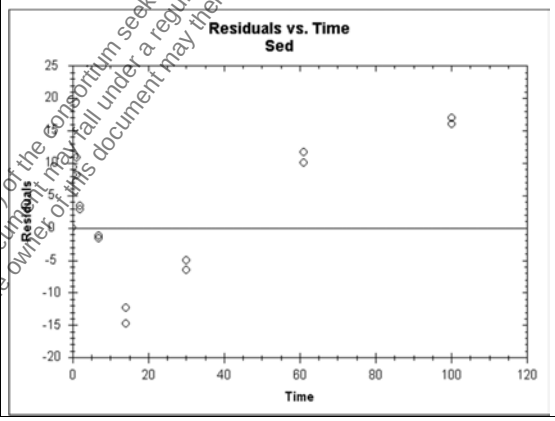


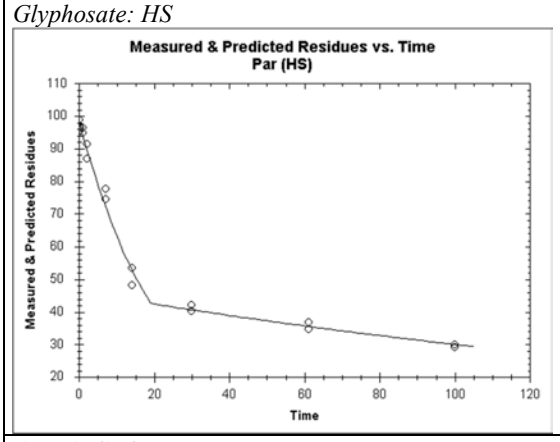
Table 7.2.2.3-39: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation

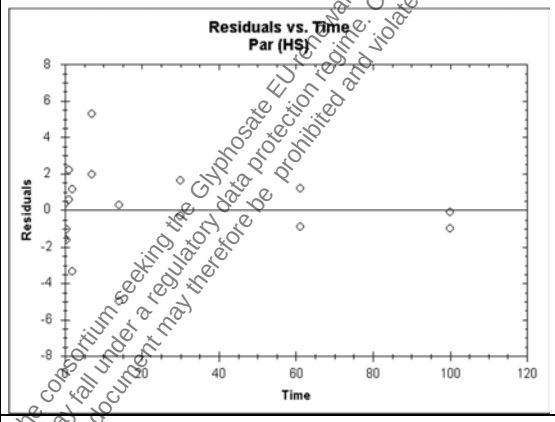
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: HS	Good	98.4	k_1: 0.0439 k_2: 0.0043 tb: 19.1	2.2	k_1: <0.001 k_2: <0.001	k_1: 0.0398 k_2: 0.0026	k_1: 0.0480 k_2: 0.0060	15.8	358.4	-
AMPA: SFO	Accep-table	0.0	k: 0.0442	9.4	k: <0.001	k: 0.0351	k: 0.0530	15.7	52.2	0.489 (±0.035) (from parent)
HMPA: SFO	Accep-table	0.0	k: 0.0052	22.6	k: 0.130	k: -0.0037	k: 0.0140	133.6	443.9	0.366 (±0.085) (from AMPA)

Table 7.2.2.3-39: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation

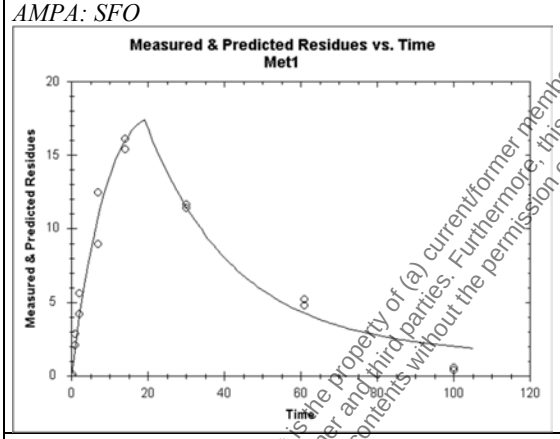
The fit of glyphosate at Level M-I degradation is comparable to that at Level P-I total system. For AMPA, both the visual and statistical fits from the SFO model are acceptable. For HMPA, the visual fit is acceptable but the degradation parameter k is not significantly different from zero. As the formation of HMPA is correctly described by the model and the standard deviation of the estimated formation fraction is low, the derived formation fraction is considered reliable. **Conclusion:** A second fitting step with fixed formation fraction from AMPA to HMPA was conducted.

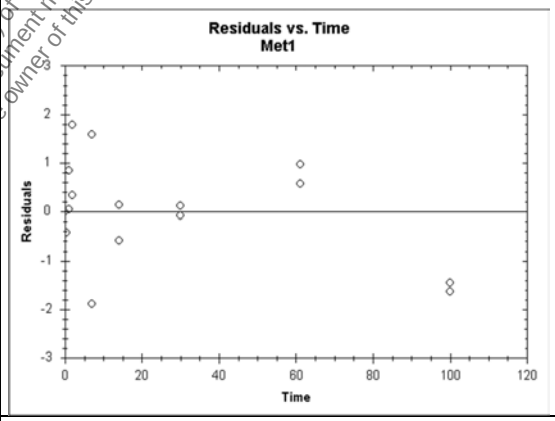
Glyphosate: HS



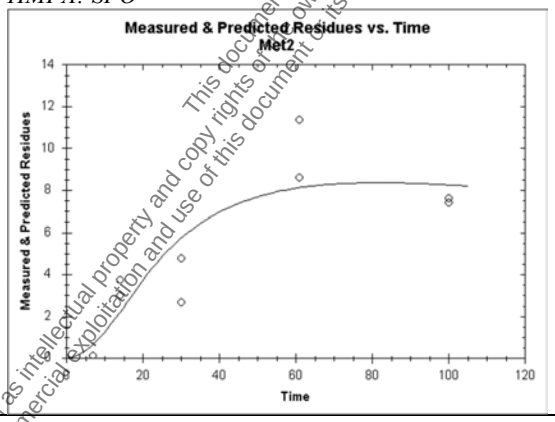


AMPA: SFO





HMPA: SFO



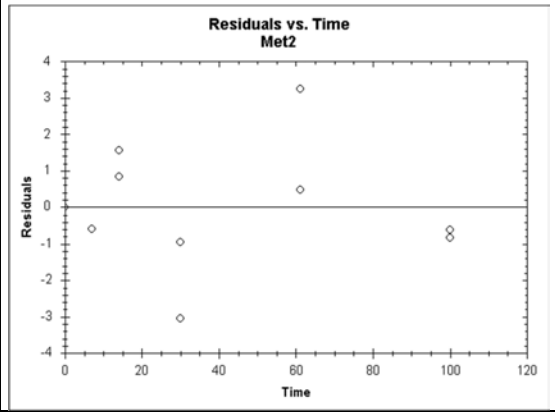


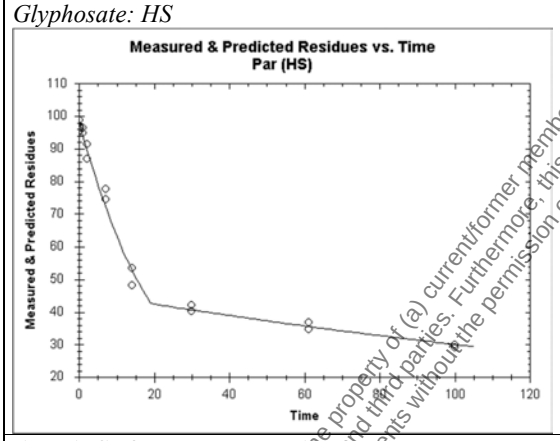
Table 7.2.2.3-40: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation (formation fraction from AMPA to HMPA fixed)

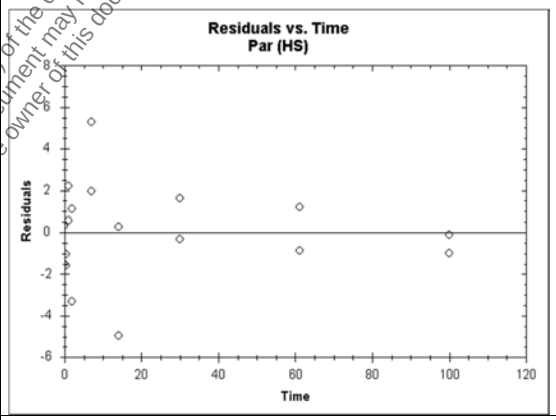
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: HS	Good	98.4	k_1: 0.0440 k_2: 0.0043 tb: 19.1	2.2	k_1: <0.001 k_2: <0.001	k_1: 0.0401 k_2: 0.0026	k_1: 0.0480 k_2: 0.0060	15.8	358.9	-
AMPA: SFO	Accep-table	0.0	k: 0.0440	9.4	k: <0.001	k: 0.0364	k: 0.0520	15.7	52.3	0.488 (±0.032) (from parent)
HMPA: SFO	Accep-table	0.0	k: 0.0054	20.5	k: 0.0157	k: 0.0006	k: 0.0100	128.8	427.8	0.366[1] (from AMPA)

The fit of glyphosate at Level M-I degradation is comparable to that at Level P-I total system degradation.
For AMPA, both the visual and statistical fits from the SFO model are acceptable.
For HMPA, both the visual and statistical fits from the SFO model are acceptable.

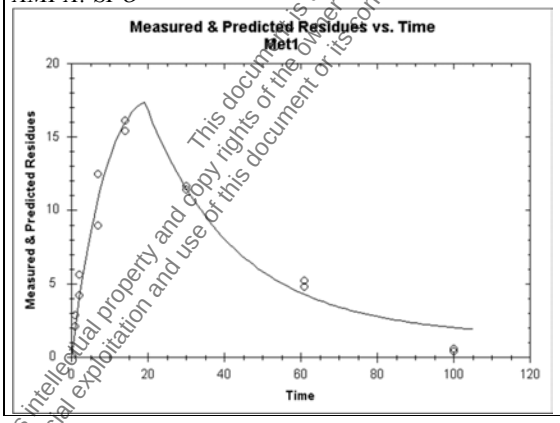
Conclusion: HS-SFO to be used for trigger endpoints of AMPA and HMPA
HS-SFO to be used for modelling endpoints of AMPA and HMPA

Glyphosate: HS





AMPA: SFO



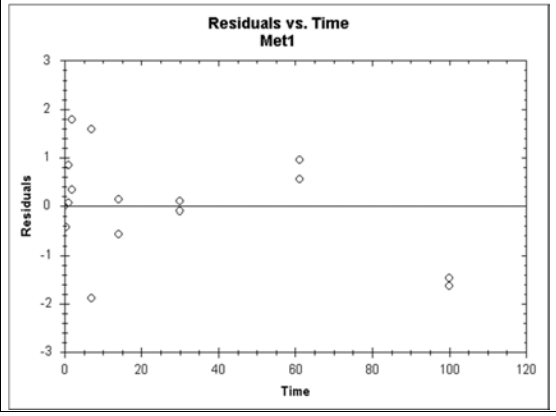
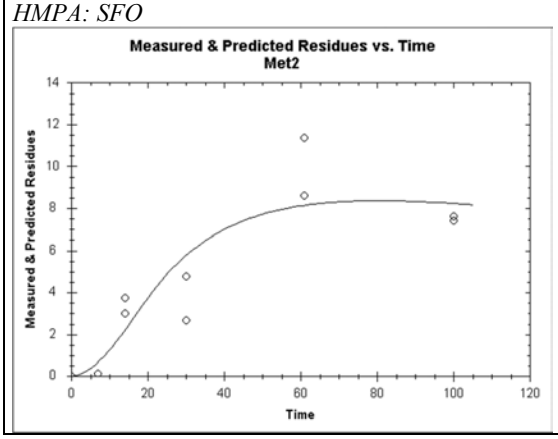


Table 7.2.2.3-40: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation (formation fraction from AMPA to HMPA fixed)

HMPA: SFO



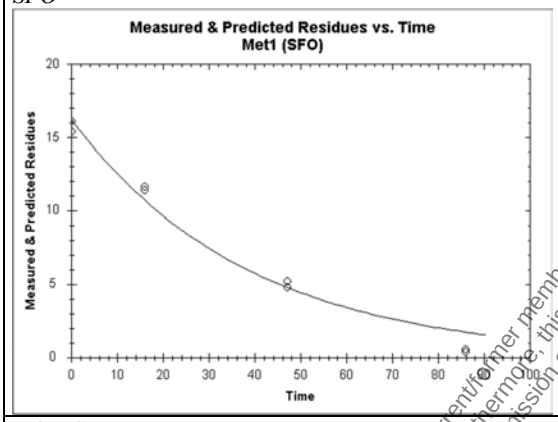
[1] Formation fraction from AMPA to HMPA was fixed to the estimated value obtained from an initial fitting step

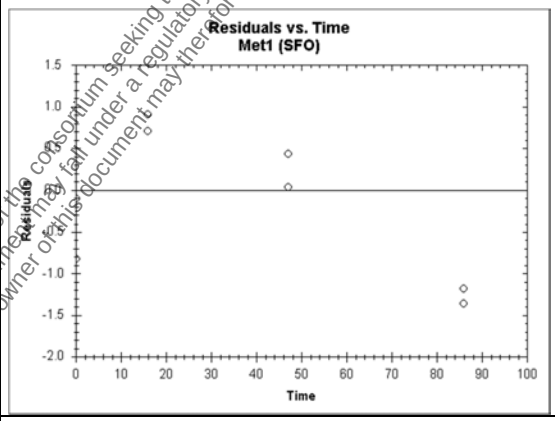
Table 7.2.2.3-41: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Bickenbach of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	16.2	k: 0.0259	7.9	k: <0.001	k: 0.0212	k: 0.0310	26.8	88.9
FOMC	Acceptable	16.2	α: 7234 β: 279100	9.9	-[1]	β: 279100	β: 279167	26.8	88.9

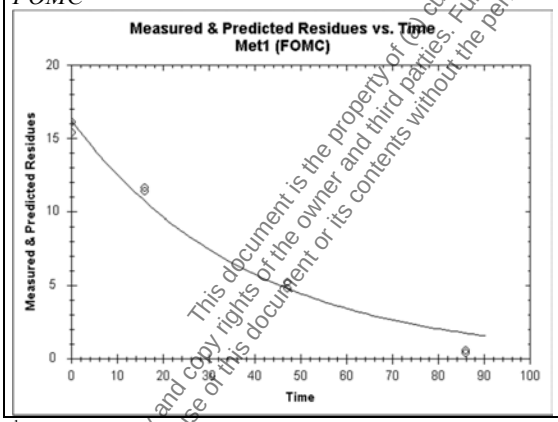
Only the SFO and FOMC model was used for evaluation due to the limited number of data points.
The visual and statistical fits from both models are acceptable but the χ^2 error resulting from the SFO model is smaller.
Thus, the SFO model is selected as the best-fit model as well as for modelling endpoints.
Conclusion: SFO to be used for trigger endpoints
SFO to be used for modelling endpoints

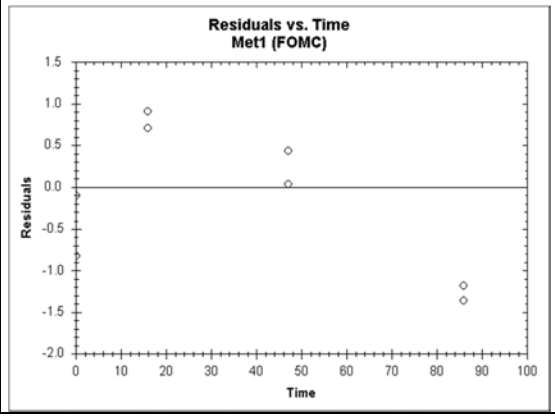
SFO





FOMC





[1] t-test not relevant for kinetic parameter β

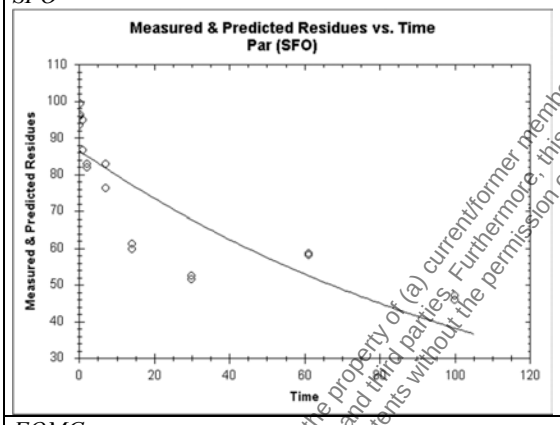
Table 7.2.2.3-42: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

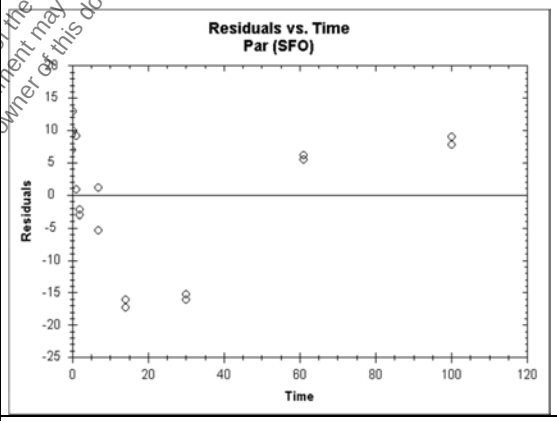
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	86.6	k: 0.0082	10.6	k: <0.001	k: 0.0052	k: 0.0110	84.7	281.3
FOMC	Acceptable	97.3	α: 0.1766 β: 1.7272	5.1	-[1]	β: -0.4493	β: 3.9040	85.7	>1000
DFOP	Acceptable	95.6	k_1: 0.1152 k_2: 0.0014 g: 0.4052	4.8	k_1: 0.007 k_2: 0.199	k_1: 0.0340 k_2:-0.0018	k_1: 0.1960 k_2: 0.0050	121.6	>1000
HS	Poor	97.2	k_1: 0.0774 k_2: 0.0044 tb: 4.3	6.9	k_1: 0.022 k_2: 0.001	k_1: 0.0091 k_2: 0.0021	k_1: 0.1460 k_2: 0.0070	85.0	447.6

Degradation of glyphosate is best described by bi-phasic models. The FOMC and DFOP models provide visually acceptable fits but the resulting parameters are not statistically reliable. Nevertheless, the DFOP model provides more reliable estimates of the DT_{50} and DT_{90} values as well as a smaller χ^2 error. Thus, the DFOP model is selected as the best-fit model.

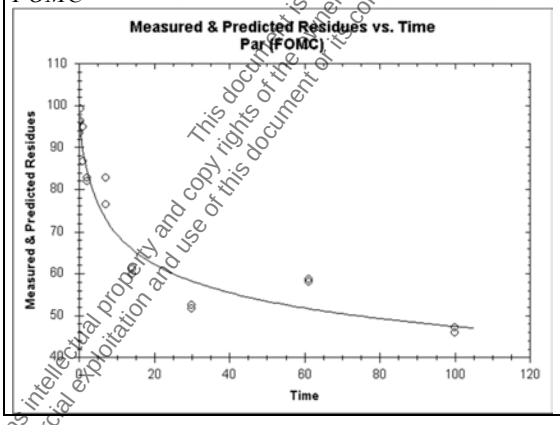
Conclusion: DFOP to be used for trigger endpoints
1000 d to be used for modelling as conservative approach

SFO





FOMC



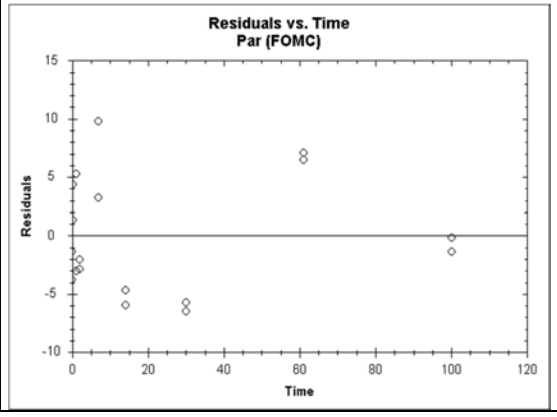
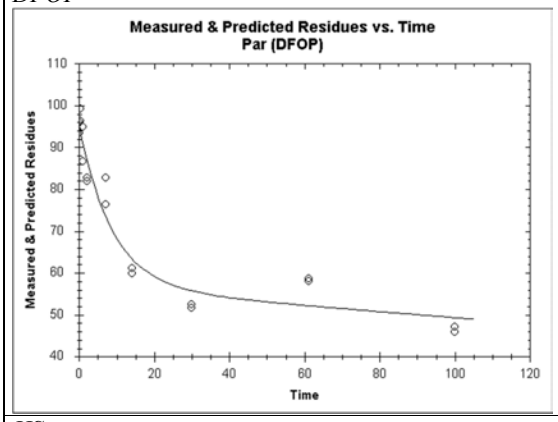
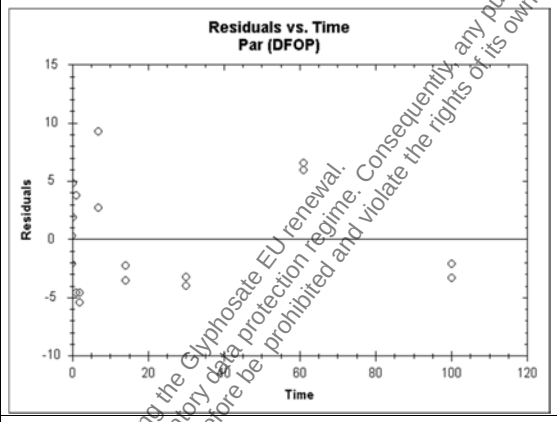


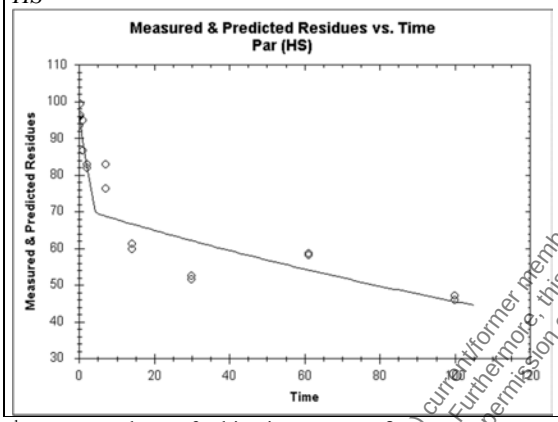
Table 7.2.2.3-42: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, total system

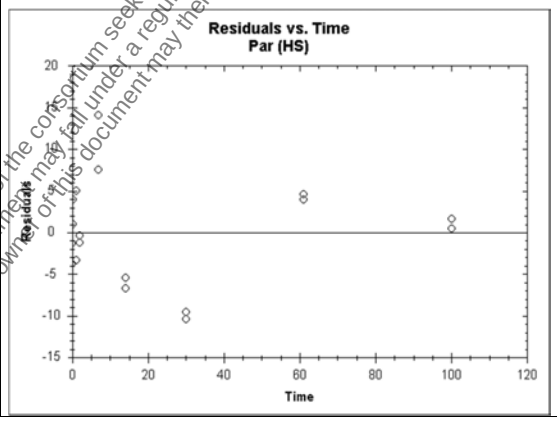
DFOP





HS





[1] t-test not relevant for kinetic parameter β

Table 7.2.2.3-43: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

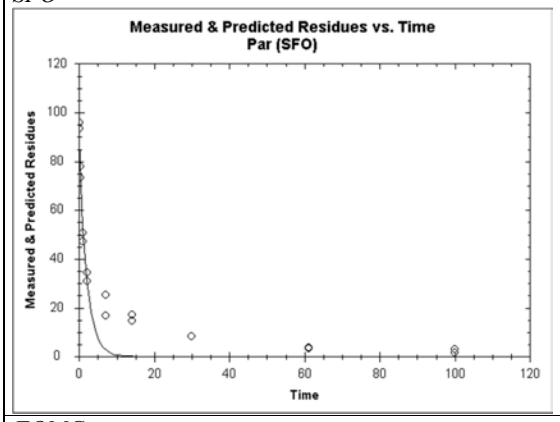
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	89.1	k: 0.4955	21.6	k: <0.001	k: 0.3130	k: 0.6780	1.4	4.6
FOMC	Good	95.3	α: 0.5818 β: 0.4649	4.9	-[1]	β: 0.2784	β: 0.6510	1.1	23.9
DFOP	Good	93.7	k_1: 1.1159 k_2: 0.0373 g: 0.7078	2.6	k_1: <0.001 k_2: <0.001	k_1:0.9006 k_2:0.0243	k_1: 1.3310 k_2: 0.0500	1.1	28.7
HS	Good	92.8	k_1: 0.665 k_2: 0.0518 tb: 1.6	5.3	k_1: <0.001 k_2: <0.001	k_1:0.5627 k_2:0.0353	k_1: 0.7670 k_2: 0.0680	1.0	25.1

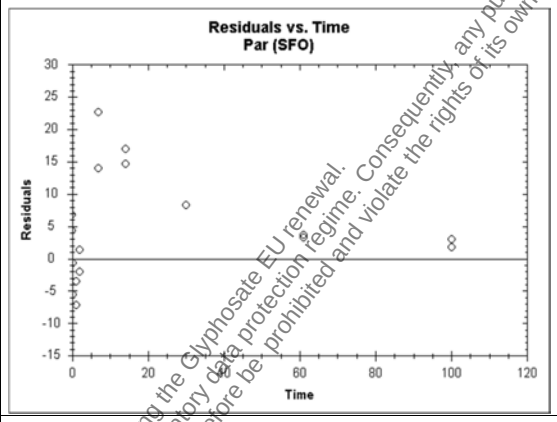
Dissipation of glyphosate is best described by bi-phasic models. All the bi-phasic models provide equally reliable and visually good results but the least χ^2 error is provided by the the DFOP model.

Conclusion: DFOP to be used for trigger endpoints
DFOP to be used for modelling endpoints

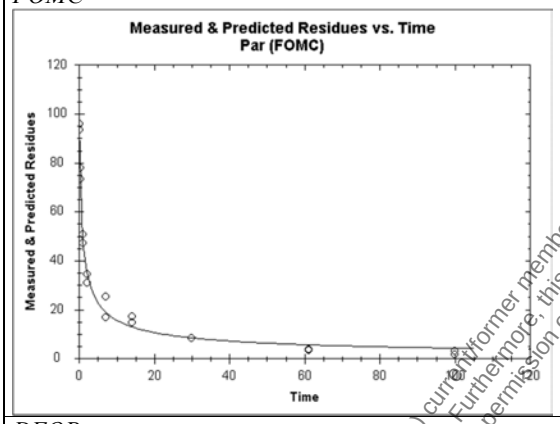
Table 7.2.2.3-43: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

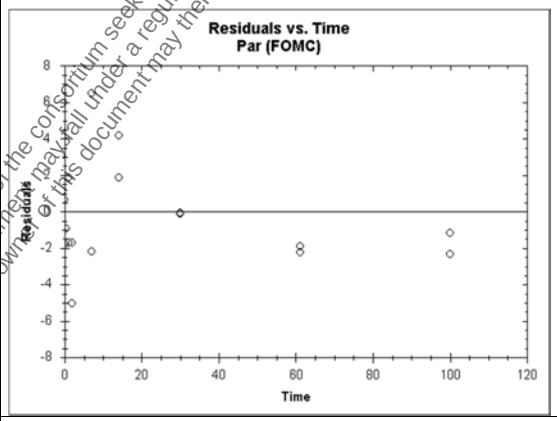
SFO



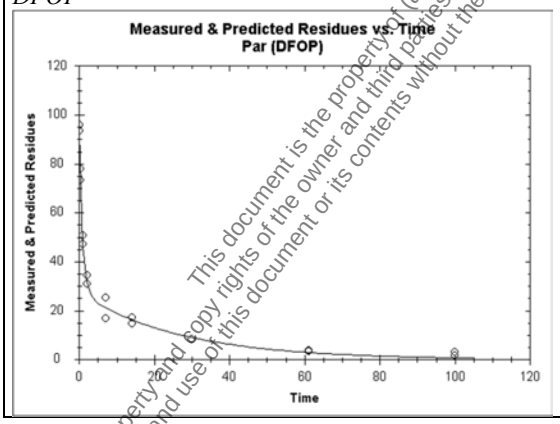


FOMC





DFOP



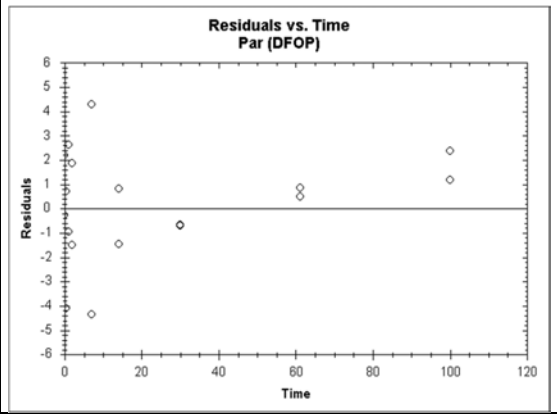
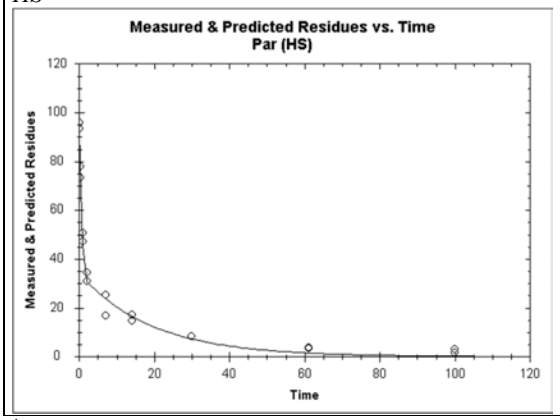
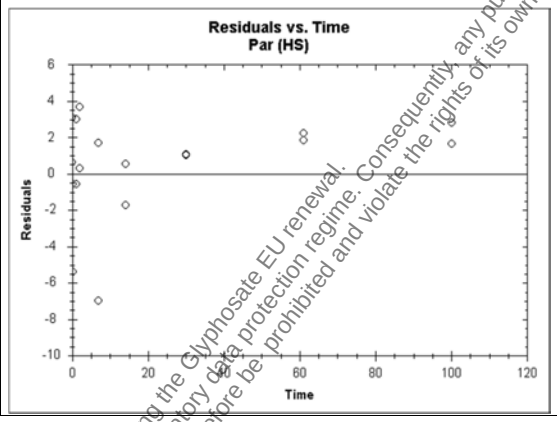


Table 7.2.2.3-43: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, water phase

HS



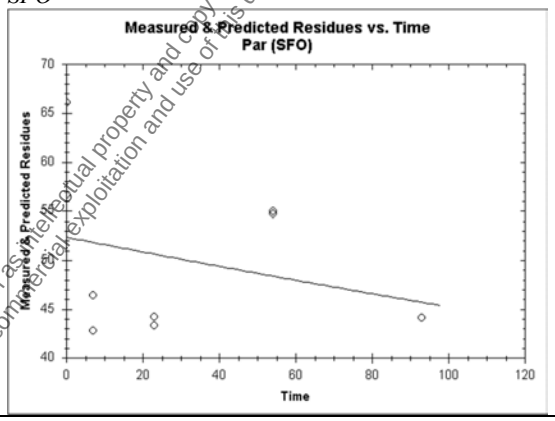


[1] t-test not relevant for kinetic parameter β

Table 7.2.2.3-44: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	52.3	k: 0.0015	10.8	k: 0.181	k: -0.0015	k: 0.0040	473.4	>1000
FOMC	Poor	61.4	α: 0.0012 β: 0	Inf	-[1]	β: NA[2]	β: NA[b]	>1000	>1000
DFOP	Poor	61.4	k_1: 11.3 k_2: 2.22×10^{-14} g: 0.2362	9.5	k_1: 0.5 k_2: 0.5	k_1: NA[2] k_2:-0.0028	k_1: NA[2] k_2: 0.0030	>1000	>1000
HS	Poor	52.3	k_1: 0.0015 k_2: 2.22×10^{-14} tb: 1078	15.4	k_1: 0.218 k_2: <0.001	k_1:-0.0020 k_2: 0.0000	k_1: 0.0050 k_2: 0.0000	473.4	>1000
Conclusion:	No reliable endpoints could be derived								

SFO



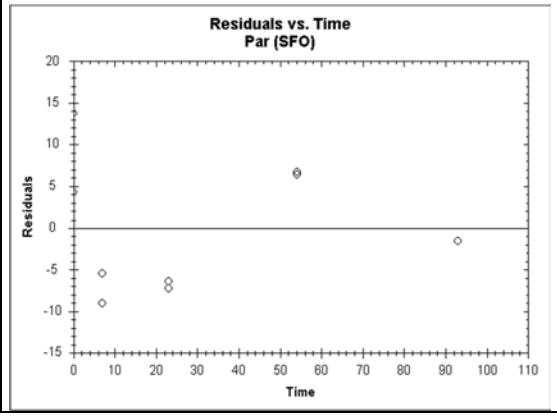
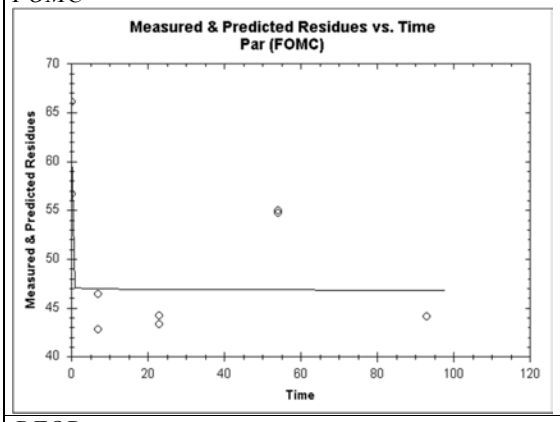
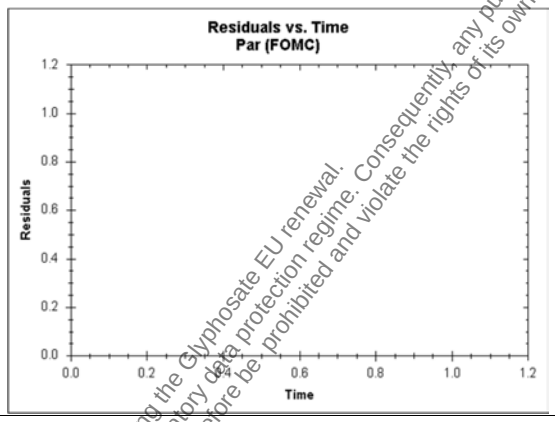


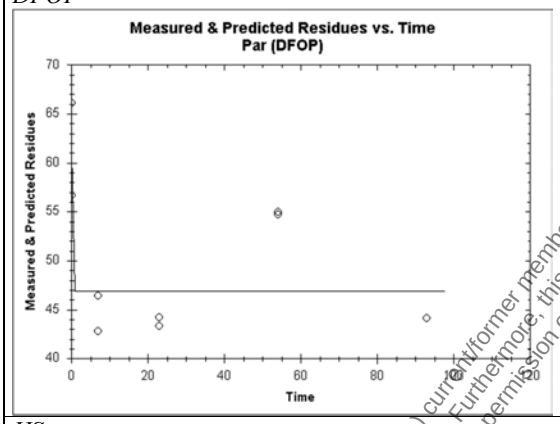
Table 7.2.2.3-44: Kinetic models and goodness-of-fit statistics of glyphosate dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-I, sediment phase

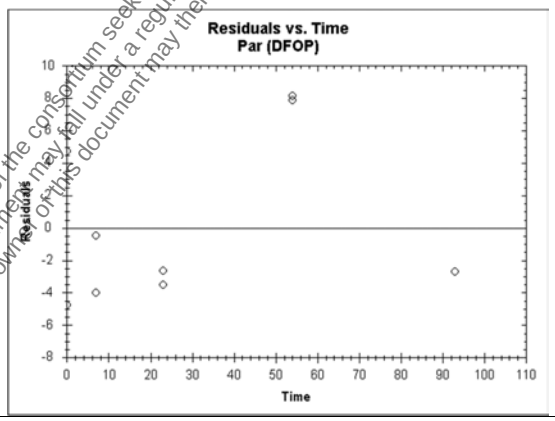
FOMC



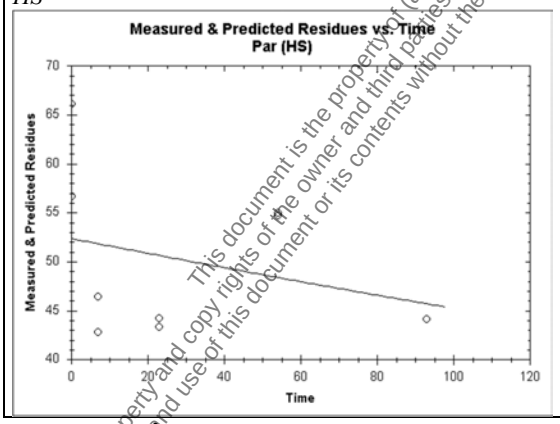


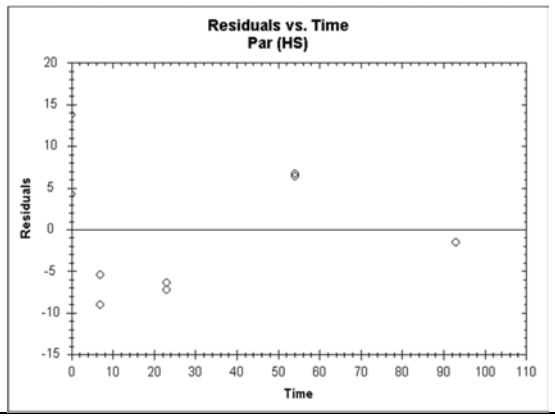
DFOP





HS





Inf = infinite; χ^2 error cannot be calculated

[1] t-test not relevant for kinetic parameter β

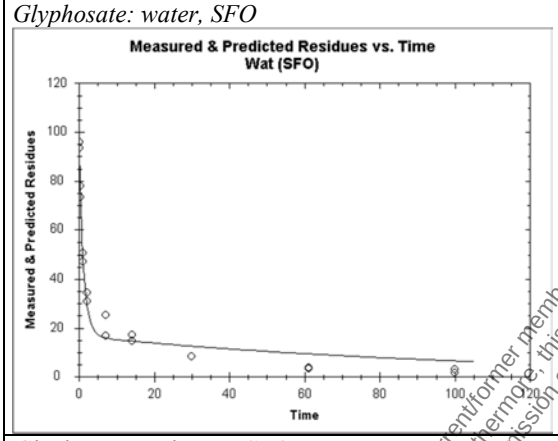
[2] Information from the KinGUI output: 'Hessian not invertible – NA was calculated for standard deviation, confidence interval and t-test'

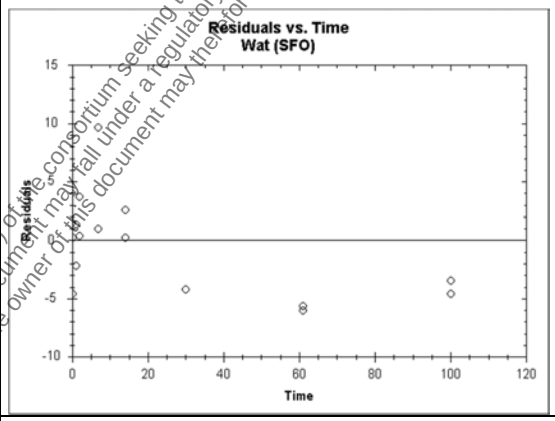
Table 7.2.2.3-45: Kinetic models and goodness-of-fit statistics of glyphosate degradation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level P-II

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Acceptable	91.9	k_{wat}: 0.0498 k_{wat_sed}: 0.6299	9.0	k_{wat}: 0.203 k_{wat_sed}: <0.001	k_{wat}: -0.0658 k_{wat_sed}: -0.4942	k_{wat}: 0.165 k_{wat_sed}: 0.766	13.9	46.3
Sediment: SFO	Poor	0.0	k_{sed}: 2.77×10^{-14} k_{sed_wat}: 0.1571	19.5	k_{sed}: 0.5 k_{sed_wat}: <0.001	k_{sed}: -0.0309 k_{sed_wat}: 0.1002	k_{sed}: 0.031 k_{sed_wat}: 0.214	>1000	>1000

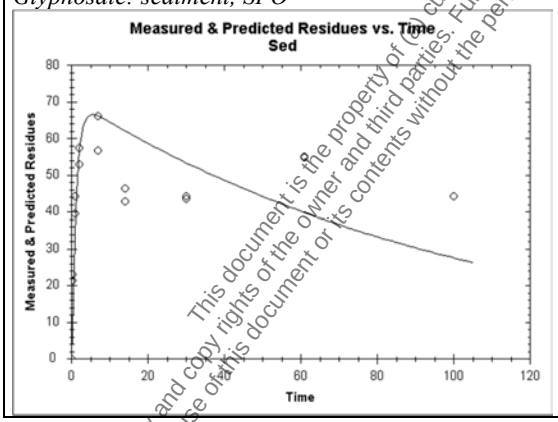
The visual fit obtained for the water phase is acceptable, but the visual fit obtained for the sediment phase is poor.
Conclusion: No further evaluation was conducted. No reliable endpoints could be derived

Glyphosate: water, SFO





Glyphosate: sediment, SFO



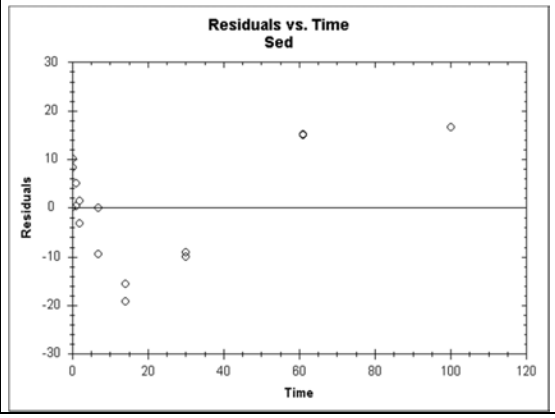


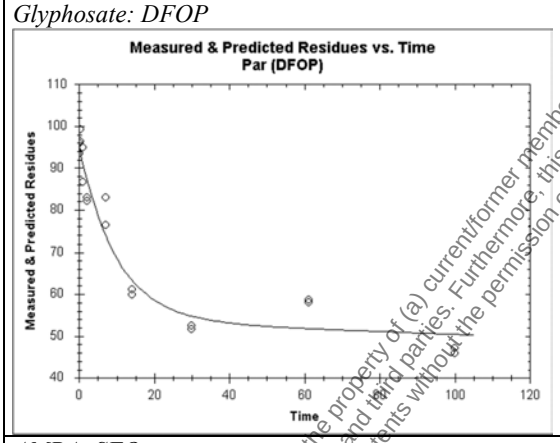
Table 7.2.2.3-46: Kinetic models and goodness-of-fit statistics of glyphosate and its metabolites AMPA and HMPA in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level M-I degradation

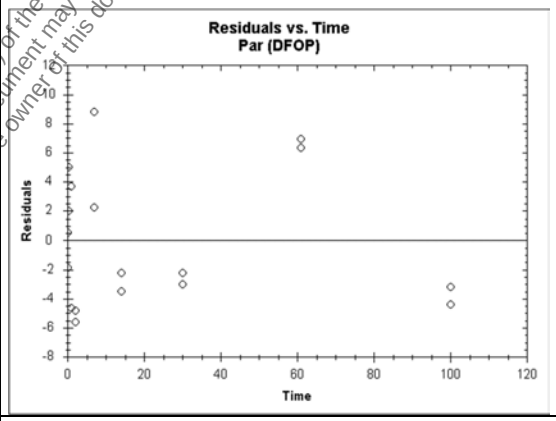
Kinetic model	Visual assess-ment	M_0	Kinetic para-meters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)	ff (± std. dev.)
Glyphosate: DFOP	Accep-table	95.4	k_1: 0.1004 k_2: 0.0006 g: 0.4370	4.9	k_1: <0.001 k_2: 0.2986	k_1: 0.0479 k_2: -0.0017	k_1: 0.1530 k_2: 0.0030	187.9	>1000	-
AMPA: SFO	Accep-table	0.0	k: 0.0788	22.4	k: <0.001	k: 0.0329	k: 0.1250	8.8	29.2	0.321 (±0.076) (from parent)
HMPA: SFO	Accep-table	0.0	k: 0.0690	39.3	k: 0.0032	k: 0.0218	k: 0.1160	10.0	33.4	0.359 (±0.159) (from AMPA)

The fit of glyphosate at level M-I degradation is comparable to that at level P-I total system.
For AMPA and HMPA, both the visual and statistical fits from the SFO model are acceptable The χ^2 values above 15 % are acceptable as measured data are overall well represented by the fit.

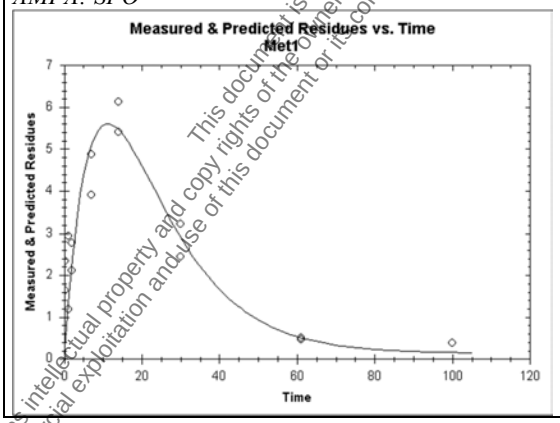
Conclusion: DFOP-SFO to be used for trigger endpoints of AMPA and HMPA
DFOP-SFO to be used for modelling endpoints of AMPA and HMPA

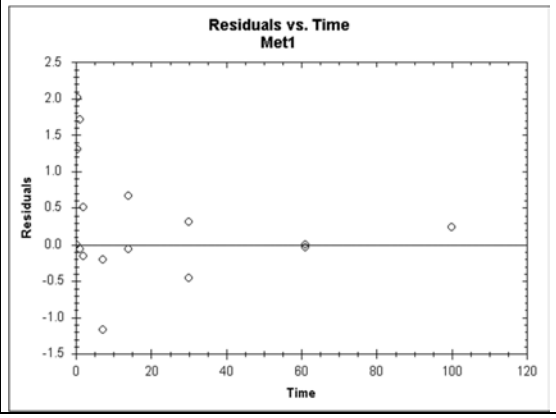
Glyphosate: DFOP



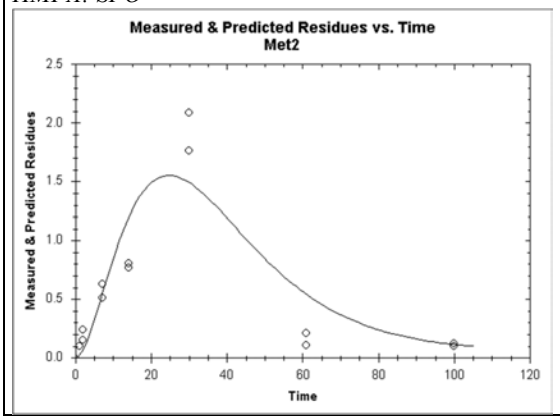


AMPA: SFO





HMPA: SFO



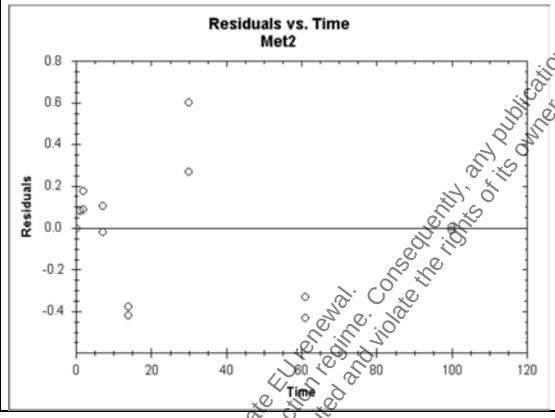


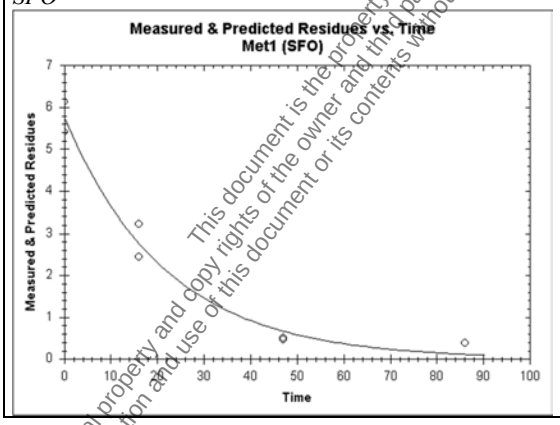
Table 7.2.2.3-47: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	5.8	k: 0.0461	5.8	k: < 0.001	k: 0.0361	k: 0.056	15.1	50.0
FOMC	Acceptable	5.8	α: 43.940 β: 940.9	7.2		β: -1013	β: 23915	15.0	50.6

Only the SFO and FOMC model were used for evaluation due to the limited number of data points.
The visual and statistical fits from the SFO model are acceptable while the statistical fit of the FOMC model is not reliable as the confidence interval of parameter β includes zero. Thus, the SFO model is selected as the best-fit model as well as for modelling endpoints.

Conclusion: SFO to be used for trigger endpoints
SFO to be used for modelling endpoints

SFO



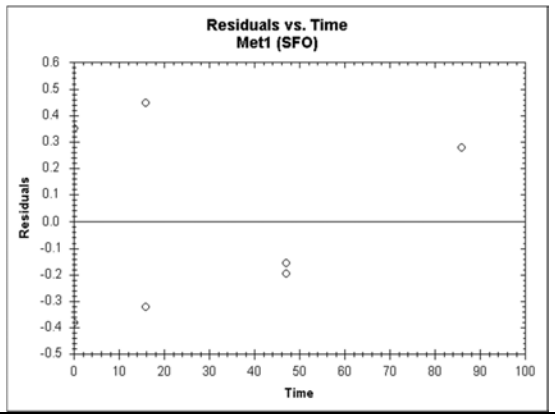
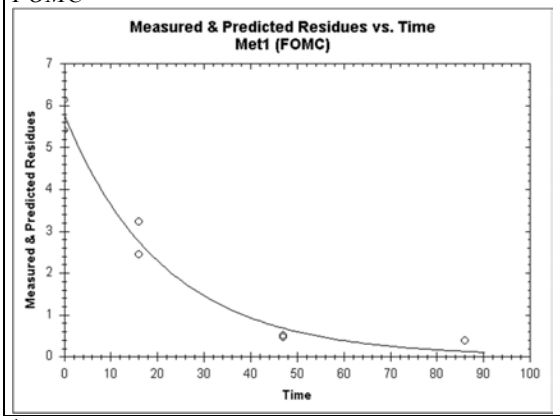
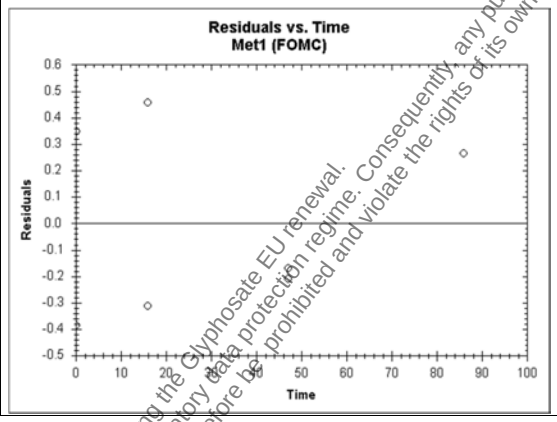


Table 7.2.2.3-47: Kinetic models and goodness-of-fit statistics of metabolite AMPA dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

FOMC





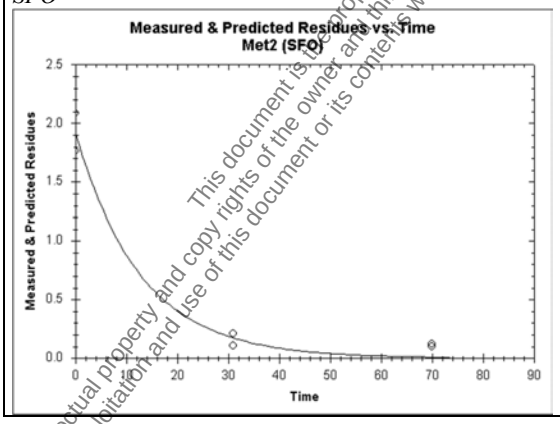
[1] t-test not relevant for kinetic parameter β

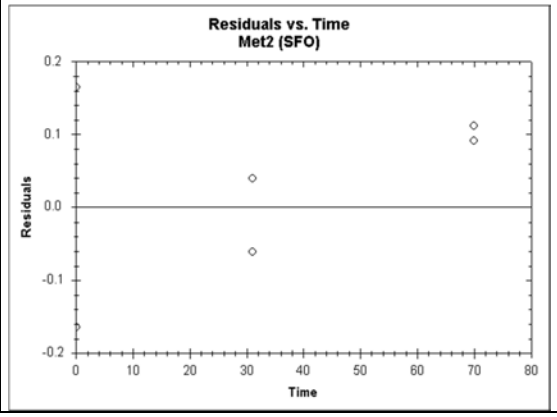
Table 7.2.2.3-48: Kinetic models and goodness-of-fit statistics of metabolite HMPA dissipation in system Unter Widdersheim of study [redacted] (1993, CA 7.2.2.3/005), Level M-I dissipation, total system & water phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	1.9	k: 0.0781	7.1	k: 0.007	k: 0.0410	k: 0.1150	8.9	29.5

Only the SFO model was used for the evaluation due to the limited number of data points.
The visual and statistical fit from the SFO model is acceptable.
Conclusion: SFO to be used for trigger endpoints
SFO to be used for modelling endpoints

SFO





[redacted] (2002, CA 7.2.2.3/020)

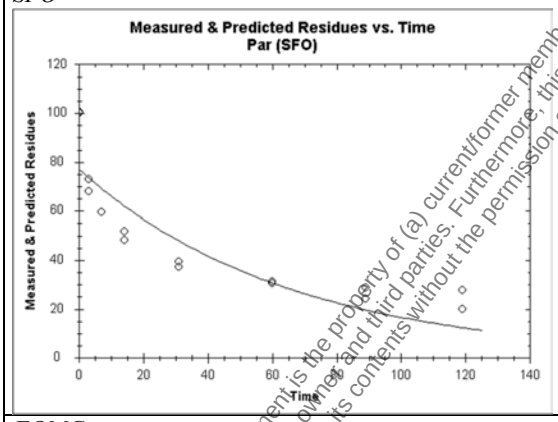
Table 7.2.2.3-49: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

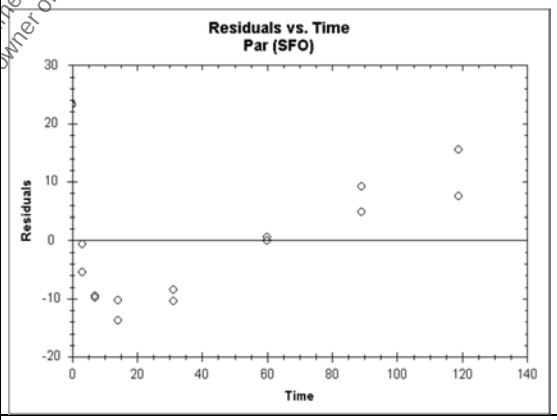
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	77.0	k: 0.0154	18.3	k: <0.001	k: 0.0094	k: 0.0210	45.1	149.8
FOMC	Good	100.0	α: 0.3317 β: 1.7846	1.6	-[1]	β: 1.1339	β: 2.4350	12.6	>1000
DFOP	Good	99.5	k_1: 0.2458 k_2: 0.0073 g: 0.4858	3.8	k_1: <0.001 k_2: <0.001	k_1: 0.1645 k_2: 0.0053	k_1: 0.3270 k_2: 0.0090	11.7	225.3
HS	Acceptable	100.3	k_1: 0.1175 k_2: 0.0091 tb: 5.0	5.8	k_1: <0.001 k_2: <0.001	k_1: 0.0830 k_2: 0.0071	k_1: 0.1520 k_2: 0.0110	16.1	193.0

Degradation of AMPA is best described by bi-phasic models. The FOMC and the DFOP models provide better visual and statistical fits than the HS model. The FOMC model provides the best visual fit and is selected as the best-fit model. Since 10 % of the initially measured substance concentration was not reached within the experimental period, the DFOP model is selected for deriving modelling endpoints.

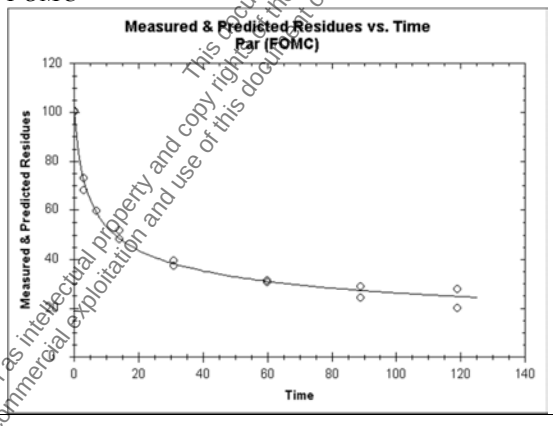
Conclusion: FOMC to be used for trigger endpoints
DFOP to be used for modelling endpoints

SFO





FOMC



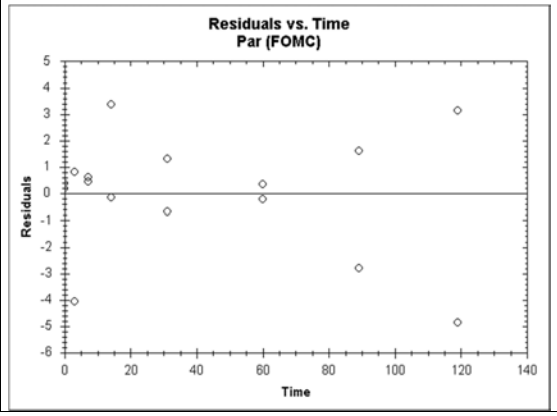
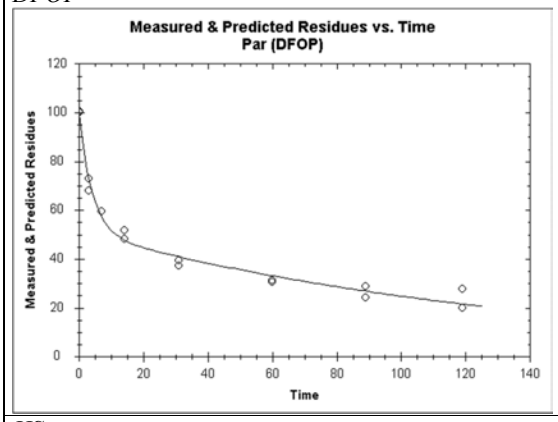
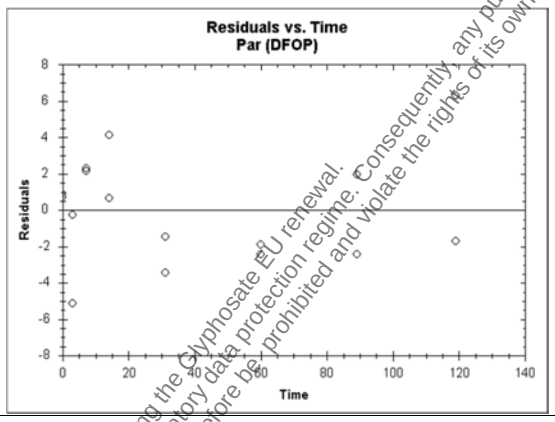


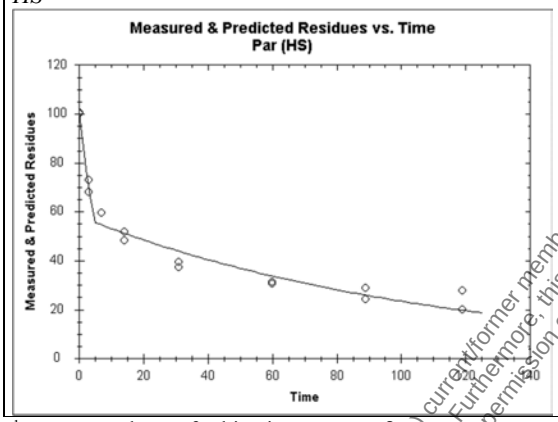
Table 7.2.2.3-49: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

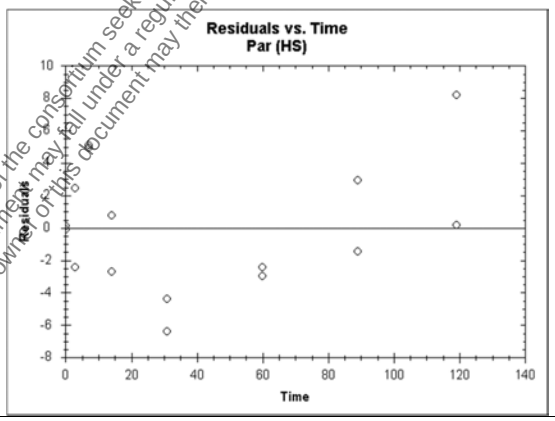
DFOP





HS





[1] t-test not relevant for kinetic parameter β

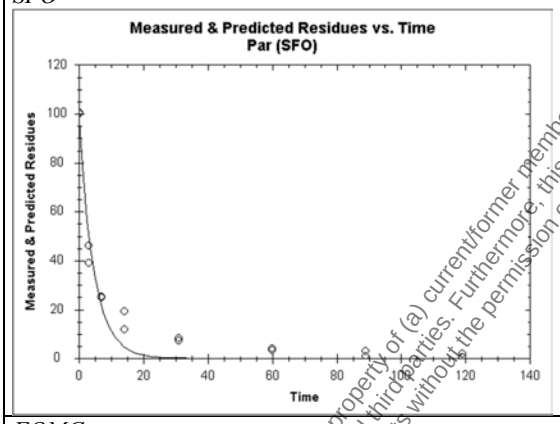
Table 7.2.2.3-50: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

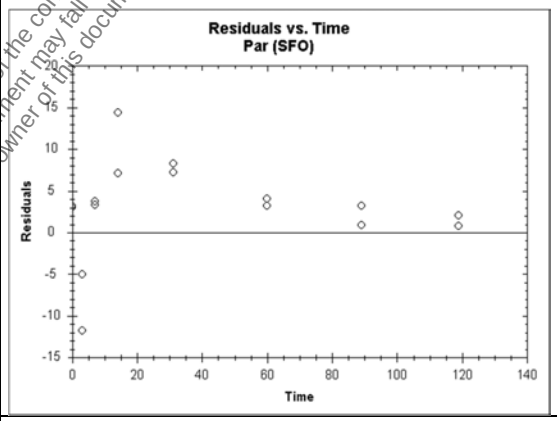
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	97.2	k: 0.2145	19.2	k: <0.001	k: 0.1685	k: 0.2610	3.2	10.7
FOMC	Good	100.3	α: 0.9301 β: 2.0284	2.1	-[1]	β: 1.2818	β: 2.7750	2.2	22.1
DFOP	Good	100.2	k_1: 0.4512 k_2: 0.0365 g: 0.7311	3.0	k_1: <0.001 k_2: <0.001	k_1: 0.3505 k_2: 0.0221	k_1: 0.5520 k_2: 0.0510	2.4	27.1
HS	Good	100.3	k_1: 0.285 k_2: 0.0455 tb: 4.7	4.3	k_1: <0.001 k_2: <0.001	k_1: 0.2555 k_2: 0.0301	k_1: 0.3140 k_2: 0.0610	2.4	26.1

Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide equally reliable and visually acceptable results but the FOMC model provides the least χ^2 error and is selected as best-fit model as well as for modelling endpoints.

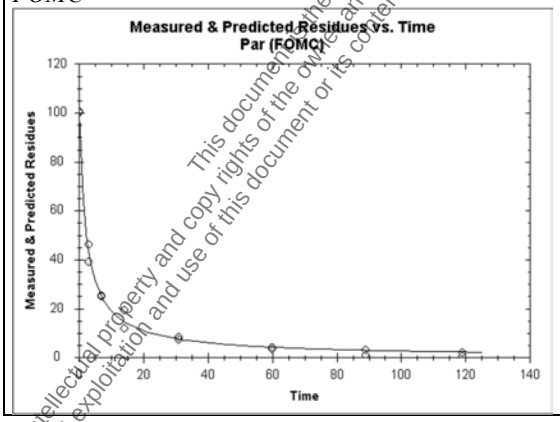
Conclusion: FOMC to be used for trigger endpoints
FOMC to be used for modelling endpoints

SFO





FOMC



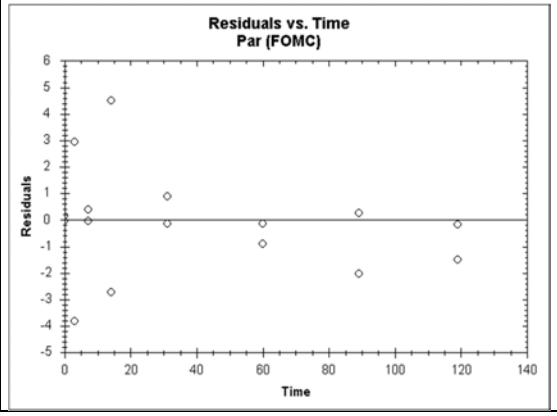
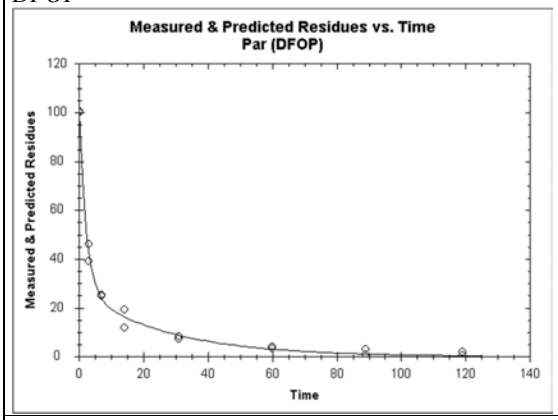
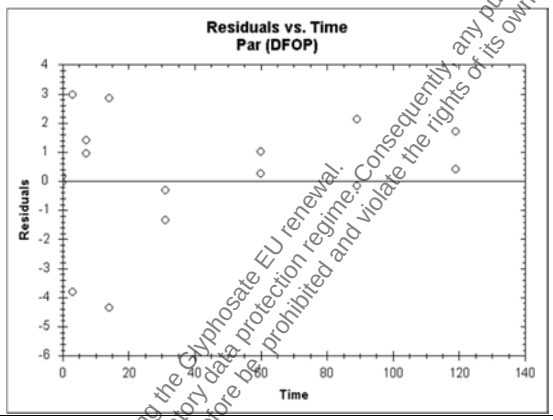


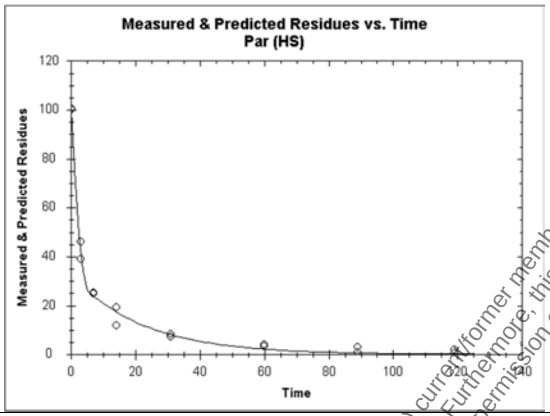
Table 7.2.2.3-50: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

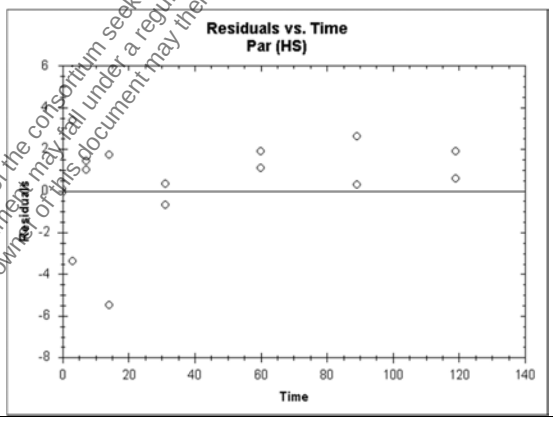
DFOP





HS





[1] t-test not relevant for kinetic parameter β

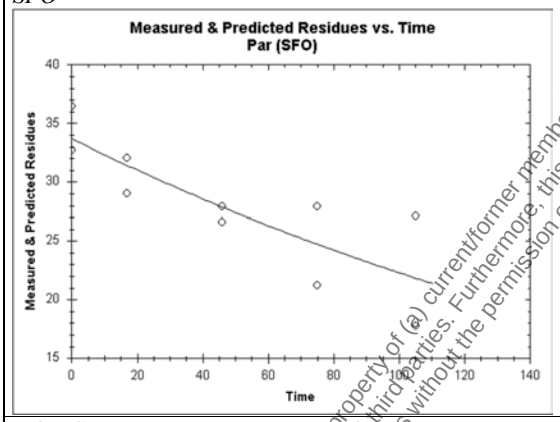
Table 7.2.2.3-51: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, sediment phase

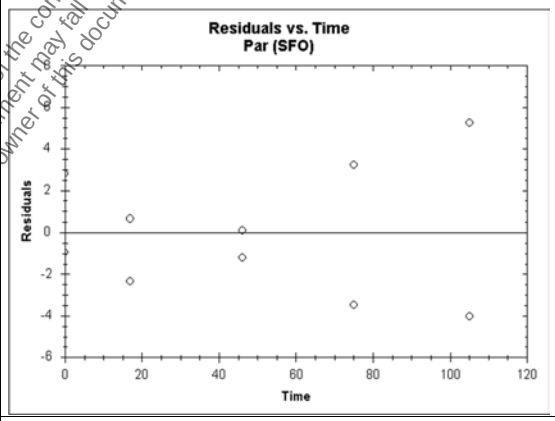
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Good	33.7	k: 0.0041	1.9	k: 0.002	k: 0.0022	k: 0.0060	168.1	558.3
FOMC	Good	34.5	α: 0.3612 β: 47.3107	0.7	-[1]	β: -194.89	β: 289.52	275.1	>1000
DFOP	Good	34.6	k_1: 0.0843 k_2: 0.0033 g: 0.0888	0.3	k_1: 0.427 k_2: 0.174	k_1: -0.7781 k_2: -0.0030	k_1: 0.9470 k_2: 0.0100	184.0	677.5
HS	Good	34.6	k_1: 0.0073 k_2: 0.0033 tb: 21.8	0.3	k_1: 0.153 k_2: 0.115	k_1: -0.0055 k_2: -0.0016	k_1: 0.0200 k_2: 0.0080	182.4	666.9

All models provide good visual fits but the statistical fits provided by the bi-phasic models are not reliable. Thus, the SFO model is selected as the best-fit model as well as for deriving modelling endpoints.

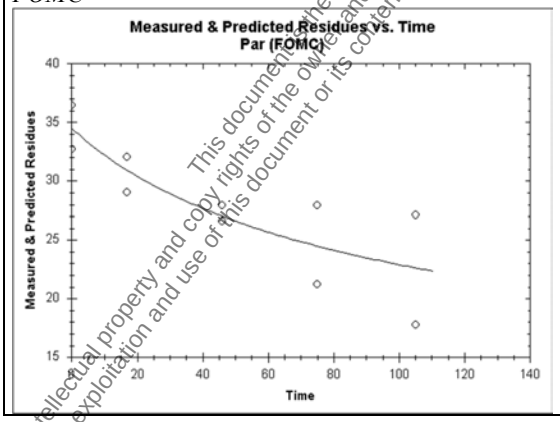
Conclusion: SFO to be used for trigger endpoints
SFO to be used for modelling endpoints

SFO





FOMC



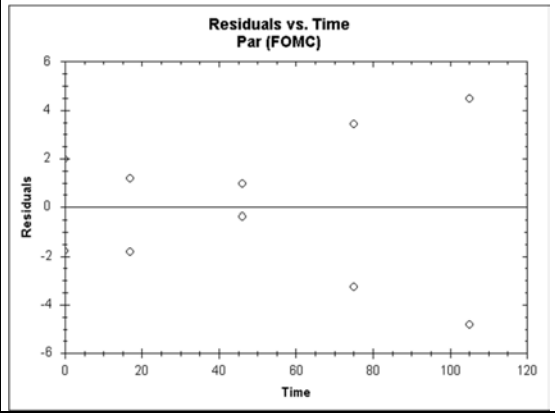
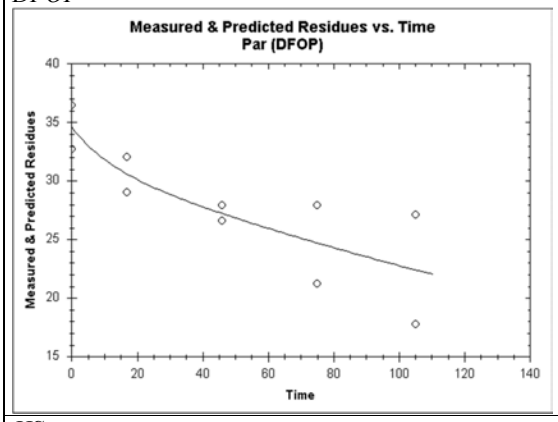
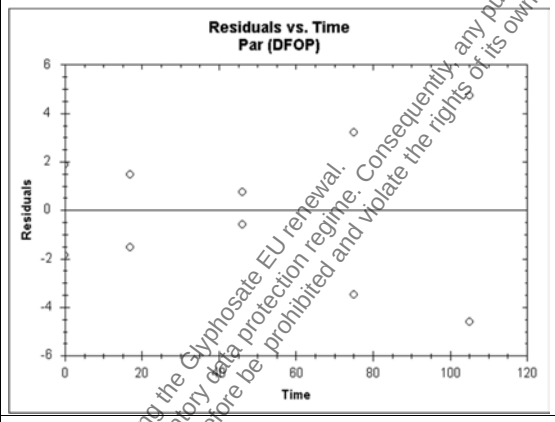


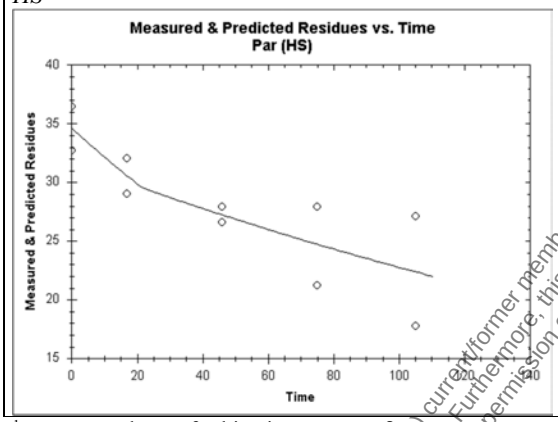
Table 7.2.2.3-51: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, sediment phase

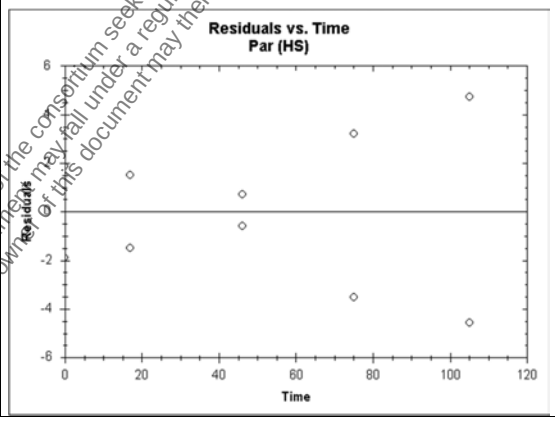
DFOP





HS





[1] t-test not relevant for kinetic parameter β

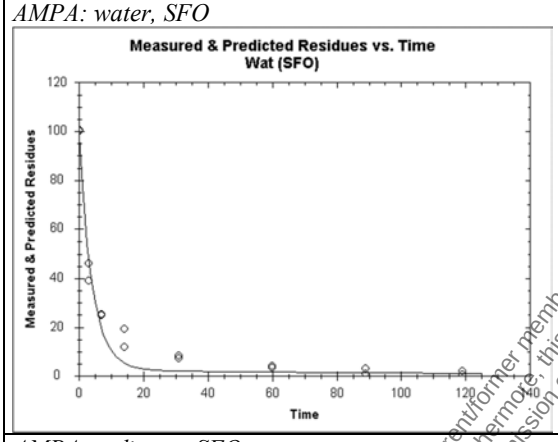
Table 7.2.2.3-52: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Rückhaltebecken of study [redacted] (2002, CA 7.2.2.3/020), Level P-II

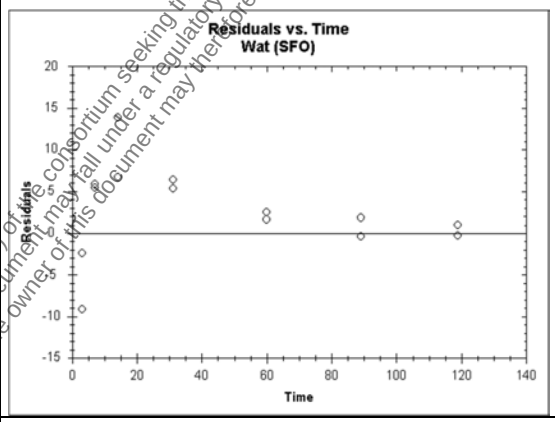
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Poor	98.5	k_{wat}: 0.1343 k_{wat_sed}: 0.1052	17.6	k_{wat}: <0.001 k_{wat_sed}: <0.001	k_{wat}: 0.0996 k_{wat_sed}: -0.0857	k_{wat}: 0.169 k_{wat_sed}: 0.125	5.1	17.2
Sediment: SFO	Acceptable	0.0	k_{sed}: 2.34×10^{-14} k_{sed_wat}: 0.0129	9.6	k_{sed}: 0.5 k_{sed_wat}: 0.199	k_{sed}: -0.0169 k_{sed_wat}: -0.0166	k_{sed}: 0.017 k_{sed_wat}: 0.042	>1000	>1000

Although the visual fit obtained for the sediment phase is acceptable, the visual fit obtained for the water phase is poor.

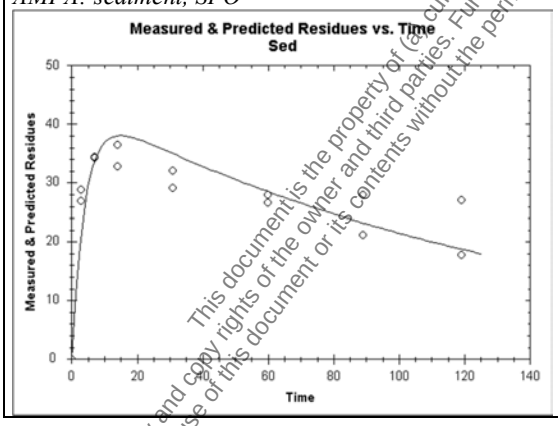
Conclusion: No further evaluation was conducted, no reliable endpoints could be derived

AMPA: water, SFO





AMPA: sediment, SFO



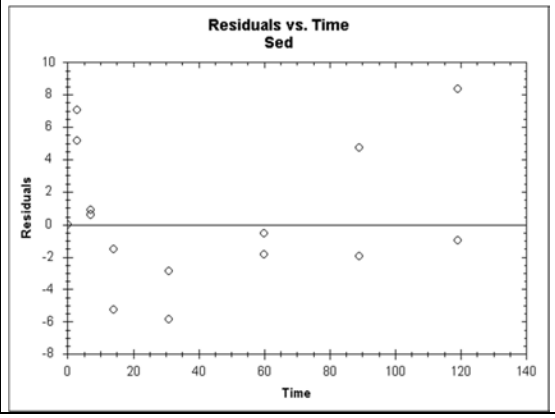


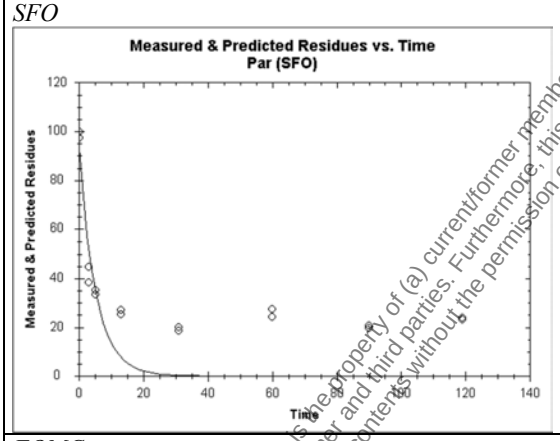
Table 7.2.2.3-53: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

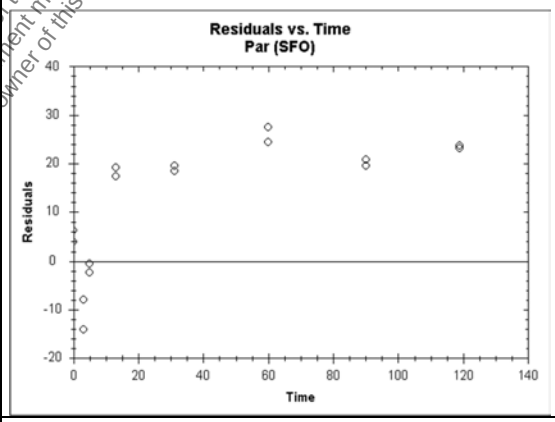
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	93.4	k: 0.1919	38.8	k: 0.003	k: 0.0730	k: 0.3110	3.6	12.0
FOMC	Acceptable	98.6	α: 0.1836 β: 0.0179	7.6	-[1]	β: -0.0196	β: 0.0550	0.8	>1000
DFOP	Good	98.4	k_1: 0.4522 k_2: 0.0008 g: 0.7532	6.2	k_1: <0.001 k_2: 0.253	k_1: 0.3606 k_2: -0.0015	k_1: 0.5440 k_2: 0.0030	2.4	>1000
HS	Acceptable	97.5	k_1: 0.2427 k_2: 0.0006 tb: 5.8	8.9	k_1: <0.001 k_2: 0.364	k_1: 0.2085 k_2: -0.0025	k_1: 0.2770 k_2: 0.0040	2.9	>1000

Degradation of AMPA is best described by bi-phasic models. The FOMC and HS models provide visually acceptable fits while the DFOP model provides a good statistical fit. However, the resulting parameters from the models are not statistically reliable. Nevertheless, the DFOP model provides more realistic estimates of the DT_{50} and DT_{90} values as well as a smaller χ^2 error. Thus, the DFOP model is selected as the best-fit model.

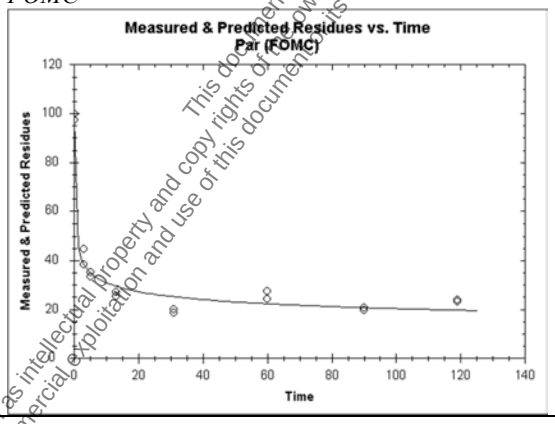
Conclusion: DFOP to be used for trigger endpoints
1000 d to be used for modelling as conservative approach

SFO





FOMC



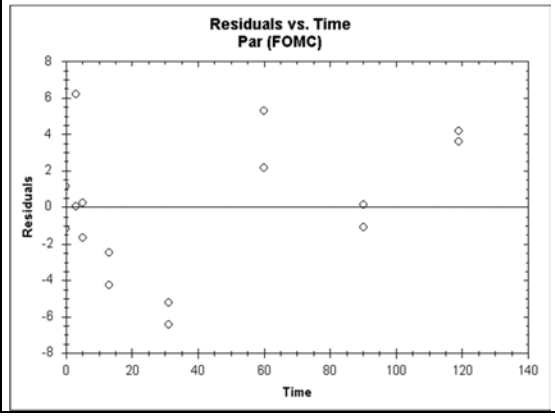
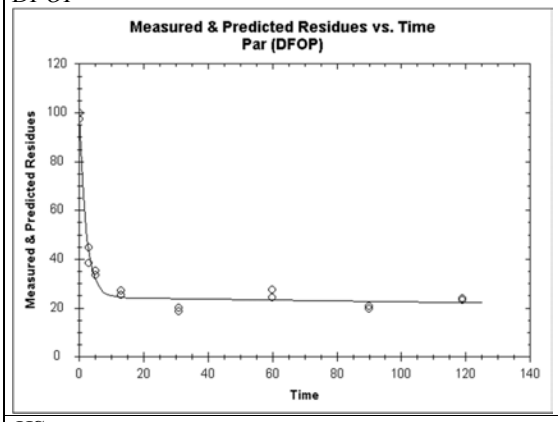
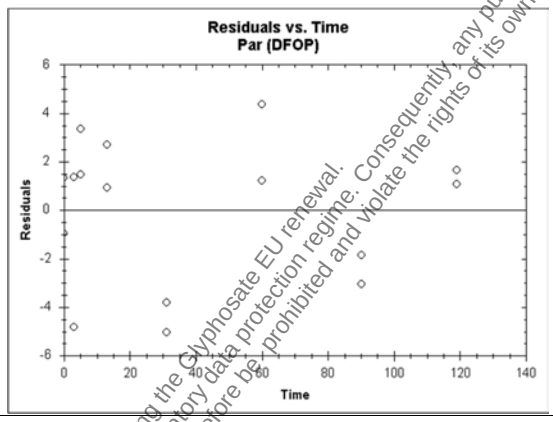


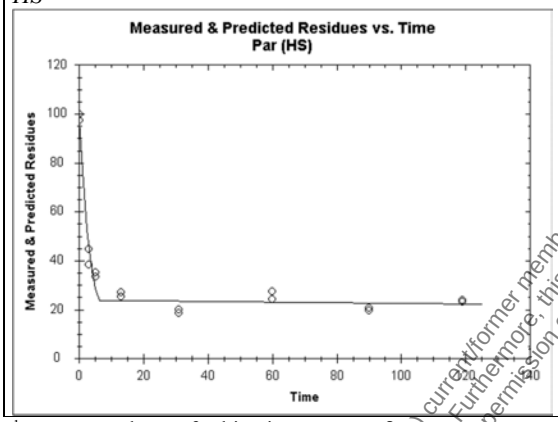
Table 7.2.2.3-53: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, total system

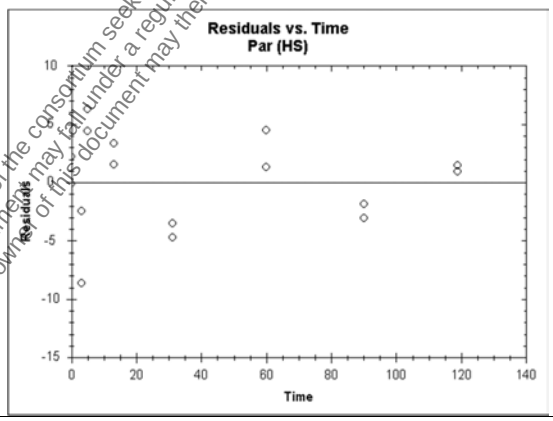
DFOP





HS





[1] t-test not relevant for kinetic parameter β

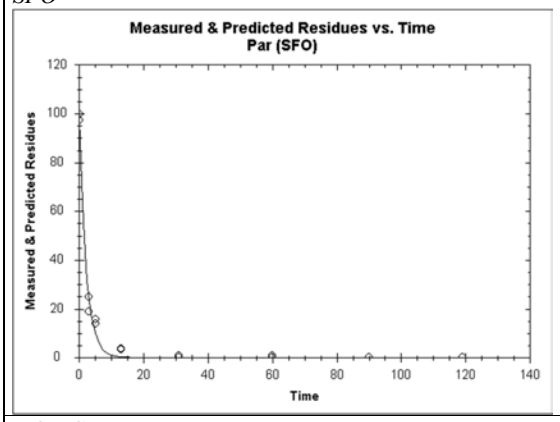
Table 7.2.2.3-54: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

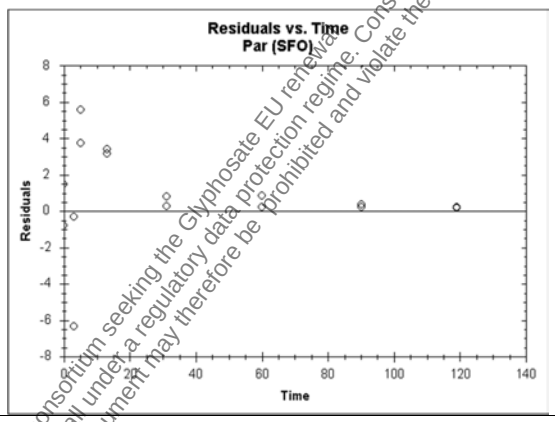
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	98.2	k: 0.4530	10.7	k: <0.001	k: 0.4085	k: 0.4970	1.5	5.1
FOMC	Good	98.5	α: 1.4858 β: 1.7767	3.2	-[1]	β: 0.6957	β: 2.8580	1.1	6.6
DFOP	Good	98.5	k_1: 1.4236 k_2: 0.1757 g: 0.6383	1.4	k_1:0.110 k_2: <0.001	k_1: -0.7326 k_2: 0.1033	k_1: 3.5800 k_2: 0.2480	0.9	7.3
HS	Good	98.2	k_1: 0.4546 k_2: 0.0943 tb: 5.8	10.7	k_1: <0.001 k_2: 0.289	k_1: 0.4114 k_2: -0.2286	k_1: 0.4980 k_2: 0.4170	1.5	5.1

Table 7.2.2.3-54: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

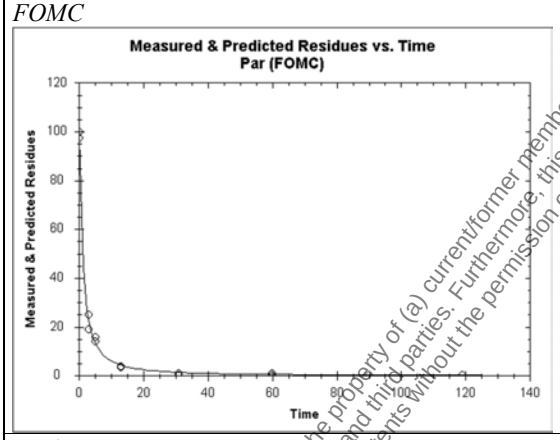
Although the visual fit of the SFO model is acceptable, the dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide equally good visual fits but the statistical parameters resulting from the DFOP and HS models do not indicate reliable fits. Thus, the FOMC model is selected as the best fit model. **Conclusion:** FOMC to be used for trigger endpoints SFO to be used for modelling endpoints

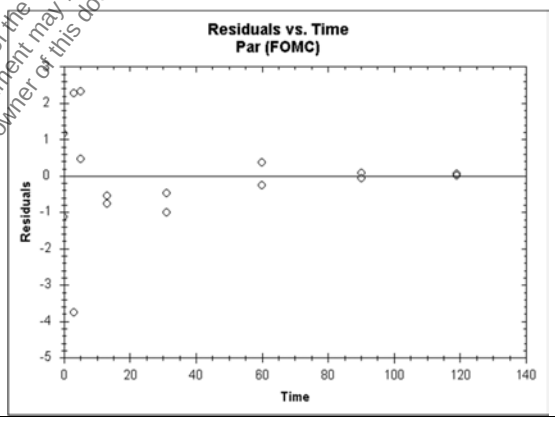
SFO



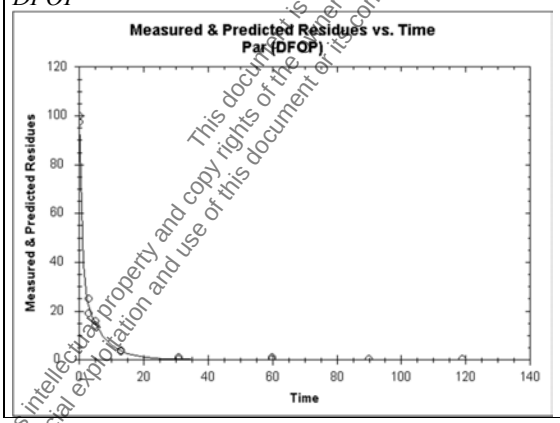


FOMC





DFOP



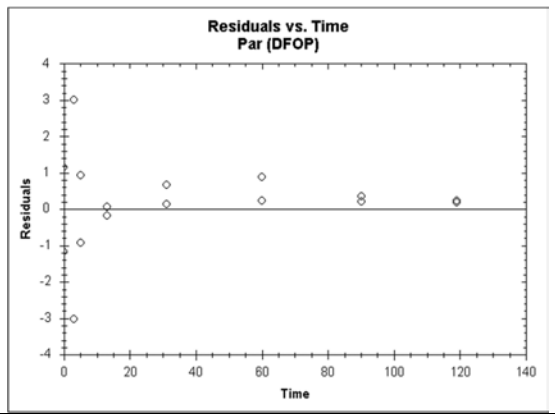
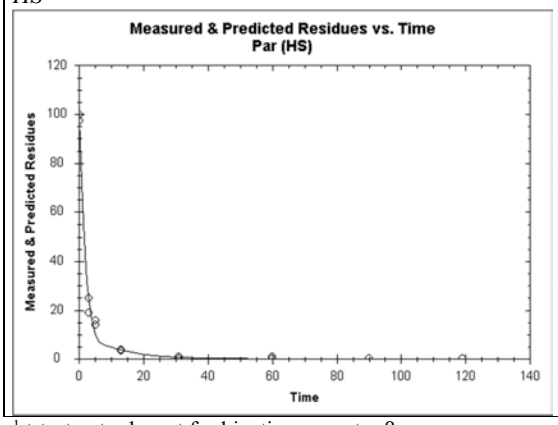
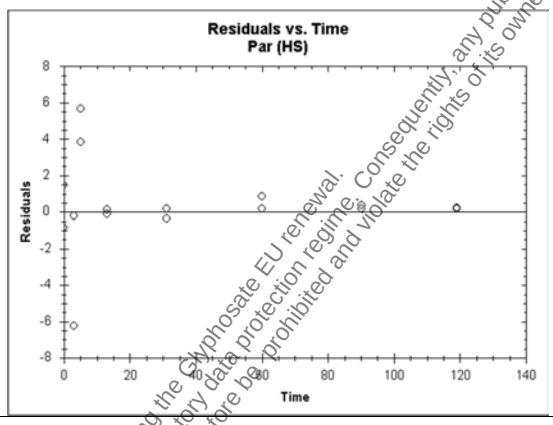


Table 7.2.2.3-54: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, water phase

HS



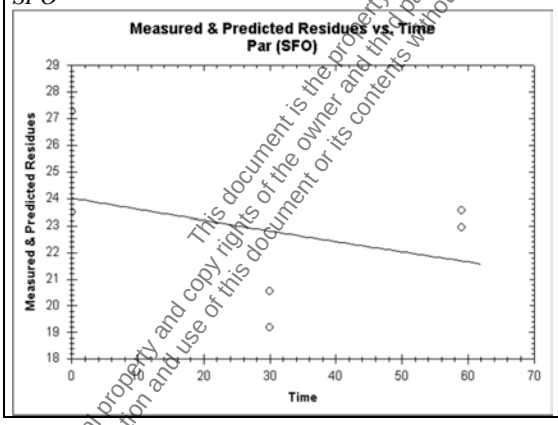


[1] t-test not relevant for kinetic parameter β

Table 7.2.2.3-55: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-I, sediment phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	24.0	k: 0.0017	8.0	k: 0.235	k: -0.0025	k: 0.0060	399.8	>1000
Only the SFO model was used for the evaluation due to the number of available data points. The model did not provide an acceptable fit. **Conclusion:** No reliable endpoints could be derived									

SFO



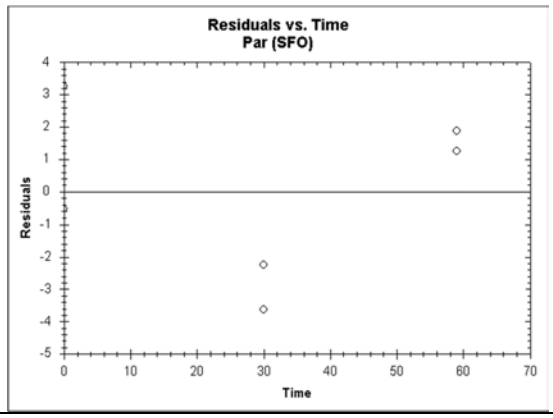


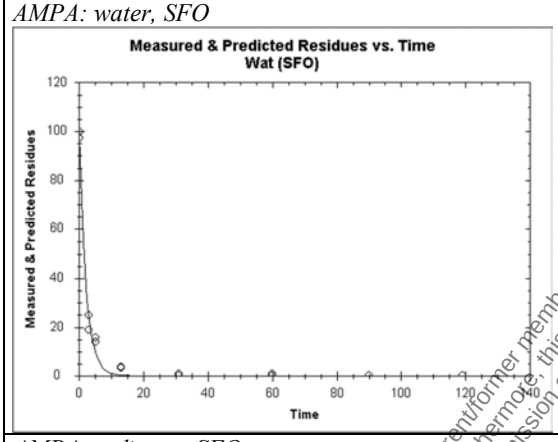
Table 7.2.2.3-56: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Schäphysen of study [redacted] (2002, CA 7.2.2.3/020), Level P-II

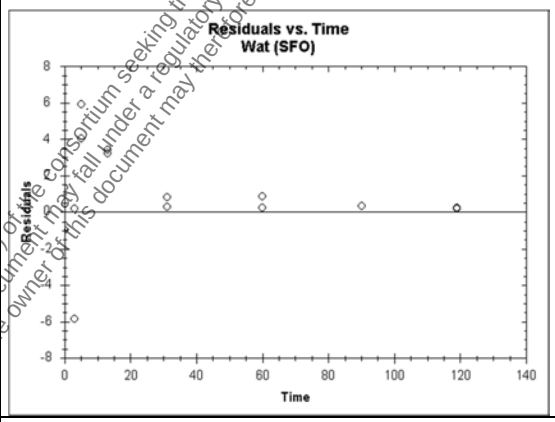
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Acceptable	98.3	k_{wat}: 0.3546 k_{wat_sed}: 0.1050	11.4	k_{wat}: <0.001 k_{wat_sed}: <0.001	k_{wat}: 0.3149 k_{wat_sed}: -0.0910	k_{wat}: 0.394 k_{wat_sed}: 0.119	2.0	6.5
Sediment: SFO	Acceptable	0.0	k_{sed}: 0.0000 k_{sed_wat}: 0.0002	8.8	k_{sed}: 0.5 k_{sed_wat}: 0.495	k_{sed}: -0.0276 k_{sed_wat}: -0.0354	k_{sed}: 0.028 k_{sed_wat}: 0.036	>1000	>1000

The visual and statistical fits obtained for the water phase are reliable but the visual fit obtained for the sediment phase is poor.

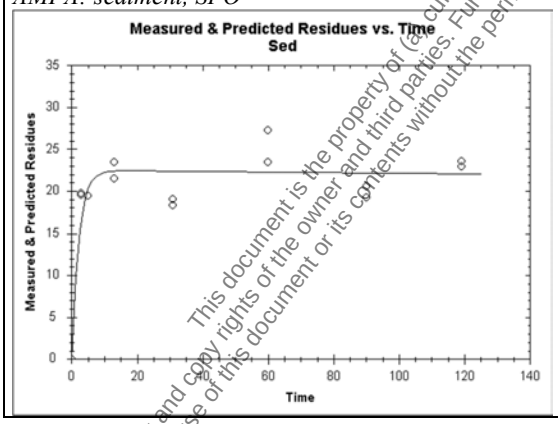
Conclusion: No reliable endpoints could be derived

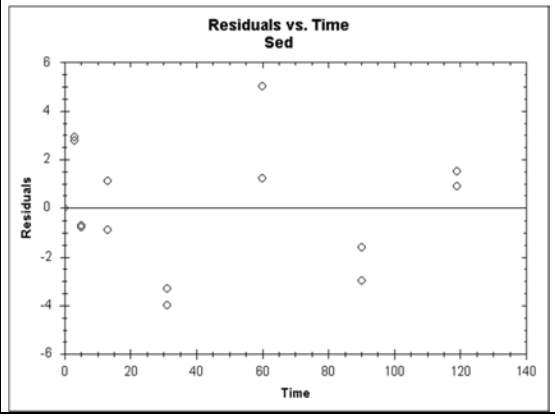
AMPA: water, SFO





AMPA: sediment, SFO





[redacted] (2003, CA 7.2.2.3/019)

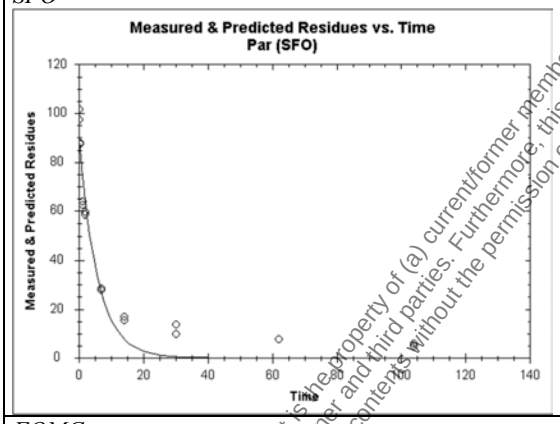
Table 7.2.2.3-57: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

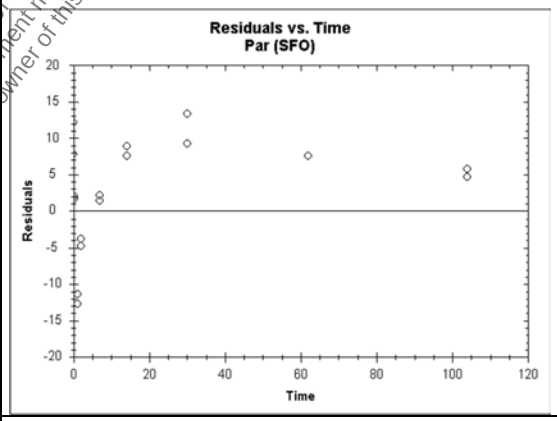
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	89.5	k: 0.1741	14.9	k: <0.001	k: 0.1229	k: 0.2250	4.0	13.2
FOMC	Good	98.5	α: 0.6972 β: 1.418	5.3	-[1]	β: 0.8552	β: 1.9810	2.4	37.1
DFOP	Good	95.9	k_1: 0.4238 k_2: 0.02 g: 0.7434	7.8	k_1: <0.001 k_2: 0.005	k_1: 0.2998 k_2: 0.0069	k_1: 0.5480 k_2: 0.0330	2.5	47.1
HS	Good	91.5	k_1: 0.2066 k_2: 0.0137 tb: 8.2	11.1	k_1: <0.001 k_2: 0.068	k_1: 0.1594 k_2: -0.0032	k_1: 0.2540 k_2: 0.0310	3.4	52.4

Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide equally reliable and visually acceptable results but the FOMC model provides the least χ^2 error and is selected as best-fit model as well as for modelling endpoints.

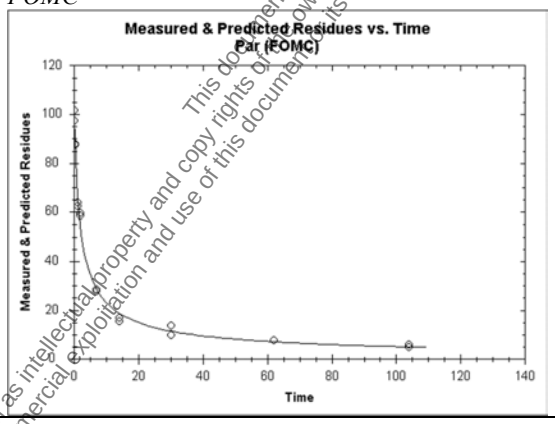
Conclusion: FOMC to be used for trigger endpoints
FOMC to be used for modelling endpoints

SFO





FOMC



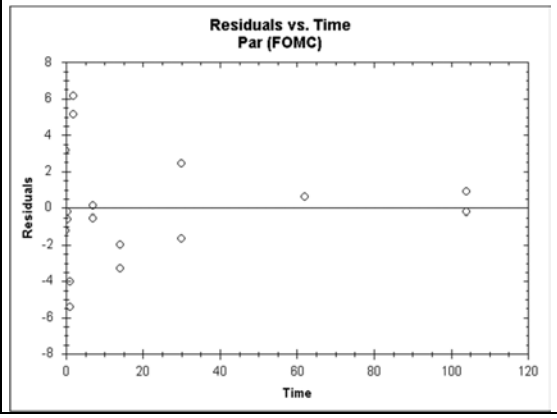
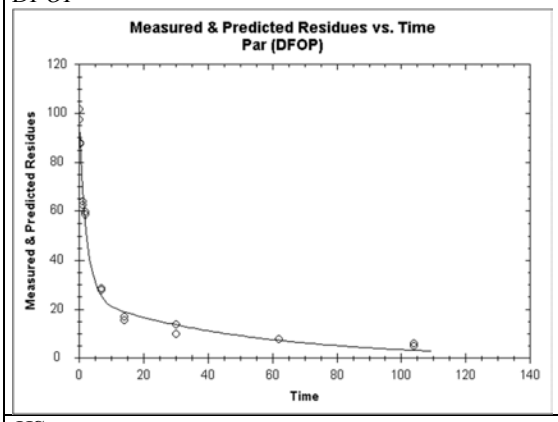
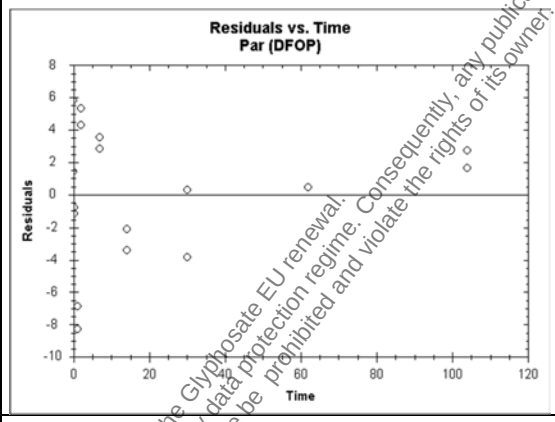


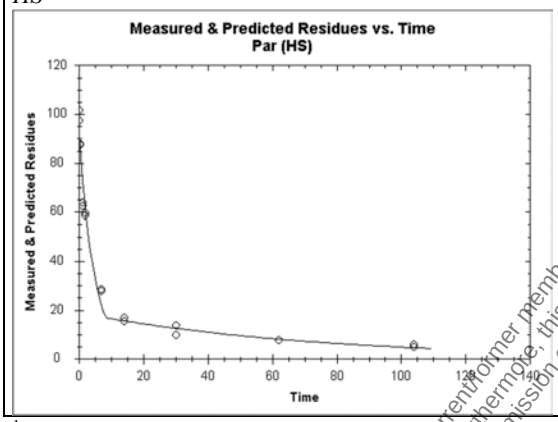
Table 7.2.2.3-57: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

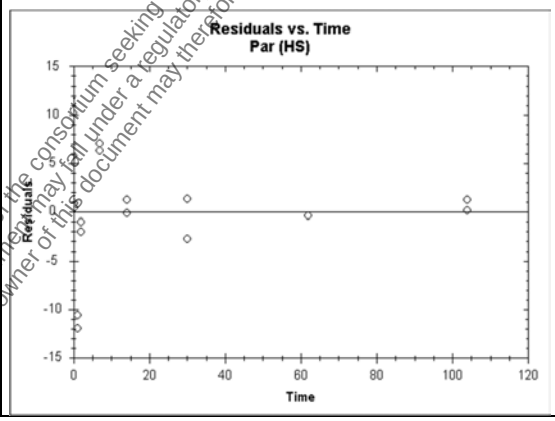
DFOP





HS





[1] t-test not relevant for kinetic parameter β

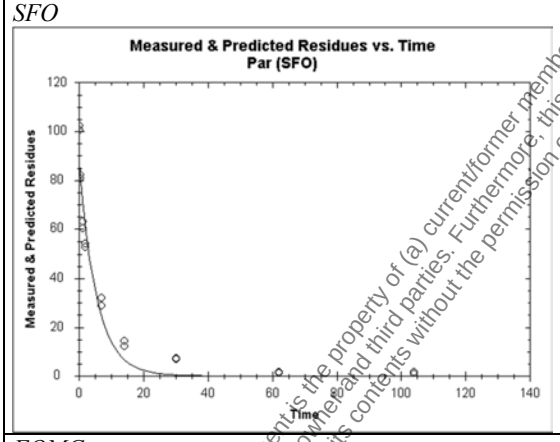
Table 7.2.2.3-58: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

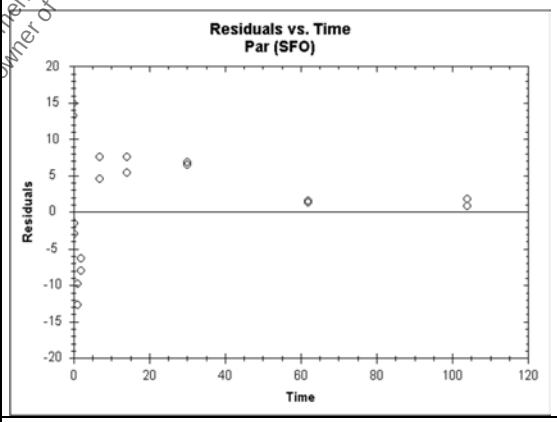
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	87.4	k: 0.1824	15.4	k: <0.001	k: 0.1291	k: 0.2360	3.8	12.6
FOMC	Good	97.4	α: 0.7981 β: 1.5317	8.0	-[1]	β: 0.6887	β: 2.3750	2.1	25.9
DFOP	Good	101.5	k_1: 2.883 k_2: 0.107 g: 0.3491	3.9	k_1: <0.001 k_2: <0.001	k_1: 1.9260 k_2: 0.0947	k_1: 3.8400 k_2: 0.1210	2.5	17.4
HS	Good	89.0	k_1: 0.2089 k_2: 0.0411 tb: 7.9	15.4	k_1: <0.001 k_2: 0.169	k_1: 0.1460 k_2: -0.0400	k_1: 0.2720 k_2: 0.1220	3.3	23.8

Dissipation of AMPA in the water phase is best described by bi-phasic models.
All bi-phasic models provide good visual fits but the statistical parameters of the HS model do not indicate a reliable fit. Since FOMC provides a slightly better visual fit than the DFOP model and 10 % of the initially measured substance concentration was reached within the experimental period, the FOMC is selected as the best-fit model for as well as for deriving modelling endpoints.

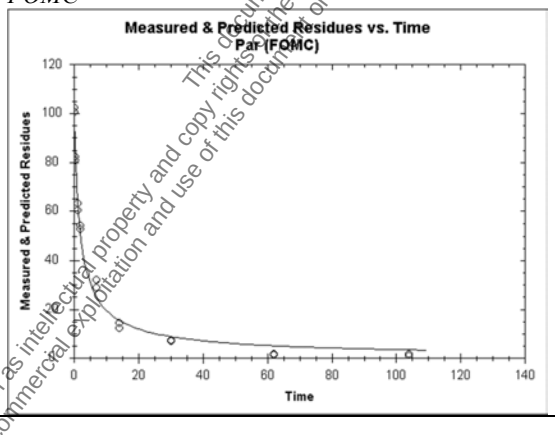
Conclusion: FOMC to be used for trigger endpoints
FOMC to be used for modelling endpoints

SFO





FOMC



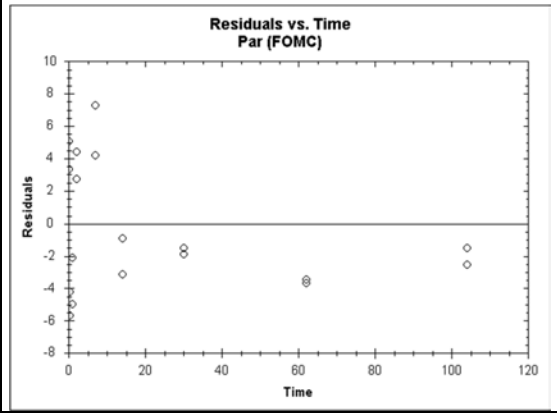
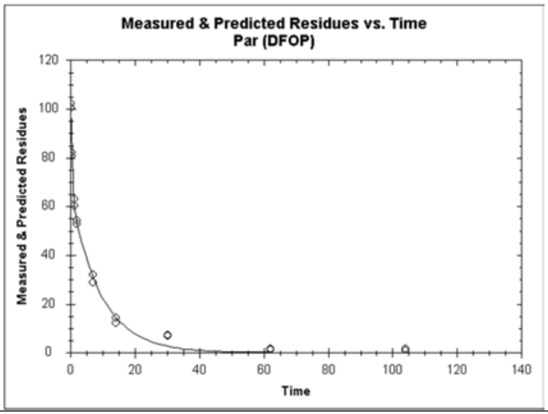
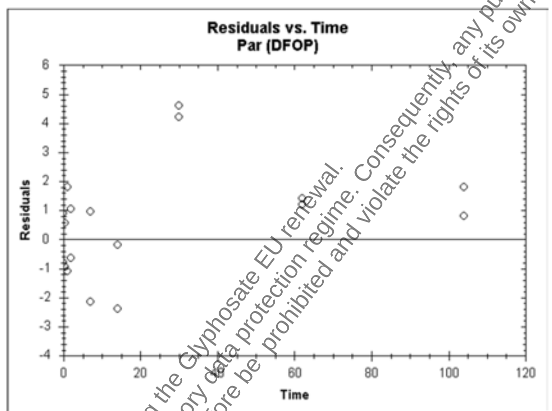
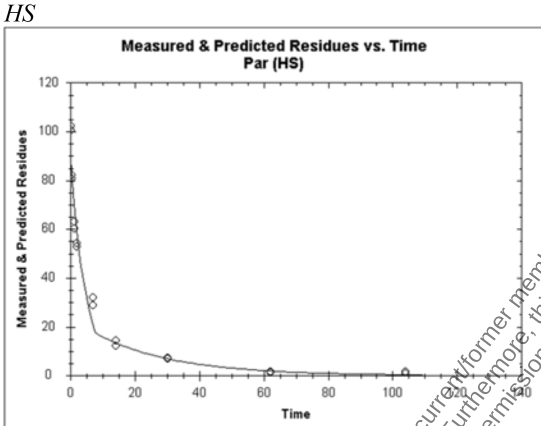
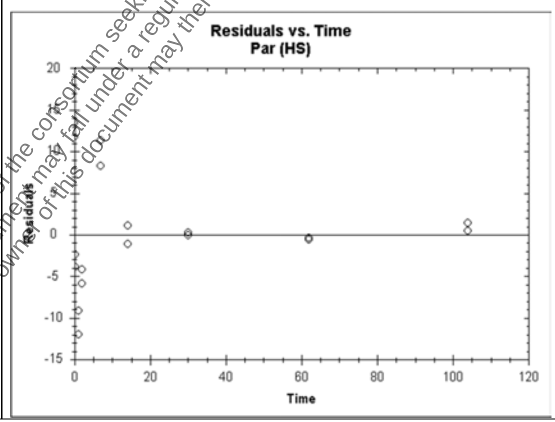


Table 7.2.2.3-58: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (2003, CA 7.2.2.3/019), Level P-I, water phase

DFOP 	
HS 	

[1] t-test not relevant for kinetic parameter β

[redacted] (1999, CA 7.2.2.3/021)

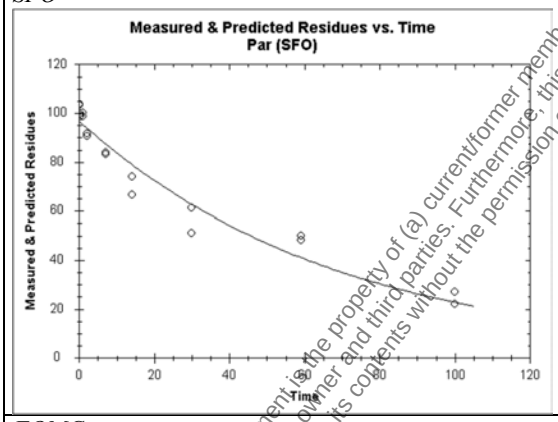
Table 7.2.2.3-59: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

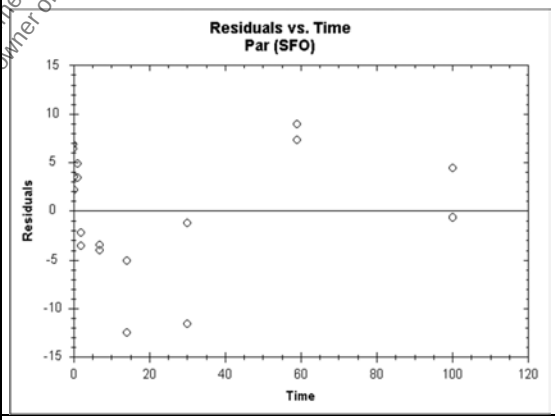
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Acceptable	96.8	k: 0.0145	5.9	k: <0.001	k: 0.0121	k: 0.0170	47.7	158.4
FOMC	Good	100.6	α: 0.7336 β: 24.7523	4.4	-[1]	β: 1.2073	β: 48.297	38.9	546.5
DFOP	Good	101.9	k_1: 0.1684 k_2: 0.0105 g: 0.2114	3.5	k_1: 0.058 k_2: <0.001	k_1: -0.0286 k_2: 0.0075	k_1: 0.3650 k_2: 0.0140	43.5	196.8
HS	Good	102.7	k_1: 0.0547 k_2: 0.0118 tb: 4.0	4.0	k_1: 0.012 k_2: <0.001	k_1: 0.0121 k_2: 0.0096	k_1: 0.0970 k_2: 0.0140	44.4	181.0

Although the visual and statistical fits of the SFO model are acceptable, the degradation of AMPA is best described by bi-phasic models. All bi-phasic models provide equally reliable and visually good results but the least χ^2 error is provided by the DFOP model.

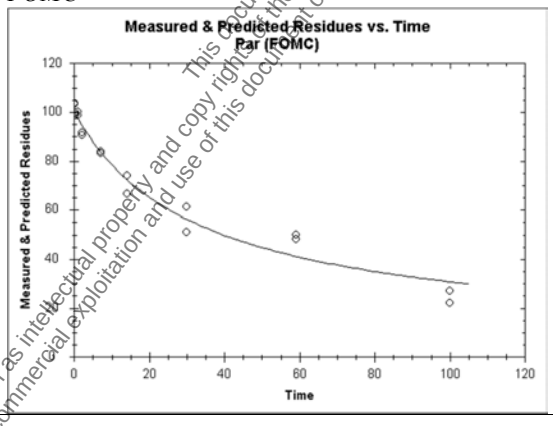
Conclusion: DFOP to be used for trigger endpoints
SFO to be used for modelling endpoints

SFO





FOMC



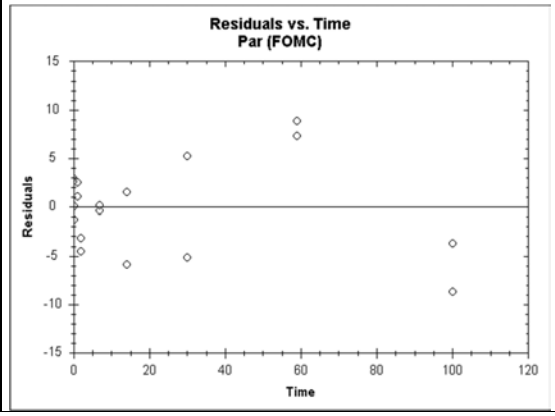
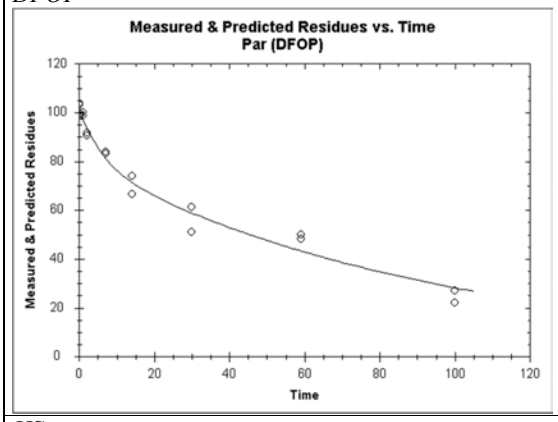
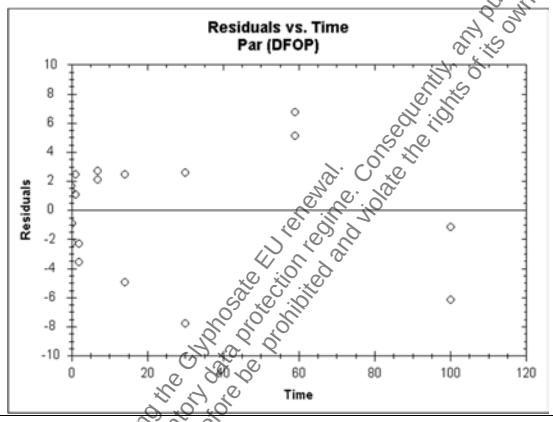


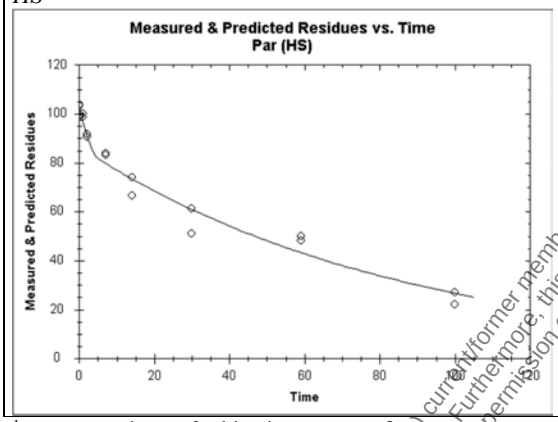
Table 7.2.2.3-59: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

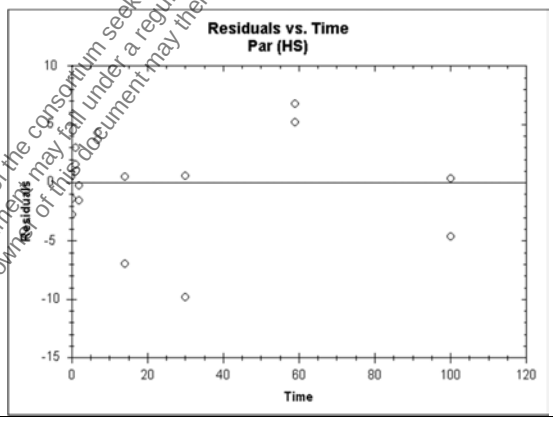
DFOP





HS





[1] t-test not relevant for kinetic parameter β

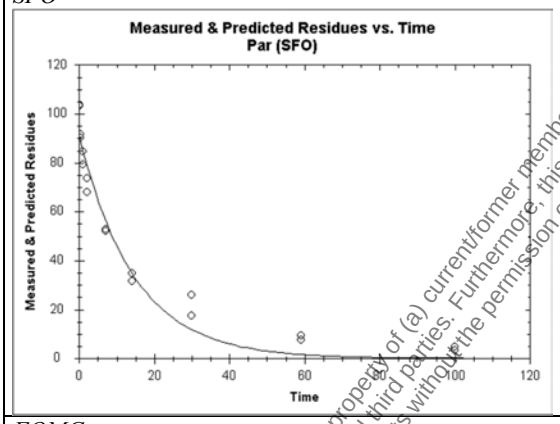
Table 7.2.2.3-60: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

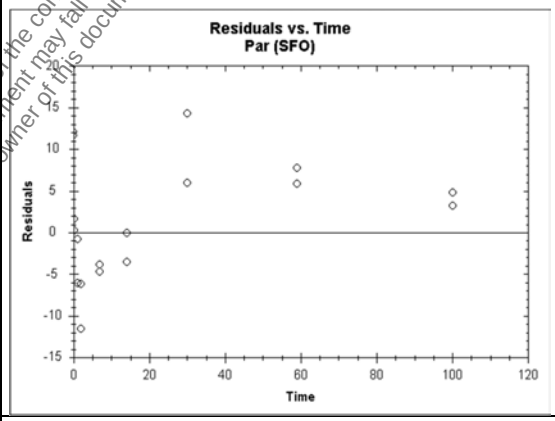
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	91.5	k: 0.0685	10.5	k: <0.001	k: 0.0527	k: 0.0840	10.1	33.6
FOMC	Good	97.6	α: 0.8827 β: 5.6835	5.7	-[1]	β: 2.0266	β: 9.3400	6.8	71.5
DFOP	Good	100.4	k_1: 0.567 k_2: 0.0361 g: 0.3765	4.5	k_1: <0.00 k_2: <0.001	k_1: 0.2831 k_2: 0.0268	k_1: 0.8510 k_2: 0.0450	6.6	50.7
HS	Good	99.4	k_1: 0.179 k_2: 0.0372 tb: 3.1	5.1	k_1: <0.001 k_2: <0.001	k_1: 0.1338 k_2: 0.0276	k_1: 0.2240 k_2: 0.0470	6.8	50.1

The dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide reliable and visually good results but the best visual fit with the least χ^2 error is provided by the DFOP model.

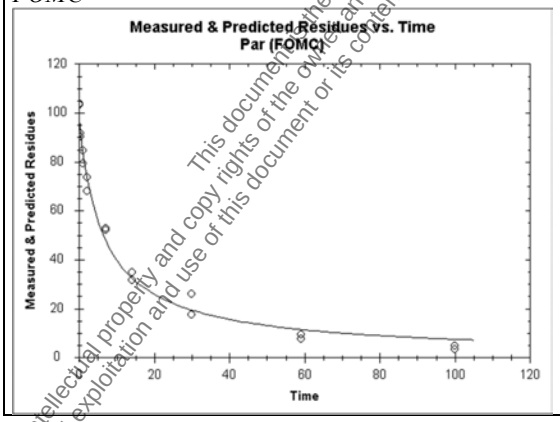
Conclusion: DFOP to be used for trigger endpoints
DFOP to be used for modelling endpoints

SFO





FOMC



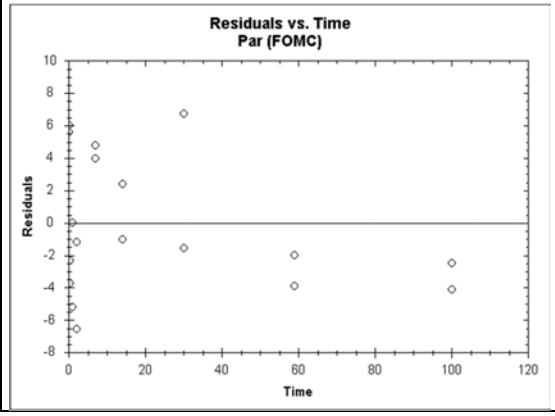
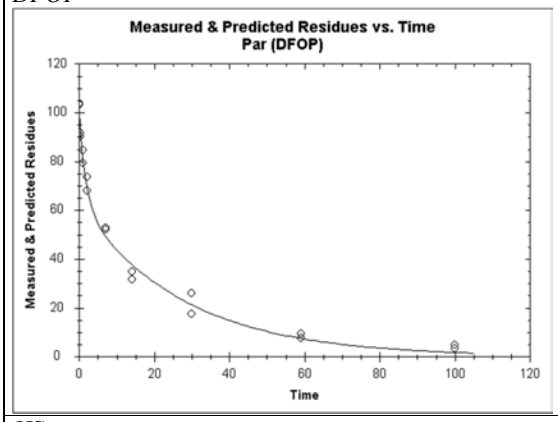
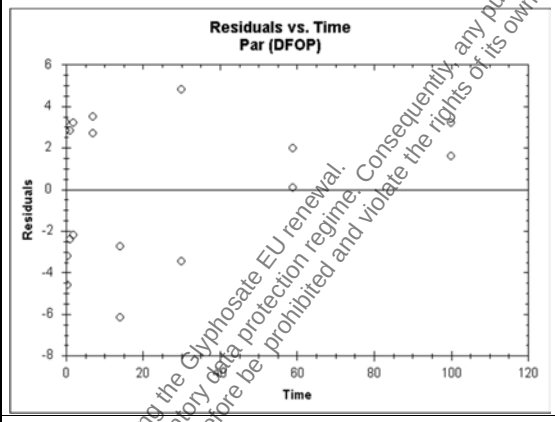


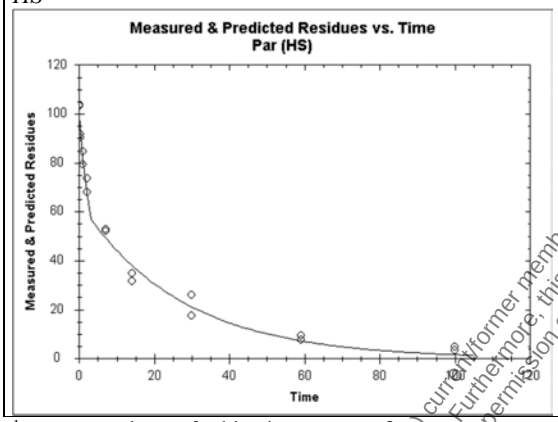
Table 7.2.2.3-60: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Bickenbach of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

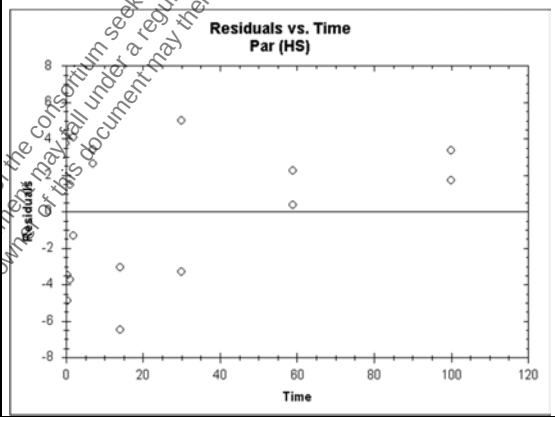
DFOP





HS





[1] t-test not relevant for kinetic parameter β

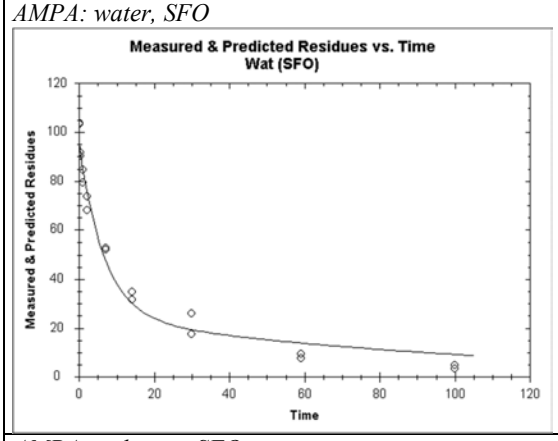
Table 7.2.2.3-61: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of study [redacted] (1999, CA 7.2.2.3/021), Level P-II

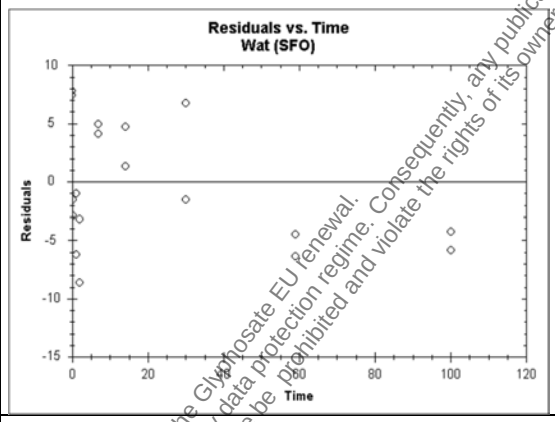
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Acceptable	95.8	k_{wat}: 0.0324 k_{wat_sed}: 0.0820	7.7	k_{wat}: 0.0041 k_{wat_sed}: <0.001	k_{wat}: 0.0100 k_{wat_sed}: -0.0611	k_{wat}: 0.055 k_{wat_sed}: 0.103	21.4	71.1
Sediment: SFO	Acceptable	0.0	k_{sed}: 2.34×10^{-14} k_{sed_wat}: 0.0469	20.3	k_{sed}: 0.5 k_{sed_wat}: 0.006	k_{sed}: -0.0159 k_{sed_wat}: -0.0213	k_{sed}: 0.016 k_{sed_wat}: 0.073	>1000	>1000

Although the visual fits obtained for the water and sediment phases are acceptable, the degradation rate in sediment is not significantly different from zero. Therefore, the statistical fit obtained for the sediment phase is not reliable.
Conclusion: No further evaluation was conducted. No reliable endpoints could be derived

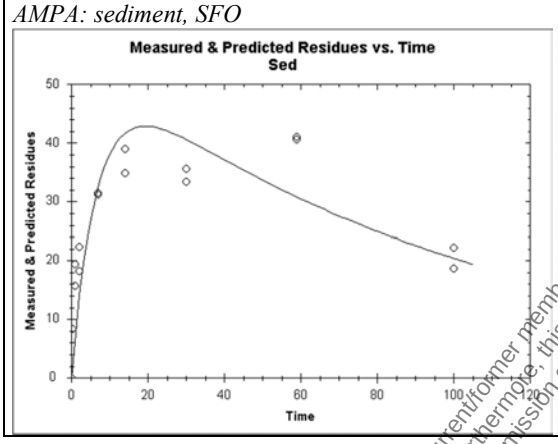
Table 7.2.2.3-61: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Bickenbach of study [redacted] (1999, CA 7.2.2.3/021), Level P-II

AMPA: water, SFO





AMPA: sediment, SFO



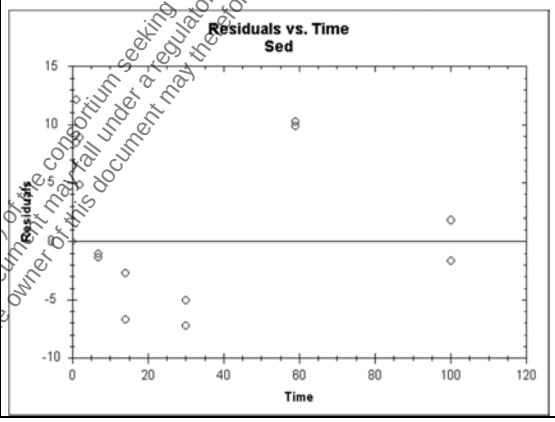


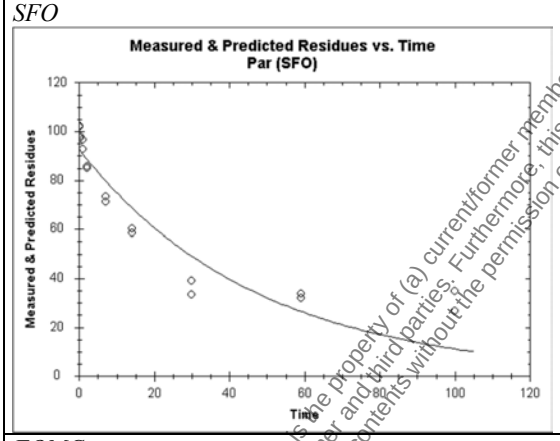
Table 7.2.2.3-62: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

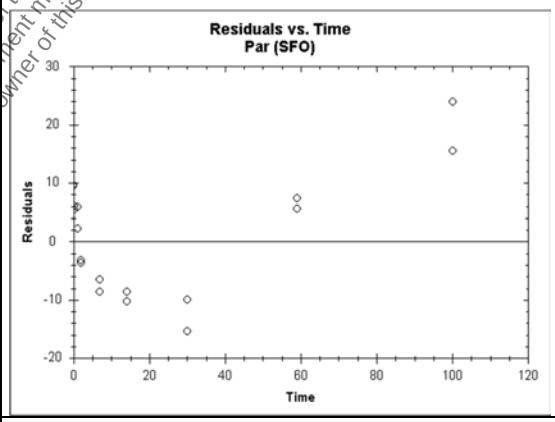
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	92.6	k: 0.0212	11.7	k: <0.001	k: 0.0150	k: 0.0270	32.7	108.5
FOMC	Good	101.2	α: 0.4410 β: 5.1794	3.9	-[1]	β: 1.9157	β: 8.4430	19.8	954.3
DFOP	Good	99.1	k_1: 0.071 k_2: 2.33×10^{-14} g: 0.6886	3.1	k_1: <0.001 k_2: 0.5	k_1: 0.0475 k_2: -0.0053	k_1: 0.0940 k_2: 0.0050	18.2	>1000
HS	Good	98.1	k_1: 0.0391 k_2: 0.0024 tb: 25.3	3.4	k_1: <0.001 k_2: 0.075	k_1: 0.0331 k_2: -0.0007	k_1: 0.0450 k_2: 0.0050	17.7	579.8

The degradation of AMPA is best described by bi-phasic models. The DFOP and HS models provide the best visual fits. However, the slow-phase degradation parameter (k_2) resulting from the DFOP model is not significantly different from zero. Thus, the HS model is selected as the best-fit model as well as for deriving modelling endpoints.

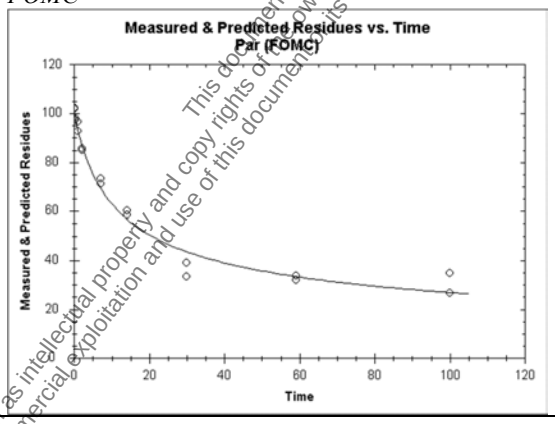
Conclusion: HS to be used for trigger endpoints
HS to be used for modelling endpoints

SFO





FOMC



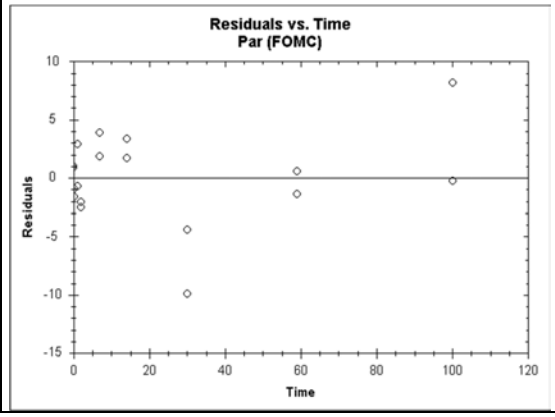
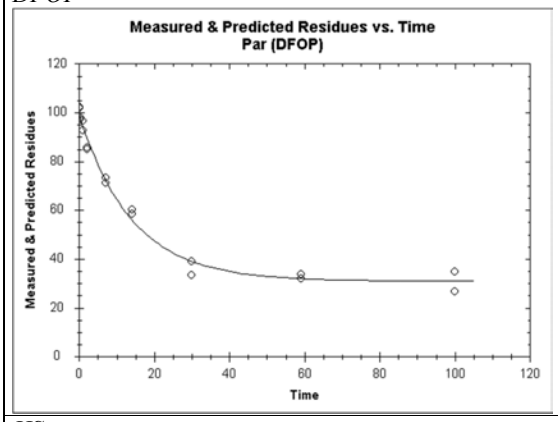
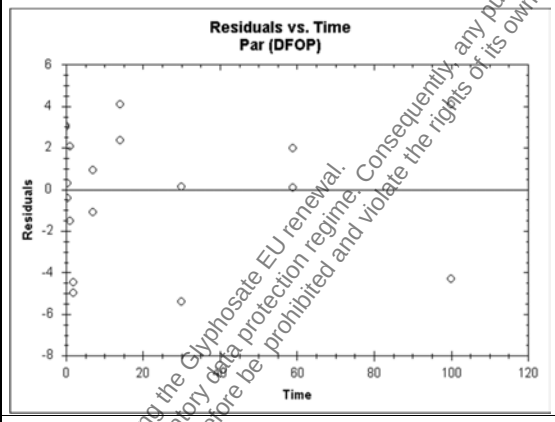


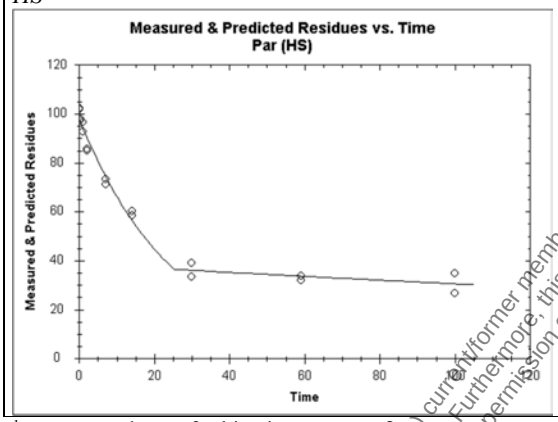
Table 7.2.2.3-62: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, total system

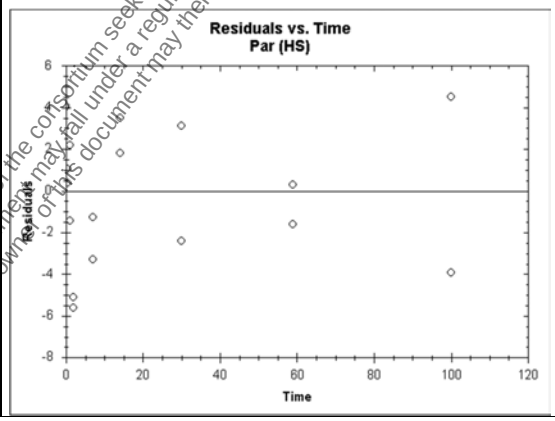
DFOP





HS





[1] t-test not relevant for kinetic parameter β

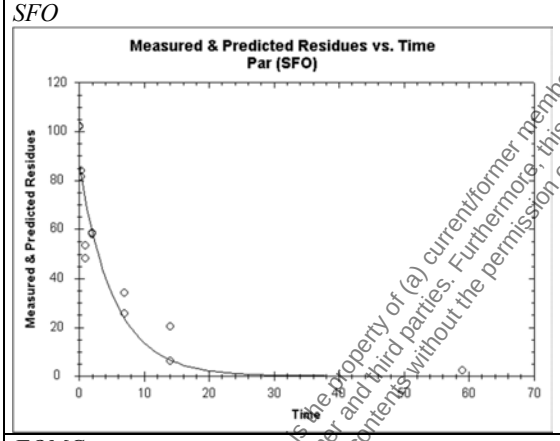
Table 7.2.2.3-63: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

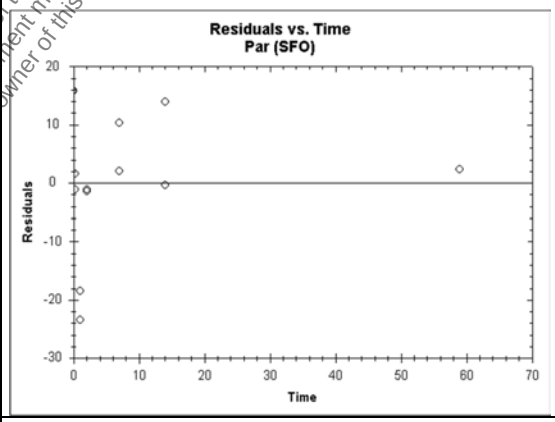
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	86.3	k: 0.1846	17.3	k: <0.001	k: 0.0996	k: 0.2700	3.8	12.5
FOMC	Poor	100.5	α: 0.5353 β: 0.6254	11.8	-[1]	β: -0.1641	β: 1.4150	1.7	45.5
DFOP	Acceptable	103.1	k_1: 3.1154 k_2: 0.1051 g: 0.3867	8.2	k_1: 0.027 k_2: <0.001	k_1: 0.3677 k_2: 0.0625	k_1: 5.8630 k_2: 0.1480	2.0	17.3
HS	Poor	87.8	k_1: 0.2099 k_2: 0.0389 tb: 7.9	19.7	k_1: 0.002 k_2: 0.385	k_1: 0.0991 k_2: -0.2138	k_1: 0.3210 k_2: 0.2920	3.3	24.6

The dissipation of AMPA in the water phase is best described by bi-phasic models.
The visual fit and the statistical parameters resulting from the FOMC and HS models do not indicate an acceptable fit. The DFOP model provides acceptable visual and statistical fit. Thus, the DFOP model is selected as the best fit model as well as for deriving modelling endpoints.

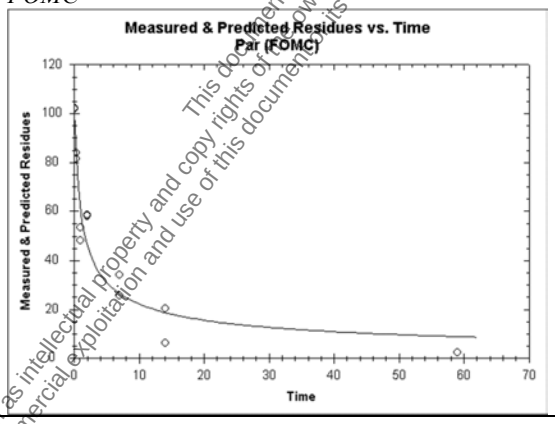
Conclusion: DFOP to be used for trigger endpoints
DFOP to be used for modelling endpoints

SFO





FOMC



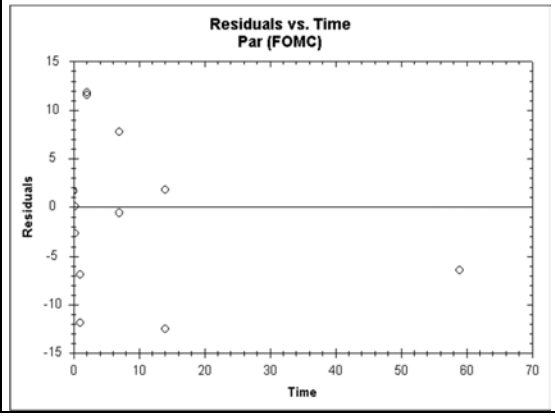
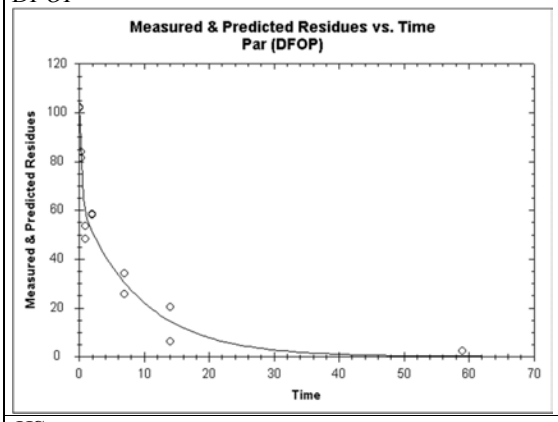
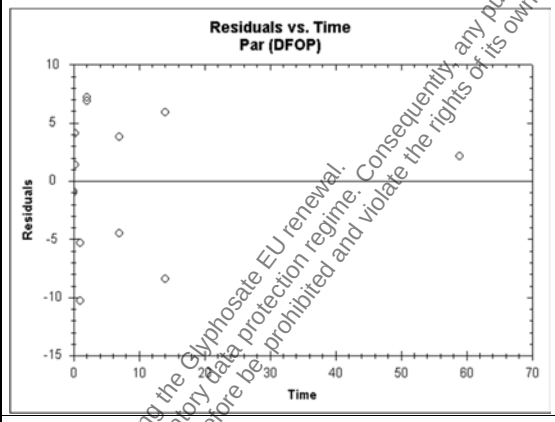


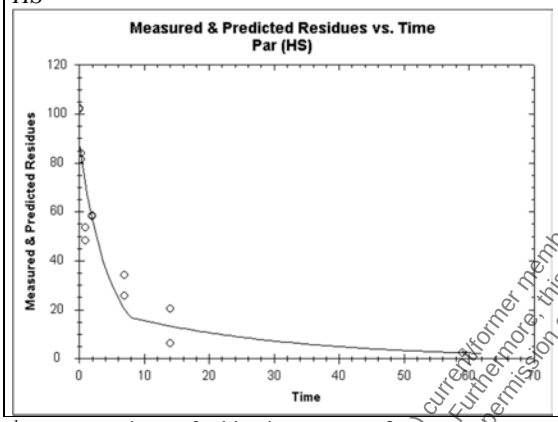
Table 7.2.2.3-63: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, water phase

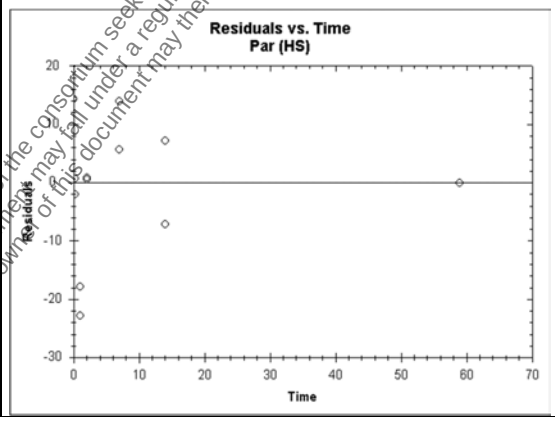
DFOP





HS





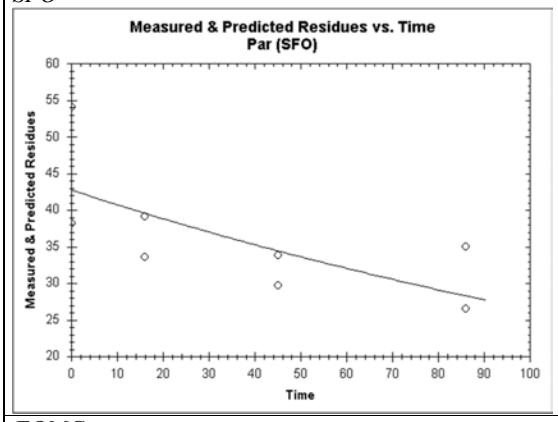
[1] t-test not relevant for kinetic parameter β

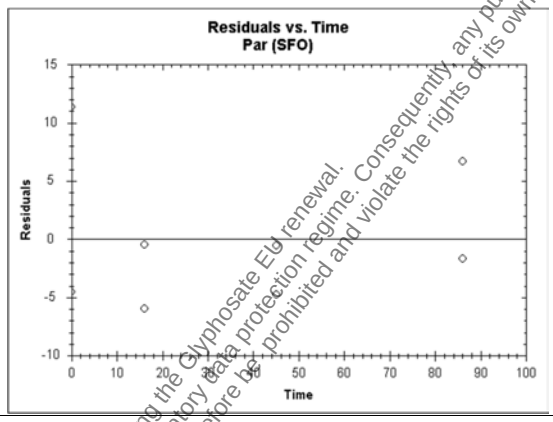
Table 7.2.2.3-64: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, sediment phase

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	42.7	k: 0.0048	6.7	k: 0.033	k: 0.0006	k: 0.0090	144.6	480.5
FOMC	Acceptable	46.2	α: 0.108 β: 1.8179	1.2	-[1]	β: -13.706	β: 17.341	>1000	>1000
Only the SFO and FOMC models were used for the evaluation due to the limited number of available data points. The SFO model did not properly describe the dissipation. Although the FOMC model provides an acceptable visual fit, the confidence interval of the parameter β is wide and includes zero. **Conclusion:** No reliable endpoints could be derived									

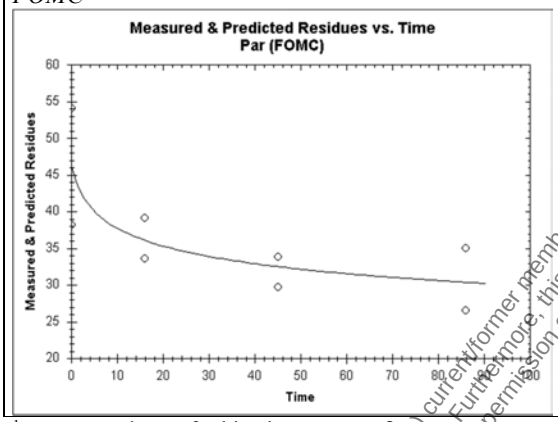
Table 7.2.2.3-64: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Unter Widdersheim of the study [redacted] (1999, CA 7.2.2.3/021), Level P-I, sediment phase

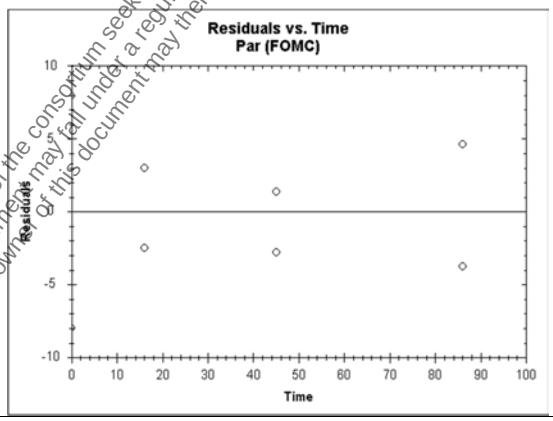
SFO





FOMC





[1] t-test not relevant for kinetic parameter β

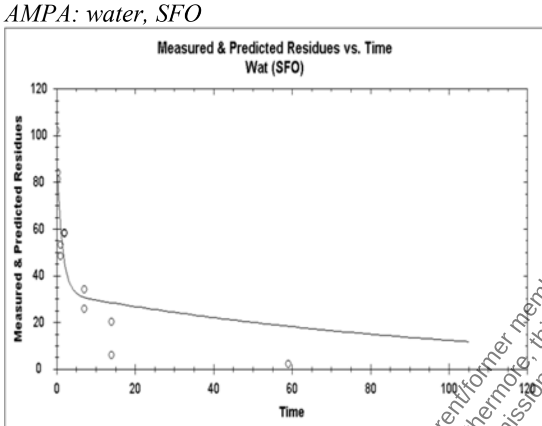
Table 7.2.2.3-65: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Unter Widdersheim of study [redacted] (1999, CA 7.2.2.3/021), Level P-II

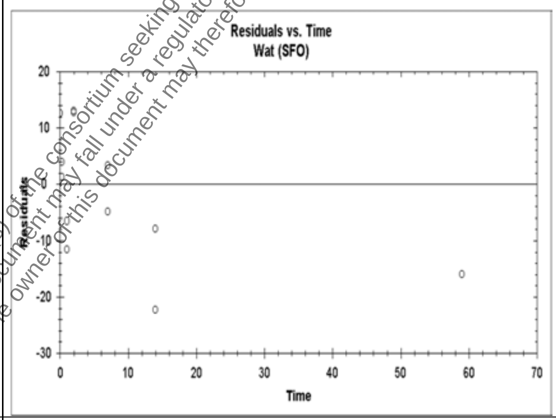
Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Poor	89.5	k_{wat}: 0.0263 k_{wat_sed}: 0.4356	20.1	k_{wat}: 0.3885 k_{wat_sed}: <0.001	k_{wat}: -0.1536 k_{wat_sed}: -0.2501	k_{wat}: 0.206 k_{wat_sed}: 0.621	26.4	87.7
Sediment: SFO	Acceptable	0.0	k_{sed}: 2.34×10^{-14}0 k_{sed_wat}: 0.2671	21.7	k_{sed}: 0.5 k_{sed_wat}: 0.0062	k_{sed}: -0.1100 k_{sed_wat}: -0.0722	k_{sed}: 0.110 k_{sed_wat}: 0.462	>1000	>1000

Although the visual fit obtained for the sediment phase is acceptable, the statistical fit is not and the visual fit obtained for the water phase is poor.

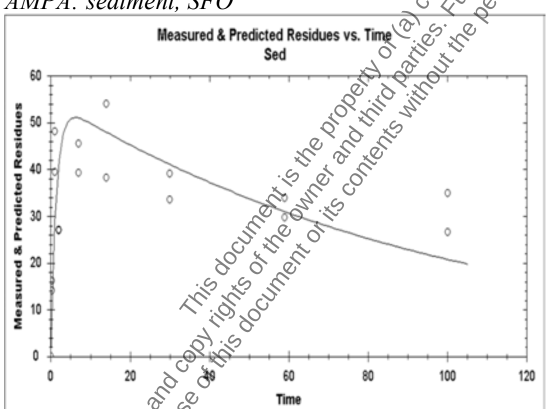
Conclusion: No further evaluation was conducted. No reliable endpoints could be derived

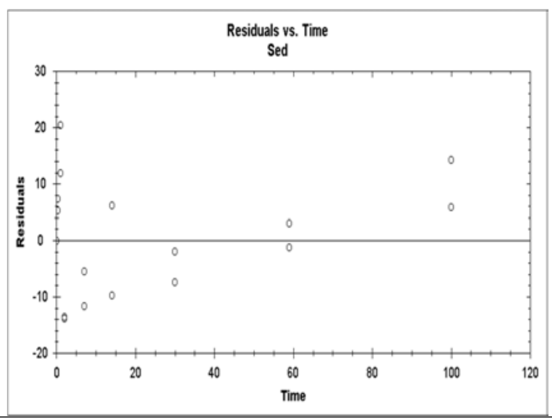
AMPA: water, SFO





AMPA: sediment, SFO





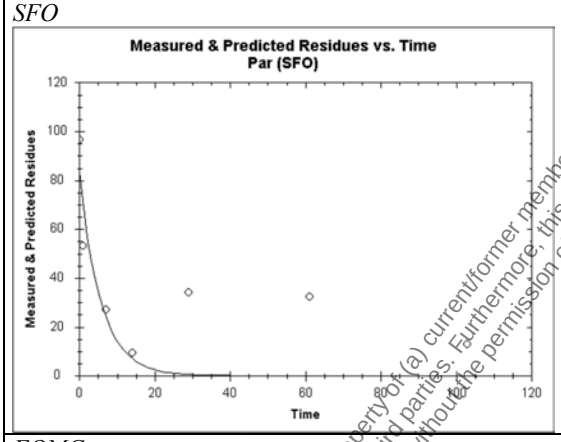
[redacted] (2004, CA 7.2.2.3/018)

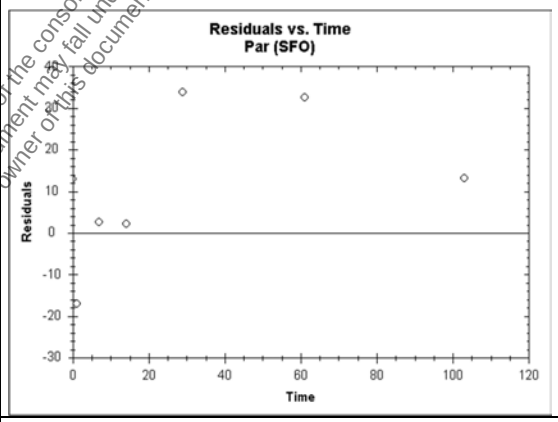
Table 7.2.2.3-66: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, total system

Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	83.5	k: 0.1757	42.1	k: 0.109	k: -0.0683	k: 0.4200	3.9	13.1
FOMC	Poor	96.7	α: 0.2234 β: 0.0488	22.1	-[1]	β: -0.2142	β: 0.3120	1.0	>1000
DFOP	Poor	96.6	k_1: 0.9354 k_2: 0.0019 g: 0.7393	21.1	k_1: 0.090 k_2: 0.407	k_1: -0.1201 k_2: -0.0126	k_1: 1.9910 k_2: 0.0160	1.2	503.7
HS	Poor	96.6	k_1: 0.5984 k_2: 0.0019 tb: 2.3	21.1	k_1: 0.060 k_2: 0.405	k_1: 0.0532 k_2: -0.0126	k_1: 1.1440 k_2: 0.0160	1.2	497.0

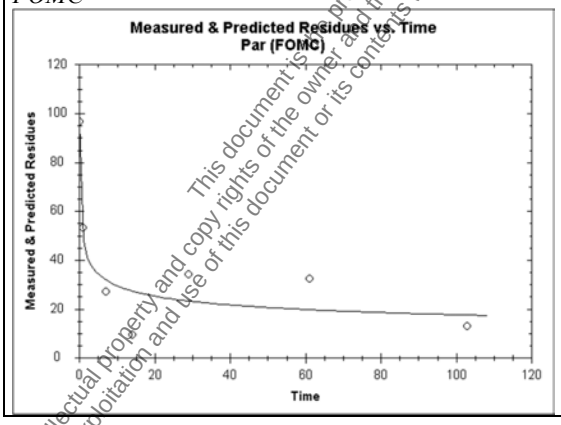
Conclusion: No reliable endpoints could be derived

SFO





FOMC



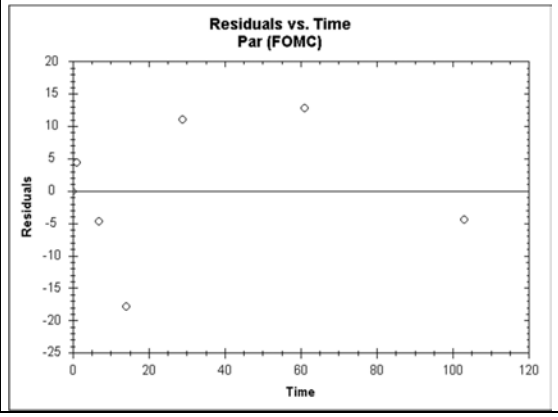
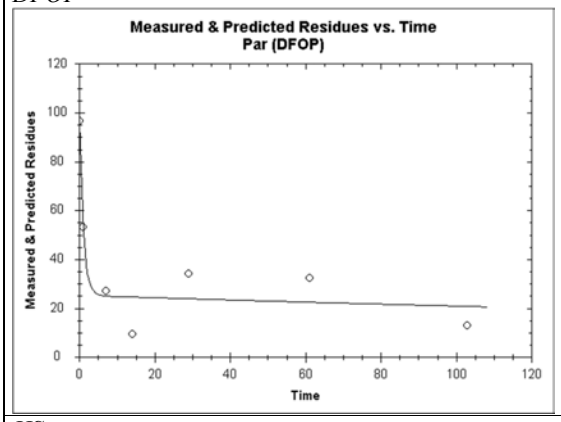
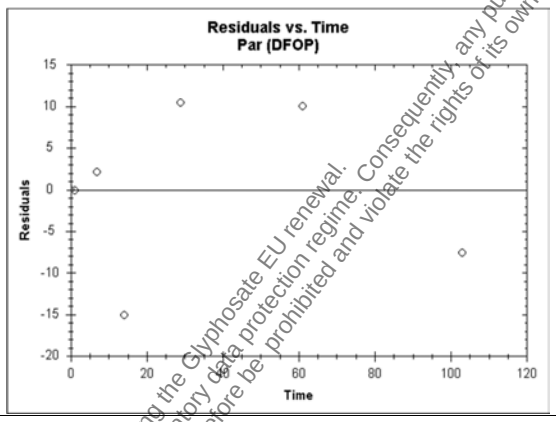


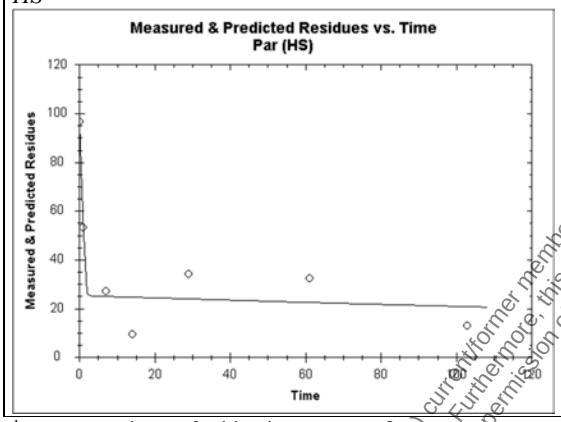
Table 7.2.2.3-66: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, total system

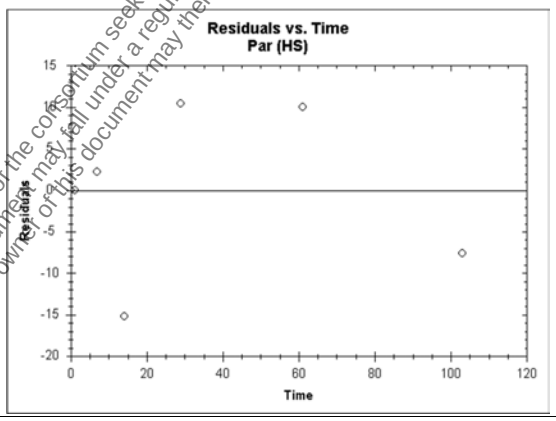
DFOP





HS





[1] t-test not relevant for kinetic parameter β

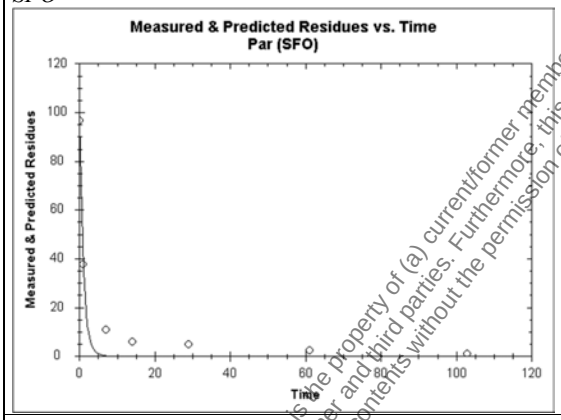
Table 7.2.2.3-67: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

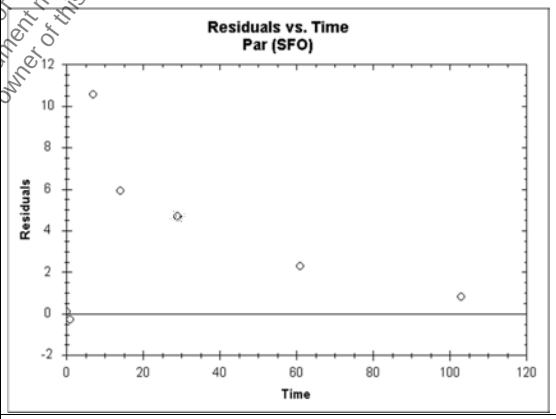
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	96.5	k: 0.9327	17.5	k: 0.001	k: 0.6052	k: 1.2600	0.7	2.5
FOMC	Good	96.6	α: 0.7584 β: 0.4064	1.8	-[1]	β: 0.3340	β: 0.4790	0.6	8.1
DFOP	Good	96.6	k_1: 1.1772 k_2: 0.0333 g: 0.8753	3.4	k_1: <0.001 k_2: 0.023	k_1: 1.0434 k_2: 0.0136	k_1: 1.3110 k_2: 0.0530	0.7	6.7
HS	Good	96.6	k_1: 0.9409 k_2: 0.0334 tb: 2.3	3.5	k_1: <0.001 k_2: >0.023	k_1: 0.8704 k_2: 0.0136	k_1: 1.0110 k_2: 0.0530	0.7	6.7

Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide good visual fits and reliable statistical parameters. Since FOMC provides the least χ^2 error and 10 % of the initially measured substance concentration was reached within the experimental period, the FOMC model is selected as the best-fit model as well as for deriving modelling endpoints.

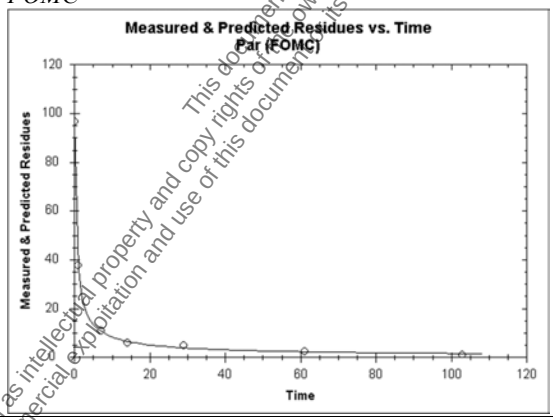
Conclusion: FOMC to be used for trigger endpoints
FOMC to be used for modelling endpoints

SFO





FOMC



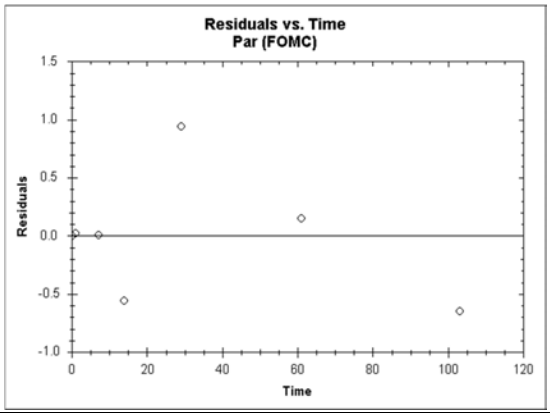
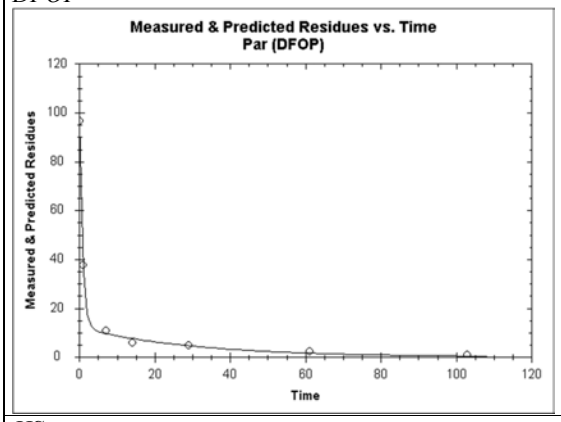
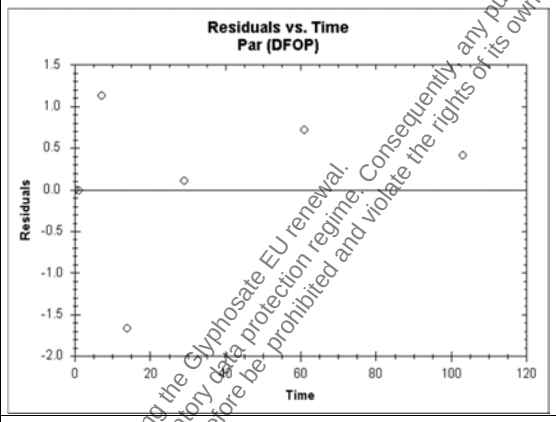


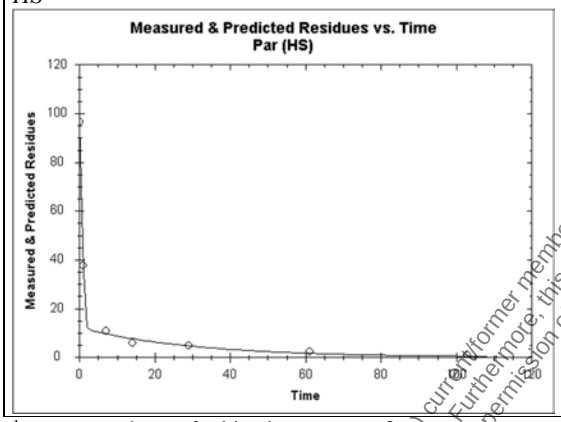
Table 7.2.2.3-67: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree A of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

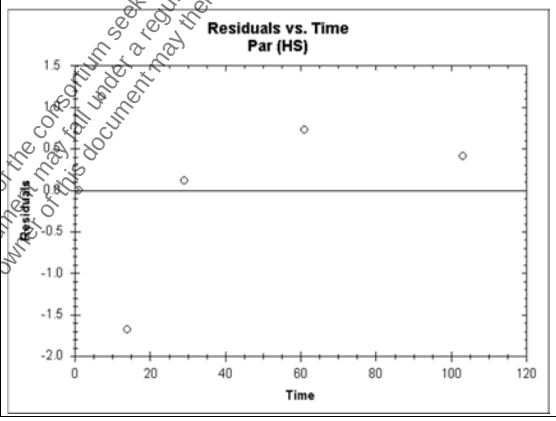
DFOP





HS





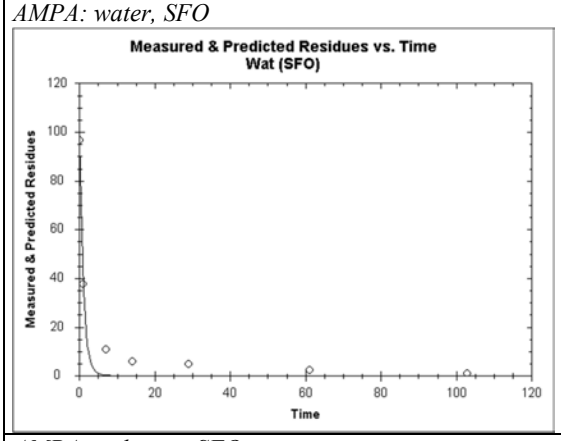
[1] t-test not relevant for kinetic parameter β

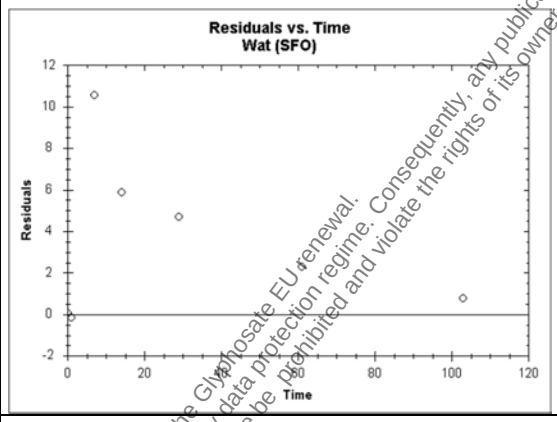
Table 7.2.2.3-68: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of study [redacted] (2004, CA 7.2.2.3/018), Level P-II

Kinetic model	Visual assess-ment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
Water: SFO	Poor	96.6	k_{wat}: 0.7478 k_{wat_sed}: 0.1884	18.9	k_{wat}: <0.001 k_{wat_sed}: 0.0303	k_{wat}: 0.4450 k_{wat_sed}: -0.0162	k_{wat}: 1.051 k_{wat_sed}: 0.361	0.9	3.1
Sediment: SFO	Poor	0.0	k_{sed}: 2.24×10^{-14} k_{sed_wat}: 0.0009	42.5	k_{sed}: 0.5 k_{sed_wat}: 0.4970	k_{sed}: -0.1898 k_{sed_wat}: -0.2356	k_{sed}: 0.190 k_{sed_wat}: 0.237	>1000	>1000
The visual and statistical fits obtained for the water phase and sediment phase are not acceptable. **Conclusion:** No further evaluation was conducted. No reliable endpoints could be derived									

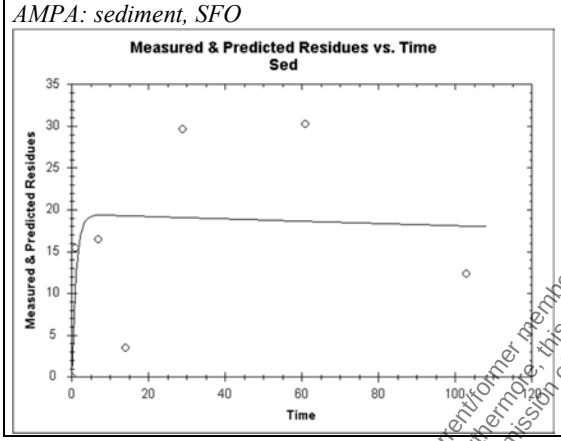
Table 7.2.2.3-68: Kinetic models and goodness-of-fit statistics of AMPA degradation in system Manningtree A of study [redacted] (2004, CA 7.2.2.3/018), Level P-II

AMPA: water, SFO





AMPA: sediment, SFO



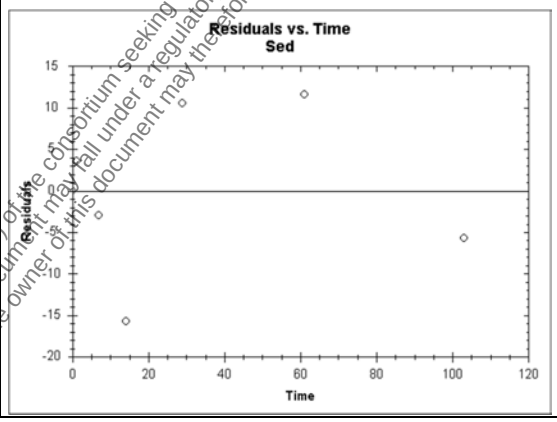


Table 7.2.2.3-69: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree B of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

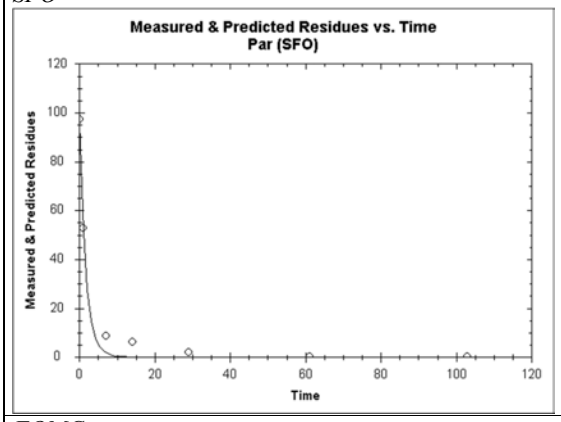
Kinetic model	Visual assessment	M_0	Kinetic parameters	χ^2 error (%)	Prob > t (10 % level)	Lower CI (95 %)	Upper CI (95 %)	DT_{50} (d)	DT_{90} (d)
SFO	Poor	96.4	k: 0.5747	12.1	k: <0.001	k: 0.4021	k: 0.7470	1.2	4.0
FOMC	Good	97.3	α: 1.4791 β: 1.93	3.4	-[1]	β: 1.2645	β: 2.5960	1.2	7.2
DFOP	Good	97.2	k_1: 0.7311 k_2: 0.0592 g: 0.8689	1.3	k_1: <0.001 k_2: 0.004	k_1: 0.6921 k_2: 0.0415	k_1: 0.7700 k_2: 0.0770	1.1	6.2
HS	Good	97.2	k_1: 0.6122 k_2: 0.0623 tb: 3.6	1.0	k_1: <0.001 k_2: 0.002	k_1: 0.5951 k_2: 0.0486	k_1: 0.6290 k_2: 0.0760	1.1	5.5

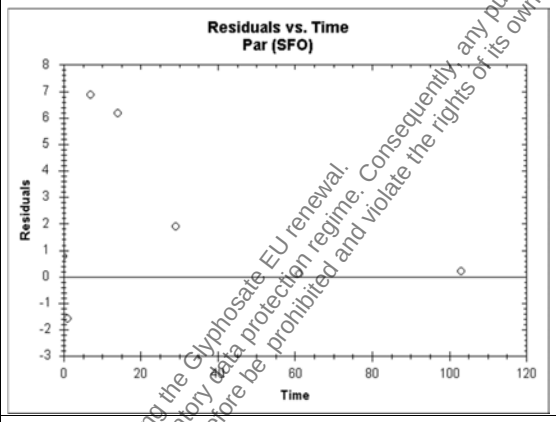
Dissipation of AMPA in the water phase is best described by bi-phasic models. All bi-phasic models provide good visual fits and reliable statistical parameters but the HS model provies the least χ^2 error. Thus, the HS model is selected as the best-fit model as well as for deriving modelling endpoints.

Conclusion: HS to be used for trigger endpoints
HS to be used for modelling endpoints

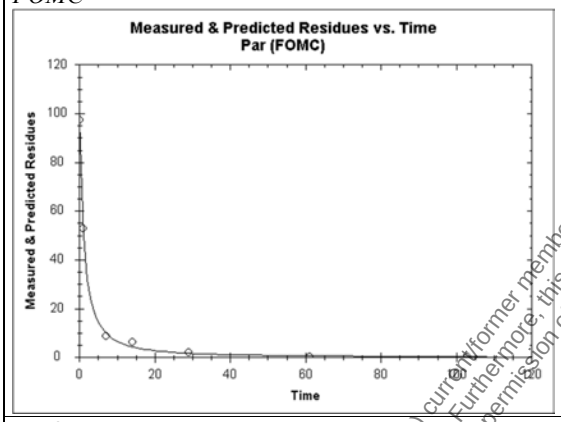
Table 7.2.2.3-69: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree B of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

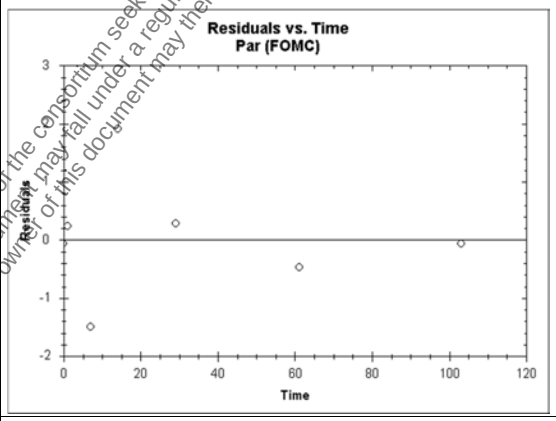
SFO



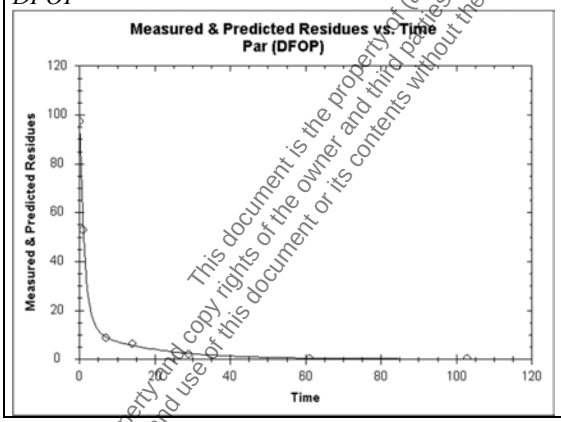


FOMC





DFOP



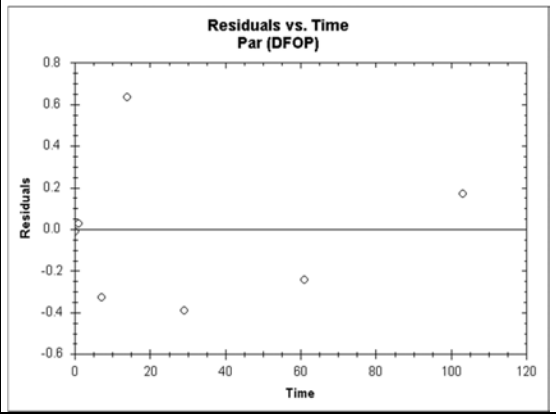
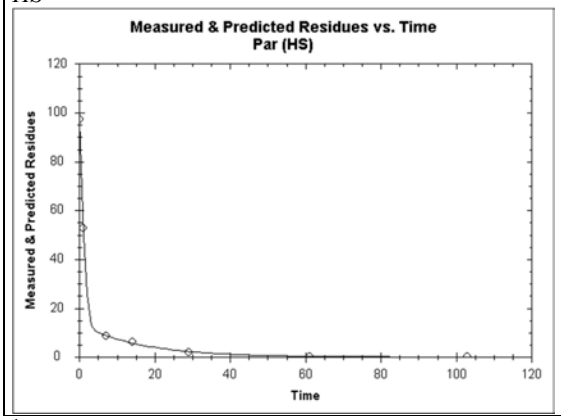
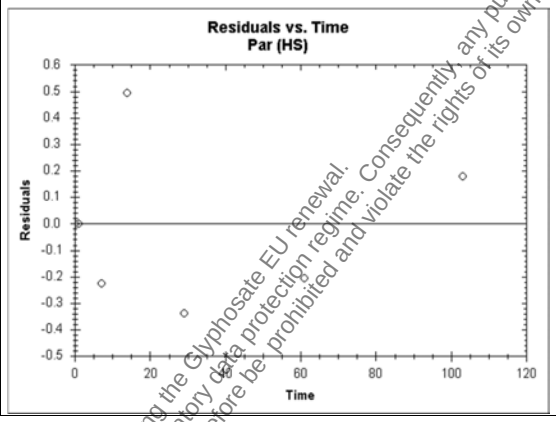


Table 7.2.2.3-69: Kinetic models and goodness-of-fit statistics of AMPA dissipation in system Manningtree B of the study [redacted] (2004, CA 7.2.2.3/018), Level P-I, water phase

HS





[1] t-test not relevant for kinetic parameter β

Overview of trigger and modelling endpoints

No reliable endpoints could be derived at Level P-II. A summary of trigger and modelling endpoints for glyphosate and its metabolites AMPA and HMPA is given in the tables below:

Table 7.2.2.3-70: Degradation and dissipation in water / sediment systems: trigger endpoints of glyphosate, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	DT_{50} (d)[1]	DT_{90} (d)[1]	χ^2 error (%)	Kinetic model
Total system								
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	8.4	45.6	2.7	FOMC
	Putah	8.4	7.5	20	195.8	902.3	4.4	DFOP
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	15.8	329.4	2.2	HS
	Unter Widdersheim	8.6	7.68	20	121.6	>1000	4.8	DFOP
Water phase								
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	5.0	22.7	2.3	DFOP
	Putah	8.4	7.5	20	7.9	78.2	10.0	FOMC
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	2.0	22.2	5.2	DFOP
	Unter Widdersheim	8.6	7.68	20	1.1	28.7	2.6	DFOP

Table 7.2.2.3-70: Degradation and dissipation in water / sediment systems: trigger endpoints of glyphosate, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	DT_{50} (d)[1]	DT_{90} (d)[1]	χ^2 error (%)	Kinetic model
Sediment phase								
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	33.9	112.6	8.4	SFO
	Putah	8.4	7.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	158.7	965.3	3.6	DFOP
	Unter Widdersheim	8.6	7.68	20	-[3]	-[3]	-[3]	-[3]

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[3] No acceptable fit obtained and no endpoints could be derived

Table 7.2.2.3-71: Degradation and dissipation in water / sediment systems: modelling endpoints of glyphosate, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	SFO DT_{50} (d)[1]	χ^2 error (%)	Kinetic model
Total system							
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	9.7	5.3	SFO
	Putah	8.4	7.5	20	301.4[2]	4.4	DFOP
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	144.4[2]	2.2	HS
	Unter Widdersheim	8.6	7.68	20	1000[3]	4.8	DFOP
Water phase							
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	5.9	8.5	SFO
	Putah	8.4	7.5	20	23.6[4]	10.0	FOMC
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	6.7[4]	5.2	DFOP
	Unter Widdersheim	8.6	7.68	20	8.6[4]	2.6	DFOP
Sediment phase							
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	33.9	8.4	SFO
	Putah	8.4	7.5	20	-[5]	-[5]	-[5]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	346.6[2]	3.6	DFOP
	Unter Widdersheim	8.6	7.68	20	-[6]	-[6]	-[6]

[1] DT_{50} = $DegT_{50}$ for total system but $DisT_{50}$ for water and sediment phase
[2] Calculated from slow-phase degradation rate (k_2) as 10 % of the initial amount was not reached within experimental period
[3] The estimated degradation rate is not significantly different from zero, default $DegT_{50}$ of 1000 d to be used
[4] Back-calculated from DT_{90}/3.32 as 10 % of the initial amount was reached within experimental period
[5] No evaluation could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[6] No acceptable fit obtained and no endpoints could be derived

Table 7.2.2.3-72: Degradation and dissipation in water / sediment systems: trigger endpoints of AMPA, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	DT_{50} (d)[1]	DT_{90} (d)[1]	χ^2 error (%)	Kinetic model
Total system								
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	12.6	>1000	1.6	FOMC
	Schäphysen	8.0	7.34	20	2.4	>1000	6.2	DFOP
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.5	7.3	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	43.5	196.8	3.5	DFOP
	Unter Widdersheim	8.2	7.6	20	17.7	579.8	3.4	HS
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	-[3]	-[3]	-[3]	-[3]
	Manningtree B	7.1	6.3	20	-[4]	-[4]	-[4]	-[4]
Water phase								
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	2.2	22.1	2.1	FOMC
	Schäphysen	8.0	7.34	20	1.1	6.6	3.2	FOMC
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	2.4	37.1	5.3	FOMC
	Unter Widdersheim	8.5	7.3	20	2.1	25.9	8.0	FOMC
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	6.6	50.7	4.5	DFOP
	Unter Widdersheim	8.2	7.6	20	2.0	17.3	8.2	DFOP
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	0.6	8.1	1.8	FOMC
	Manningtree B	7.1	6.3	20	1.1	5.5	1.0	HS
Sediment phase								
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	168.1	558.3	1.9	SFO
	Schäphysen	8.0	7.34	20	-[3]	-[3]	-[3]	-[3]
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.5	7.3	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	-[5]	-[5]	-[5]	-[5]
	Unter Widdersheim	8.2	7.6	20	-[3]	-[3]	-[3]	-[3]
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	-[5]	-[5]	-[5]	-[5]
	Manningtree B	7.1	6.3	20	-[4]	-[4]	-[4]	-[4]

[1] DT_{50} = $Deg T_{50}$ for total system but $Dis T_{50}$ for water and sediment phase
[2] The data of the sediment phase and the total system were not considered in the kinetic evaluation
[3] No acceptable fit obtained and no endpoints could be derived
[4] Due to experimental problems, the total system and the sediment phase were not considered in the kinetic evaluation
[5] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration

Table 7.2.2.3-73: Degradation and dissipation in water / sediment systems: modelling endpoints of AMPA, Level P-I

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	SFO DT$_{50}$ (d)^{1}	χ^2 error (%)	Kinetic model
Total system							
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	95.0^{2}	3.8	DFOP
	Schäphysen	8.0	7.34	20	1000^{3}	6.2	DFOP
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	-^{4}	-^{4}	-^{4}
	Unter Widdersheim	8.5	7.3	20	-^{4}	-^{4}	-^{4}
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	47.7	5.9	SFO
	Unter Widdersheim	8.2	7.6	20	288.8^{2}	3.4	HS
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	-^{5}	-^{5}	-^{5}
	Manningtree B	7.1	6.3	20	-^{6}	-^{6}	-^{6}
Water phase							
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	6.7^{7}	2.1	FOMC
	Schäphysen	8.0	7.34	20	1.5	10.7	SFO
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	11.2^{7}	5.3	FOMC
	Unter Widdersheim	8.5	7.3	20	7.8^{7}	8.0	FOMC
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	15.3^{7}	4.5	DFOP
	Unter Widdersheim	8.2	7.6	20	5.2^{7}	8.2	DFOP
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	2.4^{7}	1.8	FOMC
	Manningtree B	7.1	6.3	20	1.7^{7}	1.0	HS
Sediment phase							
[redacted] (2002, CA 7.2.2.3/020)	Rückhaltebecken	8.7	7.64	20	168.1	1.9	SFO
	Schäphysen	8.0	7.34	20	-^{5}	-^{5}	-^{5}
[redacted] (2003, CA 7.2.2.3/019)	Bickenbach	8.5	7.0	20	-^{4}	-^{4}	-^{4}
	Unter Widdersheim	8.5	7.3	20	-^{4}	-^{4}	-^{4}
[redacted] (1999, CA 7.2.2.3/021)	Bickenbach	8.3	7.7	20	-^{8}	-^{8}	-^{8}
	Unter Widdersheim	8.2	7.6	20	-^{5}	-^{5}	-^{5}
[redacted] (2004, CA 7.2.2.3/018)	Manningtree A	7.2	7.6	20	-^{8}	-^{8}	-^{8}
	Manningtree B	7.1	6.3	20	-^{6}	-^{6}	-^{6}

[1] DT$_{50}$ = DegT$_{50}$ for total system but DisT$_{50}$ for water and sediment phase

[2] Calculated from slow-phase degradation rate (k_2) as 10 % of the initial amount was not reached within experimental period

[3] The estimated degradation rate is not significantly different from zero, default DegT$_{50}$ of 1000 d to be used

[4] The data of the sediment phase and the total system were not considered in the kinetic evaluation

[5] No acceptable fit obtained and no endpoints could be derived

[6] Due to experimental problems, the total system and the sediment phase were not considered in the kinetic evaluation

[7] Back-calculated from DT$_{90}$/3.32 as 10 % of the initial amount was reached within experimental period

[8] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration

Table 7.2.2.3-74: Degradation in water / sediment systems: trigger and modelling endpoints of AMPA, Level M-I, degradation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Formation fraction	χ^2 error (%)	Kinetic model
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	172.8	573.9	0.339 (from parent)	7.0	FOMC-SFO
	Putah	8.4	7.5	20	-[1]	-[1]	-[1]	-[1]	-[1]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	15.7	52.3	0.488 (from parent)	9.4	HS-SFO
	Unter Widdersheim	8.6	7.68	20	8.8	29.2	0.321 (from parent)	22.4	DFOP-SFO

[1] No acceptable fit obtained and no endpoints could be derived

Table 7.2.2.3-75: Dissipation in water / sediment systems: trigger and modelling endpoints of AMPA, Level M-I dissipation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DisT_{50}$ (d)	$DisT_{90}$ (d)	χ^2 error (%)	Kinetic model
Total system								
[redacted] (1999, CA 7.2.2.3/002)	Cache[1]	8.2	8.1	20	224.6	746.2	3.2	SFO
	Putah	8.4	7.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	26.8[3]	88.9[3]	7.9	SFO
	Unter Widdersheim	8.6	7.68	20	15.1[3]	50.0[3]	5.8	SFO
Water phase								
[redacted] (1999, CA 7.2.2.3/002)	Cache	8.2	8.1	20	53.8	178.8	6.1	SFO
	Putah	8.4	7.5	20	-[2]	-[2]	-[2]	-[2]
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	26.8	88.9	7.9	SFO
	Unter Widdersheim	8.6	7.68	20	15.1	50.0	5.8	SFO

[1] No evaluations could be conducted for the sediment phase due to the limited number of data points available after the peak concentration
[2] No evaluations could be conducted for any compartment at Level M-I dissipation due to the limited number of data points available after the peak concentration
[3] Since AMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system

Table 7.2.2.3-76: Degradation in water / sediment systems: trigger and modelling endpoints of HMPA, Level M-I, degradation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DegT_{50}$ (d)	$DegT_{90}$ (d)	Formation fraction	χ^2 error (%)	Kinetic model
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	128.8	427.8	0.366 (from AMPA)	20.5	HS-SFO
	Unter Widdersheim	8.6	7.68	20	10.0	33.4	0.359 (from AMPA)	39.3	DFOP-SFO

Table 7.2.2.3-77: Dissipation in water / sediment systems: trigger and modelling endpoints of HMPA, Level M-I dissipation

Study	Water / sediment system	pH water phase	pH sed	t. (°C)	$DisT_{50}$ (d)	$DisT_{90}$ (d)	χ^2 error (%)	Kinetic model
Total system & water phase[1]								
[redacted] (1993, CA 7.2.2.3/005)	Bickenbach	8.6	7.8	20	-[2]	-[2]	-[2]	-[2]
	Unter Widdersheim	8.6	7.68	20	8.9	29.5	7.1	SFO

[1] Since HMPA was not detected in sediment in the study, evaluations at Level M-I dissipation were performed for the water phase only, which are also applicable for the total system
[2] No evaluations could be conducted at Level M-I dissipation due to the limited number of data points available after the peak concentration

3. Assessment and conclusion

Assessment and conclusion by applicant:
The kinetic evaluation was performed according to the current guidances without any deviations. Thus, the provided endpoints can be used for risk assessment.

Assessment and conclusion by RMS:

Water/sediment studies with glyphosate as test item

1. Information on the study

Data point:	CA 7.2.2.3/002
Report author	[redacted]
Report year	1999
Report title	Glyphosate-Trimesium: Degradation of ^{14}C-PMG Labelled Compound in Natural Water-Sediment Systems Under Laboratory Conditions
Report No	RR 99-039B
Document No	
Guidelines followed in study	BBA Guideline Part IV, 5-1 SETAC "Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides", 8.2
Deviations from current test guideline	From OECD 308: - samples were incubated in an desiccator and air was drawn into the desiccator and not to each vessel individually - water:sediment ratio about 2:1 instead of 3:1 to 4:1 - CO_2-free air was used, - residues of glyphosate and AMPA reported for water and total system, but not for sediment
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate-trimesium, labelled in the phosphonomethyl-glycine (PMG) anion, was investigated in two water/sediment systems under aerobic conditions in the dark in the laboratory at 20 °C for 100 days. Since the glyphosate molecule was radiolabelled and thus subject of analysis, this summary is only using the term glyphosate.

The following two water/sediment systems were used: Cache, a loamy sand, and Putah, a silty loam. The amount of organic matter in the sediments was 0.6 and 2.1 % and the pH was 7.5 and 8.1. The pH in the aqueous layer was 7.6 and 7.7.

The test was performed in flow-through systems connected to two 1 N NaOH traps to collect carbon dioxide.

The test substance was applied to the surface water in each jar to give a nominal initial concentration of 3.3 mg/L of glyphosate-trimesium in the water column, equivalent to a single surface application of 9 kg/ha of glyphosate-trimesium being evenly distributed to a depth of 30 cm.

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 3, 7, 14, 30, 58 and 100 days after treatment (DAT). The NaOH traps were assayed and changed at each sampling interval, or approximately every two weeks, which ever was the sooner.

Mean material balances ranged from 86.3 to 100.4 % of applied radioactivity (% AR) for the Cache water/sediment system, and from 91.1 to 102.7 % AR for the Putah water/sediment system.

The amount of glyphosate in the water decreased from 0 DAT to 100 DAT from 98.85 to 0.83 % AR in system Cache and from 100.62 to 5.12 % AR in system Putah.

The amount of glyphosate in sediment extracts of system Cache increased from 0.54 % AR at 0 DAT to 15.88 % AR at 3 DAT and decreased to 3.68 % AR at 100 DAT. The amount of glyphosate in sediment extracts of system Putah increased from 0.69 % AR at 0 DAT to 58.22 % AR at 100 DAT.

The amount of glyphosate in the total system decreased from 0 DAT to 100 DAT from 99.39 to 4.51 % AR in system Cache and from 101.30 to 63.34 % AR in system Putah.

One major degradation product, aminomethylphosphonic acid (AMPA), formed primarily by microbial degradation of the parent, was found in both water/sediment systems over the course of the incubation. In the Cache total system, levels of AMPA were found to be highest at 30 to 58 DAT reaching up to 27 % AR and decreased to 21.8 % AR at 100 DAT. Maximum amounts of AMPA in water and sediment extracts of system Cache were 10.31 % AR (30 DAT) and 18.70 % AR (58 DAT), respectively.

In the Putah total system, levels of AMPA were also found to be highest at 30 to 58 DAT, reaching 5.26 % AR at 58 DAT and decreased to 3.57 % AR at 100 DAT. Maximum amounts of AMPA in water and sediment extracts of system Putah were 1.45 % AR (58 DAT) and 3.81 % AR (58 DAT), respectively.

No other metabolites were detected above 3 % AR at any time.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate-trimesium (radiolabelled phosphonomethyl-glycine anion)
Lot No.:	3350-149
Specific activity:	1927.7 MBq/mmol (52.1 mCi/mmol)
Radiochemical purity:	>99 %
Chemical purity:	Not reported

2. Test System:

The sediments were prepared for use in the study by sieving to 2 mm and by thorough mixing to provide homogeneous samples. The water was sieved through a 0.2 mm sieve and stored in polypropylene buckets lined with plastic bags. The water and sediment samples were stored at approximately 4 °C until all the water/sediment incubation jars had been set-up. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-78: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Cache	Putah
Country	United States of America	United States of America
Sediment:		
Textural Class (USDA)	Loamy sand	Silt loam
Sand [50 µm – 2 mm] (%)	76	26
Silt [2 µm – 50 µm] (%)	22	54
Clay [< 2 µm] (%)	2	20
pH [1]	8.1	7.5

Table 7.2.2.3-78: Characteristics of test water/sediment systems

Organic matter (%)	0.6	2.1
Cation exchange capacity (meq/100 g)	11.7	22.0
Microbial biomass (mg C/100g)		
Before application	20.3	29.7
Study end (100 DAT)	15.1	13.9
Water:		
pH	8.2	8.4
Dissolved O_2 at surface (mg/L)	10.2	10.0
Dissolved O_2 5 cm above sediment (mg/L)	10.2	9.8
Redox potential (mV)	587	608

DAT = Days after treatment, USDA: United States Department for Agriculture
[1] Medium not reported

B. STUDY DESIGN

1. Experimental conditions

The wet sediments were dispensed into cylindrical glass jars (237 mL) and the associated natural waters were added, 120 mL of Cache water and 130 mL of Putah water. The Cache test systems contained 75.7 g sediment (dry weight) and Putah test systems contained 58.9 g sediment (dry weight). In both the Cache and Putah system the average depth of settled sediment was 3.0 cm and the average depth of the surface water was 6.0 cm.

The test vessels were placed in a desiccator and CO_2-free air was drawn slowly into the desiccator over the surface in the jars to maintain the aerobic status of the water. Air entering the system was passed through a water hydrator and 1 N NaOH scrubber. After leaving the test vessels the air was passed through two traps containing 100 mL of 1 N NaOH to collect carbon dioxide.

The test systems were incubated in the dark in a constant temperature room at 20 ± 2°C. The water/sediment systems were pre-incubated at 20°C ± 2°C for 19 days (Putah) and 20 days (Cache) prior to treatment to allow equilibration.

The test substance was applied to the surface water in each jar to give a nominal initial concentration of 3.3 mg/L of glyphosate-trimesium in the water column, equivalent to a single surface application of 9 kg/ha of glyphosate-trimesium being evenly distributed to a depth of 30 cm. After application the test vessels (except 0 DAT), were closed with trap attachments.

Test systems were incubated under aerobic conditions in the dark for 100 days at 20°C. During acclimatization and incubation pH value, oxygen saturation and redox potential of the water layer and the redox potential of the sediment layer were monitored in additional untreated test vessels.

2. Sampling

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 3, 7, 14, 30, 58 and 100 days after treatment (DAT). The surface waters were analysed by LSC and HPLC on the day they were sampled (except 14 DAT HPLC analysis, which was run within 7 days). All sediment samples were extracted on the designated sampling day and analysed by LSC within 2 days and by HPLC within 15 days. The NaOH traps were assayed and changed at each sampling interval, or approximately every two weeks, which ever was the sooner.

3. Analytical procedures

For each system the water column from above the sediment was transferred by suction to a 250 ml polypropylene centrifuge bottle without disturbing the sediment. Afterwards, the water was acidified with 50 mL of 0.5 M KH_2PO_4 and sparged for 30 minutes by pulling air through the water and on through two 1N NaOH traps to remove and trap volatile degradates and carbonate/carbon dioxide. Following the sparging, the volume of the acidified and CO_2-free water was measured and an aliquot was analysed by LSC. Small volumes (about 1 mL) of the acidified water samples were filtered and analysed by HPLC and TLC. Prior to acidification, small aliquots of selected water samples were removed for HPLC analysis.

The sediment was also acidified (and extracted) with 50 mL of 0.5 M KH_2PO_4 and sparged for 30 minutes to purge and trap volatile degradates and carbonate/carbon dioxide in a manner similar to the water. The acidified sediment was transferred into polypropylene centrifuge bottles and extracted by shaking for half an hour on a wrist action shaker. The extract was separated from the sediment by centrifugation, the volume measured and an aliquot analysed by LSC. The sediment was extracted 3-5 times and the extracts were combined for further analyses by HPLC. Selected extracts were analysed by TLC.

To quantify non-extractable residues (NER), extracted sediments were dried with acetone (50 mL) by shaking and centrifugation. The acetone layer was decanted, the volume measured and an aliquot analysed by LSC. The extracted sediments were left in the fume hood in open centrifuge bottles to dry. The radioactivity in the dry sediment was quantified by combustion/LSC. All sample calculations were corrected for combustion efficiency. Mean combustion efficiency was 98.0 % for the study samples.

The limit of detection (LOD) for both the LSC and LSC/combustion methods was twice the background signal, corresponding to 0.001 ppm. The limit of quantitation for HPLC/RAM is twice the background signal, equalling a peak height greater than 20 cpm above background.

The sodium hydroxide trap solutions generated during sample sparging were analysed by LSC. The identification of CO_2 in the sodium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

Glyphosate and its metabolite were identified by co-chromatography with reference items.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 8.0 and 8.9 in system Cache and between 7.3 and 7.7 for system Putah. The oxygen saturation in the water phase ranged between 51 and 84 % in system Cache and between 33 and 48 % in system Putah. The redox potential of the water was between 55 and 198 mV for system Cache and between 155 and 282 mV for system Putah. The redox potential of the sediment was between 57 and 187 mV in system Cache and between -132 and 14 mV for system Putah.

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in water/sediment systems are summarised in Table 7.2.2.3-79 to Table 7.2.2.3-82.

Table 7.2.2.3-79: Distribution of radioactivity in Cache water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

Fraction	Replicate	DAT									
		0	0.25	1	2	3	7	14	30	58	100
$^{14}CO_2$ (Aq NaOH)	Mean	0.0 [1]	0.0 [1]	2.3	5.2	6.5	15.3	24.6	27.5	37.9	48.0
Surface water	A	98.2	88.4	75.7	67.9	62.1	44.7	30.2	18.4	10.2	5.1
	B	100.9	88.0	72.8	69.3	63.7	45.1	31.4	18.8	10.3	5.1
	Mean	99.6	88.2	74.3	68.6	62.9	44.9	30.8	18.6	10.3	5.1
Sediment extract	A	0.6	7.3	11.4	14.9	20.1	21.6	21.8	27.2	23.9	23.3
	B	0.5	8.1	15.4	17.4	17.8	21.0	23.1	28.6	24.0	24.1
	Mean	0.50	7.7	13.4	16.2	18.9	21.4	22.5	27.9	23.9	23.7
Acetone (drying)	A	<0.1	0.1	0.2	0.2	0.2	0.5	0.7	0.6	0.7	0.8
	B	0.1	0.1	0.2	0.2	0.2	0.4	0.5	0.6	0.6	0.6
	Mean	<0.1	0.1	0.2	0.2	0.2	0.4	0.6	0.6	0.7	0.7
Non-extractable residues (NER)	A	0.2	2.2	5.7	7.0	8.2	11.8	11.7	12.4	12.5	13.9
	B	0.2	2.2	7.7	7.1	9.5	12.2	12.4	11.7	14.6	13.1
	Mean	0.2	2.2	6.7	7.1	8.9	12.0	12.1	12.1	13.5	13.5
Mass balance	A	99.0	98.0	95.1	95.6	97.2	94.4	89.1	89.4	85.0	89.3
	B	101.7	98.4	98.5	98.9	97.5	93.5	91.9	84.0	87.6	92.5
	Mean	100.4	98.2	96.9	97.3	97.4	94.0	90.6	86.7	86.3	91.0

DAT: Days after treatment
[1] Sparging and trapping was not performed on the 0 and 0.25 DAT samples.
$^{14}CO_2$ consists of both radioactivity trapped during incubation and radioactivity from the water/sediment compartments that was volatilized on acidification of water and sediment samples. The amount of radioactivity recovered in the post-desiccator NaOH traps was divided by the number of test vessels in the desiccator over the trapping period to determine the radioactivity evolved as CO_2 per jar.

Table 7.2.2.3-80: Distribution of radioactivity in Putah water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

Fraction	Replicate	DAT									
		0	0.25	1	2	3	7	14	30	58	100
$^{14}CO_2$ (Aq NaOH)	Mean	0.0 [1]	<0.1 [1]	3.8	0.8	2.2	2.0	3.9	5.2	5.7	5.9
Surface water	A	102.6	91.9	75.0	77.5	64.2	61.3	35.1	20.0	13.2	5.8
	B	100.4	92.9	66.7	76.6	64.2	61.6	33.6	22.7	10.2	5.5
	Mean	101.5	92.4	70.8	77.1	64.2	61.5	34.3	21.3	11.7	5.6
Sediment extract	A	0.7	5.4	12.8	12.6	20.0	22.5	37.9	57.0	59.7	60.5
	B	0.7	5.9	14.3	14.2	21.5	21.6	36.0	60.8	64.7	64.2
	Mean	0.7	5.7	13.6	13.4	20.7	22.1	37.0	58.9	62.2	62.3
Acetone (drying)	A	<0.1	<0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.2
	B	<0.1	<0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.3
	Mean	<0.1	<0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.3
Non-extractable residues (NER)	A	0.6	2.9	8.0	6.6	11.6	12.5	17.7	15.4	19.4	17.1
	B	0.5	2.95	8.9	6.9	10.4	10.0	15.8	15.0	21.1	16.2
	Mean	0.5	2.9	8.4	6.7	11.0	11.2	16.7	15.2	20.3	16.7
Mass balance	A	103.9	100.2	98.2	97.5	98.3	98.5	94.1	98.2	98.7	89.5
	B	101.6	101.8	95.2	98.6	98.1	95.5	90.3	103.5	101.4	92.0
	Mean	102.7	101.0	96.7	98.1	98.2	97.0	92.2	100.8	100.1	91.1

Table 7.2.2.3-80: Distribution of radioactivity in Putah water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

DAT: Days after treatment
[1] Sparging and trapping was not performed on the 0 and 0.25-DAT samples.
$^{14}CO_2$ consists of both radioactivity trapped during incubation and radioactivity from the water/sediment compartments that was volatilized on acidification of water and sediment samples. The amount of radioactivity recovered in the post-desiccator NaOH traps was divided by the number of test vessels in the desiccator over the trapping period to determine the radioactivity evolved as CO_2 per jar.

Table 7.2.2.3-81: Degradation of [^{14}C]glyphosate in Cache water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Compartment	Replicate	DAT 0	0.25	1	2	3	7	14	30	58	100
Glyphosate	Water	A	97.63	87.38	74.26	66.24	59.9	39.51	21.98	7.34	1.53	0.79
		B	100.06	87.17	71.54	67.13	61.27	39.82	22.22	8.3	1.61	0.87
		Mean	*98.85*	*87.28*	*72.90*	*66.69*	*60.59*	*39.67*	*22.10*	*7.82*	*1.57*	*0.83*
	Sediment	A	*0.54*	*6.71*	*10.68*	*12.8*	*16.98*	*14.1*	*11.27*	*9.45*	*3.03*	*4.06*
		B	*0.54*	*7.48*	*14.38*	*15.31*	*14.77*	*14.36*	*12.35*	*10.3*	*3.76*	*3.3*
		Mean	*0.54*	*7.10*	*12.53*	*14.06*	*15.88*	*14.23*	*11.81*	*9.88*	*3.40*	*3.68*
	Total system	A	98.17	94.09	84.94	79.04	76.88	53.61	33.25	16.79	4.56	4.85
		B	100.6	94.65	85.92	82.44	76.04	54.18	34.57	18.6	5.37	4.17
		Mean	*99.39*	*94.37*	*85.43*	*80.74*	*76.46*	*53.90*	*33.91*	*17.70*	*4.97*	*4.51*
AMPA	Water	A	0.24	0.33	1.30	1.62	2.19	5.24	8.07	10.52	8.07	3.69
		B	0.66	0.46	1.30	2.17	2.45	5.30	8.93	10.10	8.08	3.97
		Mean	*0.45*	*0.40*	*1.30*	*1.90*	*2.32*	*5.27*	*8.50*	*10.31*	*8.08*	*3.83*
	Sediment	A	*0.00*	*0.58*	*0.72*	*1.85*	*2.99*	*7.08*	*9.86*	*16.45*	*19.19*	*17.02*
		B	*0.00*	*0.62*	*0.98*	*1.86*	*2.67*	*6.26*	*9.99*	*17.08*	*18.20*	*18.92*
		Mean	*0.00*	*0.60*	*0.85*	*1.86*	*2.83*	*6.67*	*9.93*	*16.77*	*18.70*	*17.97*
	Total system	A	0.24	0.91	2.02	3.47	5.18	12.32	17.93	26.97	27.26	20.71
		B	0.66	1.08	2.28	4.03	5.12	11.56	18.92	27.18	26.28	22.89
		Mean	*0.45*	*1.00*	*2.15*	*3.75*	*5.15*	*11.94*	*18.43*	*27.08*	*26.77*	*21.80*

DAT: Days after treatment
Values calculated during dossier preparation are given in *italics*

Table 7.2.2.3-82: Degradation of [^{14}C]glyphosate in Putah water/sediment system under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Compartment	Replicate	DAT									
			0	0.25	1	2	3	7	14	30	58	100
Glyphosate	Water	A	101.59	90.68	74.05	76.63	63.52	60.24	34.02	18.64	11.45	5.26
		B	99.64	91.77	65.68	75.39	63.28	60.74	32.47	22.11	9.04	4.97
		Mean	*100.62*	*91.23*	*69.87*	*76.01*	*63.40*	*60.49*	*33.25*	*20.38*	*10.25*	*5.12*
	Sediment	A	*0.68*	*5.43*	*12.08*	*12.59*	*18.88*	*21.18*	*36.29*	*52.18*	*54.28*	*56.51*
		B	*0.69*	*5.9*	*13.36*	*13.78*	*20.17*	*20.69*	*34.56*	*37.77*	*60.15*	*59.93*
		Mean	*0.69*	*5.67*	*12.72*	*13.19*	*19.53*	*20.94*	*35.43*	*54.98*	*57.22*	*58.22*
	Total system	A	102.27	96.11	86.13	89.22	82.4	81.42	70.31	70.82	65.73	61.77
		B	100.33	97.67	79.04	89.17	83.45	81.43	67.03	79.88	69.19	64.9
		Mean	*101.30*	*96.89*	*82.59*	*89.20*	*82.93*	*81.43*	*68.67*	*75.35*	*67.46*	*63.34*
AMPA	Water	A	0.41	0.8	0.96	0.81	0.64	1.1	1.08	1.32	1.78	0.54
		B	0.37	0.69	0.89	0.9	0.86	0.82	1.11	0.58	1.12	0.5
		Mean	*0.39*	*0.75*	*0.93*	*0.86*	*0.75*	*0.96*	*1.10*	*0.95*	*1.45*	*0.52*
	Sediment	A	*0.00*	*0.00*	*0.75*	*0.00*	*0.89*	*0.91*	*1.6*	*4.01*	*4.37*	*3.13*
		B	*0.00*	*0.00*	*0.9*	*0.49*	*0.98*	*0.61*	*1.04*	*2.44*	*3.25*	*2.96*
		Mean	*0.00*	*0.00*	*0.83*	*0.25*	*0.94*	*0.76*	*1.32*	*3.23*	*3.81*	*3.05*
	Total system	A	0.41	0.8	1.71	0.81	1.53	2.01	2.68	5.33	6.15	3.67
		B	0.37	0.69	1.79	1.39	1.84	1.43	2.15	3.02	4.37	3.46
		Mean	*0.39*	*0.75*	*1.75*	*1.10*	*1.69*	*1.72*	*2.42*	*4.18*	*5.26*	*3.57*

DAT: Days after treatment
Values calculated during dossier preparation are given in *italics*

B. MASS BALANCE

Mean material balances ranged from 86.3 to 100.4 % of applied radioactivity (% AR) for the Cache water/sediment system, and from 91.1 to 102.7 % AR for the Putah water/sediment system.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 100 DAT from 99.6 to 5.1 % AR in the Cache water/sediment system, and from 101.5 to 5.6 % AR in the Putah water/sediment system.

The amount of radioactivity extractable from the sediment increased from 0 DAT to 100 DAT from 0.5 to 23.7 % AR in the Cache water/sediment system, and from 0.7 to 62.3 % AR in the Putah water/sediment system.

The amount of radioactivity in the total system decreased from 0 DAT to 100 DAT from 100.1 to 28.8 % AR in the Cache water/sediment system, and from 102.2 to 67.9 % AR in the Putah water/sediment system.

Levels of non-extractable residues (NER) in the sediment increased gradually to maxima of 13.5 % in the Cache system and 20.3 % in the Putah system at 58 DAT. The levels remained similar by 100 DAT in Cache system, but lower (16.7 %) in Putah system.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (100 DAT) were 48.0 and 5.9 % AR in the Cache and Putah systems, respectively. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in the water decreased from 0 DAT to 100 DAT from 98.85 to 0.83 % AR in system Cache and from 100.62 to 5.12 % AR in system Putah.

The amount of glyphosate in sediment extracts of system Cache increased from 0.54 % AR at 0 DAT to 15.88 % AR at 3 DAT and decreased to 3.68 % AR at 100 DAT. The amount of glyphosate in sediment extracts of system Putah increased from 0.69 % AR at 0 DAT to 58.22 % AR at 100 DAT.

The amount of glyphosate in the total system decreased from 0 DAT to 100 DAT from 99.39 to 4.51 % AR in system Cache and from 101.30 to 63.34 % AR in system Putah.

One major degradation product, aminomethylphosphonic acid (AMPA), formed primarily by microbial degradation of the parent, was found in both water/sediment systems over the course of the incubation. In the Cache total system, levels of AMPA were found to be highest at 30 to 58 DAT reaching up to 27.1 % AR (30 DAT) and decreased to 21.8 % AR at 100 DAT. Maximum amounts of AMPA in water and sediment extracts of system Cache were 10.3 % AR (30 DAT) and 18.7 % AR (58 DAT), respectively.

In the Putah total system, levels of AMPA were also found to be highest at 30 to 58 DAT, reaching 5.26 % AR at 58 DAT and decreased to 3.57 % AR at 100 DAT. Maximum amounts of AMPA in water and sediment extracts of system Putah were 1.45 % AR (58 DAT) and 3.81 % AR (58 DAT), respectively.

No other metabolites were detected above 3 % AR at any time.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in Anagu, 2020, CA 7.2.2.3/001.

III. CONCLUSIONS

Glyphosate dissipated rapidly from surface water in natural water/sediment systems incubated in the dark at 20°C. The rapid initial loss of glyphosate from the surface waters was most likely due to binding to the sediment. This behaviour is consistent with the adsorptive properties of glyphosate.

The binding property of glyphosate was particularly evident in Putah sediment which was higher in the organic matter content. The strong absorptive property of glyphosate rendered it unavailable for the microbial degradation in the Putah system. The majority of the ^{14}C residue recovered from the multiple extractions of Putah sediment was determined to be glyphosate.

The only major metabolite of glyphosate detected in the water/sediment systems was aminomethylphosphonic acid (AMPA). In the Cache systems, AMPA reached maximum levels of 27.0 % of applied radioactivity by 30 DAT and declined to 21.8 % of the applied radioactivity at 100 DAT. In the Putah system, AMPA reached the maximum level of 5.3 % of the applied radioactivity by 58 DAT and declined to 3.6 % by 100 DAT.

A total of 48.0 % of the applied radioactivity in the Cache water/sediment system and 5.9 % in the Putah water/sediment system was mineralised to ^{14}C-carbon dioxide during the course of the incubation. No other individual radiolabelled compound amounted to more than 3 % of the applied radioactivity.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was conducted consistent with the current guideline with minor deviations.

No detailed information on further degradates is given beyond the statement that 'no other radiolabelled compounds amounted to more than 3 % of the applied radioactivity at any time during the incubation.' This is supported by the fact that the sum of glyphosate and AMPA in terms of % AR is nearly the same as the total radioactivity.

Samples were incubated in a desiccator and CO_2-free air was drawn into the desiccator.

Residues of glyphosate and AMPA are reported for water and total system with no separate values for sediment. Values for sediment were calculated upon dossier preparation and do not differ significantly from the amount of radioactivity extracted from the sediment.

Mass balance for Cache samples was below 90 % for some samples (85 and 87 % on 58 DAT, one replicate on 14, 30 and 100 DAT). Since the mass balance was slightly below 90 % and only for a few samples, this is considered negligible.

These deviations are considered to not influence the overall outcome of the study.

Therefore, the study and its data are considered valid to address the data point..

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.2.3/003
Report author	[redacted]
Report year	1997
Report title	[14C-PMG]Glyphosate-trimesium: Aquatic sediment degradation
Report No	RR97-066B
Document No	
Guidelines followed in study	US EPA 162-4
Deviations from current test guideline	From OECD 308: - Mass balances below 90 % AR for all sampling intervals except day zero (68 - 83 %) - Traps only for CO_2 and not for other volatiles - For samples processing, water and sediment were transferred into centrifuge bottles and centrifuged; according to the current guideline water should be decanted without disturbing the sediment- inconsistencies with peak identification
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate-trimesium, labelled in the phosphonomethyl-position, was investigated in two water/sediment systems under aerobic conditions in the dark in the laboratory at 20 ± 1.5 °C for 52 days. Since the glyphosate molecule was radiolabelled and thus subject of analysis, this summary is only using the term glyphosate.

The sediments of the aquatic test systems were characterized as loamy sand from the Cache Creek location and clay loam from the Putah Creek location. The amount of organic matter in the sediments ranged from 0.49 to 1.4 % and the pH in the sediments ranged from 8.0 to 8.1.

The test was performed in flow-through systems connected to a tube of 1 M NaOH to collect carbon dioxide.

The application rate was 2 mg glyphosate-trimesium a.s./L which is equivalent to a use rate of 9000 g glyphosate-trimesium/ha (6000 g glyphosate/ha) evenly distributed to a depth of 30 cm.

Duplicate samples from each system were processed and analysed at 3, 10, 13, 17, 24, 32, and 52 days after treatment (DAT). The NaOH traps were assayed at each sampling time or at about every week, whichever came first, to determine the amount of carbon dioxide.

Mean mass balances ranged from 68.3 to 99.9 % of applied radioactivity (% AR) for system Cache Creek location and from 72.4 to 99.9 % AR for system Putah Creek.

Maximum amounts of carbon dioxide reached at study end (52 DAT) were 19.3 and 12.9 % AR in the Cache Creek and Putah Creek systems, respectively.

The amount of radioactivity in the surface water decreased from 0 DAT to 52 DAT from 98.7 to 9.8 % AR in system Cache Creek system and from 98.7 to 10.3 % AR in system Putah Creek.

The amount of radioactivity extractable from the sediment increased from 0.0 % AR at 0 DAT to a maximum of 56.1 % AR at 3 DAT then decreased to 22.5 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, the amount of radioactivity extractable from the sediment increased from 0.0 % AR at 0 DAT to a maximum of 63.3 % AR at 3 DAT then decreased to 33.7 % AR at 52 DAT.

The amount of non-extractable residues (NER) increased from 1.2 % AR at 0 DAT to 16.7 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, the amount of NER increased from 1.2 % AR at 0 DAT to a maximum of 19.8 % AR at 17 DAT then decreased to 15.6 % AR at 52 DAT.

The amount of glyphosate in the total test system (water and sediment) decreased from 90.1 % AR at 0 DAT to 1.2 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, the amount of glyphosate in the total test system (water and sediment) decreased from 98.1 % AR at 0 DAT to 8.3 % AR at 52 DAT.

Besides carbon dioxide, aminomethylphosphonic acid (AMPA) was detected in the water/sediment systems. AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 32 DAT then decreased to 24.0 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 52 DAT.

Levels of a metabolite assigned to N-methyl-AMPA increased from 0.2 % AR at 0 DAT to a maximum of 14.5 % AR at 13 DAT then decreased to 5.5 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, N-methyl AMPA levels increased from 0.4 % AR at 0 DAT to a maximum of 13.0 % AR at 32 DAT then decreased to 8.7 % AR at 52 DAT.

The degradation of glyphosate under the study conditions was primarily microbially mediated.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]glyphosate-trimesium (labelled in the phosphonomethyl-position)
Lot No.: 3048-281
Specific activity: 51 Ci/mol (674,000 dpm/µg of glyphosate)
Radiochemical purity: 99 % by HPLC
Chemical purity: Not provided

2. Test System:

The sediments were sieved to ≤2 mm. The water and sediment were stored at 4 °C under aerobic conditions for eight weeks before the start of the experiment. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-83: Characteristics of water/sediment test systems

Parameter		**Results**	
System		Cache Creek	Putah Creek
Location		United States of America	United States of America
Sampling depth for	Water	Mid-stream	Mid-stream
	Sediment	1–3 meters from the bank	1–3 meters from the bank
Water			
pH		8.4/8.4	8.3/8.3
Total hardness (mg/L)		134	240
Total alkalinity (mg/L)		100/100	203/202
Chemical oxygen demand (mg/L)		5/4	15/12
Sediment			
Textural Class [1]		Loamy sand/Sand	Clay loam
Sand (%)		87.0/89.1	30.7/30.4
Silt (%)		4.2/4.1	34.5/36.8
Clay (%)		8.8/6.8	34.8/32.8
pH [2]		8.1/8.0	8.0
Organic matter (%)		0.49	1.41/1.36
Cation exchange capacity (meq/100 g)		9.14/8.78	23.83/24.34
Dry matter content (%)		76.1	53.8

[1] Classification system not reported
[2] Medium not stated
Two aliquots of both test systems were characterized

Biomass results indicated that the two water-sediment systems were microbially active at the start of the test incubation period, and that a similar pattern of activity remained at the end of the test period.

B. STUDY DESIGN

1. Experimental conditions

The flow-through test system consisted of a glass vessel connected via tubing to a vacuum system. Air entering the system was first moistened by bubbling through a column of distilled water. The water-sediment systems were pre-incubated at 22 °C for 38 days prior to treatment with the test substance to allow equilibration, as determined by assessment of redox potential, pH and dissolved oxygen levels. Following

application of the radiolabelled test compound, the effluent air from each series of water/sediment systems was drawn through a tube of sodium hydroxide to absorb any $^{14}CO_2$ produced.

The wet sediments were dispensed into cylindrical glass vessels and the associated natural waters were added to a total volume of 150 ml. The Cache Creek test systems contained 79 g sediment (dry weight basis) and the Putah Creek test systems contained 46 g sediment (dry weight basis). For each system, the depth of settled sediment was between 2 and 2.5 cm and the depth of the surface water was approximately 6 cm. Throughout the equilibration period water levels were maintained at 150 mL in the systems by the addition, as necessary, of the appropriate river water.

The application rate was 2 mg glyphosate-trimesium/L in the water phase, which is equivalent to a use rate of 9000 g glyphosate-trimesium/ha (6000 g glyphosate/ha) evenly distributed to a depth of 30 cm.

Test systems were incubated under aerobic conditions in the dark for 52 days at 20 ± 1.5 °C.

Sterile systems were prepared to distinguish between microbial (biotic) and abiotic degradation of the test substance

2. Sampling
Duplicate samples from each system were processed and analyzed at 3, 10, 13, 17, 24, 32 and 52 days after treatment (DAT). The NaOH traps were assayed at each sampling time or about every week, whichever came first. Sterile samples were processed and analysed at 10 and 52 DAT. Only results of 52 DAT are presented in this summary. Although duplicate samples were analyzed, only mean values were reported.

3. Analytical procedures
At each sampling interval, the water/sediment systems were transferred into centrifuge bottles and centrifuged. Afterwards, the water was decanted. Water samples were analysed directly by liquid scintillation counting (LSC).

Sediment samples were extracted four times (3 DAT samples were extracted three times) with ammonium hydroxide for 30 minutes by shaking followed by centrifugation. Fine suspended solids formed in the ammonium hydroxide extracts. The ammonium hydroxide extracts containing suspended solids were combined. The resulting suspension was treated with 0.1 M potassium phosphate monobasic and pH was adjusted to pH 2 using concentrated phosphoric acid. Samples from 3, 10, and 52 DAT were made acidic to pH 3-4 with concentrated hydrochloric acid prior to treatment with phosphate buffer. Subsequently, these suspensions were shaken for one minute and centrifuged. The supernatants were decanted and aliquots were taken for determination of radioactivity by LSC. Subsamples of the precipitates were assayed by combustion followed by LSC. Residues in water and sediment extracts were quantified by HPLC/radiodetection.

On removal, the radioactivity in the sodium hydroxide traps was quantified by LSC. The amount of radioactivity recovered in the sodium hydroxide traps was divided by the number of water-sediment systems in-line over the trapping period to determine evolved radioactivity per vessel.

Radioactivity in extracted sediments were determined by combustion/LSC.

Glyphosate and metabolites in the surface water and sediment extracts were characterized by co-chromatography using HPLC and, for selected samples, TLC.

Samples were stored at approximately -20 °C.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study (including pre-equilibration) between 7.8 and 8.4 in system Cache Creek and between 7.9 and 8.4 for system Putah Creek. The oxygen saturation in the water phase ranged between 61.9 and 76.2 % in system Cache Creek and between 61.9 and 85.7 % in system Putah Creek. The redox potential of the water was between 278 and 427 mV for system Cache Creek and between 300 and 450 mV for system Putah Creek. The redox potential of the sediment was between 170 and 352 mV in system Cache Creek and between 231 and 403 mV for system Putah Creek.

Radioactive mass balance and distribution of glyphosate and metabolites in water/sediment system extracts are summarized in the tables below.

Table 7.2.2.3-84: Amount of radioactivity in Cache Creek under aerobic conditions (mean values of two replicates, expressed as percent of applied radioactivity)

Fraction	DAT								
	0	3	10	13	17	24	32	52	52 sterile
Surface water	98.7	14.6	22.5	22.6	21.3	22.9	18.6	9.8	23.8
Sediment extractable	0.0	56.1	42.1	39.9	35.0	24.8	27.4	22.5	37.3
Non-extractable residues	1.2	11.1	9.5	12.4	13.1	15.3	15.5	16.7	20.5
CO_2	0.0	0.1	1.5	1.6	8.2	11.1	6.7	19.3	2.2
Mass balance	99.9	81.9	75.6	76.5	77.5	74.1	68.3	68.3	83.8

DAT: Days after treatment

Table 7.2.2.3-85: Amount of radioactivity in Putah Creek under aerobic conditions (mean values of two replicates, expressed as percent of applied radioactivity)

Fraction	DAT								
	0	3	10	13	17	24	32	52	52 sterile
Surface water	98.7	2.3	9.7	8.6	7.5	13.6	16.5	10.3	1.2
Sediment (extractable)	0.0	63.3	53.3	53.4	45.1	46.9	43.3	33.7	62.8
Non-extractable residues	1.2	17.0	15.9	16.0	19.8	13.2	10.7	15.6	27.0
CO_2	0.0	0.0	1.0	2.5	2.5	2.8	3.5	12.9	3.1
Mass balance	99.9	82.6	79.9	80.5	74.9	76.4	74.0	72.4	94.3

DAT: Days after treatment

Table 7.2.2.3-86: Degradation of [^{14}C]glyphosate in Cache Creek under aerobic conditions (of two replicates, expressed as percent of applied radioactivity)

Component	DAT								
	0	3	10	13	17	24	32	52	52 sterile
Glyphosate									
Surface water	90.1	4.4	4.3	2.0	1.1	0.4	0.5	0.0	15.2
Sediment (extractable)	NA	39.0	26.2	20.4	13.8	5.1	7.9	1.2	29.9
Total system	90.1	43.5	30.4	22.4	14.9	5.5	8.4	1.2	45.1
AMPA									
Surface water	NA	2.6	5.3	6.3	6.5	9.3	7.4	4.0	3.9
Sediment (extractable)	0.0	10.9	12.7	15.9	18.4	13.2	17.9	19.9	6.3
Total system	NA	13.5	18.1	22.2	24.9	22.5	25.3	24.0	10.2
N-methyl AMPA[1]									
Surface water	0.2	7.1	12.3	13.5	13.0	12.3	9.9	5.1	3.6
Sediment (extractable)	NA	0.6	0.8	1.0	0.9	0.8	1.0	0.5	0.3
Total system	0.2	7.7	13.2	14.5	13.9	13.0	10.9	5.5	3.9

[1] The peak assigned to N-methyl-AMPA was more likely due to $14CO_2$ not accounted for by the trapping system as discussed in [redacted] (1999), CA 7.2.2.3/002 and Expert Statement on this summary
DAT: Days after treatment; NA: Extracts were below 1 % AR and not analysed

Table 7.2.2.3-87: Degradation of [^{14}C]glyphosate in Putah Creek under aerobic conditions (mean values of two replicates, expressed as percent of applied radioactivity)

Component	DAT								
	0	3	10 [1]	13	17	24	32	52	52 sterile
Glyphosate									
Surface water	98.1	0.2	0.0	0.0	0.0	0.1	0.3	0.0	0.2
Sediment (extractable)	NA	54.7	36.7	37.4	27.1	28.2	21.3	8.3	51.2
Total system	98.1	54.9	36.7	37.4	27.1	28.3	21.6	8.3	51.4
AMPA									
Surface water	0.0	0.1	3.0	0.6	0.6	1.3	4.0	1.8	0.1
Sediment (extractable)	NA	3.3	11.2	13.0	14.6	17.2	20.3	23.5	8.9
Total system	0.0	3.4	14.2	13.6	15.1	18.5	24.3	25.3	9.0
N-methyl AMPA[2]									
Surface water	0.4	1.9	6.7	7.7	6.7	11.8	11.7	8.1	0.8
Sediment (extractable)	NA	1.5	1.3	1.0	1.2	0.6	1.3	0.6	1.5
Total system	0.4	3.4	8.0	8.7	7.9	12.3	13.0	8.7	2.3

[1] Calculations based on a single replicate due to sample loss.
[2] The peak assigned to N-methyl-AMPA was more likely due to $14CO_2$ not accounted for by the trapping system as discussed in [redacted] (1999), CA 7.2.2.3/002 and Expert Statement on this summary
DAT: Days after treatment; NA: Extracts were below 1 % AR and not analyzed

B. MASS BALANCE

Mean mass balances ranged from 68.3 to 99.9 % of applied radioactivity (% AR) for system Cache Creek location and from 72.4 to 99.9 % AR for system Putah Creek.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the surface water decreased from 0 DAT to 52 DAT from 98.7 to 9.8 % AR in system Cache Creek system and from 98.7 to 10.3 % AR in system Putah Creek.

The amount of radioactivity extractable from the sediment increased from 0.0 % AR at 0 DAT to a maximum of 56.1 % AR at 3 DAT then decreased to 22.5 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, the amount of radioactivity extractable from the sediment increased from 0.0 % AR at 0 DAT to a maximum of 63.3 % AR at 3 DAT then decreased to 33.7 % AR at 52 DAT.

The amount of non-extractable residues (NER) increased from 1.2 % AR at 0 DAT to 16.7 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, the amount of NER increased from 1.2 % AR at 0 DAT to a maximum of 19.8 % AR at 17 DAT then decreased to 15.6 % AR at 52 DAT.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (52 DAT) were 19.3 and 12.9 % AR in the Cache Creek and Putah Creek systems, respectively.

E. TRANSFORMATION OF THE TEST ITEM

In the Cache Creek system, the amount of glyphosate in the total test system (water and sediment) decreased from 90.1 % AR at 0 DAT to 1.2 % AR at 52 DAT. In the water layer, it decreased from 90.1 % AR at 0 DAT to 0.0 % AR at 52 DAT. In the sediment, it decreased from 39.0 % AR at 3 DAT to 1.2 % AR at 52 DAT. In the Putah Creek system, the amount of glyphosate in the total test system (water and sediment) decreased from 98.1 % AR at 0 DAT to 8.3 % AR at 52 DAT. In the water layer, it decreased from 98.1 % AR to 0.0 % AR at 10 DAT. In the sediment, it decreased from 54.7 % AR at 3 DAT to 8.3 % AR at 52 DAT.

Besides carbon dioxide, two metabolites, aminomethylphosphonic acid (AMPA) and N-methylaminophosphoic acid (N-methyl AMPA), were detected in the water/sediment systems.

AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 32 DAT then decreased to 24.0 % AR at 52 DAT in the Cache Creek total system. In the water layer, it increased to 9.3 % AR at 24 DAT and decreased to 4.0 % AR at 52 DAT. In the sediment layer, it increased to 19.9 % AR at 52 DAT. In the Putah Creek system, AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 52 DAT. In the water layer, it increased from 0.0 % AR at 0 DAT to 4.0 % AR at 32 DAT and decreased to 1.8 % AR at 52 DAT. In the sediment layer, it increased to 23.5 % AR at the end of the study at 52 DAT.

Levels of a metabolite assigned to N-methyl-AMPA increased from 0.2 % AR at 0 DAT to a maximum of 14.5 % AR at 13 DAT then decreased to 5.5 % AR at 52 DAT in the Cache Creek total system. In the water layer it increased to 13.5 % AR at 13 DAT and decreased to 5.1 % AR at 52 DAT. In the sediment layer it increased to 1.0 % AR at 13 DAT and decreased to 0.5 % AR at 52 DAT. In the Putah Creek total system, N-methyl AMPA levels increased from 0.4 % AR at 0 DAT to a maximum of 13.0 % AR at 32 DAT then decreased to 8.7 % AR at 52 DAT. In the water layer, it increased to 11.8 % AR at 24 DAT and decreased to 8.1 % AR at 52 DAT. In the sediment, it increased to 1.3 % AR at 32 DAT and decreased to 0.6 % AR at 52 DAT.

Comparison with sterile samples shows that the degradation of glyphosate under the study conditions was primarily microbially mediated.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted] 2020, CA 7.2.2.3/001.

III. CONCLUSIONS

Glyphosate dissipated rapidly from surface water in natural water/sediment systems incubated in the dark at 20 °C. More than 90 % of the applied [^{14}C]glyphosate-trimesium is lost from the surface water in less than three days. The rapid initial loss of glyphosate from the surface waters was probably due to binding to the sediment and is consistent with the adsorption properties of glyphosate. Levels of glyphosate in the surface waters had fallen to below the detection limit after incubation for 52 days, in both water-sediment systems under the study conditions.

Besides carbon dioxide, aminomethylphosphonic acid (AMPA) was detected in the water/sediment systems. AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 32 DAT then decreased to 24.0 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, AMPA levels increased from 0.0 % AR at 0 DAT to a maximum of 25.3 % AR at 52 DAT.

Levels of a metabolite assigned to N-methyl-AMPA increased from 0.2 % AR at 0 DAT to a maximum of 14.5 % AR at 13 DAT then decreased to 5.5 % AR at 52 DAT in the Cache Creek system. In the Putah Creek system, N-methyl AMPA levels increased from 0.4 % AR at 0 DAT to a maximum of 13.0 % AR at 32 DAT then decreased to 8.7 % AR at 52 DAT.

No other individual radiolabelled degradate accounted for more than 1 % of the applied dose in either system.

Maximum amounts of carbon dioxide reached at study end (52 DAT) were 19.3 and 12.9 % AR in the Cache Creek and Putah Creek system, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Pre-equilibration of the test systems was 38 days thus slightly exceeding 4 weeks as given by the guideline. Nevertheless, pH, oxygen content and redox potential were monitored throughout the study and thus, the validity is not affected.

Mass balances were below 90 % AR (i.e. 68 - 83 %) for all samples except day zero.

Early sampling points like 1 and 2 DAT were not sampled. This limits the possibility of kinetic evaluation of the data. Additonally, mean values of two replicates are reported and no individual values are available.

For sample processing, water and sediment were transferred into centrifuge bottles and centrifuged; according to the current guideline water should be decanted without disturbing the sediment. Thus, the distribution residues between water and sediment may be affected.

The study by [redacted] (1999, CA 7.2.2.3/002) used the same sediments and thus repeated the study performed by [redacted] (1997, CA 7.2.2.3/003). In [redacted] (1999, CA 7.2.2.3/002), there is a comment that the identity of the peak assigned to N-methyl-AMPA in this study was more likely to be $^{14}CO_2$ not accounted for. In [redacted] (1999, CA 7.2.2.3/002), the potential presence of $^{14}CO_2$ in water and sediment was taken care for in work-up by acidification/additional trapping by NaOH and significant amounts $^{14}CO_2$ were released this way from water/sediment systems.

Labelling in Figure 11 of original report (HPLC chromatogram of 10 DAT in Putah Creek water; see Figure 7.2.2.3-2 below) is inconsistent with the findings in Table 7.2.2.3-87 above.

The study is considered invalid.

Assessment and conclusion by RMS:

Expert Statement – Assessment on validity

The Glyphosate Renewal Group found that the study ([redacted] 1997, CA 7.2.2.3/003) has major shortcomings and should not be considered for use in environmental risk assessments. The reasoning is based on the following:

- Poor mass balances in both test systems make data unacceptable for rate of glyphosate dissipation determinations
- The metabolite reported as *N*-methyl AMPA in both systems is actually carbonate, and is therefore not to be considered a metabolite for risk assessment
- Inconsistencies with peak identification

Rationales supporting these points are discussed below.

The mass balances in the 1997 study ([redacted] 1997, CA 7.2.2.3/003) for all time intervals in both test systems from Day 3 through the end of the study on Day 52 are well below current guidance regarding mass balance acceptance criteria; thus making data from the study unacceptable for rate of glyphosate dissipation determinations. The OECD 308 Aerobic and Anaerobic Transformation in Aquatic Sediment Systems guideline states, "Recoveries should range from 90 % to 110 % for labelled chemicals (6) and from 70 % to 110 % for non-labelled chemicals." The study was conducted with ^{14}C-labelled glyphosate and except for the Day 0 samples, which averaged 99.9 % in both test systems, mass balances were significantly below 90 % from Day 3 through the end of the study on Day 52. In addition, mass balances generally decreased during the study. For the Putah Creek system, the average mass balance was 82.6 % on Day 3 and 72.4 % on Day 52. A similar result was obtained for the Cache Creek system with an average mass balance of 81.9 % on Day 3 and 68.3 % on Day 52 (see Table 7.2.2.3-84 and Table 7.2.2.3-85).

No explanation for the low mass balances is reported. Because glyphosate, AMPA, and any other likely metabolites are highly water soluble, significant losses of radioactive residues to the test vessels is highly unlikely and has not been observed in other environmental fate studies. The only other reasonable explanation for the low mass balances is that $^{14}CO_2$ was not fully accounted for in the test systems.

There are three primary ways $^{14}CO_2$ might not have been fully accounted for in the study: leaks in both systems, inefficient trapping of $^{14}CO_2$ in the NaOH traps, or $^{14}CO_2$ entrained in the waters and/or sediment as carbonate that was partially or completely lost during processing of samples for analysis. It seems unlikely that losses would have occurred through leaks or inefficient trapping, but neither possibility can be completely ruled out.

Because no efforts appear to have been taken, losses of entrained $^{14}CO_2$ also cannot be ruled out.

Zeneca clearly recognized that $^{14}CO_2$ could have been entrained in the waters and sediments as they conducted a follow up study two years later. The study involved test systems from the same sources as the original study, but the study design was modified from the original study to account for entrained $^{14}CO_2$ in both the waters and sediments ([redacted] 1999, CA 7.2.2.3/002). The waters were acidified with 0.5 M KH_2PO_4 and sparged by pulling air through them into two 1 M NaOH traps to trap evolved $^{14}CO_2$. The sediments were extracted with aqueous 0.5 M KH_2PO_4 and evolved $^{14}CO_2$ was trapped in the same manner as the acidified water samples. Incorporating these precautions, mass balances were >90 % for all time intervals except for the Day 30 and Day 58 intervals in the Cache test system which were 86.7 % and 86.3 %, respectively (Table 7.2.2.3-79 and Table 7.2.2.3-80 in Bowler and Johnson 1999, CA 7.2.2.3/002).

To show that the radioactive material sparged from waters and extracts was $^{14}CO_2$, Zeneca applied a standard approach used to test for $^{14}CO_2$. Aliquots of the NaOH traps were treated with $BaCl_2$ to precipitate $BaCO_3$. Analysis of the supernatants by LSC showed levels of radioactivity just above background. This provided clear evidence that $^{14}CO_2$ was entrained in the waters and sediment extracts.

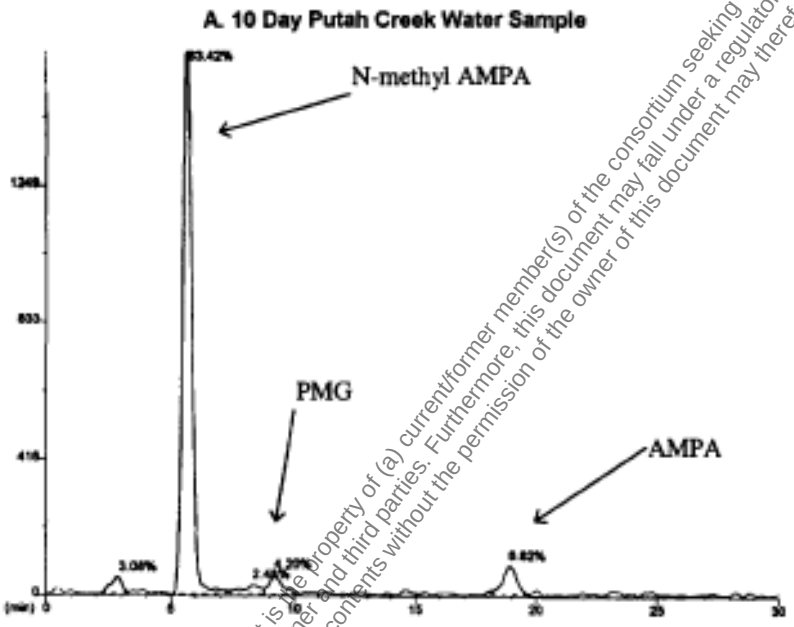
The poor mass balances obtained in [redacted] (1997, CA 7.2.2.3/003) can clearly be attributed in large part, if not completely, to losses of entrained $^{14}CO_2$ in the test systems waters and sediments based

on results from [redacted] (1999, CA 7.2.2.3/002). However, even with this explanation for the poor mass balances, the data are not appropriate for dissipation rate determinations of glyphosate in either test system in the 1997 study.

The Metabolite Identified as *N*-methyl AMPA is Actually Carbonate

The peak eluting at approximately 5.5 minutes in the chromatogram in Figure 7.2.2.3-2 (Figure 11 in original study) from [redacted] (1997, CA 7.2.2.3/003) was misidentified as *N*-methyl AMPA based on misinterpretation of the available data. Instead, the Glyphosate Renewal Group concludes that the peak actually corresponds to carbonate based on results from [redacted] (1999, CA 7.2.2.3/002) as well as an assessment of chromatographic properties obtained in [redacted] (1997, CA 7.2.2.3/003). In addition, the labelling in Figure 7.2.2.3-2 is inconsistent with the percentages of % total radioactivity in Table 7.2.2.3-87 (summary of [redacted] 1997, CA 7.2.2.3/003).

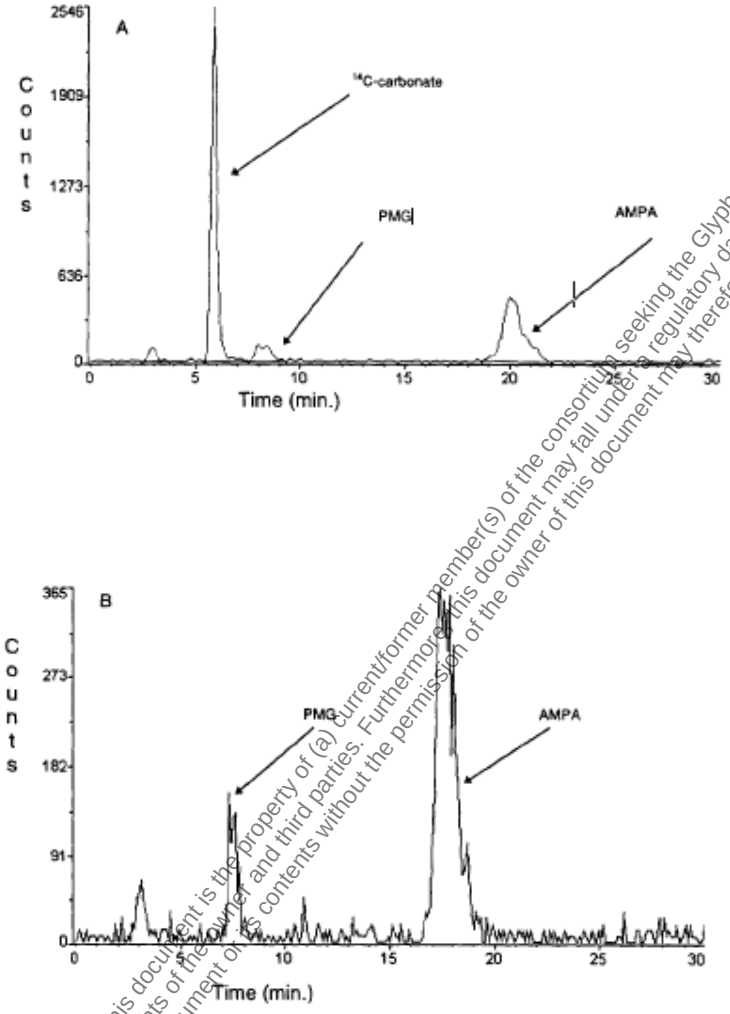
Figure 7.2.2.3-2: HPLC Chromatogram of Day 10 Putah Creek Water ([redacted] 1997, CA 7.2.2.3/003)



As described in the previous section, [redacted] (1999, CA 7.2.2.3/002) showed that $^{14}CO_2$ was entrained in the waters and sediment extracts of both test systems. To provide additional evidence for the presence of $^{14}CO_2$ in waters, a sample of the Day 58 Cache Creek water was analyzed by HPLC before and after acidification (Figure 7.2.2.3-3, Figure 11A in original report). The chromatogram obtained before acidification (Figure 7.2.2.3-3) contains a 5.5-minute peak along with peaks identified as PMG and AMPA. The chromatogram obtained after acidification (Figure 7.2.2.3-3, Figure 11B) does not contain the 5.5-minute peak and only shows PMG and AMPA as identified peaks. This result provides compelling information that the 5.5-minute peak is carbonate. Furthermore, because the chromatograms (Figure 7.2.2.3-2) from the [redacted] (1997, CA 7.2.2.3/003) were essentially obtained under the same HPLC conditions (flow rates differed by 0.1 mL/min between the two studies), it can be concluded that the 5.5-minute peak in that study is also carbonate.

Figure 7.2.2.3-3 HPLC Chromatograms of Day 58 Cache Creek Water from the 1999 Aquatic Sediment Study Before and After Acidification ([redacted] 1999, CA 7.2.2.3/002)

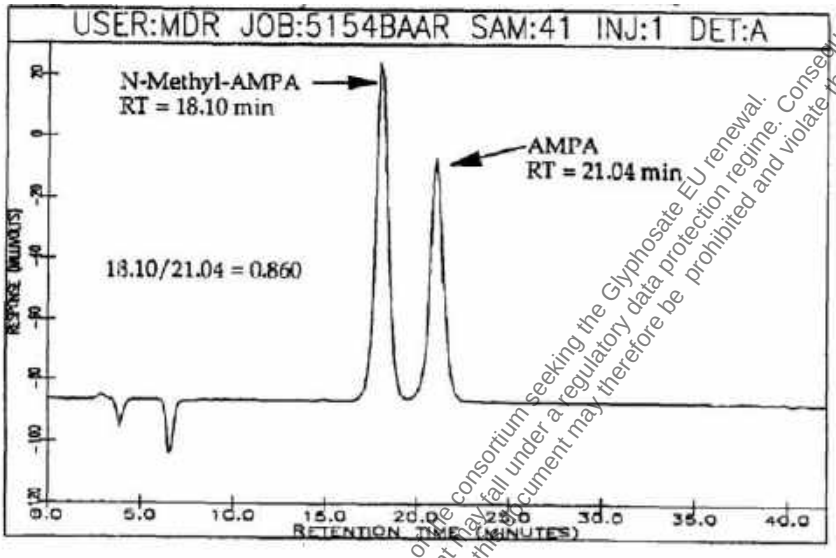
Figure 11 HPLC chromatograms of 58 Days Cache Creek surface water before (A) and after acidification and sparging (B)



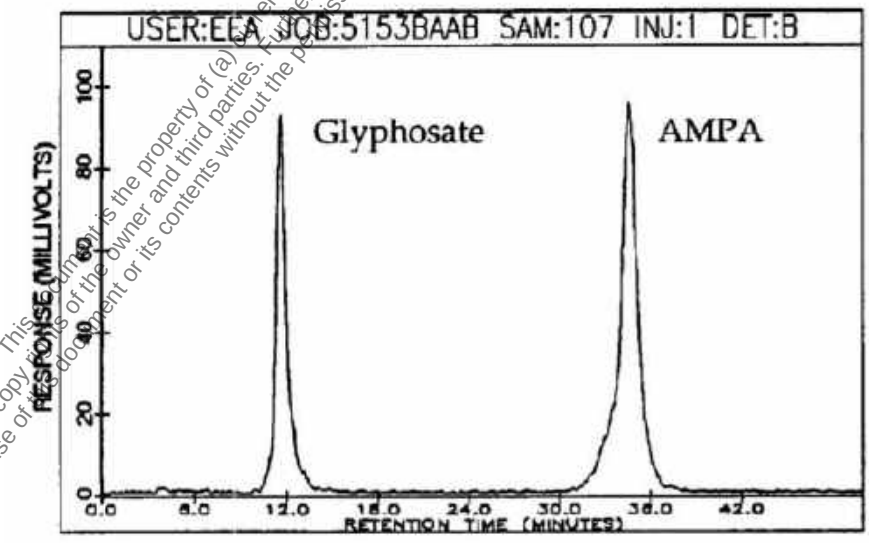
The chromatographic properties expected for *N*-methyl AMPA and carbonate under the strong cation exchange column conditions used in [redacted] (1997, CA 7.2.2.3/003) are another consideration. A retention time of 5.5 minutes is unreasonable for *N*-methyl AMPA, but it is reasonable for the retention time of carbonate. *N*-Methyl AMPA is structurally similar to AMPA with the only difference being a methyl group on nitrogen. Based on the structural similarities, one would expect comparable retention times. Evidence supporting this expectation is found in the top chromatogram in Figure 7.2.2.3-4 from the metabolism study with glyphosate-tolerant soybeans ([redacted] 1994, *Monsanto Report MSL-13520,* see M-CA Section 6, CA 6.2.1/022). The chromatogram was obtained on a cation exchange column using a mobile phase comparable to the one used in the two aquatic sediment studies. As can be seen, the two analytes are well retained with *N*-methyl AMPA eluting at 18.1 min and AMPA eluting at 21.0 min. In contrast, the bottom chromatogram in Figure 7.2.2.3-4 shows that glyphosate elutes at a much earlier time (~12 min) than AMPA (~34 min), but with the same relative elution order as in the aquatic sediment studies.

Figure 7.2.2.3-4 Analysis of N-Methyl AMPA, AMPA, and Glyphosate Reference Standards on a Cation Exchange Column

Analysis of N-Methyl AMPA and AMPA Reference Standards on a Cation Exchange Column Using a 0.005 M KH_2PO_4 / 4 % Methanol Mobile Phase Adjusted to pH 2 (Figure 73B in [redacted] 1994, Monsanto Report MSL 13520, see M-CA Section 6, CA 6.2.1/022)



Analysis of [^{14}C]AMPA and [^{14}C]Glyphosate Reference Standards on a Cation Exchange Column Using a 0.005 M KH_2PO_4 / 4 % Methanol Mobile Phase Adjusted to pH 2 (Figure 73B in [redacted] 1994, Monsanto Report MSL 13520, see M-CA Section 6, CA 6.2.1/022)

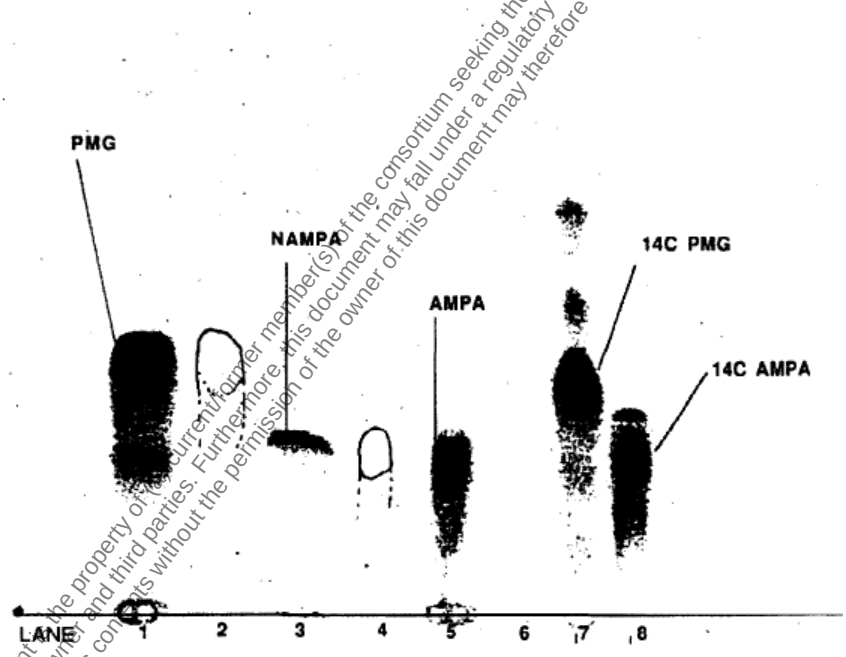


Lastly, the two different conditions used for TLC analyses in [redacted] (1997, CA 7.2.2.3/003) do not provide convincing evidence supporting the identification of *N*-methyl AMPA (Figure 7.2.2.3-5). In fact, a reasonable case can be made that regions associated with *N*-methyl AMPA in the analyses conducted are actually due to AMPA.

The TLC result in Figure 7.2.2.3-6 shows a radioactive region in lane 3 designated as *N*-methyl AMPA from a Day 32 water sample, a region in lane 4 corresponding to the *N*-methyl AMPA reference standard, and a radioactive region in lane 5 corresponding to AMPA from a Day 24 sediment extract sample. As can be seen, the leading front of each region migrated to the same extent, which means there was essentially no separation of *N*-methyl AMPA and AMPA under the conditions used for elution. In addition, the shape of the radioactive region in lane 3 assigned as *N*-methyl AMPA is much different than that for the *N*-methyl AMPA reference standard in lane 4. A reasonable explanation for this is that the radioactive material actually corresponds to the low level of AMPA (~4.0 %) in the sample, while the more predominant ^{14}C-carbonate residue in the sample (~11.7 %) was lost during TLC under acidic conditions in the open system.

Figure 7.2.2.3-5 TLC on Silica Gel Using MeOH/H_2O/NH_4OH/ Trichloroacetic Acid (65/21/14/0.45) ([redacted] 1997, CA 7.2.2.3/003)

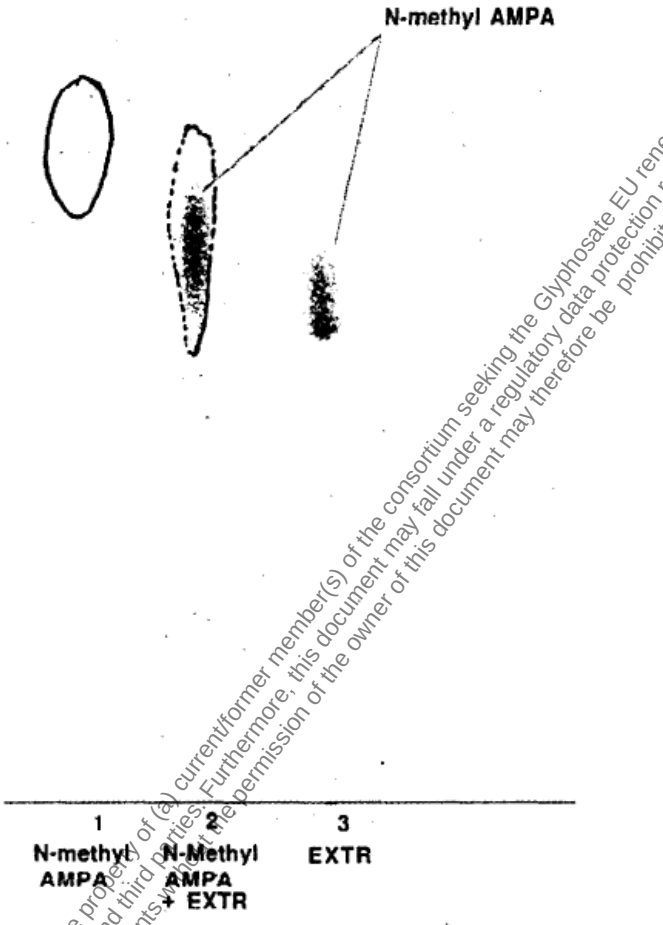
Figure 9. Co-chromatography of Selected Water Samples and Sediment Extracts with cold PMG and N-Methyl AMPA standards.



Lane 1. Co Chromatography of 72Hr Putah Creek Sediment Extract with Cold PMG Standard
Lane 2 Cold PMG Standard
Lane 3 Co-Chromatography of 32 Day Putah Creek Water Sample with Cold N-Methyl-AMPA.
Lane 4. Cold N-Methyl-AMPA
Lane 5 Co-Chromatography of 24 Day Cache Creek Sediment Extract with Cold AMPA Standard.
Lane 6. Cold AMPA Standard, low concentration, not visualized. AMPA identified above
Lane 7. ^{14}C PMG Standard
Lane 8. ^{14}C AMPA Standard

Figure 7.2.2.3-6 TLC on Silica Gel Using MeOH/50 mM NH_4HCO_3 at pH 3.7 (40/60) ([redacted] 1997, CA 7.2.2.3/003)

Figure 10. TLC Co-Chromatography of 32 Day Water Sample with N-Methyl-AMPA standards in Method 2.



The TLC result for the Day 32 water in Figure 7.2.2.3-6, which involved a different mobile phase than the one in Figure 7.2.2.3-5, is also generally consistent with the presence of AMPA instead of *N*-methyl AMPA. Lanes 2 and 3 contain a radioactive component that does not migrate to the same extent as the *N*-methyl AMPA reference standard in lane 1. Furthermore, the *N*-methyl AMPA reference standard cospotted with the Day 32 water sample in lane 2 migrated well beyond the radioactive component in the water sample. As with the explanation for the TLC result in Figure 7.2.2.3-5, a reasonable explanation for the results in Figure 7.2.2.3-6 is that the radioactive material actually corresponds to the AMPA in the sample, while the more predominant ^{14}C-carbonate residue was lost during TLC under acidic conditions.

The poor mass balance recoveries in the 1997 aquatic sediment study make the data unacceptable for rate of glyphosate dissipation determinations. The poor mass balances are due to losses of entrained $^{14}CO_2$ during sample processing. The peak identified as *N*-methyl AMPA in the 1997 study is actually carbonate.

1. Information on the study

Data point:	CA 7.2.2.3/004
Report author	[redacted]
Report year	1996
Report title	Degradation and metabolism of glyphosate in two water/sediment systems under aerobic conditions - laboratory test
Report No	96138/01-CUWS
Document No	
Guidelines followed in study	BBA Guideline Part IV, 5-1
Deviations from current test guideline	From OECD 308: - Mass balances were <90 % AR at some sampling points - Acetone/water extracts were discarded because they contained <5 % AR - Acclimation time prior to application not stated - Water:sediment ratio between 3:1 and 2:1 - LOD of the chromatographic method not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate, labelled in the phosphonomethyl-position, was investigated in two water/sediment systems under aerobic conditions in the dark in the laboratory at 20 ± 2°C for 120 days.

The following two water/sediment systems were used: thee pond system, a loamy silt, and the creek system, a sand. The amount of organic matter in the sediments ranged from 0.20 to 5.69 % and the pH ranged from 6.64 to 7.85. The pH in the aqueous layer ranged from 7.85 to 8.26.

The test was performed in a gas flow systems connected to an ethylene glycol trap to collect organic volatiles, two soda lime traps and one NaOH trap to collect carbon dioxide.

The test item was applied to the water surface in each flask to give a nominal initial application of 691.2 µg glyphosate/ 80 cm^2, equivalent to 4.32 kg/ha. The test item was applied to each test system as a mixture of radiolabelled and unlabelled glyphosate, resulting in 185 kBq [^{14}C]glyphosate (15.6 µg) and 0.676 mg unlabelled glyphosate per test system.

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 7, 14, 29, 58, 98 and 120 days after treatment (DAT). Traps for volatiles were exchanged at the date of sampling or after 28±2 days, whichever was shorter.

Mean material balances ranged from 90.69 to 109.30 % of applied radioactivity (% AR) for the pond water/sediment system and from 79.89 to 101.08 % AR for the creek water/sediment system.

Maximum amounts of carbon dioxide reached at study end (120 DAT) were 14.77 and 30.08 % AR in the pond and creek systems, respectively.

The amount of radioactivity in the water decreased from 0 DAT to 58 DAT from 84.00 to 1.55 % AR and increased to 3.79 % AR at 120 DAT in the pond water/sediment system. In the creek water/sediment system, the amount of radioactivity in the water decreased from 92.31 % AR at 0 DAT to 20.57 % AR at 120 DAT.

The amount of radioactivity extractable from the sediment increased from 0 DAT to 58 DAT from 14.16 to 93.13 % AR and decreased to 77.90 at 120 DAT in the pond water/sediment system. In the creek water/sediment system, the amount of radioactivity extractable from the sediment increased from 0 DAT to 29 DAT from 8.74 to 36.20 % AR and decreased to 31.76 at 120 DAT.

Levels of non-extractable residues (NER) in the sediment increased gradually to maxima of 29.46 % at 58 DAT in the pond system and 24.83 % at 29 DAT in the creek system. The levels dropped to 17.15 and 12.47 % by 120 DAT in the pond and creek system, respectively.

The amount of glyphosate in the water decreased from 0 DAT to 58 DAT from 70.57 to 0.24 % AR and showed a slightly higher amount of 1.83 % AR at 120 DAT in the pond system. The amount of glyphosate in the water decreased from 0 DAT to 120 DAT from 85.90 to 0.00 % AR in the creek system.

The amount of glyphosate in sediment extracts of pond system increased from 3.60 % AR at 0 DAT to 40.00 % AR at 29 DAT and decreased to 27.10 % AR at 97 DAT (29.80 at 120 DAT). The amount of glyphosate in creek system sediment extracts increased from 5.60 % AR at 0 DAT to 9.40 % AR at 2 DAT and decreased to 0.00 % AR at 97 DAT.

The amount of glyphosate in the total system decreased from 0 DAT to 97 DAT from 74.17 to 28.14 % AR at 97 DAT in the pond system and from 91.5 to 0.0 % AR at 120 DAT in the creek system.

The major degradation product, aminomethylphosphonic acid (AMPA), was found in both water/sediment systems over the course of the incubation. In the pond total system, the level of AMPA was found to be highest at 120 DAT with 16.34 % AR. Maximum amounts of AMPA in water and sediment extracts of pond system were 1.97 % AR (7 DAT) and 15.7 % AR (120 DAT), respectively.

In the creek total system, the level of AMPA was found to be highest at 120 DAT, with 24.0 % AR. Maximum amounts of AMPA in water and sediment extracts of the creek system were 10.34 % AR (58 DAT) and 15.9 % AR (120 DAT), respectively. No other metabolites were detected above 0.1 % AR at any time in water or sediment extracts of both test system.

Additionally, extractable residues called non-chromatographable residues (NCRs) occurred in the course of work-up that were quantified, but could not be characterised analytically. NCR thus consisted of radioactivity lost during sample preparation prior to HPLC analysis. These precipitates were formed during concentration of extracts and were not re-dissolvable in further processing or did not elute from chromatographic columns. NCR were formed up to 24.83 % AR in the water of the creek system and 16.91 % AR in the water of the pond system. In sediment, NCR originating from extracts were 7.40 and 20.80 % AR in maximum in creek and pond systems, respectively.

Glyphosate degraded with a total system DT_{50} of 71 ± 24 days in the pond system and 10 ± 2 days in the creek system according to the Timme and Frehse method. The DT_{50} in the water phase was 2 days in the pond system and 10 days in the creek system.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate, free acid (labelled in the phosphonomethyl-position)
GAB No.:	95138
Batch. No.:	25A
Code:	CFA.745
Specific activity:	2000 MBq/mmol (54 mCi/mmol; 11.7 MBq/mg)
Radiochemical purity:	98.3 %
Chemical purity:	Not reported

Identification:	glyphosate technical (non-radiolabelled)
GAB No.:	96159
Batch. No.:	80240496
Code:	96/N-272
Chemical purity:	99 %

2. Test System:

Water and sediment were sampled from two different locations, e.g. pond and river, known not to be submitted to discharges of effluents or near human activity. Water was sampled down to a depth of 10 to 30 cm and the sediment was sampled from the top 20 cm of each system. The water/sediment systems were stored for approximately 14 days at approximately 4°C and afterwards prepared for acclimatisation. The sediments were sieved to ≤2 mm and the water was sieved through a 0.2 mm sieve. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-88: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Pond (Bauschlott)	Creek (Ottenhofen)
Country	Germany	Germany
Sediment		
Textural Class	Loamy silt	Sand
Sand (%)	9.8	97.2
Silt (%)	79.1	1.7
Clay (%)	11	1.1
pH [1]	6.64	7.85
Organic carbon (%)	3.31	0.11
Organic matter [2] (%)	5.69	0.20
Cation exchange capacity (meq/100 g)	22.1	4.3
Redox potential (mV)	-192	208
Microbial biomass (µg C/ g dry matter)		
Before application	1017 ± 25	121 ± 3
Study end	1024 ± 24	214 ± 5
Water phase (at the time of sampling)		
pH	8.26	7.85
Oxygen concentration (mg/L)	15.6	11.3
Redox potential (mV)	88	90

[1] Medium not reported
[2] Calculated from organic carbon according to OM = OC x 1.72
DAT = Days after treatment, USDA: United States Department for Agriculture

B. STUDY DESIGN

1. Experimental conditions

The study was performed with a closed gas flow system using 1000 mL all-glass metabolism flasks containing about 500 mL ± 40 mL water and approx. 230 g wet pond sediment (dry weight approx. 130 g) and 360 g creek sediment (dry weight approx. 308 g), respectively. In both systems, the height of the water column was about 6 cm and the sediment layer was approximately 2.5 cm thick. The systems were ventilated discontinuously for at least 60 min per day with CO_2 free, moistened air. After leaving the test vessels the air was passed through a trapping system for organic volatiles (ethylene glycol), two solid phase traps (soda lime) and one liquid trap (NaOH) to collect $^{14}CO_2$.

The test systems were incubated in the dark in a constant temperature room at 20 ± 2°C. The water/sediment systems were pre-incubated until an equilibrium based on measured variables in the water layer was reached.

The test item was applied to the water surface in each flask to give a nominal initial application of 691.2 µg glyphosate/ 80 cm^2, equivalent to 4.32 kg/ha. The test item was applied to each test system as a mixture of radiolabelled and unlabelled glyphosate, resulting in 185 kBq [^{14}C]glyphosate (15.6 µg) and 0.676 mg unlabelled glyphosate per test system.

Test systems were incubated under aerobic conditions in the dark for 120 days at 20 ± 2°C.

2. Sampling

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 7, 14, 29, 58, 98 and 120 days after treatment (DAT). Traps for volatiles were exchanged at the date of sampling or after 28 ± 2 days, whichever was shorter.

3. Analytical procedures

During acclimatization and at each sampling point pH value, oxygen saturation and redox potential of the water layer and the redox potential of the sediment layer were monitored.

For each system the water column from above the sediment was poured out and filtered. The radioactivity in the water was analysed by LSC. The sediment was extracted at ambient temperature three times with 1 M NH_4OH for 1 hour and one further time with acetone/water (50/50, v/v). The radioactivity in the water/acetone extracts was analysed by LSC and the extracts were discarded as they contained <5 % AR at all sampling intervals. The water and NH_4OH extracts were worked-up as described in "Rückstandsanalytik von Pflanzenschutzmitteln" (GAB SOP 12.3.2-1). The radioactivity in the sample that did not remain on the columns or precipitate during sample preparation was determined by LSC. The solution was concentrated by evaporation under vacuum and otherwise prepared for an analytical determination of the radioactivity.

The amounts of glyphosate and AMPA in water and sediment extracts were quantified by HPLC.

The recovery rates for the extraction of the sediment, the overlaying water phases and the sample preparation via ion exchangers were determined prior to sample analysis for both water/sediment systems were determined by spiked sediment samples taken through the entire work-up and clean-up procedure. Recoveries obtained for analysis of spiked sediment extracts were 72 % for Pond sediment and 97 % for Creek sediment. Recoveries obtained for analysis of spiked water samples were 88 % for Pond sediment and 100 % for Creek sediment.

To quantify non-extractable residues (NER), extracted sediments were combusted and the radioactivity was determined by LSC.

The sodium hydroxide and ethylene glycol trap solutions were analysed by LSC. The $^{14}CO_2$ collected in the solid soda lime traps was stripped by leading in of acidic gas and collection in a NaOH trap solution, which was analysed by LSC afterwards.

Extracts containing more than 5 % AR were characterised by HPLC and co-chromatography of available reference compounds.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water during the study was between 7.26 and 7.88 in the pond system and between 7.45 and 9.05 for creek system. The oxygen concentration in the water phase ranged between 0.9 and 4.0 mg/L in the pond system and between 0.9 and 8.2 mg/L in creek system. The redox potential of the water was between 80 and 222 mV for the pond system and between 80 and 224 mV for the creek system. The redox potential of the sediment was between -180 and -70 mV in the pond system and between -18 and 202 mV for the creek system.

Radioactive mass balance and distribution of [^{14}C]glyphosate and metabolites in water/sediment systems are summarised in Table 7.2.2.3-89 to Table 7.2.2.3-94.

Table 7.2.2.3-89: Mean distribution of radioactivity in pond water/sediment system (expressed as percent of applied radioactivity)

Compound	DAT									
	0	0.25	1	2	7	14	29	58	97	120
Water	84.00	73.01	52.78	41.55	37.23	19.27	12.43	1.55	3.08	3.79
Sediment	14.16	26.77	52.89	62.32	71.87	86.35	91.67	93.13	77.95	77.90
Volatiles	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.03
Carbon dioxide	0.00	0.04	0.04	0.06	0.19	0.55	0.70	6.70	9.63	14.77
Total recovery	98.16	99.83	105.72	103.94	109.30	106.18	104.81	101.40	90.69	96.50

Table 7.2.2.3-90: Mean distribution of radioactivity in creek water/sediment system (expressed as percent of applied radioactivity)

Compound	DAT									
	0	0.25	1	2	7	14	29	58	97	120
Water	92.31	91.71	86.09	81.42	72.11	66.19	49.91	38.75	22.99	20.57
Sediment	8.74	9.33	12.99	19.88	25.55	26.36	36.20	35.78	30.54	31.76
Volatiles	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.04	0.04
Carbon dioxide	0.00	0.03	0.08	0.26	1.26	2.57	7.99	12.33	26.32	30.08
Total recovery	101.05	101.08	99.17	101.57	98.93	95.13	94.11	86.88	79.89	82.45

Table 7.2.2.3-91: Content of Glyphosate and its metabolites in the water phase in the pond water/sediment system (expressed as µg/flask and % of the applied radioactivity)

Compound		DAT									
		0	0.25	1	2	7	14	29	58	97	120
NCR [1]	%	11.84	16.91	11.72	8.82	7.85	3.29	2.44	1.13	1.55	1.32
	µg	81.8	116.9	81.0	60.9	54.2	22.7	16.9	7.9	10.7	9.1
Glyphosate	%	70.57	55.18	39.57	31.36	27.42	14.32	8.20	0.24	1.04	1.83
	µg	487.6	381.3	273.5	216.7	189.5	99.0	56.7	1.6	7.2	12.7
AMPA	%	1.59	0.93	1.49	1.38	1.97	1.67	1.79	0.12	0.49	0.64
	µg	11.0	6.4	10.3	9.5	13.6	11.5	12.4	0.8	3.4	4.4
Metabolite 2	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
	µg	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Sum	%	84.00	73.02	52.78	41.56	37.24	19.28	12.43	1.56	3.08	3.79
	µg	580.4	504.6	364.8	287.1	257.3	133.2	86.0	10.8	21.3	26.2

[1] Non chromatographable residues: Activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatography columns or not redissolvable precipitates

Table 7.2.2.3-92: Content of Glyphosate and its metabolites in the sediment in the pond water/sediment system (expressed as µg/flask and % of the applied radioactivity)

Compound		DAT									
		0	0.25	1	2	7	14	29	58	97	120
Bound Residues	%	2.60	2.84	10.06	11.62	19.85	25.12	24.46	29.46	16.13	17.15
	µg	18.0	19.6	69.5	80.3	137.2	173.6	169.0	203.6	111.5	118.5
NCR [1]	%	6.80	12.30	18.10	11.40	14.90	19.40	16.60	20.00	20.80	15.20
	µg	47.0	84.8	124.8	78.5	103.2	134.1	115.1	137.9	143.4	105.0
Glyphosate	%	3.60	8.80	19.90	33.40	31.60	35.50	40.00	33.00	27.10	29.80
	µg	24.9	60.8	137.4	231.1	218.2	245.6	276.7	228.1	187.6	206.0
AMPA	%	1.20	2.90	4.90	5.90	5.50	6.30	10.50	10.70	13.90	15.70
	µg	8.1	19.8	33.8	40.8	38.1	43.4	72.6	74.0	95.2	108.8
Sum	%	14.20	26.84	52.96	62.32	71.85	86.32	91.56	93.16	77.93	77.85
	µg	98.0	185.0	365.5	430.7	496.7	596.7	633.4	643.6	538.7	538.3

[1] Non chromatographable residues: Activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatography columns or not redissolvable precipitates

Table 7.2.2.3-93: Content of Glyphosate and its metabolites in the water phase in the creek water/sediment system (expressed as µg/flask and % of the applied radioactivity)

Compound		DAT									
		0	0.25	1	2	7	14	29	58	97	120
NCR [1]	%	6.42	14.20	10.68	7.82	12.99	16.20	24.83	22.39	16.62	12.47
	µg	44.4	98.1	73.8	54.0	89.8	112.0	171.6	154.7	114.9	86.2
Glyphosate	%	85.90	77.51	72.78	69.89	52.97	42.54	16.69	6.03	1.43	0.00
	µg	593.5	535.6	502.9	483.0	366.0	294.0	115.3	41.7	9.9	0.0
AMPA	%	0.00	0.00	2.63	3.71	6.16	7.45	8.39	10.34	4.95	8.10
	µg	0.0	0.0	18.2	25.6	42.6	51.5	58.0	71.5	34.2	56.0
Sum	%	92.32	91.71	86.09	81.42	72.12	66.19	49.91	38.76	23.00	20.57
	µg	637.9	633.7	594.9	562.6	498.4	457.5	344.9	267.9	159.0	142.2

[1] Non chromatographable residues: Activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatography columns or not redissolvable precipitates

Table 7.2.2.3-94: Content of Glyphosate and its metabolites in the sediment in the creek water/sediment system (expressed as µg/flask and % of the applied radioactivity)

Compound		DAT									
		0	0.25	1	2	7	14	29	58	97	120
Bound Residues	%	1.10	1.30	2.05	2.65	4.83	4.12	9.81	10.43	8.02	9.49
	µg	7.6	9.0	14.2	18.3	33.4	28.5	67.8	72.1	55.4	65.6
NCR [1]	%	1.40	1.50	2.10	4.20	4.90	6.90	7.10	6.30	7.40	6.40
	µg	10.0	10.4	14.2	28.7	33.5	47.4	48.8	43.5	51.4	43.9
Glyphosate	%	5.60	5.10	6.90	9.40	8.90	6.60	7.20	6.30	0.00	0.00
	µg	38.7	35.5	47.8	64.7	61.2	45.8	49.6	43.4	0.0	0.0
AMPA	%	0.60	1.40	2.00	3.70	7.00	8.70	12.20	12.80	15.10	15.90
	µg	4.2	9.6	13.7	25.7	48.5	60.5	84.0	88.3	104.3	110.0
Sum	%	8.70	9.30	13.05	19.95	25.63	26.32	36.31	35.83	30.52	31.79
	µg	60.5	64.5	89.9	137.4	176.6	182.2	250.2	247.3	211.1	219.5

[1] Non chromatographable residues: Activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatography columns or not redissolvable precipitates

B. MASS BALANCE

Mean material balances ranged from 90.69 to 109.30 % of applied radioactivity (% AR) for the pond water/sediment system and from 79.89 to 101.08 % AR for the creek water/sediment system.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 58 DAT from 84.00 to 1.55 % AR and increased to 3.79 % AR at 120 DAT in the pond water/sediment system. In the creek water/sediment system, the amount of radioactivity in the water decreased from 92.31 % AR at 0 DAT to 20.57 % AR at 120 DAT.

The amount of radioactivity extractable from the sediment increased from 0 DAT to 58 DAT from 14.16 to 93.13 % AR and decreased to 77.90 at 120 DAT in the pond water/sediment system. In the creek water/sediment system, the amount of radioactivity extractable from the sediment increased from 0 DAT to 29 DAT from 8.74 to 36.20 % AR and decreased to 31.76 at 120 DAT.

Levels of non-extractable residues (NER) in the sediment increased gradually to maxima of 29.46 % at 58 DAT in the pond system and 24.83 % at 29 DAT in the creek system. The levels dropped to 17.15 and 12.47 % by 120 DAT in the pond and creek system, respectively.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (120 DAT) were 14.77 and 30.08 % AR in the pond and creek systems, respectively.

E. TRANSFORMATION OF THE TEST ITEM

The amount of glyphosate in the water decreased from 0 DAT to 58 DAT from 70.57 to 0.24 % AR and showed a slightly higher amount of 1.83 % AR at 120 DAT in the pond system. The amount of glyphosate in the water decreased from 0 DAT to 120 DAT from 85.90 to 0.00 % AR in the creek system.

The amount of glyphosate in sediment extracts of pond system increased from 3.60 % AR at 0 DAT to 40.00 % AR at 29 DAT and decreased to 27.10 % AR at 97 DAT (29.80 at 120 DAT). The amount of glyphosate in creek system sediment extracts increased from 5.60 % AR at 0 DAT to 9.40 % AR at 2 DAT and decreased to 0.00 % AR at 97 DAT.

The amount of glyphosate in the total system decreased from 0 DAT to 97 DAT from 74.17 to 28.14 % AR at 97 DAT in the pond system and from 91.5 to 0.0 % AR at 120 DAT in the creek system.

The major degradation product, aminomethylphosphonic acid (AMPA), was found in both water/sediment systems over the course of the incubation. In the pond total system, the level of AMPA was found to be highest at 120 DAT with 16.34 % AR. Maximum amounts of AMPA in water and sediment extracts of pond system were 1.97 % AR (7 DAT) and 15.7 % AR (120 DAT), respectively.
In the creek total system, the level of AMPA was found to be highest at 120 DAT, with 24.0 % AR. Maximum amounts of AMPA in water and sediment extracts of the creek system were 10.34 % AR (58 DAT) and 15.9 % AR (120 DAT), respectively. No other metabolites were detected above 0.1 % AR at any time in water or sediment extracts of both test system.

Additionally, non-chromatographable residues (NCRs) were quantified. This radioactivity is defined as activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatographic columns or not redissolvable precipitates. These NCRs were formed with an extend to 24.83 % AR in the water of the creek system and 16.91 % AR in the water of the pond system. In sediment extracts these NCR amounted to 7.40 and 20.80 % AR in creek and pond systems, respectively.

F. KINETICS
Glyphosate degraded with a total system DT_{50} of 71±24 days in the pond system and 10±2 days in the creek system, calculated using the Timme, time and Frehse method. The DT_{50} in the water phase was 2 days in the pond system and 10 days in the creek system.

III. CONCLUSIONS

The parent compound is degraded in the creek water phase and in the pond and creek sediment phase. Disappearance in the pond system was primarily caused by a continuous transfer from the water phase to the sediment phase, probably caused by sorption processes.

In the sediment, rising amounts of degradation products indicated that the degradation process was still in progress after 120 d in both systems. The main fractions were the uncharacterized group of bound residues (pond system), the soluble or extractable group of non-chromatographable residues and AMPA. The nature of the possible structures in the uncharacterised groups are given by the structure of the glyphosate itself. Glyphosate and its metabolites may be transferred to biological substrates as proteins, sugars and humidic acids.

Total mineralisation to carbon dioxide was important for pond (15 % AR) and creek (30 % AR), volatiles were negligible. In the pond system the pure degradation leads to a long degradation time for 90 % of the parent compound up to approx. Two years in the water/sediment system. Nevertheless, degradation and metabolisation were still in progress.

Two metabolites were detected. Metabolite 1 was identified as Aminomethylphosphonoic acid (AMPA). Metabolite 2 was not identified as it only appeared in samples taken from the pond system (58 d) with an amount of less than 0.1 % of the applied activity. The amount of organic volatiles was in any case below 0.1 % of the applied activity. No other metabolites were found except to the bound residues, the non-chromatographable part of the extracts and carbon dioxide.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study showed some deviations to the current guidelines. Mass balances were below 90 % AR for a number of sampling points in both systems. The acclimation period prior to application was not reported. The water/sediment ratio was between 3:1 and 2:1.

Additionally, non-chromatographable residues (NCRs) were quantified. This radioactivity is defined as activity lost during sample preparation prior to HPLC analysis either not binding and removable from chromatographic columns or not redissolvable precipitates. These NCRs were formed with an extend to 24.83 % AR in the water of the creek system and 16.91 % AR in the water of the pond system. In sediment extracts these NCR amounted to 7.40 and 20.80 % AR in creek and pond systems, respectively.

In conclusion, the study was considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.2.3/005
Report author	[redacted]
Report year	1993
Report title	Determination of the Degradability and Persistence of ^{14}C-Glyphosate in the Water/Sediment-System.
Report No	ET01SE01
Document No	
Guidelines followed in study	BBA Guideline Part IV 5-1
Deviations from current test guideline	From OECD 308: - Mean material balances lower than 90 % AR starting for all samplingsfrom 14 DAT onwards (losses explained by insufficient trapping of volatiles) - ratio of sediment to water not reported - processing recovery for some water samples below 90 %, but more than 80 %
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

Data point:	CA 7.2.2.3/006
Report author	[redacted]
Report year	1995
Report title	Amendment to the final report - Determination of the Degradability and Persistence of ^{14}C-Glyphosate in the Water/Sediment-System - Report on the additional metabolite identification
Report No	ET01SE01
Document No	
Guidelines followed in study	None
Deviations from current test guideline	- following re-analysis of water phases for metabolite identification, storage conditions were not reported for the approx. 6 months period between experimental completion including reporting and issue of the amendment
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate, labelled in the phosphonomethyl-position, was investigated in two water/sediment systems under aerobic conditions in the dark in the laboratory at 20 ± 2°C for 100 days.

The following two water/sediment systems were used: a sandy system (Bickenbach) and a loamy system (Unter Widdersheim). The amount of organic matter of the sediment was 1.17 and 7.24 %, respectively. The pH of the sediment was 7.68 and 7.80, respectively, while the pH of the water was 8.65 and 8.47, respectively.

The test was performed in static test systems, consisting of flasks filled with water and sediment in a way that the thickness of the sediment was 2 to 2.5 cm and the thickness of the water layer was 6 cm. Glass tubes filled with two layers of soda lime and glass wool were used to collect carbon dioxide and other volatiles.

The application rate was 230 µg to the water/sediment system, corresponding to the highest recommended application rate of 3600 g glyphosate/ha.

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 7, 14, 30, 61 and 100 days after treatment (DAT).

Mean material balances ranged from 80.79 to 98.83 % of applied radioactivity (% AR) for the sandy water/sediment system Bickenbach and from 76.92 to 100.5 % AR for the loamy water/sediment system Unter Widdersheim.

Maximum amounts of carbon dioxide were 23.48 % AR at 100 DAT in the sandy water/sediment system Bickenbach and 19.37 % AR at 61 DAT in the loamy water/sediment system Unter Widdersheim. Organic volatiles determined were ≤0.1 % AR for both test systems at all sampling points.

The amount of radioactivity in the water decreased from 0 DAT to 100 DAT from 92.47 to 8.27 % AR for the sandy water/sediment system Bickenbach and from 87.81 to 3.09 % AR for the loamy water/sediment system Unter Widdersheim.

The amount of radioactivity extractable from the sediment increased from 0 DAT to 100 DAT from 5.26 to 29.24 % AR for the sandy water/sediment system Bickenbach and from 6.65 to 44.15 % AR for the loamy water/sediment system Unter Widdersheim.

The amount of non-extractable residues (NER) increased from 0 DAT to 100 DAT from 0.07 to 22.01 % AR for sandy water/sediment system and from 0.20 to 13.61 % AR for the loamy water/sediment system Unter Widdersheim.

The amount of [^{14}C]glyphosate in water decreased from 0 DAT to 100 DAT from 92.47 to 0.27 % AR for water/sediment system Bickenbach and from 83.80 to 2.42 % AR for water/sediment system Unter Widdersheim.

The amount of [^{14}C]glyphosate in sediment extracts increased from 0 DAT to 7 DAT from 5.26 to 53.08 % AR, before declining to 29.24 % AR at 100 DAT for water/sediment system Bickenbach and increased from 0 DAT to 7 DAT from 6.65 to 61.36 % AR, before declining to 44.15 % AR at 100 DAT for water/sediment system Unter Widdersheim.

The amount of [^{14}C]glyphosate in the total system decreased from 0 DAT to 100 DAT from 97.73 to 29.50 % AR for water/sediment system Bickenbach and from 90.41 to 46.57 % AR for water/sediment system Unter Widdersheim.

Two metabolites were identified in the water phase of both test systems. AMPA was detected with a maximum amount of 15.74 % AR at 14 DAT in water/sediment system Bickenbach, decreasing to 0.48 % AR at 100 DAT. HMPA was detected with a maximum amount of 9.97 % AR at 61 DAT in water/sediment system Bickenbach and decreased to 7.52 % AR at 100 DAT. No other metabolites were detected in water above 5 % AR at any time. No metabolites were detected at any timepoint in sediment extracts of both test systems.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate (labelled in the phosphonomethyl-position)
Lot No.:	1071-83-6
Specific activity:	12.3 MBq/mg
Radiochemical purity:	98.9 % by HPLC, >97.7 % by TLC
Chemical purity:	Not provided

2. Test System:

The sediment was sieved to ≤2 mm and the water was sieved to ≤0.2 mm. Water and sediment were stored at 4 ± 2 °C for 8 days. During this time the sediment was shaken periodically and the water was purged with air to avoid anaerobic conditions. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-95: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Water/Sediment I	Water/Sediment II
Location	Bickenbach	Unter Widdersheim
Country	Germany	Germany
Sediment:		
Textural Class (DIN)	Sand	Loam
Sand (%)	82.3	15.0
Silt (%)	11.8	75.0
Clay (%)	5.9	10.0
pH [1]	7.80	7.68
Organic matter (%)	1.17	7.24
Organic carbon [2] (%)	0.68	4.20
Cation exchange capacity (mval/kg dry weight)	762	1030
Redox Potential (mV)	331	162
Microbial biomass (mg C/100 g dry weight)		
Study begin (0 DAT)	21.7	80.2
Study end (100 DAT)	2.8	10.1
Water:		
pH at sampling	8.65	8.47
pH at day 0	8.6	8.6
Total organic carbon (mg/L)	5.52	3.90
Redox Potential (mV)	527	493
Oxygen saturation (%)	131	104

DAT = Days after treatment, DIN: Deutsches Institut für Normung e.V. (German Institute for Standardization)
[1] Medium not reported
[2] Calculated during dossier preparation using the equation: OC = OM/1.724

B. STUDY DESIGN

1. Experimental conditions

The test was performed in static test systems, consisting of 250-mL glass flasks filled with water and sediment in a way that the thickness of the sediment was 2 to 2.5 cm and the thickness of the water layer was 6 cm with a total volume of 190 mL. Glass tubes filled with two layers of soda lime and glass wool were used to collect carbon dioxide and other volatiles. After set-up of the test systems they were acclimatized at the experimental conditions (shaken at 20 ± 2 °C) for 5 days, until an equilibrium of oxygen content, redox potential and pH value had set.

Additionally, sterile samples were prepared by autoclaving and analysed after 100 days.

The study application rate corresponded to the highest recommended use rate of 3600 g a.s./ha. 230 µg of [^{14}C]glyphosate was applied to each test system. Immediately after the application of the test chemical, small glass tubes filled with paraffin covered glass wool, were put up on top of the test container. During incubation, samples were shaken without mixing water and sediment.

Test systems were incubated under aerobic conditions in the dark for 100 days at 20 ± 2 °C.

2. Sampling

Duplicate test systems were processed and analysed 0, 0.25, 1, 2, 7, 14, 30, 61 and 100 days after treatment (DAT).

3. Analytical procedures

The determination of radioactivity was performed with the liquid scintillation counters (LSC). For each type of sample (e.g. water, sediment, extract of sediment) the blank value was subtracted. All analyses were conducted in triplicate.

At each sampling interval, water and sediment were separated by decantation without centrifugation. The decanted water phase was adjusted with deionized water to a final volume of 200 mL. For the determination of radioactivity by LSC, aliquots between 100 µL and 1000 µL were used. A suitable sample volume within the above mentioned range was used in order to minimize the error according to the "2-Sigma method". For the determination of the blank value, deionized water corresponding to the sample volumes, was mixed with 12 mL of scintillator.

The sediment samples were extracted four times with 150 mL of 0.5 N sodium hydroxide solution each for a period of 10 minutes. Afterwards the samples were centrifuged for 10 minutes at 4000 rpm and the combined extracts were adjusted to a final volume of 650 mL with deionized water. Aliquots of between 500 µL and 1000 µL were mixed with 12 mL scintillator and the total radioactivity was measured. A suitable sample volume within the above mentioned range was used in order to minimize the error according to the "2-Sigma method". Aliquots of 0.5 N sodium hydroxide solution corresponding to the sample volumes, were mixed with 12 mL of scintillator and used as controls.

The amount of non-extractable residues from sediment was determined by combustion. For the determination of the non-extractable amount of radioactivity from water, 3 mL of the water phase were extracted with 3 mL ethylacetate and measured by LSC.

Extracts and sediment were stored at -25 ± 15 °C until analysis.

For preparation of analysis water, aliquots were evaporated to dryness. In pre-experiments it could be shown, that the recoveries for this work-up step were >80 %. The residue was dissolved in a mixture of 750 µL methanol and 500 µL deionized water and 200 µL of 1 M disodiumphosphate buffer were added. After centrifugation at 3000 rpm for 5 min, an aliquot of the liquid phase was evaporated to a final volume of 100 µL. An aliquot of 10 µL was spotted onto a TLC plate. The mobile phase for the TLC was methanol/water/trichloroacetic acid/ammonia/glacial acetic acid (40 mL/ 60 mL/ 3.5 g/ 5 mL/ 2 mL).

For sediment extracts an aliquot was acidified with 150 µL glacial acetic acid and 50 µL were spotted on a TLC plate.

[^{14}C]glyphosate and metabolite AMPA were initially identified in study samples by thin layer chromatography (TLC) with reference items. In the course of the addendum, subsequent identification of an unknown metabolite was performed on selected concentrated water samples (30 DAT of system Unter Widdersheim and 61 DAT of system Bickenbach) by one-dimensional thin layer chromatography (1D-TLC) and two-dimensional thin layer chromatography (2D-TLC) co-spotted with reference standards.

The hydrophobized glass wool was removed from the glass tube and was extracted one after another with 5 mL hexane, 5 mL chloroform and 5 mL methanol for one minute using a vibro-fix. The extracts were combined and adjusted with a mixture of hexane/chloroform and methanol (1/1/1 v /v /v) to a final volume of 15 mL. This solvent mixture was also used for measuring blank values. After adding the scintillator aliquots of 1 mL of the combined extracts were measured. 1 mL of the solvent mixture was used as control value.

The two soda lime layers were removed from the glass tube and transferred quantitatively into a liberation apparatus for the determination of the CO_2 absorbed. Hydrochloric acid was added through a dropping funnel to slowly liberate the CO_2 from the soda lime. The liberated CO_2 was carried by a nitrogen stream into a vessel, which was filled with a cocktail of scintillator and absorber. The total radioactivity was determined by LSC.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 8.6 and 9.2 for the sandy system and between 8.6 and 8.9 for the loamy system. The oxygen content in the water phase ranged between 8.1 and 8.5 mg/L in the sandy system and between 7.8 and 8.8 mg/L in the loamy system. The

redox potential of the water was in the highly positive range with values between 300 and 351 mV for both test systems. The redox potential of the sediment (mean) of the sandy system was 6 mV at 0 DAT, dropped to approx. -84 mV at 2 DAT and increased then to approx. 100 mV at 100 DAT. The redox potential of the sediment of the loamy system was -98 mV during the total incubation time and increased to approx. 92 mV at 100 DAT.

Radioactive mass balance and distribution of glyphosate and metabolites in water/sediment systems are summarised in Table 7.2.2.3-96 to Table 7.2.2.3-101.

Table 7.2.2.3-96: Amount of radioactivity in water/sediment system Bickenbach under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Water	A	91.51	81.04	66.04	51.89	35.96	34.06	24.24	17.31	8.22
	B	93.42	80.29	66.30	57.33	35.69	31.20	25.78	15.52	8.31
	Mean	92.47	80.69	66.17	54.61	35.83	32.63	25.01	16.42	8.27
Sediment extract	A	5.29	14.75	33.23	39.16	52.83	38.60	34.46	35.65	29.73
	B	5.23	16.04	30.56	39.62	53.32	36.14	32.86	34.05	28.74
	Mean	5.26	15.40	31.90	39.39	53.08	37.37	33.66	34.85	29.24
Non-extractable residues (NER)	A	0.07	0.18	0.63	0.98	2.76	4.56	8.71	16.37	26.03
	B	0.06	0.19	0.60	0.96	2.80	4.86	8.72	17.77	17.99
	Mean	0.07	0.19	0.62	0.97	2.78	4.71	*8.72* [1]	17.07	22.01
Organic volatiles	A	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.01
	B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
	Mean	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.01
CO_2	A	0.06	0.04	0.16	0.22	3.05	6.27	12.34	19.84	21.53
	B	0.05	0.04	0.10	0.22	3.34	5.89	11.13	20.63	25.42
	Mean	0.06	0.04	0.13	0.22	3.20	6.08	11.74	20.24	23.48
Mass Balance	A	96.93	96.01	100.1	92.25	94.60	83.49	79.75	89.18	85.52
	B	98.76	96.56	97.56	98.13	95.15	78.09	78.49	87.98	80.47
	Mean	97.86	96.32	98.83	95.19	94.89	80.79	*78.92* [1]	88.59	83.01

DAT: Days after treatment
[1] These values were calculated during summary preparation, as the values given in the report (16.31 and 86.72 %) were obviously not the mean values of the two corresponding replicates.

Table 7.2.2.3-97: Amount of radioactivity in water/sediment system Unter Widderheim under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Water	A	88.29	80.38	50.51	37.41	21.23	20.94	13.28	3.89	2.67
	B	87.33	74.88	51.92	33.32	30.81	23.85	12.79	4.38	3.51
	Mean	87.81	77.63	51.22	35.37	26.02	22.40	13.04	4.14	3.09
Sediment extract	A	5.01	21.29	39.52	52.83	66.09	46.38	43.37	54.76	44.14
	B	8.29	23.08	44.28	57.31	56.62	42.84	44.22	55.02	44.15
	Mean	6.65	22.19	41.90	55.07	61.36	44.61	43.80	54.89	44.15
Non-extractable residues (NER)	A	0.10	0.65	1.51	2.80	4.92	6.12	10.78	11.51	13.30
	B	0.30	0.60	1.93	1.88	5.61	6.66	10.10	11.40	13.91
	Mean	0.20	0.63	1.72	2.34	5.27	6.39	10.40	11.46	13.61
Organic volatiles	A	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.01	0.01
	B	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.01
	Mean	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.01	0.01
CO_2	A	0.08	0.05	0.20	0.42	2.69	4.49	10.58	19.04	17.21
	B	0.00	0.06	0.13	0.41	2.27	5.10	8.78	19.57	18.46
	Mean	0.04	0.06	0.17	0.42	2.48	4.80	9.68	19.37	17.84
Mass Balance	A	93.48	102.4	91.84	93.46	94.93	77.93	78.01	89.21	77.33
	B	95.92	98.62	98.39	92.92	94.81	78.45	75.89	90.37	80.04
	Mean	94.70	100.5	95.13	93.20	95.13	78.20	76.92	89.87	78.70

DAT: Days after treatment

Table 7.2.2.3-98: Degradation of [14C]glyphosate in water of water/sediment system Bickenbach under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Glyphosate	A	91.51	81.04	63.18	47.68	21.52	14.92	5.86	1.10	0.20
	B	93.42	80.29	64.22	51.68	24.37	12.10	9.39	0.62	0.33
	Mean	92.47	80.67	63.70	49.68	22.95	13.51	7.63	0.86	0.27
AMPA	A	nd	nd	2.86	4.21	12.45	15.39	11.41	4.83	0.39
	B	nd	nd	2.08	5.65	8.98	16.10	11.61	5.23	0.56
	Mean	nd	nd	2.47	4.93	10.72	15.74	11.51	5.03	0.48
HMPA [1]	A	nd	nd	nd	nd	nd	3.75	2.67	11.37	7.63
	B	nd	nd	nd	nd	nd	3.01	4.78	8.58	7.41
	Mean	nd	nd	nd	nd	nd	3.38	3.72	9.97	7.52

DAT: Days after treatment
nd: Not detected
AMPA: Aminomethyl-phosphoric acid
HMPA: (Hydroxymethyl)-phosphonic acid
[1] The metabolite HMPA was identified by TLC co-chromatography in the course of the addendum.

Table 7.2.2.3-99: Degradation of [^{14}C]glyphosate in water of water/sediment system Unter Widderheim under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Glyphosate	A	83.92	78.03	47.17	34.41	16.77	14.78	8.30	3.31	1.83
	B	83.68	73.24	50.74	31. 06	25.43	17.07	8.25	3.66	3.02
	Mean	83.80	75.64	48.95	32.74	21.10	15.92	8.27	3.48	2.42
AMPA	A	4.37	2.35	2.95	2.77	3.91	5.41	3.22	0.47	0.39
	B	3.65	1.64	1.18	2.11	4.88	6.14	2.45	0.51	0.39
	Mean	4.01	1.99	2.07	2.44	4.40	*5.78* [1]	2.83	0.49	0.39
HMPA [2]	A	nd	nd	nd	0.24	0.63	0.81	1.76	0.11	0.12
	B	nd	nd	nd	0.15	0.51	0.77	2.09	0.21	0.10
	Mean	nd	nd	nd	0.20	0.57	0.79	1.93	0.16	0.11

DAT: Days after treatment
AMPA: Aminomethyl-phosphoric acid
HMPA: (Hydroxymethyl)-phosphonic acid
[1] This value was calculated during summary preparation, as the value given in the report (8.84 %) was obviously not the mean values of the two corresponding replicates.
[2] The metabolite HMPA was identified by TLC co-chromatography in the course of the addendum.

Table 7.2.2.3-100: Percentage radioactivity of the parent compound in extract samples of system Bickenbach and system Unter Widderheim (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Bickenbach										
Glyphosate	A	5.29	14.75	33.23	39.16	52.83	38.60	34.46	35.65	29.73
	B	5.23	16.04	30.56	39.62	53.32	36.14	32.86	34.05	28.74
	Mean	5.26	15.40	31.90	39.39	53.08	37.37	33.66	34.85	29.24
Unter Widderheim										
Glyphosate	A	5.01	21.29	39.52	52.83	66.09	46.38	43.37	54.76	44.14
	B	8.29	23.08	44.28	57.31	56.62	42.84	44.22	55.02	44.15
	Mean	6.65	22.19	41.90	55.07	61.36	44.61	43.80	54.89	44.15

DAT: Days after treatment

Table 7.2.2.3-101: Percentage radioactivity of the parent compound in the total system (sum of sediment extracts and water) of system Bickenbach and system Unter Widderheim (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	61	100
Bickenbach										
Glyphosate	A	96.80	95.79	96.41	86.84	74.35	53.50	40.32	36.75	29.93
	B	98.65	96.33	94.78	91.30	77.69	48.24	42.25	34.67	29.07
	Mean	97.73	96.06	95.59	89.07	76.02	50.87	41.29	35.71	29.50
Unter Widderheim										
Glyphosate	A	88.93	99.32	86.69	82.86	82.86	61.16	51.67	58.07	45.97
	B	91.97	96.32	95.02	82.05	76.30	59.91	52.47	58.68	47.17
	Mean	90.41	97.82	90.86	82.46	79.47	60.54	52.07	58.38	*46.57* [1]

DAT: Days after treatment
[1] This value was calculated during summary preparation, as the value given in the report (51.07 %) was obviously not the mean value of the two corresponding replicates.

B. MASS BALANCE

Mean material balances ranged from 80.79 to 98.83 % of applied radioactivity (% AR) for the sandy water/sediment system Bickenbach and from 76.92 to 100.5 % AR for the loamy water/sediment system Unter Widdersheim. Material balances below 90 % may be caused by the formation of volatile metabolites.

The material balance for sterile samples at day 100 was 94.1 % for system Bickenbach and 93.5 % for system Unter Widdersheim.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 100 DAT from 92.47 to 8.27 % AR for the sandy water/sediment system Bickenbach and from 87.81 to 3.09 % AR for the loamy water/sediment system Unter Widdersheim.

The amount of radioactivity extractable from the sediment increased from 0 DAT to 100 DAT from 5.26 to 29.24 % AR for the sandy water/sediment system Bickenbach and from 6.65 to 44.15 % AR for the loamy water/sediment system Unter Widdersheim.

The amount of non-extractable residues (NER) increased from 0 DAT to 100 DAT from 0.07 to 22.01 % AR for sandy water/sediment system and from 0.20 to 13.61 % AR for the loamy water/sediment system Unter Widdersheim.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide were 23.48 % AR at 100 DAT in the sandy water/sediment system Bickenbach and 19.37 % AR at 61 DAT in the loamy water/sediment system Unter Widdersheim. Organic volatiles determined were ≤0.1 % AR for both test systems at all sampling points.

E. TRANSFORMATION OF THE TEST ITEM

The amount of [^{14}C]glyphosate in water decreased from 0 DAT to 100 DAT from 92.47 to 0.27 % AR for water/sediment system Bickenbach and from 83.80 to 2.42 % AR for water/sediment system Unter Widdersheim.

The amount of [^{14}C]glyphosate in sediment extracts increased from 0 DAT to 7 DAT from 5.26 to 53.08 % AR, before declining to 29.24 % AR at 100 DAT for water/sediment system Bickenbach and increased from 0 DAT to 7 DAT from 6.65 to 61.36 % AR, before declining to 44.15 % AR at 100 DAT for water/sediment system Unter Widdersheim.

The amount of [^{14}C]glyphosate in the total system decreased from 0 DAT to 100 DAT from 97.73 to 29.50 % AR for water/sediment system Bickenbach and from 90.41 to 46.57 % AR for water/sediment system Unter Widdersheim.

Two metabolites were identified in the water phase of both test systems. AMPA was detected with a maximum amount of 15.74 % AR at 14 DAT in water/sediment system Bickenbach, decreasing to 0.48 % AR at 100 DAT. HMPA was detected with a maximum amount of 9.97 % AR at 61 DAT in water/sediment system Bickenbach and decreased to 7.52 % AR at 100 DAT. No other metabolites were detected in water above 5 % AR at any time. No metabolites were detected at any timepoint in sediment extracts of both test systems.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted] 2020, CA 7.2.2.3/001.

3. Assessment and conclusion

Assessment and conclusion by applicant: The study is conducted consistent with the current guideline, showing minor deviations. The mass balance shows values below 90 % for several sampling points, explained by the formation and loss of volatile metabolites. The exact amount of sediment per test vessel is not provided, but the relative ratio of water to sediment (2-2.5 cm sediment layer and 6 cm water layer). Although the storage conditions for re-analysis of water samples are not provided in the amendment, the 1D-TLC results show that the chromatographic pattern is the same as in the main study and no additional spots were observed. In conclusion, the deviations do not influence the overall results and general outcome of the study. Therefore, the study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.2.3/007
Report author	[redacted]
Report year	1993
Report title	Water/sediment biodegradation of [^{14}C]-glyphosate
Report No	IMW-R93/033
Document No	
Guidelines followed in study	Dutch Regulations for Biocides G.2.1.
Deviations from current test guideline	From OECD 308: - Procedural recoveries were low for water and sediment extracts following freeze drying prior to TLC analysis (i.e. 51 to 98 %, mean about 73 %, as calculated from tables in report). Following freeze-drying and attempts to re-suspend residues, "radioactivity adhered irreversibly to plastic storage bottles" - Low recovery of test item at 0 DAT of 56 % AR in total TNO systems and 92 % AR for Kromme Rijn system. In TNO systems, an unidentified compound occurred at about 46 % AR at 0 DAT. In the Kromme Rijn system, 92 % AR corresponded to glyphosate and 11 % AR of that unidentified compound. Unknown occurrence was explained in the report by "potential formation of complexes between glyphosate and water soluble humic acids" - Particle size for sieving of sediment and water is not reported - Temperature was between 22 and 24 °C for 75 h during study - Microbial biomass characterised by toxicity and viability test - Study duration slightly below 100 d (13 weeks), only 4 sampling time points - The redox potential of the water was not determined during the study - No parameters (pH, oxygen and redox) of the sediment were determined during the study

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The degradation of [^{14}C]glyphosate, labelled in the phosphonomethyl-position, was investigated in two water/sediment systems under aerobic conditions in the dark in the laboratory at 20 ± 2 °C for 13 weeks.

The following two water/sediment systems were used: TNO and Kromme Rijn. The amount of organic matter of the sediment was 12.4 % and 2.5 %, respectively. The pH of the sediment was 7.3 and 7.4, respectively, while the pH of the water was 9.3 and 7.7, respectively.

The test was performed in static systems, consisting of flasks filled with water and sediment in a way that the thickness of the sediment was about 2 cm, overlaid with 166 mL or 182 mL of water. The flasks were closed with a screw cap from which a carbon dioxide trap (a scintillation vial) was suspended, filled with 10 M NaOH.

The application was 170.7 kBq [^{14}C]glyphosate and 0.20 mg unlabelled glyphosate to each test system. Duplicate test systems were processed and analysed 0, 2, 4, 8 and 13 weeks after treatment. Carbon dioxide traps were collected after 2, 4, 8 and 13 weeks after treatment.

Mean material balances ranged from 92.9 to 104.8 % AR for the TNO water/sediment system and from 87.1 to 104.4 % AR for the Kromme Rijn water/sediment system.

Maximum amounts of carbon dioxide were 5.9 % AR after 13 weeks in the water/sediment system TNO and 25.6 % AR after 13 weeks in the water/sediment system Kromme Rijn.

The amount of radioactivity in the water decreased from 0 weeks after treatment to 13 weeks after treatment from 96.9 to 0.1 % AR for the TNO water/sediment system and from 94.2 to 0.6 % AR for the Kromme Rijn water/sediment system.

The amount of radioactivity extractable from the sediment of the TNO water/sediment system increased from 0 weeks after treatment to 8 weeks after treatment from 6.2 to 54.0 % AR and decreased then to 52.6 % AR at 13 weeks after treatment. The amount of radioactivity extractable from the sediment of the Kromme Rijn water/sediment system increased from 0 weeks after treatment to 2 weeks after treatment from 9.3 to 63.1 % AR and decreased then to 30.4 % AR at 13 weeks after treatment.

The amount of non-extractable residues (NER) increased from 0 weeks after treatment to 13 weeks after treatment from 1.6 to 35.0 % AR for the TNO water/sediment system and from 0.8 to 30.4 % AR for the Kromme Rijn water/sediment system.

The amount of glyphosate in water decreased from 52 % AR at 0 weeks to 4 % AR at 4 weeks after treatment to not detectable at 13 weeks after treatment for water/sediment system TNO and from 84 % AR at 0 weeks after treatment to 5 % AR at 2 weeks after treatment to not detectable at 4 weeks after treatment for water/sediment system Kromme Rijn water/sediment system.

The amount of glyphosate in sediment extracts increased from 4 % AR at 0 weeks after treatment to 54 % AR at 8 weeks after treatment and slightly decreased to 53 % AR at 13 weeks after treatment for

water/sediment system TNO. For water/sediment system Kromme Rijn, the amount of glyphosate in the sediment extracts increased from 8 % AR at 0 weeks after treatment to 63 % AR at 2 weeks after treatment and declined to 30 % AR after 13 weeks after treatment.

The amount of glyphosate in the total system decreased from 56 % AR at 0 weeks after treatment to 53 % AR at 13 weeks after treatment for water/sediment system TNO and from 92 % AR at 0 weeks after treatment to 30 % AR at 13 weeks after treatment for water/sediment system Kromme Rijn.

One unknown metabolite, which was mostly present in the water phase, was detected in both test systems. This metabolite was only detected at 0 weeks after treatment and it was suggested, that this was an artefact caused by formation of a complex of glyphosate with water soluble humic acids which resulted in a different behaviour on the cellulose TLC plates. No other metabolite in water or sediment was detected with >1 % AR.

The DT_{50} in the TNO water/sediment system was reported to be 17.7 weeks, best described by a reaction of a root second order. In Kromme Rijn water/sediment system, the DT_{50} was reported to be 4.4 weeks, best described by a reaction of a root first order.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]glyphosate (labelled in the phosphonomethyl-position)
Lot No.:	Code CFA.745, batch 17
Specific activity:	12.3 MBq/mg
Radiochemical purity:	98.6 % by TLC
Chemical purity:	Not provided

Identification:	glyphosate (non-radiolabelled
Lot No.:	F92/-/086
Chemical purity:	99 %

2. Test System:
The sediments were allowed to settle and then sieved to remove coarse particles. Water samples were filtered through a paper filter to remove water fleas and large particles. Water and sediment were stored refrigerated until used. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-102: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Water/Sediment I	Water/Sediment II
Location	TNO Zuidpolder	Kromme Rijn
Country	The Netherlands	The Netherlands
Sediment:		
Sand (>50 µm) (%)	39.0	79.6
Silt (2 µm – 50µm) (%)	34.2	11.1
Clay (≤2 µm) (%)	26.8	9.3
pH (KCl)	7.3	7.4
Organic matter (%)	12.4	2.5
Organic carbon [1] (%)	7.19	1.45
Cation exchange capacity (meq/100 g dry weight)	35.0	7.8
Total N (g/100 g dry weight)	0.509	0.118
Total P_2O_5 (g/100 g dry weight)	440	242
Water:		

Table 7.2.2.3-102: Characteristics of test water/sediment systems

pH [2]	9.3	7.7
Oxygen content (mg/L) [2]	15.3	7.0

[1] Calculated during dossier preparation using the equation: OC = OM/1.724
[2] Measured in lab after sampling

B. STUDY DESIGN

1. Experimental conditions

The test was performed in static test systems, consisting of 250-mL cylindrical flasks (biometer flasks) filled with water and sediment in a way that the thickness of the sediment was about 2 cm. For the TNO system, 54.2 g of wet sediment (20 g dry solids) and 166 mL water were used. For the Kromme Rijn system, 48.4 g of wet sediment (30 g dry solids) and 182 mL water were used. The flasks were closed with a screw cap from which a carbon dioxide trap, filled with 10 M NaOH, was suspended.

After set-up of the test systems they were pre-incubated on a rotary shaker for 14 days at 20 ± 2 °C in the dark.

The test item was applied to each test system as a mixture of radiolabelled and unlabelled glyphosate in 100 µL aqueous solution, resulting in 170.7 kBq [^{14}C]glyphosate and 0.20 mg unlabelled glyphosate per test system.

Samples were incubated for 13 weeks on a rotary shaker in the dark at 20 ± 2°C. Thereby an aerobic environment in the upper section was achieved while maintaining an undisturbed anaerobic sediment.

Additionally, four flasks for toxicity and viability test of each sediment were prepared. To two flasks of each sediment glyphosate was added at a concentration of 1 mg/L. To all flasks about 40 kBq radiolabelled and unlabelled sodium acetate was added to reach a final concentration of 100 mg/L.

2. Sampling

Duplicate test systems were processed and analysed 0, 2, 4, 8 and 13 weeks after treatment. The contents of the flasks of week 0 were analysed about one hour after addition of the test compound. Carbon dioxide traps were collected after 2, 4, 8 and 13 weeks after treatment. At the same time the trapping solution was replaced with fresh NaOH in the biometer flasks which were not sacrificed.

3. Analytical procedures

The pH and oxygen concentrations were measured in the flasks that were sacrificed for analysis.

At each sampling interval, water and sediment were separated by decantation of the water through a plug of cotton wool in a glass funnel. If the aqueous phase contained >2.5 % AR they were freeze-dried.

$^{14}CO_2$ dissolved in the aqueous phase was determined by adding 18 % hydrochloric acid to an aliquot (10 mL) of the sample in a closed system.

Sediment samples were extracted by shaking for 5 min with 0.5 M ammonium hydroxide solution. Cotton wool plug and the funnel, used for decanting the water phase, were rinsed with 0.5 M ammonium hydroxide and the plug was squeezed out. This ammonium hydroxide was added to the sediment and the solvent was removed from the sediment by centrifugation. Sediments were extracted with varying amount of 0.5 M ammonium hydroxide, until the extract contained <5 % AR. All extracts were pooled. Extracts were freeze-dried if they contained >2.5 % AR.

The determination of radioactivity in liquid samples (water, sediment extracts, volatile traps) was performed by liquid scintillation counter (LSC).

Radioactivity in the solids (non-extractable residues) after drying at room temperature were determined by combustion of the samples.

Freeze-dried residues of the aqueous phases and the extracts of the solids were extracted with 0.5 M ammonium hydroxide and 18 % hydrochloric acid. The recoveries after freeze-drying of the aqueous phases and the extracts of the solids were not very high, because part of the radioactivity adhered irreversibly to the plastic bottles used for freeze-drying the fractions.

The amounts of glyphosate and its metabolites were determined by TLC in the various concentrated phases with the use of reference compounds. Plates were developed in isobutyric acid:water:1-propanol:concentrated ammonium hydroxide:2-propanol:1-butanol (500:95:70:20:15:15) with 0.24 g of sodium-EDTA.

II. RESULTS AND DISCUSSION

A. DATA

Only small differences between the blank and the flasks with addition of 1 mg/L glyphosate were measured in the toxicity and viability test. Mean values differed by 1.8 % AR for the TNO system and by 0.5 % AR for the Kromme Rijn system. Radioactive mass balances of the carbon dioxide traps in the toxicity and viability test are summarised in Table 7.2.2.3-103.

Table 7.2.2.3-103: Amount of carbon dioxide evolved in the toxicity and viability test incubated with sodium acetate (expressed as percent of applied radioactivity)

System		Replicate	Time (weeks)						
			0	2	4	8	13	In H_2O	Sum
TNO	Blank	A	8.1	8.3	14.0	5.5	7.6	0.8	44.3
		B	9.2	8.0	13.2	6.1	6.3	0.9	43.6
		Mean	*8.7*	*8.2*	*13.6*	*5.8*	*7.0*	*0.9*	*44*
	+GLY	A	9.0	6.9	11.3	6.5	6.6	1.1	41.4
		B	8.7	7.0	13.5	5.7	7.3	0.9	43.1
		Mean	*8.9*	*7.0*	*12.4*	*6.1*	*7.0*	*1*	*42.3*
Kromme Rijn	Blank	A	14.6	17.9	24.3	6.7	2.3	0.9	66.8
		B	16.5	19.6	23.4	4.9	2.0	0.7	67.1
		Mean	*15.6*	*18.8*	*23.9*	*5.8*	*2.2*	*0.8*	*66.9*
	+GLY	A	18.1	19.1	25.8	5.8	1.3	1.1	71.2
		B	14.9	16.2	21.7	5.3	2.3	1.2	61.7
		Mean	*16.5*	*17.7*	*23.8*	*5.6*	*1.8*	*1.2*	*66.4*

Blank = Nothing added
+GLY = 0.198 mg of glyphosate added in 30 µL of water
In H_2O = Carbon dioxide remaining in the aqueous phases after 13 weeks
Values calculated in the course of this summary are given in *italics*

During the biodegradation test the pH varied between 7.4 and 9.1 in the TNO system and between 7.1 and 8.6 in the Kromme Rijn system (individual values of replicates). The oxygen content in the water phase ranged between 7.5 and 8.7 mg/L in the TNO system and between 6.8 and 8.8 in the Kromme Rijn system (individual values of replicates). Radioactive mass balance and distribution of glyphosate and metabolites in water/sediment systems extracts are summarised in Table 7.2.2.3-104 to Table 7.2.2.3-107.

Table 7.2.2.3-104: Amount of radioactivity in water/sediment system TNO (expressed as percent of applied radioactivity)

Compound	Replicate	Time (weeks)				
		0	2	4	8	13
CO_2 trap	A	0.0	0.2	0.6	2.9	4.8
	B	0.0	0.1	0.5	2.1	4.2
	Mean	0.0	0.2	0.6	2.5	4.5
CO_2 in H_2O	A	0.0	1.2	1.7	0.6	0.6
	B	0.0	1.0	1.2	0.8	2.0
	Mean	0.0	1.1	1.4	0.7	1.3
CO_2 Sum	A	0.0	1.4	2.2	3.5	5.4
	B	0.0	1.1	1.7	2.9	6.2
	Mean	0.0	1.2	2.0	3.2	5.9
H_2O	A	97.2	13.5	5.1	0.1	0.2
	B	96.6	17.0	3.8	0.2	0.0
	Mean	96.9	15.2	4.4	0.2	0.1
Solid	A	6.7	52.0	49.7	56.5	53.6
	B	5.8	52.5	54.5	51.6	51.6
	Mean	6.2	52.2	52.1	54.0	52.6
Non-extractable residues	A	1.7	28.6	35.0	34.7	33.4
	B	1.4	24.7	33.7	38.1	36.7
	Mean	1.6	26.6	34.4	36.4	35.0
Recovery	A	105.7	95.5	92.1	94.7	92.5
	B	103.8	95.3	93.7	92.8	94.5
	Mean	104.8	95.4	92.9	93.8	93.5

CO_2 trap = Results of carbon dioxide measurements in the trap
CO_2 in H_2O = Carbon dioxide in the aqueous phase
CO_2 sum = Sum of the carbon dioxide measurements
H_2O = Radioactivity in the aqueous phase (excluding CO_2)
Solids = Extractable radioactivity in the solids (sediment)

Table 7.2.2.3-105: Amount of radioactivity in water/sediment system Kromme Rijn (expressed as percent of applied radioactivity)

Compound	Replicate	Time (weeks)				
		0	2	4	8	13
CO_2 trap	A	0.0	5.9	10.5	19.7	22.5
	B	0.0	6.0	10.3	19.0	24.9
	Mean	0.0	6.0	10.4	19.4	23.7
CO_2 in H_2O	A	0.0	8.2	8.2	2.6	1.8
	B	0.0	9.6	5.1	2.3	2.1
	Mean	0.0	8.9	6.6	2.4	2.0
CO_2 Sum	A	0.0	14.1	18.7	22.2	24.3
	B	0.0	15.6	15.5	21.4	27.0
	Mean	0.0	14.8	17.1	21.8	25.6
H_2O	A	95.9	2.5	0.3	0.5	0.8
	B	92.5	5.7	2.3	0.6	0.4
	Mean	94.2	4.1	1.3	0.6	0.6
Solid	A	8.4	61.9	50.9	42.3	30.8
	B	10.2	64.3	51.0	43.0	30.0
	Mean	9.3	63.1	51.0	42.6	30.4
Non-extractable residues	A	0.7	20.5	24.8	26.9	32.3
	B	1.0	11.7	19.9	25.6	28.6
	Mean	0.8	16.1	22.4	26.2	30.4

Table 7.2.2.3-105: Amount of radioactivity in water/sediment system Kromme Rijn (expressed as percent of applied radioactivity)

Compound	Replicate	Time (weeks)				
		0	2	4	8	13
Recovery	A	105.1	98.9	94.7	92.0	88.2
	B	103.8	97.4	88.6	90.6	86.0
	Mean	104.4	98.2	91.6	91.3	87.4

CO_2 trap = Results of carbon dioxide measurements in the trap
CO_2 in H_2O = Carbon dioxide in the aqueous phase
CO_2 sum = Sum of the carbon dioxide measurements
H_2O = Radioactivity in the aqueous phase (excluding CO_2)
Solids = Extractable radioactivity in the solids (sediment)

Table 7.2.2.3-106: Amount of radioactivity in water/sediment system TNO (mean values of two replicates, expressed as mean percent of applied radioactivity)

Phase	Rf	Time (weeks)				
		0	2	4	8	13
Aqueous phase (freeze-dried)	0.0 (Glyphosate)	52	14	4	nd	nd
	0.1	-	-	<1	nd	nd
	0.2	-	<1	-	nd	nd
	0.4	-	<1	-	nd	nd
	0.5	-	<1	-	nd	nd
	0.9	44	-	-	nd	nd
Extracts of the solids (freeze-dried)	0.0 (Glyphosate)	4	51	52	54	53
	0.9	2	1	-	-	-
Sum of non-volatile radiolabelled compounds	0.0 (Glyphosate)	56	66	56	54	53
	0.1	-	-	<1	-	-
	0.2	-	<1	-	-	-
	0.4	-	<1	-	-	-
	0.5	-	<1	-	-	-
	0.9	46	1	-	-	-

nd: Not determined
- Not detected

Table 7.2.2.3-107: Amount of radioactivity in water/sediment system Kromme Rijn (mean values of two replicates, expressed as mean percent of applied radioactivity)

Phase	Rf	Time (weeks)				
		0	2	4	8	13
Aqueous phase freeze-dried	0.0 (Glyphosate)	84	5	nd	nd	nd
	0.4	-	<1	nd	nd	nd
	0.5	-	<1	nd	nd	nd
	0.8	-	<1	nd	nd	nd
	0.9	10	-	nd	nd	nd
Extracts of the solids (freeze-dried)	0.0 (Glyphosate)	8	63	51	42	30
	0.9	1	-	-	-	-
Sum of non-volatile radiolabelled compounds	0.0 (Glyphosate)	92	66	51	42	30
	0.4	-	<1	-	-	-
	0.5	-	<1	-	-	-
	0.8	-	<1	-	-	-
	0.9	11	-	-	-	-

nd: not determined
- not detected

B. MASS BALANCE
Mean material balances ranged from 92.9 to 104.8 % AR for the TNO water/sediment system and from 87.1 to 104.4 % AR for the Kromme Rijn water/sediment system.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES
The amount of radioactivity in the water decreased from 0 weeks after treatment to 13 weeks after treatment from 96.9 to 0.1 % AR for the TNO water/sediment system and from 94.2 to 0.6 % AR for the Kromme Rijn water/sediment system.

The amount of radioactivity extractable from the sediment of the TNO water/sediment system increased from 0 weeks after treatment to 8 weeks after treatment from 6.2 to 54.0 % AR and decreased then to 52.6 % AR at 13 weeks after treatment. The amount of radioactivity extractable from the sediment of the Kromme Rijn water/sediment system increased from 0 weeks after treatment to 2 weeks after treatment from 9.3 to 63.1 % AR and decreased then to 30.4 % AR at 13 weeks after treatment.

The amount of non-extractable residues (NER) increased from 0 weeks after treatment to 13 weeks after treatment from 1.6 to 35.0 % AR for the TNO water/sediment system and from 0.8 to 30.4 % AR for the Kromme Rijn water/sediment system.

D. VOLATILE RADIOACTIVITY
Maximum amounts of carbon dioxide were 5.9 % AR at 13 weeks after treatment in the TNO water/sediment system and 25.6 % AR at 13 weeks in the Kromme Rijn water/sediment system.

E. TRANSFORMATION OF THE TEST ITEM
The amount of glyphosate in water decreased from 52 % AR at 0 weeks to 4 % AR at 4 weeks after treatment to not detectable at 13 weeks after treatment for water/sediment system TNO and from 84 % AR at 0 weeks after treatment to 5 % AR at 2 weeks after treatment to not detectable at 4 weeks after treatment for water/sediment system Kromme Rijn water/sediment system.

The amount of glyphosate in sediment extracts increased from 4 % AR at 0 weeks after treatment to 54 % AR at 8 weeks after treatment and slightly decreased to 53 % AR at 13 weeks after treatment for water/sediment system TNO. For water/sediment system Kromme Rijn, the amount of glyphosate in the sediment extracts increased from 8 % AR at 0 weeks after treatment to 63 % AR at 2 weeks after treatment and declined to 30 % AR after 13 weeks after treatment.

The amount of glyphosate in the total system decreased from 56 % AR at 0 weeks after treatment to 53 % AR at 13 weeks after treatment for water/sediment system TNO and from 92 % AR at 0 weeks after treatment to 30 % AR at 13 weeks after treatment for water/sediment system Kromme Rijn.

One unknown metabolite, which was mostly present in the water phase, was detected in both test systems. This metabolite was only detected at 0 weeks after treatment and it was suggested, that this was an artefact caused by formation of a complex of glyphosate with water soluble humic acids which resulted in a different behaviour on the cellulose TLC plates. No other metabolite in water or sediment was detected with >1 % AR.

F. KINETICS
The DT_{50} in the TNO water/sediment system was reported to be 17.7 weeks, best described by a reaction of a root second order. In Kromme Rijn water/sediment system, the DT_{50} was reported to be 4.4 weeks, best described by a reaction of a root first order.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Besides several minor deviations and shortcomings the study shows the following two major deficiencies.

Procedural recoveries were low for water and sediment extracts following freeze-drying prior to TLC analysis (i.e. 51 to 98 %, mean about 73 %, as calculated from tables in report). Following freeze-drying and attempts to re-suspend residues, "radioactivity adhered irreversibly to plastic storage bottles".

Low recovery of test item was observed at 0 DAT of 56 % AR in total TNO systems and 92 % AR for Kromme Rijn system. In TNO systems, an unidentified compound occurred at about 46 % AR at 0 DAT. In the Kromme Rijn system, 92 % AR corresponded to glyphosate and 11 % AR of that unidentified compound. Unknown occurrence was explained in the report by "potential formation of complexes between glyphosate and water soluble humic acids".

In consequence the amounts of glyphosate determined in this study are not considered reliable.

Therefore, the study is onsidered invalid.

Assessment and conclusion by RMS:

Further information on justifying invalidity
Extracts with >2.5 % AR were freeze dried for further TLC analysis. The recovery of this workup was not reported within the report. Calculated recovery based on reported radioactivity before and after freeze-drying and re-suspending can be found below.

Table 7.2.2.3-108: Recovery after freeze-drying and re-suspending of the aqueous phases and the extracts of solids in the TNO water/sediment system

Phase		Time (weeks)				
		0	2	4	8	13
Aqueous phases	**Replicate 1**					
	H_2O	97.2	13.5	5.1	0.1	0.2
	After	79.7	10.0	5.0	nd	nd
	Recovery	*82.0*	*74.1*	*98.0*	*nd*	*nd*
	Replicate 2					
	H_2O	96.6	17.0	3.8	0.2	0.0
	After	81.8	15.0	3.7	nd	nd
	Recovery	*84.7*	*88.2*	*97.4*	*nd*	*nd*
Extracts of the solids	**Replicate 1**					
	H_2O	6.7	52.0	49.7	56.5	53.6
	Pool	3.8	32.0	32.7	38.2	29.9
	Recovery	*56.7*	*61.5*	*65.8*	*67.6*	*55.8*
	Replicate 2					
	H_2O	5.8	52.5	54.5	51.6	51.6
	Pool	3.0	32.7	37.0	33.6	29.9
	Recovery	*51.7*	*62.3*	*67.9*	*65.1*	*58.0*

H_2O = Aqueous phase
Pool = Sum of 0.5 M NH_4OH extracts of the solids
After = After freeze-drying and resuspending
nd = Not determined
Values calculated in the course of this summary are given in *italics*

Table 7.2.2.3-109: Recovery after freeze-drying and re-suspending of the aqueous phases and the extracts of solids in the Kromme Rijn water/sediment system

Phase		Time (weeks)				
		0	2	4	8	13
Aqueous phases	**Replicate 1**					
	H_2O	95.9	2.5	0.3	0.5	0.8
	After	83.0	nd	nd	nd	nd
	Recovery	*86.6*	*nd*	*nd*	*nd*	*nd*
	Replicate 2					
	H_2O	92.5	5.7	2.3	0.6	0.4
	After	78.8	6.9	nd	nd	nd
	Recovery	*85.2*	*121.1*	*nd*	*nd*	*nd*
Extracts of the solids	**Replicate 1**					
	Pool	8.4	61.9	50.9	42.3	30.8
	After	6.3	43.7	39.0	31.7	22.6
	Recovery	*75.0*	*70.6*	*76.6*	*74.9*	*73.4*
	Replicate 2					
	Pool	10.2	64.3	51.0	43.0	30.0
	After	7.1	44.3	38.9	33.2	20.8
	Recovery	*69.6*	*68.9*	*76.3*	*77.2*	*69.3*

H_2O = Aqueous phase
Pool = Sum of 0.5 M NH_4OH extracts of the solids
After = After freeze-drying and resuspending
nd = Not determined
Values calculated in the course of this summary are given in *italics*

Workup recovery of the freeze-drying ranged from 51.7 to 98.0 % and from 68.9 to 121.1 % in the TNO and Kromme Rijn water/sediment system, respectively. Recoveries for the freeze-drying ranged from 74.1 to 98.0 % for work up of the aqueous phase and from 51.7 to 77.2 % for the work up of the extracts of the solids. Overall mean workup recovery of the freeze-drying was 74.5 %.

Low recoveries, especially for the sediment workup, indicate a substantial loss of radioactivity during freeze drying. It cannot be clearly proven, that this loss can be attributed equally to the parent substance and its metabolites. The report itself did not discuss this issue.

Therefore, the poor procedural recoveries during workup justify to consider the study invalid.

1. Information on the study

Data point:	CA 7.2.2.3/008
Report author	[redacted]
Report year	1991
Report title	(^{14}C)-Sulfosate: Degradation in ditch waters and their associated hydrosoils
Report No	6589-38/127
Document No	
Guidelines followed in study	None
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)

Short description of study design and observations:

Study type: Water/sediment
Test item: ^{14}C-PMG anion (glyphosate, radiochemical purity 97.1 %, specific radioactivity 974.77 Bq/µg)
^{14}C-TMS cation (radiochemical purity 98.2 %, specific radioactivity 1173 Bq/µg))
Test system: Old Basing and Carrick Hill
Soil type: Silty clay loam (Old Basing)
Sandy loam (Carrick Hill)
pH: Old Basing: 7.4
Carrick Hill: 6.9
Organic matter:
Old Basing: 26.3 %
Carrick Hill: 1.6 %
Sediment was sieved to 5 mm.
Degradation of ^{14}C- PMG anion and ^{14}C-TMS cation was assessed in two water/sediment systems at 20°C, illuminated 12 h/dark 12°h for a duration of 91 days. Only results for the PMG anion (glyphosate) are considered here.

Application rate:1.6 mg/L (^{14}C-PMG anion)
Test design: static system with borosilicate glass cylinders
Volatiles trapping:
CO_2: Two ethanolamine trap
Organic volatiles, non-polar: One trap containing 2 % liquid paraffin in xylene
Organic volatiles, polar: One trap containing ethanediol
Additional volatile trap: One Polyurethane foam bung
Incubation: Exposed to a 12 h fluorescent lighting and 12 h dark regime at 20 °C
Sampling: 0, 3, 7, 14, 30, 60 and 91 DAT (duplicate samples)

Workup:
The contents of each unit were mixed thoroughly by manual shaking (5 to 10 mins), then centrifuged (4300 x g, 20 mins) and supernatants were decanted. Sediment was extracted with 0.37 M ammonia.
Samples (water or sediment extracts) containing insufficient radioactivity were concentrated by ultracentrifugation followed by freeze-drying of the supernatant for 48 h. Samples were reconstructed in 0.1 M formic acid.
Storage:
Loss of radioactivity during storage (ca. 4 months at ca. -18 °C) determined on one exemplary 7-day water sample per test sytem was 77.3 % for Old Basing and 52.9 % for Carrick Hill.

Analysis of radioactivity:
Water: LSC
Extracts: LSC
NER: Combustion/LSC
Volatiles: LSC
Identification of radioactive residues: TLC with reference standard

Levels of radioactivity in associated water samples were often <5 %, so many samples were not analysed. For those samples that were analysed, significant losses of radioactivity (72 to 85 %) occurred during sample concentration. A preliminary experiment showed that <5 % of ^{14}C-glyphosate (anion or cation labelled) was lost during this procedure, thus,

	the losses were presumably not ^{14}C-glyphosate. Further losses (34 to 58 %) occurred from the TLC plate between sample application and sample analysis. The result of the combined loss of radioactivity was that radioactivity on the TLC plate at the time of analysis accounted for only ca. 1 % of applied radioactivity for probably all but day 0 samples. For sediment extracts, the loss from the TLC plate between sample application and sample analysis was not analysed directly. It was assumed that
Short description of results:	^{14}C-PMG anion (glyphosate): Recovery of radioactivity: 68.04-97.29 % AR Losses of radioactivity may be due to the formation of volatile compounds (e.g. dimethyl sulphide or methane) which are not absorbed by the trapping reagents employed in this study. Mineralisation: Old Basing: 4.17 % AR at 91 DAT Carrick Hill: 22.12 % AR at 91 DAT Other volatiles: Polar organic volatiles: Old Basing: 0.2 % AR at 91 DAT Carrick Hill: 0.1 % AR at 91 DAT Non-polar organic volatiles: Old Basing: 0.06 % AR at 91 DAT Carrick Hill: 0 % AR at 91 DAT Extractable radioactivity: Old Basing: Water: 2.78 % AR at 0 DAT, 2.37 % AR at 91 DAT Sediment: 73.26 % AR at 0 DAT, 41.29 % AR at 91 DAT Carrick: Water: 13.86 % AR at 0 DAT, 0.93 % AR at 91 DAT Sediment: 57.09 % AR at 0 DAT, 29.71 % AR at 91 DAT Non-extractable radioactivity: Old Basing: 13.73 % AR at 0 DAT, max 37.00 % AR at 91 DAT Carrick Hill: 10.98 % AR at 0 DAT, max 17.92 at 14 DAT, 14.74 % AR at 91 DAT Transformation of test item: Due to the high losses of radioactivity during work-up of the water phase, the radioactivity on the TLC plate at the time of analysis accounted for only ca. 1 % of applied radioactivity. Thus, degradation can only be assessed relatively as percentage of TLC plate radioactivity and not given in % of applied radioactivity. Old Basing: Water (7 DAT): Glyphosate: 27 % of plate radioactivity AMPA: 16 % of plate radioactivity Other degradates (two compounds): 51 % of plate radioactivity Sediment (7 DAT): Glyphosate: 80 % of plate radioactivity AMPA: 15 % of plate radioactivity Carrick Hill: Water:

	Glyphosate: 92 % of plate radioactivity at 0 DAT, 31 % of plate radioactivity at 7 DAT AMPA: 2 % of plate radioactivity at 0 DAT, 52 % of plate radioactivity at 7 DAT Other degradates (three compounds): 15 % of plate radioactivity at 7 DAT Sediment: Glyphosate: 91 % of plate radioactivity at 0 DAT, 31 % of plate radioactivity at 91 DAT AMPA: 3 % of plate radioactivity at 0 DAT, 65 % of plate radioactivity at 7 DAT DT_{50} for glyphosate was determined to be >100 days for Old Basing system and 35 days for Carrick Hill system.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid based on the following deficiencies: - Incubation followed a 12 h fluroescent light and 12 h dark regime, i.e. not in full darkness - Mass balances below 75 % AR in System 2 and below 90 % in System 1 - Work-up procedure disturbed distribution of radioactivity between sediment and water (water and sediment mixed, then centrifuged) - Only samples with >5 % AR were analysed by TLC - Procedural losses during extract concentration, frozen storage and between application to TLC plates and analysis - for TLC analysis, significant (72 to 85 %) losses occurred during sample concentration; only ca. 1 % AR on the TLC plate at the time of for probably all but day 0 samples - TLC results not available for all sampling points - Degradation products not reported as % AR - acclimation for eight weeks - sediment was sieved to 5 mm
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.2.2.3/009
Report author	[redacted]
Report year	1990
Report title	Aerobic aquatic metabolism of [^{14}C]Glyphosate
Report No	MSL-10576
Document No	
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Section 162-4
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Category study in AIR 5 dossier (L docs)	Category 3b

Data point:	CA 7.2.2.3/010
Report author	[redacted]
Report year	1992

Report title	Addendum to MSL-10576 Aerobic aquatic metabolism of [^{14}C] Glyphosate
Report No	MSL-10576
Document No	
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Section 162-4
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Category study in AIR 5 dossier (L docs)	Category 3b

Short description of study design and observations:	Study type: Water/sediment Test item: ^{14}C-labeled glyphosate (radiochemical purity 98.8 %, specific radioactivity 3.98 mCi/mMole) Test water: Pond water (Fayette County, Kentucky) Test sediment: Pond bottom (Fayette County, Kentucky) Soil type: Silty Clay Loam Organic matter: 0.9 % pH: Water: 7.3, sediment: 6.6 Test system: 20 g sediment (dry weight) and 100 mL pond water in Erlenmeyer flasks, equipped with inlet and outlet tubes Application: 1 mL aqueous solution, resulting concentration 4.1 mg/kg, flasks swirled to mix Test design: Incubation at approximately 25 °C, flushed with oxygen Volatiles trapping: CO_2: 10 % NaOH trapping solution Organic volatiles: ethylene glycol trapping solution Sampling: 0, 1, 3, 7, 10, 15, 20, 24 and 30 DAT, duplicate samples Work up: Water and sediment were transferred completely to centrifuge bottles, centrifugation, decantation; extraction of sediment with 0.5 N KOH two or three times (20 min), samples from 3, 7, 10, 15, 20, 24 and 30 DAT were subsequently extracted with 0.03 M EDTA one to three times Analysis of radioactivity: Water: LSC Extracts: LSC NER: combustion/LSC Volatiles: LSC Identification of radioactive residues: radio HPLC with reference standards
Short description of results:	Recovery of radioactivity: 78.3-104.8 % (single values) pH during study: 5.9-7.0 Dissolved oxygen during study: 5.0-19.5 mg/L Mineralisation (maximum CO_2 at 24 DAT, mean): 24.3 % AR Other volatiles (maximum at 24 DAT, mean): 4.8 % AR Radioactivity in water (mean): 1.2 at 0 DAT Radioactivity in KOH extracts (mean): 98.9 % AR at 0 DAT Radioactivity in EDTA extracts (mean): 4.0 % AR at 24 DAT Non extractable radioactivity (mean): 7.2 % AR at 30 DAT Transformation of the test item in total system (mean): 0 DAT: 93.0 % AR Glyphosate

	3.3 % AR AMPA 0.4 % AR Unknown A 2.5 % AR Unknown B 1.1 % AR others 30 DAT: 22.2 % AR Glyphosate 22.7 % AR AMPA 1.5 % AR Unknown A 2.2 % AR Unknown B 1.0 % AR others Max values of metabolites: AMPA: 24.8 % AR (20 DAT) Unknown A: 1.9 % AR (20 DAT) Unknown B: 2.6 % AR (24 DAT) Others: 1.1 % AR (0 DAT) It was stated in the amendment that Unknown A and B may not be the product of microbial degradation but have been derived from AMPA by another mechanism such as radiolysis. The half-life of glyphosate was estimated to about 14.4 days.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The studies are considered invalid based on the following discrepancies: - Closed vessels with headspace of oxygen instead of atmospheric air - Work-up procedure disturbed distribution between sediment and water (water and sediment transferred to centrifuge bottles and centrifuged) - Short test duration (30 d, 22 % of ^{14}C glyphosate still remaining) - After application, test vessels were swirled to mix - Less than 50 g dry weight of sediment were used per sample - Mass balance below 90 % for some sampling intervals (77-105 %, 85 % on 30 DAT) - No acclimation period - Microbial biomass was not determined - Sample storage time prior to analysis not reported - Redox potential not measured during study

1. Information on the study

Data point:	CA 7.2.2.3/011
Report author	[redacted]
Report year	1990
Report title	Anaerobic aquatic metabolism of [^{14}C] Glyphosate
Report No	MSL-10577
Document No	
Guidelines followed in study	EPA Guidelines, Subdivision N, Section 162-3
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Category study in AIR 5 dossier (L docs)	Category 3b

Data point:	CA 7.2.2.3/012
Report author	[redacted]
Report year	1992
Report title	Addendum to MSL-10577

	Anaerobic aquatic metabolism of [^{14}C] Glyphosate
Report No	MSL-10577
Document No	
Guidelines followed in study	EPA Guidelines, Subdivision N, Section 162-3
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not accepted in RAR (2015)
Category study in AIR 5 dossier (L docs)	Category 3b

Short description of study design and observations:

Study type: water/sediment, anaerobic
Test item: [^{14}C] Glyphosate (radiochemical purity 98.8 %)
Test water: Pond water (Fayette County, Kentucky)
Test sediment: Pond bottom (Fayette County, Kentucky)
Soil type: Silty Clay Loam
Organic matter: 0.9 %
pH: Water:7.3, sediment: 6.6

An anaerobic water/sediment experiment was conducted for 365 days.

Application rate: 3.87 mg/kg
Test design: Static system with Erlenmeyer flasks flushed with nitrogen
Volatiles trapping:
CO_2: 10 % NaOH trap
Organic volatiles: Ethylene glycol trap
Incubation: In darkness at mean 25.4 ± 0.84 °C (20-27 °C)
Sampling: 0, 1, 4, 7, 15, 29, 60, 90, 180, 270 and 365 DAT, duplicate samples

Workup:
Water and sediment were transferred completely to centrifuge bottles and centrifuged. Supernatant water was decanted and sediment extracted
0-90 DAT: Extracted with 50 mL 0.5 N KOH (30 min) and 100 mL 0.5 N KOH (overnight)
180 DAT: Extracted three times with 50 mL 0.5 N NH_4OH (30 min), twice with 50 mL 0.5 N KOH (1 h) and 100 mL 0.5 N KOH (overnight)
270 and 365 DAT: Extracted twice with 50 mL 0.5 N KOH (30 min) and 100 mL 0.5 N KOH (overnight)
All successive extractions for each sample were pooled.
15 and 29 DAT: Subsequently extracted with 0.03 M EDTA

Analysis of radioactivity:
Water: LSC
Extracts: LSC (combined extracts)
NER: Combustion/LSC
Volatiles: LSC
Identification of radioactive residue: HPLC

Short description of results:

Recovery of radioactivity: 69.6-104.3 % AR (single values)

An additional test was performed to investigate the loss of radioactivity during incubation. Therefore, new test vessels were used and 4.15 ppm [^{14}C]glyphosate was incubated with 20 g sediment and 100 mL water for 6 months. Recoveries of the additional test were between 91.7-103.2 %. Thus, it is considered to be proven that the loss of radioactivity during the degradation was due to a loss of $^{14}CO_2$.

pH during study: 5.7-6.2
Dissolved oxygen during study: 1.4-3.7 mg/L
Mineralisation: max. 35.0 % AR at 365 DAT (single value)
Other volatiles: max. 3.7 % AR at 270 DAT (single value)
Radioactivity in water (mean values): 7.5 % AR at 0 DAT
Radioactivity in KOH (mean values): 93.6 % AR at 0 DAT, 40.1 % AR at 365 DAT
Radioactivity in EDTA extracts (mean values): 5.5 % AR at 15 DAT
Non-extractable radioactivity (mean values): 2.3 % AR at 0 DAT, 3.9 % AR at 365 DAT
Transformation of the test item in total system:
0 DAT (mean values):

- 95.3 % AR Glyphosate
- 3.8 % AR AMPA
- 0.5 % AR Unknown A
- 1.0 % AR Unknown B
- 1.0 % AR others

365 DAT (only one replicate available):

- 20.3 % AR Glyphosate
- 17.7 % AR AMPA
- 0.6 % AR Unknown A
- 1.0 % AR Unknown B
- 0.5 % AR others

Max values of metabolites (mean values):

- AMPA: 25.3 % AR (7 DAT)
- Unknown A: 1.1 % AR (7 DAT)
- Unknown B: 3.8 % AR (29 DAT)
- Others: 1.3 % AR (180 DAT)

It was stated in the amendment that Unknown A and B may not be the product of microbial degradation but have been derived from AMPA by another mechanism such as radiolysis.

The half-life of glyphosate was estimated to about 208 days.

Honegger (1992) discussed and recalculated the half-life using nonlinear first order kinetics, due to poor fit of the data points in the original report. The half-life of glyphosate in the calculation in the addendum was estimated to be 8.1 days.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	The studies are considered invalid based on the following discrepancies: - Anaerobic study; no data requirementd - Work-up procedure disturbed the sediment (water and sediment transferred to centrifuge bottles and centrifuged) - Water and sediment extracts were pooled prior to HPLC analysis - Test vessels were sealed - No acclimation prior to application - Test vessels were swirled to mix after application - Low mass balance (70-100 %): attributed to loss of $^{14}CO_2$ - Less than 50 g dry weight of sediment were used - Incubation temperature not controlled (20-27 C) - Long test duration: 365 d, but 7 sampling intervals analysed till 90 DAT - Sample storage time prior to analysis not reported - Microbial biomass was not determined - Redox potential not measured during study

1. Information on the study

Data point:	CA 7.2.2.3/013
Report author	[redacted]
Report year	1992
Report title	Review of the aquatic metabolism of Glyphosate.
Report No	Addendum to PTRL 366 and PTRL 367
Document No	
Guidelines followed in study	see CA 7.2.2.3/009 and CA 7.2.2.3/011
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	This addendum discusses the half-lives of glyphosate in water/sediment systems calculated in the reports PTRL 366 (aerobic aquatic metabolism) and PTRL 367 (anaerobic aquatic metabolism). The DT_{50} was calculated assuming pseudo first order kinetics to be 14.4 days and 208 days in PTRL 366 and PTRL 367, respectively. The degradation rate of glyphosate was re-calculated in this addendum, as the degradation was found to be better described by non-linear first order kinetics.
Short description of results:	Using non-linear first order kinetics, the DT_{50} was determined to be 6.48 and 8.12 days and DT_{90} was determined to be 107 and 6630 days in PTRL 366 and PTRL 367, respectively. However, the DT_{90} value for the anaerobic aquatic metabolism study was extrapolated from the data and the confidence interval for this value (6630 days) was quite large (0-24,400 days).
Reasons why the study is not considered relevant/reliable or not considered as key study:	Addendum to two invalid studies
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.2.2.3/014
Report author	[redacted]
Report year	1988
Report title	Aquatic dissipation of glyphosate and AMPA in water and soil sediment following application of glyphosate in irrigated crop and forestry uses
Report No	MSL-8332
Document No	
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Reference Number 164-2 of Subdivision N.
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: Water/sediment field study Test item: Rodeo® on irrigation water Accord® on forestry sites Two experiments were conducted: An application of the test item to two irrigation sources and an aerial application of the test item to a forestry site. Test systems: <u>Irrigation water:</u> <u>Non-flowing farm pond Clarence, Missouri:</u> pH sediment: 4.8-7.2 organic matter sediment: 1.5-2.1 % sediment texture: loam – clay loam <u>Flowing irrigation ditch Ephrata, Washington:</u> pH sediment: 6.3-6.8 organic matter sediment: 0.9-1.7 % sediment texture: sandy loam <u>Forestry sites (8.1 ha each and each containing a flowing stream and a pond water source):</u> <u>Chassell, Michigan:</u> pH sediment: 4.8-5.0 organic matter sediment: 2.5-2.6 % sediment texture: sandy loam <u>Corvallis, Oregon:</u> pH sediment: 5.6-5.8 organic matter sediment: 4.1-7.2 % sediment texture: clay loam – sandy clay loam <u>Cuthbert, Georgia:</u> pH sediment: 5.4-5.6 organic matter sediment: 0.4-0.8 % sediment texture: sandy loam <u>Irrigation water experiment:</u> Application rate: not stated Test design: Rodeo® was applied as a 1.5 % v/v solution to the edge of the irrigation source with backpack or tractor-mounted sprayer. Water from these sources was used to irrigate alfalfa, corn, grass and lettuce. Irrigation water and sediment located under treated areas was analysed. Water samples were collected from the treated area, the sprinkler pump and the sprinkler head. Sampling: Water: 0, 1, 3, 7, 14, 30, 49 (only Clarence), 55 (only Ephrata)°DAT

	Sediment: 0, 1, 3, 7, 14, 30, 60, 120, 180, 365°DAT Forestry site experiment: Application rate: 4.2 kg/ha Test design:Accord® was sprayed over the forest by helicopter. Pond and stream water samples and pond and stream sediments samples were analysed. Sampling: Water: 0, 1, 3, 7, 14, 28/30 DAT Sediment: approx. 0, 1, 3, 7, 14, 30, 60, 120, 180, 365°DAT Analytical procedures for both experiments: Workup: water samples were acidified and evaporated, sediment was extracted with 0.5 N KOH, centrifuged, acidified with HCl to pH 2 and filtered, chelated with Chelex 100 resin in the Fe(III) form, eluted with HCl. Iron was removed using anion exchange resin; concentration to dryness, samples redissolved in HPLC mobile phase containing EDTA Analysis: analysis by HPLC-PCR using fluorometric detection
Short description of results:	Irrigation water experiment: Maximum glyphosate: In Water: Clarence (Non-flowing): *Treated area*: 21.3 ppm at 0 DAT; 0.46 ppm at 1 DAT *Intake area*: 0.318 ppm at 1 DAT *Sprinkler head*: 0.125 ppm at 7 DAT Ephrata (Flowing): *Treated area*: <0.001 ppm *Intake area*: <0.001 ppm *Sprinkler head*: <0.001 ppm In Sediment: Clarence: 11.20 ppm at 0 DAT; 1.17 ppm at 1 DAT Ephrata: <0.05 ppm at all samplings Maximum AMPA: In Water: Clarence (Non-flowing): *Treated area*: 0.134 ppm at 0 DAT; 0.049 ppm at 1 DAT *Intake area*: 0.019 ppm at 14 DAT *Sprinkler head*: 0.021 ppm at 15 DAT Ephrata (Flowing): *Treated area*: <0.001 ppm *Intake area*: <0.001 ppm *Sprinkler head*: <0.001 ppm at all samplings In Sediment: Clarence: 1.23 ppm at 14 DAT Ephrata: <0.05 ppm Half-lives for Clarence were estimated as 6.3-9.26 days for pond water and 72.72-346.99 days for pond sediment. Forestry site experiment: Pond water samples: Maximum glyphosate: In Water: Chassell: 1.68 ppm at 0 DAT Corvallis: 0.091 ppm at 0 DAT

	Cuthbert: 0.985 ppm at 0 DAT In Sediment: Chassell: 2.11 ppm at 60 DAT Corvallis: 20.19 ppm at 28 DAT Cuthbert: 0.26 ppm at 0 DAT Maximum AMPA: In Water: Chassell: 0.035 ppm at 3 DAT Corvallis: 0.002 ppm at 0 DAT Cuthbert: 0.014 ppm at 0 DAT In Sediment: Chassell: 1.53 ppm at 30 DAT Corvallis: 1.95 ppm at 28 DAT Cuthbert: 0.13 ppm at 321 DAT <u>Flowing stream water samples:</u> Maximum glyphosate: In Water: Chassell: 1.237 ppm at 0 DAT Corvallis: 0.035 ppm at 0 DAT Cuthbert: 0.031 ppm at 0 DAT In Sediment: Chassell: 0.69 ppm at 7 DAT Corvallis: 0.11 ppm at 180 DAT Cuthbert: 0.18 ppm at 1 DAT Maximum AMPA: In Water: Chassell: 0.01 ppm at 0 DAT Corvallis: 0.002 ppm at 1 DAT Cuthbert: <0.001 ppm In Sediment: Chassell: 0.38 ppm at 14 DAT Corvallis: 0.18 ppm at 63 DAT Cuthbert: <0.05 at all samplings
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid based on the following discrepancies: - By its design, the study is not a water/sediment study but an outdoor study investigating the dissipation of glyphosate in water and sediment following residues from irrigation (farm pond & irrigation ditch), forest pond water & stream sources water at different locations in the US after edge of field application (irrigation sources) or forestry treatment - By the used study design it cannot be distinguished between processes of dilution, adsorption and degradation - No information of actual application rate (e.g. trough quantification of losses during application), thus the detected amount of glyphosate and AMPA cannot be related to the applied amount - Pesticide history of test systems not reported - Application of the formulated product (Rodeo® or Accord®) and not the active substance - sSamples were deep-frozen prior start of analytical procedures; storage length not reported
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.2.2.3/015
Report author	[redacted]
Report year	1979
Report title	Glyphosate dissipation in water following aquatic use of Roundup® in the U.K.
Report No	MLL-30038
Document No	
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: Water/sediment field study with overspray application to water Test item: Roundup® Two experiments were conducted with a duration of 32 days: Roundup® was applied to flowing water, near Wessex, Chippenham (UK) and to non-flowing water near Boston, Lincolnshire (UK) Test systems: Wessex (flowing water): canal was approximately 15 m wide with a depth of 1.5 m in the center and 0.5 m near the sides; water flow between sampling stations 1 and 2 was 2.25 min, 1.75 min between stations 3 and 4 and 2.5 min between stations 5 and 6. Boston (non-flowing water): two non-flowing farm drainage canals; water depth varied from 0.25 to 0.375 m pH: Wessex: Water: 6.9-7.5 (mean 7.2) Hydrosoil: 7.1 Boston: Water: 6.4-7.7 (mean 6.7) Hydrosoil: 6.6-7.4 (mean 6.9) Water temperature: Wessex: 14-16°C (mean 14.9°C) Boston: 7.2-15.5°C (mean 13.2°C) % Dry matter: Wessex: 0.3-1.0 % (mean 0.5 %) Boston: 0.4-1.4 % (mean 0.8 %) Application rate: 3.6 kg glyphosate/ha Test design: Flowing water: Roundup® sprayed over the channel with a knapsack sprayer Non-flowing water: Roundup® sprayed over the channel using a tractor mounted sprayer Sampling: 0.5, 1, 4, 8 h after treatment and 1, 2, 4, 8, 16, 32 DAT Collected by scooping top water layer and by using a vacuum system for sampling hydrosoils from canal bottom Samples were obtained from three replicate sampling points Samples were deep-frozen prior start of analytical procedures

	Workup: Hydrosoil samples were mixed with 30 mL of 0.5 M NH_4OH prior to filtration, glyphosate and aminomethylphosphonic acid (AMPA) recovered from samples by concentration on an anion exchange column, fractionation an a cation exchange column, derivatization to the N-trifluoroacetyl methyl esters Analysis: Gas-liquid chromatography using a phosphorous specific flame photometric detector; the detection limit was 0.005 mg/kg
Short description of results:	Flowing water: Recovery of the test item: 85-100 % Maximum glyphosate: In water 0.24 mg/kg at 30 min after treatment In hydrosoil: 0.006 mg/kg at 1 h after treatment Maximum AMPA: In water: <LOD In hydrosoil: <LOD 8 hours after application, no more glyphosate could be detected neither in the hydrosoils nor in the water samples taken at the application point Non-flowing water: Recovery of the test item:80-100 % Maximum glyphosate: In water 1.7 mg/mg at 4 h after treatment In hydrosoil: 0.03 mg/kg at 4 h after treatment Maximum AMPA: In water: 0.07 mg/kg at 4 DAT In hydrosoil: <LOD Glyphosate content dissipated below detection limit in less than 8 days in those hydrosoils; AMPA fully dissipated 16 days after treatment; the half-life for the dissipation of glyphosate in water was calculated to be 0.36 days using the nonlinear model of Gustafson and Holden
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid based on the following discrepancies: - field study with overspray application to water - with this setup it cannot be distinguished between dilution, adsorption and degradation - no information of actual application rate (e.g. trough quantification of losses during application), thus the detected amount of glyphosate and AMPA cannot be related to the applied amount - pesticide history of test systems not reported - application of the formulated product (Roundup®) and not the active substance - samples were deep-frozen prior start of analytical procedures; storage length not reported
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.1.1.3/016
Report author	[redacted]
Report year	1978
Report title	Photodegradation and anaerobic aquatic metabolism of Glyphosate, N-phosphonomethylglycine
Report No	MSL-0598
Document No	
Guidelines followed in study	None
GLP	No, GLP was not compulsory at the time the study was performed
Previous submission	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: Water/sediment (anaerobic) Test item: [^{14}C]-labelled glyphosate (specific activity 10.12 mC/mM, 98-99 % radiochemical purity) Test water and sediment: Natural water and sediment from lake number 34, Busch Wildlife Area, Weldon Springs, Missouri pH (water): 6.6 pH (sediment): 7.3 (medium not stated) Organic matter: 1.4 % Sediment was sieved with a 4 mesh sieve. One anaerobic water sediment experiment was conducted with natural water and sediment. Application rate: 150 µg was added to each flask (0.1 ppm) Test design: Anaerobic metabolism flasks were filled with 100 mL water and 50 mL of sediment, flushed with with nitrogen for 10 min, closed and incubated in the dark at 30 °C. The test substance was applied after 35 days. After application of glyphosate the flasks were flushed with nitrogen again and fitted with a carbon dioxide trap. Volatiles trapping: CO_2: ascarite trap Organic volatiles: no trapping Incubation: 30°C, gassed with nitrogen Sampling: 0, 1, 2, 3, 4 and 6 weeks after treatment Workup: Flasks were terminated by separating the sediment and water by centrifugation and subsequently extracting the sediment two times with 0.5 N NH_4OH. Analysis of radioactivity: Water: LSC Extracts: LSC/HPLC NER: combustion/LSC Volatiles: LSC Identification of radioactive residues: HPLC/radiodetection co-chromatography with reference items

Short description of results:	Recovery of radioactivity: 80.3-104.5 % Mineralisation: 10.4 % AR after 6 weeks Other volatiles: not measured Extractable radioactivity: 28.5 % AR after 6 weeks Radioactivity in water: 8.1 % AR after 0 weeks, 2.3 % AR after 6 weeks Non-extractable radioactivity: 29.4 % AR after 0 weeks, 40.7 % AR after 6 weeks Transformation of test item in sediment extracts (HPLC analysis): Glyphosate: 44.0 % AR after 0 weeks, 9.8 % AR after 6 weeks AMPA: 23.0 % AR after 0 weeks, 18.7 % AR after 6 weeks
Reasons why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid based on the following discrepancies: - Anaerobic incubation (no data requirement) - Incubation at 30 °C - Water was not analysed - Recovery of radioactivity below 90 % for several samplings - Water and sediment were separated by centrifugation which disturbed the sediment - Only 8 % of radioactivity in water at time zero and 29 % non-extractable residues, indication of work-up issues resulting in fast dissipation to the sediment - No proof of stability during application - Only single samples were incubated - Recovery of glyphosate at time zero far below 90 %
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point:	CA 7.2.2.3/017
Report author	[redacted]
Report year	1972
Report title	The degradation and metabolism of MON-0573 in river and lake bottom sediments and surface water
Report No	276
Document No	
Guidelines followed in study	US EPA guidelines for registering pesticides, draft 5-1-71, Section II – Degradation studies in water containing suspended solids, and Section III – Degradation studies in bottom sediments, draft 5-1-72
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: Water/sediment Test item: methane-^{14}C-labeled MON-0573 (N-(phosphonomethyl)glycine; glyphosate), radiochemical purity 96.5 %, specific radioactivity 8.51 mCi/mMol Test water/sediment: Mississippi River (75 feet from the shore, swift current) Illinois River (3 feet from the shore, moderate current) Missouri River (close to the shore, slow current) Springfield Lake, Illinois (30 feet from the shore) pH: Water samples: 8.20-8.55 Mississippi Sediment: 7.75

Illinois Sediment: 7.65
Missouri Sediment: 7.85
Springfield Sediment: 7.60

Degradation in collected water and sediment was assessed in separated experiments. A degradation study containing sediment, distilled water and the test substance and a degradation study containing the test water and the test substance were performed. Experiments were carried out with a duration of 14 days for the sediment experiment and 45 days for the water experiment in a shaker at 30 °C. In parallel control vessels were incubated with ^{14}C-sucrose to determine the evolution of $^{14}CO_2$ without presence of the test item.

Sediment experiment:

Test system:	bottom sediment was mixed for 20 min with a Hobart mixer, aliquots (10 g dry weight) were weighed into a funnel and flushed into the flasks with distilled water (95/100 mL)
Application:	1 mL NH_4CO_3 solution containing 0.5 mg of ^{14}C-glyphosate
Test design:	closed static system with sealed Erlenmeyer flasks shaken at 180 rpm at each sampling point, CO_2 collection apparatus was attached, and air was flushed through the systems
Volatiles trapping:	
CO_2:	apparatus containing ascarite (NaOH, glass wool and drierite ($CaSO_4$) attached to the flask by glass ground joints
Organic volatiles:	none
Incubation:	at 30°C
Sampling:	0, 4, 7 and 14 DAT
Work up:	water was separated from sediment by centrifugation; sediment was washed with 25 mL water, suspended by vigorous shaking followed by centrifugation; after lyophilisation, sediment was extracted three times with 40 mL of 0.5 N NH_4OH; samples were combusted prior to and after extraction with NH_4OH
Analysis of radioactivity:	
Extracts:	LSC
NER:	combustion/LSC
Volatiles:	LSC
Identification of radioactive residues:	TLC/Beta Camera with reference standards

Water experiment:

Test system:	100 mL of test water were filled into the flasks
Application:	1 mL NH_4CO_3 solution containing 0.5 mg of ^{14}C-glyphosate
Test design:	static system with sealed Erlenmeyer flasks
CO_2:	apparatus containing ascarite (NaOH, glass wool and drierite ($CaSO_4$) attached to the flask by glass ground joints
Organic volatiles:	none
Incubation:	at 30°C
Sampling:	0, 4, 7, 14, 21, 28, 35 and 45 DAT

	Work up: None Analysis of radioactivity: Supernatant: LSC Volatiles: LSC Identification of radioactive residues: TLC/Beta Camera with reference standards
Short description of results:	Sediment test: Recovery of radioactivity: 82.6-94.7 % Mineralisation (cumulative CO_2 after14 days): Missouri Sediment: 41.0°%°AR Illinois Sediment: 44.6 % AR Mississippi Sediment: 41.5 % AR Springfield Sediment: 43.6 % AR Other volatiles: none Radioactivity in supernatant at 14 DAT: Missouri Sediment: 12.3 % AR Illinois Sediment: 6.9 % AR Mississippi Sediment: 10.3 % AR Springfield Sediment: 10.7 % AR Radioactivity in NH_4OH extracts at 14 DAT: Missouri Sediment: 23.8 % AR Illinois Sediment: 16.6 % AR Mississippi Sediment: 20.4 % AR Springfield Sediment: 20.0 % AR Non extractable radioactivity at 14 DAT: Missouri Sediment: 11.3 % AR Illinois Sediment: 12.7 % AR Mississippi Sediment: 20.3 % AR Springfield Sediment: 16.5 % AR Distribution of residues at 14 DAT (water/ sediment extract/ total system in % AR): Missouri Sediment: Glyphosate: 1.2 / 8.6/ 9.8 AMPA: 11.1 / 15.2 / 26.3 Illinois Sediment: Glyphosate: 0.7 / 2.6 / 3.3 AMPA: 6.2 / 14.0 / 20.2 Mississippi Sediment: Glyphosate: 0.5 / 5.9 / 6.4 AMPA: 8.4 / 14.5 / 22.9 Unknown I: 0.7 / - / 0.7 Unknonwn II: 0.7 / - / 0.7 Springfield Sediment: Glyphosate: 1.6 / 3.0 / 4.6 AMPA: 7.2 / 13.4 / 20.6 Unknown I: 0.3 / 0.6 / 0.9 Unknown II: 0.5 / 1.0 / 1.5 Water test: Recovery of radioactivity: 90.8-95.3 % Mineralisation(cumulative CO_2 after14 days): Missouri Water: 1.82 % AR Illinois Water: 1.55 % AR

	Mississippi Water: 1.49 % AR Springfield Water: 5.76 % AR Other volatiles: none Transformation of the test item at 45 DAT (in % AR): Missouri Water: glyphosate: 82.1 AMPA: 9.2 Illinois Water: glyphosate: 86.6 AMPA: 7.1 Mississippi Water: glyphosate: 86.9 AMPA: 6.9 Springfield Water: glyphosate: 70.7 AMPA: 14.3
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid based on the following discrepancies: - Separate incubation in water and sediment, i.e. no 'systems' - Incubation of sediment by adding distilled water - Test was performed at 30 °C - Sediment was extracted with NH_4OH after lyophilization - Only 4 instead of the recommended six sampling times were processed in the sediment experiment - Distribution into components only reported for the last sampling - Test duration was 14 days for sediment and 45 days for water - Oxygen saturation, pH value and redox potential during study were not reported - After sampling sediment was mixed for 20 min using a Hobard mixer - Characterisation data (pH, organic carbon, texture) of test systems not available - Recovery in the sediment experiment <90 % for one system - No LOD/LOQ reported - No acclimation period of test systems prior application - 10 g dry weight of sediment used and thus less than recommended 50 g - Range of temperature during study not reported - Single samples were investigated per sampling interval - No information whether samples were incubated in the dark
Category study in AIR 5 dossier (L docs)	Category 3b

Water/sediment studies with AMPA as test item

1. Information on the study

Data point:	CA 7.2.2.3/018
Report author	[redacted]
Report year	2004
Report title	[14C]-AMPA: Degradation and fate in water/sediment systems
Report No	SNN/03
Document No	
Guidelines followed in study	Guidelines concerning the inclusion of Active Substances in Annex I 91/414/EEC SETAC
Deviations from current test guideline	From OECD 308: - Issues in analysis of sediment extracts of Sediment B, probably caused by co-extracted matrix disrupting the ion-exchange chromatography

	- Water:sediment ratio of 2:1 by volume (instead of 3:1 to 4:1 as recommended by the guideline) - Single test systems processed at each timepoint - No storage time at -15 °C reported for water phases prior to chromatographic analysis
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]aminomethylphosphonic acid (AMPA) was investigated in two water/sediment systems under aerobic conditions in the dark in the laboratory at 20 ± 2°C for 103 days.

The following two water/sediment systems were used: Manningtree A, a clay loam sediment with pH 7.6 and an organic matter content of 5.6 % and Manningtree B, a clay loam sediment with pH 6.3 and an organic matter content of 6.0 %.

The test was performed in flow-through systems purged with moistened air and connected to two 1 M potassium hydroxide traps to collect carbon dioxide and an ethyl digol trap to collect volatile organic compounds.

The application rate was 0.51 mg AMPA/test vessel.

Single samples from each system were processed and analysed at 0, 1, 7, 14, 29, 61, and 103 days after treatment (DAT). The volatile traps were assayed at each sampling interval to determine the amount of carbon dioxide and volatile organic compounds.

Mass balances ranged from 89.3 to 96.6 % of applied radioactivity (% AR) (one exception being on 61 DAT, recovery 86.5 % AR) for Manningtree A and from 87.7 to 97.2 % AR) (one exception being on 61 DAT, recovery 81.9 % AR for Manningtree B.

In all test systems formation of volatiles increased steadily during the experimental period, the majority of which was carbon dioxide. Maximum amounts of volatiles reached at study end (103 DAT) were 9.8 and 8.2 % AR in Manningtree A and Manningtree B, respectively. The barium precipitation test confirmed the identity of volatiles from the KOH traps as carbon dioxide.

In Manningtree A, radioactivity recovered in the water decreased from 94.7 % AR at 0 DAT to 1.8 % AR at 103 DAT. Correspondingly, radioactivity in the sediment extracts increased from 1.5 % AR at 0 DAT to 65.3 % AR at 103 DAT.

In Manningtree B, radioactivity recovered in the water decreased from 96.7 % AR at 0 DAT to 0.3 % AR at 103 DAT. Correspondingly, radioactivity in the sediment extracts increased from 0.3 % AR at 0 DAT to 56.2 % AR at 103 DAT.

In system Manningtree A, non-extractable residues (NER) accounted for up to 24.5 % AR (7 DAT) and ranged between 0.4 and 24.5 % AR during the course of the study. Fractionation indicated that the majority of radioactivity was associated with the humic acid fraction.

In system Manningtree B, NER accounted for up to 40.7 % AR (29 DAT) and ranged between 0.2 to 40.7 % AR during the course of the study. Fractionation indicated that the majority of radioactivity was associated with the humic acid fraction.

Analysis of water samples by HPLC showed that the majority of the radioactivity in samples from both sediments was associated with AMPA. In the water sample from Manningtree A, AMPA decreased from 90.5 % AR at 0 DAT to 0.8 % AR at 103 DAT. A minor unidentified peak chromatographically more acidic than AMPA (P1a) was detected at 5 minutes, but this accounted for less than 1 % AR at each time point.

Analysis of water samples from Manningtree B showed a decrease in AMPA from 91.2 % AR at 0 DAT to 0.2 % AR at 103 DAT. Similarly a minor unidentified peak (P1a) was detected at 5 minutes, but this accounted for less than 0.5 % AR at each time point.

Analysis of the Manningtree A sediment extracts showed that the amount of AMPA in the sediment increased from 1.1 % AR at 0 DAT to 30.2 % AR at DAT-61 and decreased to 12.3 % AR until 103 DAT.

Analysis of the extracts from Manningtree B sediment, AMPA accounted for 22.3 % AR at 1 DAT but decreased to 0.2 % AR at 14 DAT.

One major radioactive component (designated P1a) was observed in sediments after 103 days. This component was more acidic than AMPA in the chromatographic system employed. In Manningtree B, an additional radioactive component was observed (designated P3) and this accounted for around 33 % AR at DAT-103. Due to the broad nature of the chromatographic peak it is believed that the radioactivity is associated with more than one component.

In system Manningtree A the amount of AMPA in the total system decreased from 91.6 % AR at 0 DAT to 9.4 % AR at 14 DAT, increased then to 34.3 % AR at 29 DAT and finally decreased to 13.1 % AR at 103 DAT. In system Manningtree B, the amount of AMPA in the total system decreased from 0 DAT to 103 DAT from 91.2 to 0.2 % AR.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Identification: [^{14}C]AMPA
Lot No.: RUS 0316
Specific activity: 17.65 mCi/mmol (159 µCi/mg)
Radiochemical purity: 97.8 %
Chemical purity: Not provided

2. Test System:
Sediments were sieved to ≤2 mm. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-110: Characteristics of test water/sediment systems

Parameter	**Results**	
Test system	Manningtree A	Manningtree B
Country	United Kingdom	United Kingdom
Sediment:		
Textural Class (UK)[1]	Clay loam	Clay loam
Sand (%)	48	48
Silt (%)	29	28
Clay (%)	23	24

Table 7.2.2.3-110: Characteristics of test water/sediment systems

Parameter	Results	
Test system	Manningtree A	Manningtree B
pH [2]	7.6	6.3
Organic matter (%)	5.6	6.0
Organic carbon [3] (%)	3.2	3.5
Cation exchange capacity (meq/100 g)	14.7	17.0
Water content (% dry weight)	88.9	96.3
Water content (% wet weight)	47.1	49.0
Microbial biomass (µg C/g)		
Study beginning (0 DAT)	338.6	316.3
Study end (103 DAT)	296.1	143.9
Water:		
Organic carbon (mg/L)	12.1	26.4
pH	7.2	7.1

DAT = Days after treatment, USDA: United States Department for Agriculture
[1] No details on classification system (i.e. particle size) reported
[2] Medium not reported
[3] Calculated during dossier preparation using the equation: OC = OM/1.724

B. STUDY DESIGN

1. Experimental conditions

Flow-through test system were used, consisting of a dreschel bottle (with sintered stem for uniform gas dispersion) containing water to humidify the air-flow, connected to the test vessel containing the water/sediment test system (the end of the glass tube bringing air into the test vessel was positioned just below the water surface). The test system was connected to an empty dreschel bottle followed by a dreschel bottle containing ethyl digol (to trap organic volatile compounds) and two dreschel bottles containing 1 M aqueous potassium hydroxide (KOH) solution with phenolphthalein indicator (to trap $^{14}CO_2$).

Sediment, equivalent to 55 to 60 g dry weight (ca 100 mL equivalent to 120 g wet weight) was added to each test vessel and covered with approximately 200 mL of the corresponding water. The test systems were incubated at 20 ± 2°C in darkness with an air flow-rate sufficient to achieve as close as possible to the specified water oxygen content (20 % saturation), until an equilibrium was reached with respect to the pH and oxygen content in the water and the redox potential in the water and sediment.

The application rate was 0.51 mg AMPA/test vessel.

Test systems were incubated under aerobic conditions in the dark for 103 days at 20 ± 2°C. During acclimatization and incubation pH value, oxygen saturation and redox potential of the water layer and the redox potential of the sediment layer were monitored in additional untreated test vessels.

2. Sampling

Single samples from each system were processed and analysed at 0, 1, 7, 14, 29, 61, and 103 days after treatment (DAT). The ethyl digol and KOH traps were assayed and changed on a weekly basis for the first month of the study and about ten days thereafter.

3. Analytical procedures

The sediment and water in each test vessel were separated by decanting the water from the test vessel. At each sampling interval, the radioactivity associated with dosing formulations, water, air traps and sediment extracts was determined directly by liquid scintillation counting (LSC). Water samples were stored at -15 °C prior to chromatographic analyses.

Sediments were extracted three times at room temperature with 0.5 M ammonium hydroxide for 2 hours using a shaker. Afterwards, sediments were extracted by shaking twice at room temperature for 2 hours using 1 M hydrochloric acid. Each extract was separated by centrifugation and analysed by LSC in duplicates separately. Sediment residues were air-dried and analysed by combustion/LSC.

Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification (LOQ).

Residues in water and sediment extracts were quantified by HPLC. The limit of detection was not reported.

Selected samples of extracted sediments containing >10 % applied radioactivity were further extracted with 0.5 M NaOH solution for fractionation intro humins, fulvic acid and humic acid.

The identification of CO_2 in the potassium hydroxide traps was determined by the addition of barium chloride to aliquots of the trap contents. The absence of radioactivity in the supernatant and the presence of the precipitate, $Ba^{14}CO_3$, confirmed the presence of CO_2 in the traps.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 6.9 and 7.8 in system Manningtree A and between 7.0 and 8.1 for system Manningtree B. The oxygen saturation in the water phase ranged between 10 and 18 % in system Manningtree A and between 5 and 19 % in system Manningtree B. The redox potential of the water was between 32 and 210 mV for system Manningtree A and between -44 and 239 mV for system Manningtree B. The redox potential of the sediment was between -93 and 222 mV in system Manningtree A and between -129 and 248 mV for system Manningtree B.

Radioactive mass balance and distribution of AMPA and metabolites in two water/sediment systems are summarised in Table 7.2.2.3-111 to Table 7.2.2.3-114. Fractionation of non-extractable residues into fulvic acid, humic acid in humin fractions is presented in Table 7.2.2.3-115.

Table 7.2.2.3-111: Distribution of radioactivity in water/sediment system Manningtree A under aerobic conditions (single samples, expressed as percent of applied radioactivity)

Compound	DAT						
	0	1	7	14	29	61	103
Water	94.7	38.4	11.3	6.8	5.3	2.8	1.8
Sediment Extracts	1.5	39.2	55.1	66.9	64.7	67.7	65.3
Non-extractable Residue	0.4	13.4	24.5	16.7	16.0	7.9	13.0
Carbon dioxide	n.s.	0.3	0.5	0.6	3.3	8.1	9.8
Mass balance	96.6	91.3	91.4	91.0	89.3	86.5	89.9

DAT: Days after treatment
n.s. No sample
Radioactivity in ethyl digol traps was always <0.1 % AR.

Table 7.2.2.3-112: Distribution of radioactivity in water/sediment system Manningtree B under aerobic conditions (single samples, expressed as percent of applied radioactivity)

Compound	DAT 0	1	7	14	29	61	103
Water	96.7	53.5	9.0	6.6	2.3	0.3	0.3
Sediment Extracts	0.3	25.8	48.0	50.8	47.4	62.9	56.2
Non-extractable Residue	0.2	13.0	35.3	31.6	40.7	10.7	23.0
Carbon dioxide	n.s.	0.2	0.6	2.4	3.0	8.0	8.2
Mass balance	97.2	92.5	92.9	91.4	93.4	81.9	87.7

DAT: Days after treatment
n.s. No sample
Radioactivity in ethyl digol traps was always <0.1 % AR.

Table 7.2.2.3-113: Degradation of [14C]AMPA in water/sediment system Manningtree A under aerobic conditions (single samples, expressed as percent of applied radioactivity)

Compound		DAT 0	1	7	14	29	61	103
Water	P1a	<0.1	0.3	0.4	0.7	0.5	0.5	0.7
	AMPA	90.5	37.7	10.7	5.9	4.7	2.3	0.8
	Others [1]	4.2	0.5	0.2	0.2	0.1	0.1	0.4
Sediment	P1a	0.3	21.7	34.8	53.0	24.5	31.6	31.0
	AMPA	1.1	15.4	16.4	3.5	29.6	30.2	12.3
	Others [1]	0.1	2.1	3.9	10.4	10.6	5.9	22.0
Total system	AMPA	*91.6*	*53.1*	*27.1*	*9.4*	*34.3*	*32.5*	*13.1*

DAT: Days after treatment
[1] Represents regions of radioactivity which cannot be assigned to a designated peak
Values calculated during dossier preparation are given in *italics*

Table 7.2.2.3-114: Degradation of [14C]AMPA in water/sediment system Manningtree B under aerobic conditions (single samples, expressed as percent of applied radioactivity)

Compound		DAT 0	1	7	14	29	61	103
Water	P1a	<0.1	0.3	0.3	0.3	0.2	0.1	<0.1
	AMPA	91.2	52.7	8.6	6.2	1.9	0.1	0.2
	Others [1]	5.5	0.5	0.1	0.1	0.2	0.1	0.1
Sediment	P1a	0.1	1.4	44.1	49.2	26.6	41.8	18.0
	AMPA	0.1	22.3	2.6	0.2	<0.1	<0.1	<0.1
	P3	<0.1	<0.1	<0.1	<0.1	6.1	16.6	33.2
	Others [1]	0.1	2.1	1.3	1.4	14.7	4.5	5.0
Total system	AMPA	*91.2*	*52.7*	*8.6*	*6.2*	*1.9*	*0.1*	*0.2*

DAT: Days after treatment
[1] Represents regions of radioactivity which cannot be assigned to a designated peak
Values calculated during dossier preparation are given in *italics*

Table 7.2.2.3-115: Fractionation of day 103 post extracted sediment (in percent of applied radioactivity)

Experiment	Fulvic acid	Humic acid	Humin
Manningtree A	38.8	59.5	1.8
Manningtree B	28.2	68.4	3.4

B. MASS BALANCE

Material balances ranged from 89.3 to 96.6 % of applied radioactivity (% AR) for Manningtree A (one exception being on 61 DAT, recovery 86.5 % AR) and from 87.7 to 97.2 % AR (one exception being on 61 DAT, recovery 81.9 % AR for Manningtree B).

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

In system Manningtree A, radioactivity recovered in the water decreased from 94.7 % AR at 0 DAT to 1.8 % AR at 103 DAT. Correspondingly, radioactivity in the sediment extracts increased from 1.5 % AR at 0 DAT to 65.3 % AR at 103 DAT.

In system Manningtree B, radioactivity recovered in the water decreased from 96.7 % AR at 0 DAT to 0.3 % AR at 103 DAT. Correspondingly, radioactivity in the sediment extracts increased from 0.3 % AR at 0 DAT to 56.2 % AR at 103 DAT.

In system Manningtree A, non-extractable residues (NER) accounted for up to 24.5 % AR (7 DAT) and ranged between 0.4 and 24.5 % AR during the course of the study. Fractionation indicated that the majority of radioactivity was associated with the humic acid fraction.

In system Manningtree B, NER accounted for up to 40.7 % AR (29 DAT) and ranged between 0.2 to 40.7 % AR during the course of the study. Fractionation indicated that the majority of radioactivity was associated with the humic acid fraction.

D. VOLATILE RADIOACTIVITY

In both test systems, the majority of volatiles was carbon dioxide. Maximum amounts of volatiles reached at study end (103 DAT) were 9.8 and 8.2 % AR in systems Manningtree A and Manningtree B, respectively. The barium precipitation test confirmed the identity of volatiles from the KOH traps as carbon dioxide. Radioactivity in ethyl digol traps was always ≤0.1 % AR.

E. TRANSFORMATION OF THE TEST ITEM

Analysis of water samples by HPLC showed that the majority of the radioactivity in samples from both sediments was associated with AMPA. In the water sample from Manningtree A, AMPA decreased from 90.5 % AR at 0 DAT to 0.8 % AR at 103 DAT. A minor unidentified peak chromatographically more acidic than AMPA (P1a) was detected at 5 minutes, but this accounted for less than 1 % AR at each time point.

Analysis of water samples from Manningtree B showed a decrease in AMPA from 91.2 % AR at 0 DAT to 0.2 % AR at 103 DAT. Similarly a minor unidentified peak (P1a) was detected at 5 minutes, but this accounted for less than 0.5 % AR at each time point.

Analysis of the Manningtree A sediment extracts showed that the amount of AMPA in the sediment increased from 1.1 % AR at 0 DAT to 30.2 % AR at 61 DAT and decreased to 12.3 % AR until 103 DAT. Peak P1a was also detected in the extract samples and accounted for approximately 53 % AR up to 14 DAT decreasing to ca 31 % AR by 103 DAT.

Analysis of the extracts from Manningtree B sediment, AMPA accounted for 22.3 % AR at 1 DAT but decreased to 0.2 % AR at 14 DAT. Peak P1a was also detected and accounted for ca 42 % AR up to 61 DAT. Radioactivity associated with P1a accounted for 18 % AR at 103 DAT. In this system severe problems were encountered in obtaining chromatography for these extracts. This is believed to be due to the presence of co-extracted endogenous material affecting the ion-exchange chromatography. There appears to be a further radioactive component present in this system (designated P3) and this accounted for 6.1 % AR at 29 DAT and 33.2 % AR at 103 DAT. This component was observed as a broad peak and could well be composed of several components that were unresolved due to chromatographic interference from endogenous material. Several attempts were made to improve the chromatography, including solid phase extraction dilution of the extracts with mobile phase and concentration/re-suspension in mobile phase. All attempts proved unsuccessful. Since the study was a metabolite study, not a parent glyphosate study, and the compounds were never detected in any of the available glyphosate water/sediment studies no further attempts were made to identify these breakdown products.

In system Manningtree A the amount of AMPA in the total system decreased from 91.6 % AR at 0 DAT to 9.4 % AR at 14 DAT, increased then to 34.3 % AR at 29 DAT and finally decreased to 13.1 % AR at 103 DAT. In system Manningtree B, the amount of AMPA in the total system decreased from 0 DAT to 103 DAT from 91.2 to 0.2 % AR.

F. KINETICS
Degradation kinetics were updated according to latest guidance documents and can be found in [redacted] 2020, CA 7.2.2.3/001.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Material balances at 61 DAT and 103 DAT were between 81 and 89 % for both test systems. This can be attributed to a loss of volatiles or losses during combustion. Nevertheless, the time course of the radioactivity distribution in water and sediment is reasonable and consistent for both test systems. Thus, there is no effect on the understanding of the degradation behaviour of AMPA in this study.

Issues occurred in analysis of sediment extracts of Sediment B, probably caused by co-extracted matrix disrupting the ion-exchange chromatography. Attempts to improve the chromatography (e.g. solid phase extraction) were not successful. Therefore, the rate of AMPA was only calculated for water, sediment and total system of test system Manningtree A and for water of system Manningtree B. The degradation rate of AMPA in sediment and total system of Manningtree B was not calculated.

The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.2.3/019
Report author	[redacted]
Report year	2003
Report title	Aerobic aquatic degradation of aminomethylphosphonic acid according to SETAC, part 1.8.2 (March 1995)
Report No	IF-02/00005222
Document No	
Guidelines followed in study	SETAC "Procedures for assessing the environmental fate of ecotoxity of pesticides", Part 1, 8.2
Deviations from current test guideline	From OECD 308: - Pre-equilibration of the test systems for 34 days - Limit of detection and limit of quantification of the chromatographic methods is not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]Aminomethylphosphonic acid (AMPA) was investigated in two water/sediment systems under aerobic conditions in the dark in the laboratory at 20 ± 2 °C for 104 days.

The sediments of the aquatic test systems were characterized as sand from the Bickenbach location and slight sandy loam from the Unter-Widdersheim location. The amount of organic carbon in the sediments ranged from 0.64 to 2.96 % and the pH in the sediments was 8.5.

The test was performed in flow-through systems connected to a trapping system to collect carbon dioxide and volatile organic compounds.

The application rate was 0.958 mg AMPA a.s./L which is equivalent to a use rate of 2837 g AMPA/ha.

Duplicate samples from each system were processed and analysed at 0, 0.25, 1, 2, 7, 14, 30, 62 and 104 days after treatment (DAT). The volatile traps were assayed at each sampling time or at about every 14 days, whichever came first, to determine the amount of carbon dioxide and volatile organic compounds.

Mass balances (single values) ranged from 93.8 to 103.6 % AR for system Bickenbach and from 98.6 to 106.5 % AR for system Unter-Widdersheim.

Maximum amounts of carbon dioxide reached at study end (104 DAT) were 40.1 and 21.2 % AR in the Bickenbach and Unter-Widdersheim systems respectively. Organic volatiles determined were ≤1.5 % AR for both systems at all sampling points.

The amount of radioactivity in the water decreased from 0 DAT to 104 DAT from 97.2 to 7.5 % AR in system Bickenbach and from 98.9 to 2.4 % AR in system Unter-Widdersheim.

The amount of radioactivity extractable from the sediment of system Bickenbach increased from 2.0 % AR at 0 DAT to a maximum of 33.3 % AR at 30 DAT and then decreased to 16.8 % AR at 104 DAT. In the Unter-Widdersheim system, the amount of radioactivity extractable from the sediment increased from 1.8 % AR at 0 DAT to a maximum of 65.1 % AR at 30 DAT and then decreased to 43.0 % AR at 104 DAT.

The amount of non-extractable residues (NER) in system Bickenbach increased from 0.4 % AR at 0 DAT to 31.7 % AR at 62 DAT and slightly decreased to 31.3 % AR at 104 DAT. In the Unter-Widdersheim system, the amount of NER increased from 0 DAT to 104 DAT from 0.8 to 32.8 % AR. Most of the residual radioactivity (16.7 to 26.5 % AR) was found to be bound to the humin fraction in the sediments of both locations after 104 days of incubation and is not expected to be bioavailable.

The amount of AMPA in the water decreased from 0 DAT to 104 DAT from 95.2 to 3.7 % AR in system Bickenbach and from 97.1 to 0.9 % AR in system Unter-Widdersheim.

The amount of AMPA in the sediment of system Bickenbach increased from 11.1 % AR at 0.25 DAT to 31.5 % AR at 30 DAT and decreased then to 16.2 % AR at 104 DAT. The amount of AMPA in the sediment of system Unter-Widdersheim increased from 13.7 % AR at 0.25 DAT to 63.8 % AR at 30 DAT and decreased from to 40.3 % AR at 104 DAT.

The amount of AMPA in the total decreased from 0 DAT to 104 DAT from 95.2 to 19.8 % AR in system Bickenbach and from 97.1 to 41.2 % AR in system Unter-Widdersheim.

Besides carbon dioxide, two unknown compounds were detected in the total system by HPLC. Unknown 1 was detected with a maximum amount (mean value) of 8.5 % AR at 2 DAT in the Bickenbach system and 4.4 % AR at 30 DAT in the Unter-Widdersheim system. Unknown 2 was detected with a maximum amount (mean value) of 11.2 % AR at 7 DAT in the Bickenbach system and 4.4 % AR at 30 DAT in the Unter-Widdersheim system. Additional attempts to characterize the structure of the unknown by LC/MS

failed due to the presence of matrix components. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, the compounds are not considered relevant for further evaluation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification: [^{14}C]Aminomethylphosphonic acid
Lot No.: Amersham Pharmacia CFQ12959 (Item Number BE9181)
Specific activity: 55 mCi/mmol
Radiochemical purity: 98.6 % by HPLC
Chemical purity: Not provided

2. Test System:

Sediments were sieved to ≤2 mm and water was filtered to ≤0.2 mm. Water and sediment were stored separately in the dark at 4 ± 2°C for approximately one week before acclimation of the test systems was started. Aerobic conditions of the aquatic test systems were maintained during the storage period. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-116: Characteristics of test water/sediment systems

Parameter		**Results**	
System		Bickenbach	Unter-Widdersheim
Description		Brook	Brook
Location		Bickenbach, Germany	Hungen, Germany
Sampling depth for	water	Not provided	Not provided
	sediment	15-30 cm below water surface	3-15 cm below water surface
Water			
pH		8.5	8.5
Total hardness (mmol/L)		1.88	3.22
Total organic carbon (mg/L)		1.78	2.50
Total phosphorus (mg/L)		0.06	<0.06
PO_4 (mg/L)		<0.18	<0.18
Total nitrogen (mg/L)		4.3	6.05
NO_3-N (mg/L)		3.38	4.99
NO_2-N (mg/L)		<0.02	0.04

Table 7.2.2.3-116: Characteristics of test water/sediment systems

Parameter	Results	
System	Bickenbach	Unter-Widdersheim
Sediment		
Textural Class (USDA)	Not reported	Not reported
Sand (%)	94.3	35.9
Silt (%)	5.5	41.0
Clay (%)	0.2	23.1
Textural Class (DIN)	Sand	Slight sandy loam
Sand (%)	93.8	34.0
Silt (%)	6.2	42.9
Clay (%)	0.2	23.1
pH [1]	8.5	8.5
Maximum Water Holding Capacity (MWHC) (g water/(100 g))	41.4	75.4
Organic carbon (%)	0.64	2.96
Organic matter (%)	1.10	5.10
Cation exchange capacity (mval/kg)	28.7	123
$CaCO_3$ (%)	2.07	0.36
Total phosphorus (mg/kg)	459	1250
Total nitrogen (mg/kg)	400	1700
Microbial activity at 40 % MWHC (mg C/(100 g))		
After sampling	23	24
At 104 DAT	14	15

[1] Medium not stated
USDA: United States Department for Agriculture, DIN: Deutsches Institut für Normung

B. STUDY DESIGN

1. Experimental conditions

The flow-through test system consisted of six bottles connected via tubing to a vacuum system. The first bottle was a hydration flask containing reagent water. The next Woulff'sche flask containing the treated water/sediment was connected to a security bottle. The next bottle contained 50 mL 2 N NaOH with saturation indication by cresol red for the collection of CO_2. The last bottle contained 50 mL of 2-methoxy ethanol to trap volatile organics and was connected to a vacuum pump so that moist air could be pulled through all bottles. One series of metabolism flasks consisted of two replicates per sampling date.

75 g of water saturated sediment (dry weight equivalents) and 300 mL of reagent water were added to each test vessel, corresponding to a water:sediment ratio of 4:1. The oxygen concentration was at least greater than 20 % of its saturation during the experiment. The water/sediment systems were pre-incubated for 34 days at 20 ± 2 °C in the dark, until an equilibrium based on redox potential, pH-value of water and sediment and oxygen concentration of the water was reached.

The application rate was 0.958 mg AMPA/L which is equivalent to 2837 g AMPA/ha. A test solution of [^{14}C]AMPA was prepared in water and 110 µL of this solution were applied to the surface of the water phase in each test system.

Test systems were incubated under aerobic conditions in the dark for104 days at 20 ± 2 °C.

2. Sampling
Duplicate samples from each system were processed and analysed at 0, 0.25, 1, 2, 7, 14, 30, 62, and 104 days after treatment (DAT). The 2-methoxy ethanol and NaOH traps were assayed at each sampling time or at about every 14 days, whichever came first.

3. Analytical procedures
After removal of the water phase from the test system by decantation and radioactivity in the water was analysed by liquid scintillation counting (LSC). Water samples were analysed by HPLC and TLC after concentration, if needed.

Sediment samples were extracted several times with 1 M NH_3-solution for 1 hour by shaking followed by centrifugation, until the final extraction step resulted in <5 % of applied radioactivity. The sequential extractability of radioactivity of each individual extract as well as the combined extraction solutions were radioassayed by LSC. The combined extraction solutions were adjusted to pH 2 by the addition of HCl and centrifuged again. Processed specimen extracts were analysed by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Residual radioactivity in sediments was assayed by combustion/LSC.

AMPA was identified by co-chromatography with reference items. Attempts to identify unknown fraction by LC/MS failed due to the presence of matric components.

Analysed extracts were stored in tightly closed glass storage containers at ≤ - 18 °C in the dark.

The extracted sediments of the 104 DAT samplings (air-dried and ground) were subjected to further characterization of sediment radioactivity, which remained bound to the humic and fulvic acids and the humin fraction.

Aliquots of the volatile traps were directly analysed by LSC. The identification of CO_2 in the sodium hydroxide traps was determined by precipitation of $BaCO_3$ using barium chloride.

II. RESULTS AND DISCUSSION

A. DATA
The pH value of the water remained relatively constant during the study between 7.6 and 8.0 in system Bickenbach and between 7.2 and 8.2 for system Unter-Widdersheim. The pH value of the sediment remained relatively constant during the study between 7.0 and 7.2 in system Bickenbach and between 7.2 and 7.4 for system Unter-Widdersheim. The oxygen saturation of the water ranged between 39 and 51 % for system Bickenbach and between 31 and 42 % for system Unter-Widdersheim. The redox potential of the water ranged between 137 and 162 mV for system Bickenbach and between 99 and 116 mV for system Unter-Widdersheim. The redox potential of the sediment ranged between -142 and -196 mV in system Bickenbach and between -204 and -216 mV for system Unter-Widdersheim.

Radioactive mass balance and distribution of AMPA and its degradation products in water/sediment systems are summarised in Table 7.2.2.3-117 to Table 7.2.2.3-122. Fractionation of non-extractable residues into fulvic acid, humic acid, and humin fractions is presented in Table 7.2.2.3-123.

Water and sediment extracts were analysed by HPLC and TLC. In the report, the results of both methods were presented in tables. In the main text, only the results derived from HPLC analysis were discussed. Therefore, although not clearly stated, it is assumed that HPLC was considered the quantification system while the results obtained by TLC (which are very similar to HPLC results) were considered as confirmatory. Therefore, in this summary results of HPLC and TLC are presented in tables but only results determined by HPLC are discussed further.

Table 7.2.2.3-117: Distribution of radioactivity in water/sediment system Bickenbach under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	62	104
Total water	1	94.4	88.4	69.7	65.3	41.5	27.4	13.2	12.3	6.8
	2	99.9	87.4	72.0	65.5	41.4	23.3	17.7	12.4	8.2
Sediment extractable	1	2.5	11.6	23.0	28.4	32.2	32.5	33.5	24.9	18.3
	2	1.4	10.5	25.4	29.7	33.5	33.1	33.0	25.4	15.3
Non-extractable residues (NER)	1	0.4	0.9	1.9	3.2	10.6	16.4	27.4	31.8	30.0
	2	0.4	0.8	2.2	2.7	10.7	19.4	20.9	31.6	32.5
CO_2	1	n.p.	0.1	0.2	1.7	10.1	18.0	24.4	32.7	40.1
	2	n.p.	0.1	0.2	1.7	10.1	18.0	24.4	32.7	40.1
Organic volatiles	1	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	1.5	1.5
	2	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	1.5	1.5
Mass balance	1	97.3	101.0	94.8	98.6	94.4	94.3	100.0	103.2	96.7
	2	101.7	98.8	99.8	99.6	95.7	93.8	97.5	103.6	97.6

DAT: Days after treatment, n.p.: Not performed

Table 7.2.2.3-118: Distribution of radioactivity in water/sediment system Unter-Widdersheim under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	62	104
Total water	1	99.1	83.7	67.4	57.9	33.5	17.4	12.2	2.7	2.5
	2	98.9	85.5	71.0	58.4	37.0	19.4	12.0	3.0	2.2
Sediment extractable	1	1.1	14.3	30.2	40.3	55.5	65.1	64.4	60.9	39.4
	2	2.5	14.1	30.5	38.0	53.6	60.4	65.8	57.1	46.6
Non-extractable residues (NER)	1	0.5	2.9	3.2	6.3	8.0	15.5	14.6	26.9	36.0
	2	1.0	2.9	4.3	4.7	6.5	15.4	15.9	28.5	29.5
CO_2	1	n.p.	<0.1	0.1	0.3	1.6	3.4	9.3	15.9	21.2
	2	n.p.	<0.1	0.1	0.3	1.6	3.4	9.3	15.9	21.2
Organic volatiles	1	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	0.1
	2	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	0.1
Mass balance	1	100.7	100.9	100.9	104.8	98.6	101.4	100.6	106.5	99.2
	2	102.4	102.5	105.9	101.4	98.7	98.6	103.1	104.6	99.6

DAT: Days after treatment, n.p.: Not performed

Table 7.2.2.3-119: Degradation of [^{14}C]AMPA in the water of both water/sediment systems under aerobic conditions based on HPLC results (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	62	104
Bickenbach										
AMPA	1	92.6	86.2	61.5	45.5	25.8	16.6	9.4	7.0	4.7
	2	97.7	87.4	59.9	52.2	23.8	15.3	13.1	6.6	2.6
	Mean	*95.2*	*86.8*	*60.7*	*48.9*	*24.8*	*16.0*	*11.3*	*6.8*	*3.7*
Unknown 1	1	1.9	n.d.	3.1	10.0	4.6	5.9	1.7	3.1	1.9
	2	2.2	n.d.	7.0	4.2	7.6	3.3	2.0	2.7	5.7
Unknown 2	1	n.d.	2.2	3.1	7.1	11.2	5.0	2.3	2.3	0.3
	2	n.d.	n.d.	1.4	6.1	10.1	4.8	2.8	3.3	n.d.
Total Unknown	1	1.9	2.2	8.3	19.9	15.8	10.9	4.0	5.4	2.2
	2	2.2	n.d.	12.2	13.4	17.7	8.1	4.8	6.0	5.7

Table 7.2.2.3-119: Degradation of [^{14}C]AMPA in the water of both water/sediment systems under aerobic conditions based on HPLC results (expressed as percent of applied radioactivity)

Unter-Widdersheim										
AMPA	1	97.4	79.8	62.3	51.4	28.3	10.8	5.6	1.3	0.6
	2	96.8	81.2	66.1	53.1	32.4	13.5	5.9	1.0	1.2
	Mean	*97.1*	*80.5*	*64.2*	*52.3*	*30.4*	*12.2*	*5.8*	*1.2*	*0.9*
Unknown 1	1	1.7	2.2	2.3	2.9	1.9	2.1	1.9	0.8	1.8
	2	2.2	1.8	2.5	2.4	2.1	1.9	1.7	1.3	0.8
Unknown 2	1	n.d.	1.8	2.9	3.7	3.4	4.6	4.8	0.6	0.2
	2	n.d.	2.5	2.5	2.9	2.6	4.1	4.0	0.7	0.2
Total Unknown	1	1.7	4.0	5.2	6.6	5.3	6.7	6.7	1.4	2.0
	2	2.2	4.3	5.0	5.3	4.7	6.0	6.2	2.0	1.0

DAT: Days after treatment, n.d.: not detected, n.p.: Not performed
Values calculated for this summary are given in *italics*.

Table 7.2.2.3-120: Degradation of [^{14}C]AMPA in the sediment of both water/sediment systems under aerobic conditions based on HPLC results (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	62	104
Bickenbach										
AMPA	1	n.p.	11.6	23.0	26.0	30.6	30.0	31.7	23.5	18.3
	2	n.p.	10.5	25.4	28.4	30.3	31.4	31.2	22.5	14.0
	Mean	n.p.	*11.1*	*24.2*	*27.2*	*30.5*	*30.7*	*31.5*	*23.0*	*16.2*
Unknown 1	1	n.p.	n.d.	n.d.	1.7	1.6	2.5	1.8	1.4	n.d.
	2	n.p.	n.d.	n.d.	1.1	2.3	1.7	1.9	1.8	1.3
Unknown 2	1	n.p.	n.d.	n.d.	0.5	n.d.	n.d.	n.d.	n.d.	n.d.
	2	n.p.	n.d.	n.d.	n.d.	1.0	n.d.	n.d.	1.2	n.d.
Total Unknown	1	n.p.	n.d.	n.d.	2.5	1.6	2.5	1.8	1.4	n.d.
	2	n.p.	n.d.	n.d.	1.3	3.3	1.7	1.9	3.0	1.3
Unter-Widdersheim										
AMPA	1	n.p.	13.3	30.2	39.8	55.5	62.5	64.4	57.1	36.3
	2	n.p.	14.1	30.5	37.9	53.6	58.8	63.2	52.7	44.2
	Mean	n.p.	*13.7*	*30.4*	*38.9*	*54.6*	*60.7*	*63.8*	*54.9*	*40.3*
Unknown 1	1	n.p.	n.d.	n.d.	0.6	n.d.	2.6	n.d.	2.6	3.2
	2	n.p.	n.d.	n.d.	n.d.	n.d.	1.6	2.7	2.6	2.5
Unknown 2	1	n.p.	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	1.3	n.d.
	2	n.p.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.9	n.d.
Total Unknown	1	n.p.	1.1	n.d.	0.6	n.d.	2.6	n.d.	3.9	3.2
	2	n.p.	n.d.	n.d.	0.2	n.d.	1.6	2.7	4.5	2.5

DAT: Days after treatment, n.d.: Not detected, n.p.: Not performed
Values calculated for this summary are given in *italics*.

Table 7.2.2.3-121: Degradation of [^{14}C]AMPA in the total system of both water/sediment systems under aerobic conditions based on HPLC results (expressed as percent of applied radioactivity)

Compound	Replicate	DAT 0	0.25	1	2	7	14	30	62	104
Bickenbach										
AMPA	1	92.6	97.8	84.5	71.5	56.4	46.6	41.1	30.5	23.0
	2	97.7	97.9	85.3	80.6	54.1	46.7	44.3	29.1	16.6
	Mean	*95.2*	*97.9*	*84.9*	*76.1*	*55.3*	*46.7*	*44.3*	*30.5*	*19.8*
Unknown 1	1	1.9	n.d.	3.1	11.7	6.2	8.4	3.5	4.5	1.9
	2	2.2	n.d.	7.0	5.3	9.9	5.0	3.9	4.5	7.0
Unknown 2	1	n.d.	2.2	3.1	7.6	11.2	5.0	2.3	2.3	0.3
	2	n.d.	n.d.	1.4	6.1	11.1	4.8	2.8	4.5	n.d.
Total Unknown	1	1.9	2.2	8.3	22.4	17.4	13.4	5.8	6.8	2.2
	2	2.2	n.d.	12.2	14.7	21.0	9.8	6.7	9.0	7.0
Unter-Widdersheim										
AMPA	1	97.4	93.1	92.5	91.2	83.8	73.3	70.0	58.4	36.9
	2	96.8	95.3	96.6	91.0	86.0	72.3	69.1	53.7	45.4
	Mean	*97.1*	*94.2*	*94.6*	*91.1*	*84.9*	*72.8*	*69.6*	*56.1*	*41.2*
Unknown 1	1	1.7	2.2	2.3	3.5	1.9	4.7	1.9	3.4	5.0
	2	2.2	1.8	2.5	2.4	2.1	3.5	4.4	3.9	3.3
Unknown 2	1	n.d.	2.9	2.9	3.7	3.4	4.6	4.8	1.9	0.2
	2	n.d.	2.5	2.5	2.9	2.6	4.1	4.0	2.6	0.2
Total Unknown	1	1.7	5.1	5.2	7.2	5.3	9.3	6.7	5.3	5.2
	2	2.2	4.3	5.0	5.5	4.7	7.6	8.9	6.5	3.5

DAT: Days after treatment, n.d.: Not detected
Values calculated for this summary are given in *italics*.

Table 7.2.2.3-122: Degradation of [^{14}C]AMPA in water and sediment of both water/sediment systems under aerobic conditions based on TLC results (expressed as percent of applied radioactivity)

Compound	Replicate	DAT 0	0.25	1	2	7	14	30	62	104
Bickenbach										
Water AMPA	1	94.4	87.8	63.9	58.5	27.9	16.7	9.7	7.6	4.7
	2	99.9	87.4	62.5	59.5	28.6	15.4	13.8	7.6	5.8
	Mean	*97.2*	*87.6*	*63.2*	*59.0*	*28.3*	*16.1*	*11.8*	*7.6*	*5.3*
Sediment AMPA	1	n.p.	11.0	23.0	26.1	29.0	29.3	32.0	22.5	17.7
	2	n.p.	10.1	25.4	27.6	30.7	31.3	31.7	23.4	14.2
	Mean	n.p.	*11.0*	*24.2*	*26.9*	*29.9*	*30.3*	*31.9*	*23.0*	*16.0*
Unter-Widdersheim										
Water AMPA	1	99.1	80.6	60.2	52.7	28.9	12.2	7.2	1.3	1.8
	2	98.9	82.1	63.1	54.4	32.0	14.4	6.8	1.5	0.8
	Mean	*99.0*	*81.4*	*61.7*	*53.6*	*30.5*	*13.3*	*7.0*	*1.4*	*1.3*
Sediment AMPA	1	n.p.	13.7	30.2	37.9	53.7	62.7	61.3	55.2	37.0
	2	n.p.	13.2	30.5	35.9	51.5	58.3	61.8	50.7	43.2
	Mean	n.p.	*13.5*	*30.4*	*36.9*	*52.6*	*60.5*	*61.6*	*53.0*	*40.1*

DAT: Days after treatment, n.p.: Not performed
Values calculated for this summary are given in *italics*.

Table 7.2.2.3-123: Fractionation of 104 DAT post extracted sediment (in percent of AR)

Experiment	Fulvic acid	Humic acid	Humin
Bickenbach	4.4	8.5	17.1
	4.2	7.2	20.6
Unter-Widdersheim	2.9	6.2	26.5
	3.9	8.9	16.7

B. MASS BALANCE

Mass balances (single values) ranged from 93.8 to 103.6 % AR for system Bickenbach and from 98.6 to 106.5 % AR for system Unter-Widdersheim.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 104 DAT from 97.2 to 7.5 % AR in system Bickenbach and from 98.9 to 2.4 % AR in system Unter-Widdersheim.

The amount of radioactivity extractable from the sediment of system Bickenbach increased from 2.0 % AR at 0 DAT to a maximum of 33.3 % AR at 30 DAT and then decreased to 16.8 % AR at 104 DAT. In the Unter-Widdersheim system, the amount of radioactivity extractable from the sediment increased from 1.8 % AR at 0 DAT to a maximum of 65.1 % AR at 30 DAT and then decreased to 43.0 % AR at 104 DAT.

The amount of non-extractable residues (NER) in system Bickenbach increased from 0.4 % AR at 0 DAT to 31.7 % AR at 62 DAT and slightly decreased to 31.3 % AR at 104 DAT. In the Unter-Widdersheim system, the amount of NER increased from 0 DAT to 104 DAT from 0.8 to 32.8 % AR. Most of the residual radioactivity (16.7 to 26.5 % AR) was found to be bound to the humin fraction in the sediments of both locations after 104 days of incubation and is not expected to be bioavailable.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (104 DAT) were 40.1 and 21.2 % AR in the Bickenbach and Unter-Widdersheim systems respectively. Organic volatiles were ≤1.5 % AR for both systems at all sampling points.

E. TRANSFORMATION OF THE TEST ITEM

The amount of AMPA in the water decreased from 0 DAT to 104 DAT from 95.2 to 3.7 % AR in system Bickenbach and from 97.1 to 0.9 % AR in system Unter-Widdersheim.

The amount of AMPA in the sediment of system Bickenbach increased from 11.1 % AR at 0.25 DAT to 31.5 % AR at 30 DAT and decreased then to 16.2 % AR at 104 DAT. The amount of AMPA in the sediment of system Unter-Widdersheim increased from 13.7 % AR at 0.25 DAT to 63.8 % AR at 30 DAT and decreased from to 40.3 % AR at 104 DAT.

The amount of AMPA in the total decreased from 0 DAT to 104 DAT from 95.2 to 19.8 % AR in system Bickenbach and from 97.1 to 41.2 % AR in system Unter-Widdersheim.

Besides carbon dioxide, two unknown compounds were detected in the total system by HPLC. Unknown 1 was detected with a maximum amount (mean value) of 8.5 % AR at 2 DAT in the Bickenbach system and 4.4 % AR at 30 DAT in the Unter-Widdersheim system. Unknown 2 was detected with a maximum amount (mean value) of 11.2 % AR at 7 DAT in the Bickenbach system and 4.4 % AR at 30 DAT in the Unter-Widdersheim system. Additional attempts to characterize the structure of the unknown by LC/MS failed due to the presence of matrix components. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, the compounds are not considered relevant for further evaluation.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted] 2020, CA 7.2.2.3/001.

In the report document available for the evaluation, the individual results of HPLC analysis for water and sediment phase were missing. Thus, the evaluation could only be based on results of TLC analysis. The missing data led to inconsistencies in the reporting of the amounts of AMPA in sediment extracts in the text of the study report compared to tabulated results from TLC analysis. Therefore, no kinetic evaluation was performed for the sediment phase as well as the total system of both systems and only a kinetic evaluation for the water phase is included in the current submission.

A complete report document including the results of HPLC analysis was received after completion of the kinetic evaluation. The complete data may be used to update the evaluation at a later time point.

III. CONCLUSIONS

AMPA degraded rapidly in the water phases of the two German aquatic test matrices. In the processed water of Bickenbach location, two unknown metabolites reached maximum levels of more than 10 % of the applied radioactivity. Both of the metabolites were of transient character. In the Unter-Widdersheim water phases, unknown components did not exceed 5 % of the applied radioactivity. In the processed sediment, extractable radioactivity of both test matrices, unknown components reached maximum levels of below 5 %.

AMPA was converted in both compartments, but predominately in the aerobic water phases, of the two test matrices into two unidentified degradates. The degradation of AMPA was reflected by the formation of residual residues and the formation of $^{14}CO_2$.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Pre-equilibration of the test systems was 34 days and thus, slightly exceeded the 4 weeks period given by the guideline. Nevertheless, pH, oxygen content and redox potential were monitored throughout the study and thus, the validity is not affected.

Two unknown compounds were detected in the total system with a maximum amount (mean value) of 8.5 % AR at 2 DAT and 11.2 % AR at 7 DAT, respectively. Additional attempts to characterize the structure of the unknowns by LC/MS failed due to matrix effects. As indicated by the occurrence, the components showed transient character to decrease towards study end. Being a metabolite study, the components are not considered relevant for further evaluation or risk assessment.

The study is considered valid to evaluate the degradation of AMPA in water/sediment systems.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.2.3/020
Report author	[redacted]
Report year	2002
Report title	Aminomethylphosphonic acid: fate and behaviour in water-sediment
Report No	A&M01-106
Document No	
Guidelines followed in study	BBA Guideline Part IV, 5-1
Deviations from current test guideline	From OECD 308: - Acclimation period not reported - Study duration (119 days) slightly longer than recommended (100 days) - No storage time reported for water and sediment extracts until analysis
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The degradation of [^{14}C]aminomethylphosphonic acid (AMPA), a major metabolite of glyphosate, was investigated in two aquatic sediment systems under aerobic conditions in the dark in the laboratory at 20 ± 2°C for 119 days.

The following two aquatic sediment systems were used: Rückhaltebecken and Schaephysen. The amount of organic carbon of the sediments ranged from 1.3 to 4.2 % and the pH ranged from 7.35 to 7.63.

The water-sediment systems were reconstituted in 500 mL flasks with 1.5 to 2 cm height of sediment and about 6 cm height of the water phase. After addition of the test item, the water-sediment systems were equipped with volatile traps and were passively ventilated with CO_2-free air.

The test item [^{14}C]-AMPA was applied at a concentration of 0.197 mg/L water.

Duplicate test systems were processed and analysed 0, 3, 7, 14, 31, 60, 89 and 119 days after treatment (DAT) for system Rückhaltebecken and 0, 3, 5, 13, 31, 60, 90 and 119 DAT for system Schaephysen. The sterile control was processed and analysed at 122 DAT. Volatile traps were assayed at each sampling interval.

Mass balances (single replicates) ranged from 92.0 to 102.4 % of applied radioactivity (% AR) for system Rückhaltebecken and from 92.6 to 99.9 % AR for system Schaephysen.

Maximum amounts of carbon dioxide reached at study end (119 DAT) were 27.6 and 11.8 % AR or the Rückhaltebecken and Schaephysen aquatic sediment systems, respectively. Organic volatiles determined were ≤0.0 % AR for both test systemsat all sampling points.

The amount of radioactivity in the water decreased from 0 DAT to 119 DAT from 94.8 to 5.6 % AR for system Rückhaltebecken and from 84.4 to 0.4 % AR for system Schaephysen.

The amount of radioactivity extractable from the sediment increased for system Rückhaltebecken from 5.1 % AR at 0 DAT to 57.8 % AR at 14 DAT, before decreasing to 39.0 % AR at 119 DAT. The amount of radioactivity extractable from the sediment extracts increased for system Schaephysen from 11.8 % AR at 0 DAT to 72.4 % AR at 13 DAT, before decreasing to 47.4 % AR at 119 DAT.

The amount of non-extractable residues (NER) increased from 0.4 % AR at 0 DAT to 30.1 % AR at 89 DAT, before decreasing to 25.1 % AR at 119 DAT for system Rückhaltebecken. In system Schaephysen NER increased from 0 DAT to 119 DAT from 2.4 to 39.1 % AR.

The amount of AMPA in the water decreased from from 0 DAT to 119 DAT from 92.4 to 1.4 % AR for system Rückhaltebecken and from 81.8 to 0.2 % AR for system Schaephysen.

The amount of AMPA in the sediment extract increased from 0 DAT to 14 DAT from 3.4 to 34.6 % AR before decreasing to 22.5 % AR at 119 DAT for system Rückhaltebecken. The amount of AMPA in the sediment extract of system Schaephysen increased from 0 DAT to 60 DAT from 5.1 to 25.4 % AR on before decreasing to 23.3 % AR at 119 DAT.

The amount of AMPA in the total system decreased from from 0 DAT to 119 DAT from 95.8 to 23.9 % AR for system Rückhaltebecken and from 86.9 to 23.4 % AR for system Schaephysen.

Up to three different degradation products of AMPA were detected in the water/sediment systems which were assigned to M2.5 (max. 7.0 % AR), M3.3 (max. 22.9 % AR) and M7 (max. 9.8 % AR). M3.3 was found mainly in the sediments, while the M7 occurred rather in the water phases. M3.3 could be characterised as 1-oxo-AMPA; M2.5 and M7 were not identified/characterised. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, the compounds are not considered relevant for further evaluation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]AMPA
Lot No.:	CFQ13045
Specific activity:	2.11 GBq/mmol
Radiochemical purity:	98.1 % (supplier), 98.7 % (determined at test facility)
Chemical purity:	Not reported

2. Test systems:

Sediments were sieved to ≤2 mm, water was sieved to ≤0.1 mm. The aquatic systems were were taken and stored well ventilated at 2 to 8°C in the dark for 4 days after receipt. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-124: Characteristics of test sediments

Parameter	**Results**			
Sediment	Rückhaltebecken		Schaephysen	
Country	Germany		Germany	
Textural Class (DIN)	Not reported		Not reported	
Sand (63 µm – 2 mm) (%)	10.6		86.2	
Silt (2 µm – 63 µm) (%)	83.7		9.3	
Clay (< 2 µm) (%)	5.7		4.5	
pH [1]	7.64		7.34	
Total organic carbon (% dry weight) [2]	1.3 / 1.3 / 1.4		4.2 / 3.2 / 3.2	
Cation exchange capacity (µmol/g)	142.3		172.7	
Microbial biomass (mg C/100g dry weight)	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**

Table 7.2.2.3-124: Characteristics of test sediments

Post handling (before acclimatisation period)	13.9	–	20.25	–
Start of test (after acclimatisation period)	11.21	10.89	15.85	11.70
End of test (119/123 days; incubation with AMPA)	11.81	11.71	13.05	14.04
End of test (122/124 days; control)	10.16	14.4	11.60	13.93
Water				
pH at sampling	7.1		7.3	
pH at 0 DAT [1]	8.7		8.0	
Total organic carbon (mg/L) [2]	<1 / 4 / 6		<1 / 5 / 6	

DAT = Days after treatment, DIN: Deutsches Institut für Normung
[1] Determined in test systems of day 0
[2] Total organic carbon was determnined at different time points (post handling / start of test /end of test)

B. STUDY DESIGN

1. Experimental conditions

The test systems consisted of 110 g of each sediment filled into 500-mL flasks up to a height of 1.5 to 2.0 cm and 220 g of the corresponding water phase were added to a height of about 6 cm. The systems were left at 20 ± 2°C to reach a steady state in pH, redox potential, oxygen content and clearing of the water phase.

Sterile test systems were prepared by heating the water/sediment systems on two consecutive days in an autoclave for 2 h.

The absorption/ventilation device consisted of a glass tube with a gas inlet tube filled with (from inside to outside): 1 g paraffin-covered glass wool for adsorption of volatile organic compounds (moistened with 2 % paraffin-oil in hexane), 0.2 g glass wool, 10 g soda lime for absorption of carbon dioxide from the incubation mixture, 0.2 g glass wool, 4 g soda lime for absorption of atmospheric carbon dioxide and 0.2 g glass wool.
The study application rate was calculated to be 0.197 mg AMPA/L water based on a field application rate of glyphosate of 1.8 kg/ha and the assumption that glyphosate was metabolised to AMPA to an extent of 50 %. [^{14}C]AMPA application solution was prepared in water to a final concentration of 0.438 mg/mL and dripped onto the water surface of the test systems.

Test systems were incubated under aerobic conditions in the dark for up to 119 days at 20 ± 2°C.

2. Sampling

Duplicate test systems were processed and analysed 0, 3, 7, 14, 31, 60, 89 and 119 days after treatment (DAT) for system Rückhaltebecken and 0, 3, 5, 13, 31, 60, 90 and 119 DAT for system Schaephysen. The sterile control was processed and analysed at 122 DAT. Volatile traps were assayed at each sampling interval.

3. Analytical procedures

After adding 1 mL 0.1 M NaOH to the test system, the water phase was decanted from the sediment using a folded filter. After measuring the volume, the water was filled into polyethylene flasks and stored in the dark at ≤-18°C and for further analysis. The radioactivity was determined by LSC. For HPLC analysis, samples were thawed, centrifuged and an aliquot was taken.

The formation of carbon dioxide in some samples from each test system were >20 %. According to the test protocol, the water phases from these samples were analysed for water dissolved carbon dioxide. The water phases were thawed and 50 mL were used for the liberation of carbon dioxide as described for the soda lime below.

The sediment was extracted by shaking for 5 minutes with 0.1 M NaOH followed by centrifugation. The supernatant was decanted using a folded filter. The sediment was extracted a second time using 0.1 M NaOH as described above. The NaOH-extracts were combined and analysed by LSC. The NaOH-extracted

sediment was exhaustively extracted in a soxhlet apparatus with methanol for approximately 2 h. The radioactivity of the soxhlet-extract was determined by LSC. After air-drying, aliquots of the extracted sediment were combusted.

For HPLC analysis, thawed aliquots of the NaOH extracts were acidified with 32 % hydrochloric acid and centrifuged. The methanol extracts, obtained by Soxhlet extraction were evaporated and re-dissolved in water. Then, 32 % hydrochloric acid was added and an aliquot was analysed by HPLC.

The alkaline NaOH- and MeOH-soxhlet-extracts of the sediment were dark brown and contained also the fulvic acid-, humic acid- and humin-associated radioactivity. In order to avoid precipitation on the column by using an acidic eluent, these extracts had to be acidified resulting in the loss of the humic acid associated radioactivity. The supernatant which was used for HPLC analysis represented the fulvic acid-associated radioactivity. For the system Rückhaltebecken, 60 % to 84 % of the radioactivity of the NaOH-extracts and 65 % to 95 % of the MeOH-soxhlet-extract were available for HPLC analysis and for the system Schaephysen 52 % to 95 % and 47 % to 95 % of the radioactivity of the NaOH-extracts and MeOH-soxhlet-extracts. The remaining radioactivity was associated to the humic acid fraction. The water phase was analysed without acidification.

The lower limit of quantification (LLOQ) was 500 dpm/mL for radio HPLC corresponding to 0.4 %, 0.3 % and 0.8 % (mean values) of the applied radioactivity for water, 0.1 M NaOH-extracts and MeOH-soxhlet-extracts, respectively. The recovery of the radioactivity from the HPLC-column was determined to 92 %.

The carbon dioxide adsorbed to the inner soda lime compartment was liberated by hydrochloric acid and radioactivity determined by LSC. The paraffin oil-covered quartz wool was extracted with ethyl acetate, an aliquot of the extract was analysed by LSC.

At each processing time of the incubation period oxygen content of the water, pH of the water and sediment, redox potential of the water and redox potential of the sediment were determined.

[^{14}C]AMPA and metabolites were identified by HPLC-MS, flow injection MS analysis and radio HPLC of selected samples.

II. RESULTS AND DISCUSSION

A. DATA

The pH value of the water remained relatively constant during the study between 8.40 and 9.28 in system Rückhaltebecken and between 7.70 and 8.72 for system Schaephysen. The pH value of the sediment remained relatively constant during the study between 7.57 and 8.10 in system Rückhaltebecken and between 6.90 and 7.64 for system Schaephysen. The oxygen saturation of the water ranged between 90 and 96 % for system Rückhaltebecken and between 78 and 96 % for system Schaephysen. The redox potential of the water ranged between 193 and 281 mV for system Rückhaltebecken and between 243 and 316 mV for system Schaephysen. The redox potential of the sediment ranged between -78 and -322 mV in system Rückhaltebecken and between -167 and -384 mV for system Schaephysen.

Radioactive mass balance and distribution of [^{14}C]AMPA and metabolites in water/sediment systems are summarised in the tables below.

Table 7.2.2.3-125: Degradation of [14C]AMPA in the Rückhaltebecken aquatic system under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Repli-cate	DAT 0	3	7	14	31	60	89	119	Sterile 122
Water	Mean	94.8	45.6	30.4	23.9	16.9	12.1	8.3	5.6	4.7
Sediment extractables	Mean	*5.1*	*46.6*	*55.5*	*57.8*	*54.1*	*46.2*	*36.7*	*39.0*	*61.5*
Non-extractable residues	Mean	0.4	5.2	10.4	13.2	17.2	19.7	30.1	25.1	21.9
Carbon dioxide [1]	Mean	n.t.	0.2	1.0	3.1	8.7	17.9	22.1	27.6	7.6
Volatile compounds	Mean	n.t.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Recovery	1	100.2	97.3	97.2	97.6	96.0	95.0	97.7	102.4	96.8
	2	100.4	97.9	97.4	98.2	97.8	96.7	96.6	92.0	94.6
	Mean	100.3	97.6	97.3	97.9	96.9	95.9	97.2	97.2	95.7
Distribution in water										
AMPA	1	93.36	39.27	24.96	19.17	7.36	4.04	0.88	2.06	2.40
	2	91.39	46.05	25.39	11.94	8.37	3.27	3.18	0.75	2.34
	Mean	92.4	42.7	25.2	15.6	7.9	3.7	2.0	1.4	2.4
M 2.5	Mean	1.0	1.1	0.6	0.7	0.7	0.6	1.0	0.6	1.5
M 3.3	Mean	0.5	–	–	–	–	–	–	–	0.2
M 7	Mean	– [3]	0.9	3.4	7.2	8.0	7.6	5.3	3.7	0.6
Non classified radioactivity [2]	Mean	0.9	0.9	1.2	0.4	0.3	0.6	0.5	–	–
Distribution in sediment (sum of sodium hydroxide and Soxhlet extract)										
AMPA	1	2.85	28.82	34.46	32.73	32.04	26.62	27.91	17.80	40.72
	2	3.91	26.90	34.19	36.47	29.05	27.94	21.21	27.10	45.91
	Mean	3.4	27.9	34.3	34.6	30.5	27.3	24.6	22.5	43.3
M 2.5	Mean	–	3.2	3.9	4.3	4.1	3.3	2.2	1.5	4.4
M 3.3	Mean	0.6	7.0	7.4	8.8	7.4	5.5	3.2	2.8	5.9
M 7	Mean	–	0.3		0.6	1.8	0.6	1.2	0.4	1.8
Non classified radioactivity [2]	Mean	1.1	8.4	9.9	9.7	10.3	9.5	5.5	11.6	7.0
Total system (water + sediment)										
AMPA	1	96.21	68.08	59.42	51.90	39.40	30.67	28.78	19.86	43.12
	2	95.30	72.94	59.59	48.41	37.43	31.21	24.38	27.86	48.25
	Mean	95.8	70.5	59.5	50.2	38.4	30.9	26.6	23.9	45.7
M 2.5	Mean	1.0	4.2	4.6	5.1	4.8	3.6	2.7	1.8	5.9
M 3.3	Mean	1.1	7.0	7.4	8.8	7.4	5.5	3.2	2.8	6.0
M 7	Mean		1.1	3.4	7.5	9.8	8.2	6.5	4.1	1.5
Non classified radioactivity [2]	Mean	2.0	9.4	11.1	10.1	10.6	10.1	6.0	6.2	2.5

[1] The formation of carbon dioxide in the sterile controls may be caused by the use of non-sterilised water of the application solution
[2] Non-classified radioactivity in water = non-classified radioactivity from the HPLC-analysis; non-classified radioactivity in sediment = sum of the non-classified radioactivity from the HPLC-analysis and the humic acid associated radioactivity which was not available for HPLC-analysis; non-classified radioactivity in total system = sum of the non-classified radioactivity from the HPLC-analysis of water and sediment extracts and the humic acid associated radioactivity of sediment extracts which was not available for HPLC-analysis.
[3] –: Value <LLOQ, not detected or not tested
DAT: days after treatment, n.t.: not tested
Mean values were calculated from two replicates. Values calculated during dossier preparation are given in *italics*.

Table 7.2.2.3-126: Degradation of [14C]AMPA in the Schaephysen aquatic system under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT 0	3	5	13	31	60	90	119	122 Sterile
Water	Mean	84.4	24.4	16.5	5.4	1.3	1.8	1.3	0.4	1.2
Sediment extractables	Mean	*11.8*	*55.7*	*63.3*	*72.4*	*61.1*	*56.9*	*53.0*	*47.4*	*41.8*
Non-extractable residues	Mean	2.4	13.7	16.0	18.8	27.3	30.5	32.8	39.1	37.7
Carbon dioxide [1]	Mean	n.t. [4]	0.1	0.5	2.5	6.1	9.3	10.6	11.8	16.8
Volatile compounds	Mean	n.t.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Recovery	1	97.4	93.8	96.2	99.3	98.8	99.4	98.3	99.9	98.5
	2	99.7	94.0	96.4	99.0	92.6	95.7	97.1	97.7	96.5
	Mean	98.5	93.9	96.3	99.1	95.7	97.5	97.7	98.8	97.5
Distribution in water										
AMPA	1	82.97	18.91	15.80	3.69	0.82	Not detected	<LLOQ	0.24	0.61
	2	80.53	24.94	13.95	3.46	0.30	0.88	0.36	<LLOQ	0.85
	Mean	81.8	21.9	14.9	3.6	0.6	0.9	0.4	0.2	0.7
M 2.5	Mean	0.8	0.7	0.5	–	–	–	–	–	–
M 3.3	Mean	– [3]	0.6	–	–	–	–	–	–	–
M 7	Mean	0.9	1.0	1.6	1.5	0.5	1.9	1.3	0.2	0.3
Non classified radioactivity [2]	Mean	1.4	0.5	0.4	0.4	0.3	0.5	0.5	–	–
Distribution in sediment (sum of sodium hydroxide and Soxhlet extract)										
AMPA	1	3.76	19.58	19.48	21.47	19.04	27.28	20.54	22.94	25.90
	2	6.51	19.73	19.43	23.49	18.35	23.48	19.18	23.55	22.13
	Mean	5.1	19.7	19.5	22.5	18.7	25.4	19.9	23.2	24.0
M 2.5	Mean	1.2	6.2	5.9	7.0	5.7	4.4	4.4	3.5	4.1
M 3.3	Mean	0.5	13.7	19.3	22.9	20.8	11.8	13.9	8.5	6.1
M 7	Mean	0.6	0.6	0.7	–	1.2	0.6	0.4	0.5	0.5
Non classified radioactivity [2]	Mean	4.4	15.6	17.9	20.0	14.7	14.1	14.7	11.7	7.3
Total system (water + sediment)										
AMPA	1	86.73	38.49	35.28	25.16	19.87	27.28	20.54	23.18	26.51
	2	87.04	44.67	33.38	26.95	18.65	24.36	19.54	23.55	22.97
	Mean	86.9	41.6	34.3	26.1	19.3	25.8	20.0	23.4	24.7
M 2.5	Mean	2.0	6.9	6.4	7.0	5.7	4.4	4.4	3.5	4.1
M 3.3	Mean	0.5	14.0	19.3	22.9	20.8	11.8	13.9	8.5	6.1
M 7	Mean	1.0	1.5	1.5	1.5	1.7	1.2	1.8	0.6	0.6
Non classified radioactivity [2]	Mean	5.8	16.1	18.3	20.4	15.0	14.5	15.1	11.5	6.3

[1] The formation of carbon dioxide in the sterile controls may be caused by the use of non-sterilised water of the application solution
[2] Non-classified radioactivity in water = non-classified radioactivity from the HPLC-analysis; non-classified radioactivity in sediment = sum of the non-classified radioactivity from the HPLC-analysis and the humic acid associated radioactivity which was not available for HPLC-analysis; non-classified radioactivity in total system = sum of the non-classified radioactivity from the HPLC-analysis of water and sediment extracts and the humic acid associated radioactivity of sediment extracts which was not available for HPLC-analysis
[3] –: Value <LLOQ, not detected or not tested
DAT: Days after treatment, n.t.: not tested
Mean values were calculated from two replicates. Values calculated during dossier preparation are given in *italics*.

B. MASS BALANCE

Mass balances (single replicates) ranged from 92.0 to 102.4 % of applied radioactivity (% AR) for system Rückhaltebecken and from 92.6 to 99.9 % AR for system Schaephysen.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

The amount of radioactivity in the water decreased from 0 DAT to 119 DAT from 94.8 to 5.6 % AR for system Rückhaltebecken and from 84.4 to 0.4 % AR for system Schaephysen.

The amount of radioactivity extractable from the sediment (sum of NaOH and Soxhlet extracts) increased for system Rückhaltebecken from 5.1 % AR at 0 DAT to 57.8 % AR at 14 DAT, before decreasing to 39.0 % AR at 119 DAT. The amount of radioactivity extractable from the sediment extracts increased for system Schaephysen from 11.8 % AR at 0 DAT to 72.4 % AR at 13 DAT, before decreasing to 47.4 % AR at 119 DAT.

The amount of non-extractable residues (NER) increased from 0.4 % AR at 0 DAT to 30.1 % AR at 89 DAT, before decreasing to 25.1 % AR at 119 DAT for system Rückhaltebecken. In system Schaephysen NER increased from 0 DAT to 119 DAT from 2.4 to 39.1 % AR.

D. VOLATILE RADIOACTIVITY

Maximum amounts of carbon dioxide reached at study end (119 DAT) were 27.6 and 11.8 % AR or the Rückhaltebecken and Schaephysen aquatic sediment systems, respectively. Organic volatiles determined were ≤0.0 % AR for both test systemsat all sampling points.

E. TRANSFORMATION OF THE TEST ITEM

The amount of AMPA in the water decreased from from 0 DAT to 119 DAT from 92.4 to 1.4 % AR for system Rückhaltebecken and from 81.8 to 0.2 % AR for system Schaephysen.

The amount of AMPA in the sediment extract increased from 0 DAT to 14 DAT from 3.4 to 34.6 % AR before decreasing to 22.5 % AR at 119 DAT for system Rückhaltebecken. The amount of AMPA in the sediment extract of system Schaephysen increased from 0 DAT to 60 DAT from 5.1 to 25.4 % AR on before decreasing to 23.3 % AR at 119 DAT.

The amount of AMPA in the total system decreased from from 0 DAT to 119 DAT from 95.8 to 23.9 % AR for system Rückhaltebecken and from 86.9 to 23.4 % AR for system Schaephysen.

Up to three different degradation products of AMPA were detected in the water/sediment systems which were assigned to M2.5 (max. 7.0 % AR), M3.3 (max. 22.9 % AR) and M7 (max. 9.8 % AR). M3.3 was found mainly in the sediments, while the M7 occurred rather in the water phases. M3.3 could be characterised as 1-oxo-AMPA; M2.5 and M7 were not identified/characterised. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, the compounds are not considered relevant for further evaluation.

The non-classified radioactivity in water is equal to the non-classified radioactivity from the HPLC-analysis and does not exceed 1.4 % AR at any sampling interval for both test systems. The non-classified radioactivity in sediment is reported as the sum of the non-classified radioactivity from the HPLC-analysis and the humic acid associated radioactivity which was removed from the NaOH and Soxhlet extracts by acidification prior to HPLC-analysis and reached a maximum of 20 % AR (13 DAT, system Schaephysen). As the HPLC method was able to separate compounds to <5 % AR as shown for the water samples, the majority of the non-classified radioactivity was associated to the humic acid fraction. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, no further attempts were made to identify this unclassified radioactivity.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted] 2020, CA 7.2.2.3/001.

Assessment and conclusion by applicant:
The study is conducted consistent with the current guideline, showing minor deviations. Sediments were filled into the test vessels to a height of 1.5 to 2.0 cm, being slightly below the actual requirement of 2.0 ± 0.5 cm. Since the water/sediment volume ratio was within the requirement of 3.1 and 4:1, variations regarding the height of the sediment layer are considered acceptable.

The duration of acclimation prior to application of the test item is not provided. The study duration of 119 days is slightly longer than the recommended duration of 100 days. The deviations are considered not to influence the overall outcome of the study.

The study is considered valid to evaluate the degradation of AMPA in water/sediment systems.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.2.2.3/021
Report author	[redacted]
Report year	1999
Report title	Aminomethylphosphonic acid: Water/Sediment Metabolism
Report No	MSL-19217
Document No	
Guidelines followed in study	SETAC Guideline "Procedures of assessing the environmental fate and ecotoxicity of pesticides", part 1, 8.2
Deviations from current test guideline	From OECD 308: - Sediment history not given - Sediment sampling from top 15 cm instead of top 5-10 cm - CO_2-free air used
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The degradation of [^{14}C]aminomethylphosphonic acid (AMPA) was investigated in two water/sediment systems under aerobic conditions in the dark in the laboratory at 20 ± 2°C for 100 days.

The following two water/sediment systems were used: a sandy sediment from Bickenbach and a silty-sandy loam sediment from Unter Widdersheim. The amount of organic carbon of the sediments ranged from 0.52 to 3.83 % and the pH ranged from 7.4 to 7.5.

The test was performed in flow-through systems connected to two 2 N NaOH traps to collect carbon dioxide and two methoxy ethanol traps to collect volatile organic compounds.

AMPA was applied to the water surface at a rate of 470 µg/L corresponding to a rate of 1.42 kg/ha to represent a worst-case concentration based on the maximum field rate of 4.32 kg glyphosate acid/ha and a maximum formation from glyphosate of 50 %.
Duplicate samples from each system were processed and analysed at 0, 0.25, 1, 2, 7, 14, 30, 59 and 100 days after treatment (DAT).

The mean recoveries of applied radioactivity (AR) were 103.7 % (97.4 to 106.5 % AR) for the Bickenbach system and 102.1 % (97.7 to 105 % AR) for the Unter Widdersheim system.

Significant mineralisation was observed with volatile radioactivity (identified as CO_2) representing 38.0 % AR (Bickenbach) and 29.1 % AR (Unter Widdersheim) at 100 DAT. Organic volatiles determined were ≤0.3 % AR for both systems at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

In water at 20°C, the level of applied radioactivity declined very rapidly from 101.2 % at 0 DAT, 40.0 % at 14 DAT and to 10.2 % at 100 DAT in the Bickenbach water and from 100.4 % to 19.4 % and to 2.9 % in Unter Widdersheim water at the same time points.

This decline was associated with an increasing concentration in sediment extracts to 49.0 % AR (Bickenbach) and to 67.0 % AR (Unter Widdersheim) by 100 DAT.

Non-extractable sediment residues represented 19.1 % (Bickenbach) and 24.9 % (Unter Widdersheim) of applied radioactivity at 100 DAT. When the non-extractable radioactivity at 100 DAT was further fractionated into humin, humic and fulvic acid fractions, the residual radioactivity was mainly associated with the humin fraction.

The amounts of AMPA in the water (mean of both TLC systems) decreased from 0 DAT to 100 DAT from 101.2 to 4.1 % AR in system Bickenbach and from 100.4 to 1.2 % AR in system Unter Widdersheim.

The amounts of AMPA in the sediment of system Bickenbach (mean of both TLC systems) increased from 2.0 % AR at 0 DAT to 40.8 % AR at 59 DAT and decreased to 20.5 % AR at 100 DAT. The amounts of AMPA in the sediment of system Unter Widdersheim (mean of both TLC systems) increased from 1.4 % AR at 0 DAT to 46.2 % AR at 14 DAT and decreased to 30.8 % AR at 100 DAT.

The amounts of AMPA in the total system (mean of both TLC systems) decreased from 0 DAT to 100 DAT from 102.2 to 24.5 % AR in system Bickenbach and from 101.2 to 31.4 % AR in system Unter Widdersheim.

For both test systems, the unidentified radioactivity in the water phase remained below 10 % AR at all time points for both TLC systems. For the sediment extracts, the TLC system with the best separation ("SS2") showed radioactive zones containing up to 12 % AR. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, no attempts were made to identify these breakdown products.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	[^{14}C]aminomethylphosphonic acid (AMPA)
Lot No.:	C-2266.4
Specific activity:	4.8 mCi/mmol (43.23 µCi/mg)
Radiochemical purity:	≥99 % (checked by HPLC and TLC during study conduct)
Chemical purity:	Not provided

The study was conducted with a mixture of ^{13}C- and ^{14}C-labelled AMPA, diluted with analytical grade ^{12}C-AMPA.

2. Test System:
Sediment was sampled from 1 to 15 cm below the water/sediment surface. Sediments were sieved to ≤2 mm and water was filtered to ≤0.2 mm. Water and sediment were stored separately in the dark at 4 ± 2°C for approximately one week before acclimation of the test systems was started. Aerobic conditions of the aquatic test systems were maintained during the storage period. Characteristics of the test systems are presented in the table below.

Table 7.2.2.3-127: Characteristics of test systems

Parameter		Results	
Sediment		Bickenbach	Unter Widdersheim
Country		Germany	Germany
Textural Class		Sand	Silty-sandy loam
Sand (63 µm – 2 mm) (%)		99.3	38.5
Silt (2 µm – 63 µm) (%)		3.7	45.7
Clay (< 2 µm) (%)		0.6	17.5
pH		7.4	7.5
Organic carbon (%)		0.52	3.83
Organic matter (%)		0.90	6.60
Cation exchange capacity (mval/kg)		16.1	137
Maximum Water Holding Capacity (g/100 g)		17.0	69.5
Microbial biomass (mg C/100g)			
Within the course of the study		21	27
Study end (100 DAT)		10	11
Water			
pH	At sampling: After sampling: At experimental end:	8.1 8.3 7.9	8.4 8.2 7.6
Redox-potential (mV)	At sampling: After sampling: At experimental end:	452 564 495	409 602 450
Oxygen level (mg/L)	At sampling: After sampling: At experimental end:	9.7 - 10.1	9.0 - 7.7

DAT = Days after treatment

B. STUDY DESIGN

1. Experimental conditions
The metabolism flasks were filled with a 2.5 cm thick sediment layer (approximately 250 g and 215 g water saturated sediment of systems Bickenbach or Unter Widdersheim, respectively) and the corresponding water at a water column height of about 6 cm, corresponding to approximately 300 mL of water. Flow-through systems, purged with moistened, CO_2 free air were used. To maintain aerobic conditions during the experiment, the oxygen concentration of water was above 20 % of its saturation. The test systems were connected to a security bottle, two gas washing bottles filled with 50 mL of 2 N NaOH (with saturation indication by cresol red) to absorb CO_2 from sediment respiration and $^{14}CO_2$ from the mineralisation of the test substance and two gas washing bottles filled with methoxy ethanol to collect volatile organic compounds. The sodium hydroxide trapping system was checked visually for CO_2 saturation (non-saturated: crimson/ saturated: yellow) on a weekly basis, in general. At no time did the indicator show CO_2 saturation.

Test systems were pre-incubated at 20 °C in the dark for 28 days until an equilibrium based on redox potential of water and sediment, oxygen concentration and pH-value of the water was reached.

AMPA was applied to the water surface at a rate of 470 µg/L corresponding to a rate of 1.42 kg/ha to represent a worst-case concentration based on the maximum field rate of 4.32 kg glyphosate acid/ha and a maximum formation from glyphosate of 50 %.

After application, test systems were incubated under aerobic conditions in the dark with gentle agitation of the water phase for 100 days at 20 ± 2°C.

2. Sampling
Duplicate samples from each system were processed and analysed at 0, 0.25, 1, 2, 7, 14, 30, 59 and 100 days after treatment (DAT). The volatile traps were assayed at each sampling interval to determine the amount of carbon dioxide and volatile organic compounds. For analysis, the sediment and water from each metabolism flask were separated by decantation. Thereafter, water and sediment were analysed separately. Samples were prepared, extracted and analysed immediately after sampling.

3. Analytical procedures
Surface water was separated from the sediment by decantation and directly analysed by liquid scintillation counting (LSC).

Sediments were extracted with 1 M NH_3 up to 6 times (laboratory shaker: 350 rpm/min for 12 h maximum at room temperature). The ratio of the extraction solvent and sediment was 1:1 (volume:dry weight, corresponding to 200 mL 1M NH_3 for system Bickenbach and 130 mL 1M NH_3 for system Unter Widdersheim) maximum. Before the addition of fresh solvent the slurry was centrifuged (up to 4500 rpm/10 min) and the supernatant decanted. The sequential extractability of radioactivity was checked by analysis of each individual sediment extract using LSC.

Radioactive components in water and sediment extracts were analysed by two TLC/radiodetection systems with a limit of detection 0.3 % AR. Recoveries for the analytical procedure were in the range from 94.2 to 103.7 % AR for both systems.

After sediment extraction, the remaining bound residues were assayed by combustion/LSC. In addition, extracted sediment of 100 DAT was further characterized for radioactivity bound to the humic and fulvic acids and the humin fraction.

Aliquots from the volatile traps were radioassayed at each sampling point (excluding zero-time) or approximately in 14-day intervals, whichever came first. The traps were assayed by adding aliquots of the trapping solutions directly into the liquid scintillation cocktail and counting by LSC. For the sodium hydroxide traps, the identification of $^{14}CO_2$ (trapping solution containing ≥2 % AR) was performed by precipitation of $Ba^{14}CO_3$, using a saturated aqueous solution of barium chloride.

II. RESULTS AND DISCUSSION

A. DATA
The pH value of the water remained relatively constant during the study between 7.9 and 8.3 in system Bickenbach and between 7.6 and 8.2 for system Unter Widdersheim. The redox potential of the water at study end was 495 mV for system Bickenbach and 450 mV for system Unter Widdersheim. The redox potential of the sediment at study end was -175 mV in system Bickenbach and -233 mV for system Unter Widdersheim.

Radioactive mass balance and distribution of [^{14}C]AMPA and its degradation products in water/sediment systems are summarised in Table 7.2.2.3-128 to Table 7.2.2.3-137. Fractionation of non-extractable residues into fulvic acid, humic acid and humin fractions is presented in Table 7.2.2.3-138.

The results of analysis with two TLC solvent systems were found to be very similar at each sampling interval. Therefore, further discussion refers to average values of the two TLC solvent systems.

Table 7.2.2.3-128: Distribution of radioactivity in water/sediment system Bickenbach under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Water total	A	101.8	94.7	86.1	73.3	54.7	39.2	27.1	13.2	8.4
	B	100.5	93.8	80.5	78.5	57.6	40.8	34.1	14.6	12.0
	Mean	101.2	94.3	83.3	75.9	56.2	40.0	30.6	13.9	10.2
Sediment extractables	A	1.5	8.3	15.9	24.5	39.7	46.1	46.8	51.5	30.4
	B	2.5	8.4	19.8	21.1	39.4	50.0	46.8	50.1	29.4
	Mean	2.0	8.4	17.9	22.8	39.6	48.1	46.8	50.8	29.9
Non-extractable residues	A	0.3	1.1	3.0	4.7	7.4	10.4	20.6	18.9	21.8
	B	0.2	1.6	4.0	3.8	6.6	11.6	16.8	17.9	16.3
	Mean	0.3	1.4	3.5	4.3	7.0	11.0	18.7	18.4	19.1
Sediment total	Mean	2.3	9.8	21.4	27.1	46.6	59.1	65.5	69.2	49.0
Carbon Dioxide	A	n.p.	<0.1	<0.1	0.1	1.5	8.2	12.9	20.8	36.9
	B	n.p.	<0.1	<0.1	0.2	1.4	4.5	7.9	20.4	39.1
	Mean	n.p.	<0.1	<0.1	0.2	1.5	6.4	10.4	20.6	38.0
Other Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.3
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
Mass balance	Mean	103.5	104.1	104.7	103.2	104.3	105.5	106.5	103.7	97.4

DAT: Days after treatment
n.p.: Not performed

Table 7.2.2.3-129: Distribution of radioactivity in water/sediment system Unter Widdersheim under aerobic conditions (expressed as percent of applied radioactivity)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Water total	A	100.7	86.4	57.5	66.7	39.0	30.1	2.3	3.1	3.5
	B	100.1	83.5	49.7	65.1	27.2	8.6	4.3	6.5	2.3
	Mean	100.4	85.0	53.6	65.9	33.1	19.4	3.3	4.8	2.9
Sediment extractables	A	1.3	14.4	40.2	30.9	51.3	54.6	57.9	46.1	38.2
	B	1.5	16.5	49.2	29.9	59.7	74.3	51.4	41.0	45.9
	Mean	1.4	15.5	44.7	30.4	55.5	64.5	54.7	43.6	42.1
Non-extractable residues	A	0.2	2.6	5.5	6.5	10.2	16.4	24.8	23.0	23.8
	B	0.5	3.3	6.0	7.0	12.5	13.8	22.6	24.7	25.9
	Mean	0.4	3.0	5.8	6.8	11.4	15.1	23.7	23.9	24.9
Sediment total	Mean	1.8	18.5	50.5	37.2	66.9	79.6	78.4	67.5	67.0
Carbon Dioxide	A	n.p.	<0.1	<0.1	0.3	2.7	4.1	15.4	23.4	32.6
	B	n.p.	<0.1	<0.1	0.2	4.6	7.8	20.9	27.3	25.5
	Mean	n.p.	<0.1	<0.1	0.3	3.7	6.0	18.2	25.4	29.1
Other Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mass balance	Mean	102.2	103.5	104.1	103.4	103.7	105.0	99.9	97.7	99.0

DAT: Days after treatment
n.p.: Not performed

Table 7.2.2.3-130: Degradation of [^{14}C]AMPA in water of test system Bickenbach quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	101.8	90.5	86.1	68.4	54.7	32.0	18.5	9.1	6.1
	B	100.5	89.1	80.5	72.9	57.6	35.5	26.9	10.6	3.6
	Mean	101.2	89.8	83.3	70.7	56.2	33.8	22.7	9.9	4.9
About zero	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	n.d.	0.3
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7
	Mean	-	-	-	-	-	0.2	-		0.5
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.0	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5	n.d.	n.d.
	Mean	-	-	-	-	-	-	1.8	-	-
About 0.9	A	n.d.	4.2	n.d.	5.0	n.d.	6.8	6.6	4.1	2.0
	B	n.d.	4.8	n.d.	5.7	n.d.	5.3	5.8	4.0	7.7
	Mean	-	4.5	-	5.4	-	6.1	6.2	4.1	4.9
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	101.8	92.7	83.0	67.9	50.8	31.2	16.9	5.8	3.6
	B	100.5	91.3	78.2	74.3	46.3	34.4	25.0	8.1	3.0
	Mean	101.2	92.0	80.6	71.1	48.6	32.8	21.0	7.0	3.3
About zero	A	n.d.	2.0	3.2	5.4	2.2	3.6	4.3	2.1	2.4
	B	n.d.	2.6	2.3	4.2	4.4	4.4	4.6	2.4	1.7
	Mean	-	2.3	2.8	4.8	3.3	4.0	4.5	2.3	2.1
About 0.2	A	n.d.	n.d.	n.d.	n.d.	1.7	4.4	5.9	5.3	2.5
	B	n.d.	n.d.	n.d.	n.d.	6.9	1.9	4.5	4.1	7.3
	Mean	-	-	-	-	4.3	3.2	5.2	4.7	4.9

DAT: Days after treatment
n.d.: Not detectable (calculated detection limit of 0.3 % AR)

Table 7.2.2.3-131: Degradation of [^{14}C]AMPA in sediment extracts of test system Bickenbach quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	1.5	8.3	15.9	21.2	30.6	33.2	34.3	42.0	21.3
	B	2.5	8.4	19.8	17.6	30.9	38.2	35.7	42.0	20.7
	Mean	2.0	8.4	17.9	19.4	30.8	35.7	35.0	42.0	21.0
About zero	A	n.d.	n.d.	n.d.	n.d.	2.3	2.0	3.8	n.d.	1.7
	B	n.d.	n.d.	n.d.	0.7	1.8	1.6	3.4	n.d.	1.9
	Mean	-	-	-	0.4	2.1	1.8	3.6	-	1.8
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.8	n.d.	1.9
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.3	n.d.	0.9
	Mean	-	-	-	-	-	-	2.5	-	1.4
About 0.9	A	n.d.	n.d.	n.d.	3.3	6.8	10.9	6.1	9.6	5.5
	B	n.d.	n.d.	n.d.	2.9	6.7	10.4	5.7	8.1	6.0
	Mean	-	-	-	3.1	6.8	10.7	5.9	8.9	5.8
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	n.a.	8.3	15.4	23.4	31.8	36.5	32.3	39.9	23.0
	B	n.a.	8.3	19.0	18.7	32.0	39.6	35.2	39.2	16.6
	Mean	-	8.3	17.2	21.1	31.9	38.1	33.8	39.6	19.8
About zero	A	n.a.	n.d.	0.5	1.2	4.2	6.2	6.2	6.8	7.4
	B	n.a.	0.1[1]	0.8	2.4	3.7	6.8	8.3	6.9	5.9
	Mean	-	0.1	0.7	1.8	4.0	6.5	7.3	6.9	6.7
About 0.2	A	n.a.	n.d.	n.d.	n.d.	3.7	3.5	3.8	4.8	n.d.
	B	n.a.	n.d.	n.d.	n.d.	3.8	3.6	3.3	4.1	6.9
	Mean	-	-	-	-	3.8	3.6	3.6	4.5	3.5

DAT: Days after treatment
n.d.: Not detectable (calculated detection limit of 0.3 % AR)
n.a.: Not analysed (below 5 % AR in the extract to be analysed)
[1] Reduced detection limit of 0.1 % AR

Table 7.2.2.3-132: Degradation of [^{14}C]AMPA in the total system of test system Bickenbach quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	103.3	98.8	102.0	89.6	85.3	65.2	52.8	51.1	27.4
	B	103.0	97.5	100.3	90.5	88.5	73.7	62.6	52.6	24.3
	Mean	103.2	98.2	101.2	90.1	86.9	69.5	57.7	51.9	25.9
About zero	A	n.d.	n.d.	n.d.	n.d.	2.3	2.4	3.8	n.d.	2.0
	B	n.d.	n.d.	n.d.	0.7	1.8	1.6	3.4	n.d.	2.6
	Mean	-	-	-	0.4	2.1	2.0	3.6	-	2.3
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.8	n.d.	1.9
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.7	n.d.	0.9
	Mean	-	-	-	-	-	-	4.3	-	1.4
About 0.9	A	n.d.	4.2	n.d.	8.3	6.8	17.7	12.7	13.7	7.5
	B	n.d.	4.8	n.d.	8.6	6.7	15.7	11.5	12.1	13.7
	Mean	-	4.5	-	8.5	6.8	16.7	12.1	12.9	10.6
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	101.8	101.0	98.4	91.3	82.6	67.7	49.2	45.7	26.6
	B	100.5	99.6	97.2	93.0	78.3	74.0	60.2	47.3	19.6
	Mean	101.2	100.3	97.8	92.2	80.5	70.9	54.7	46.5	23.1

Table 7.2.2.3-132: Degradation of [^{14}C]AMPA in the total system of test system Bickenbach quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

About zero	A	n.d.	2.0	3.7	6.6	6.4	9.8	10.5	8.9	9.8
	B	n.d.	2.7	3.1	6.6	8.1	11.2	12.9	9.3	7.6
	Mean	-	2.4	3.4	6.6	7.3	10.5	11.7	9.1	8.7
About 0.2	A	n.d.	n.d.	n.d.	n.d.	5.4	7.9	9.7	10.1	2.5
	B	n.d.	n.d.	n.d.	n.d.	10.7	5.5	7.8	8.2	14..2
	Mean	-	-	-	-	8.1	6.7	8.8	9.2	8.4

DAT: Days after treatment
n.d.: Not detectable (calculated detection limit of 0.3 % AR)
n.a.: Not analysed (below 5 % AR in the extract to be analysed)
[1] Reduced detection limit of 0.1 % AR

Table 7.2.2.3-133: Amounts of [^{14}C]AMPA in water, sediment extracts and total system of test system Bickenbach (mean of both TLC systems, expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Water	A	*101.8*	*91.6*	*84.6*	*68.2*	*52.8*	*31.6*	*17.7*	*7.5*	*4.9*
	B	*100.5*	*90.2*	*79.4*	*73.6*	*52.0*	*35.0*	*26.0*	*9.4*	*3.3*
	Mean	*101.2*	*90.9*	*82.0*	*70.9*	*52.4*	*33.3*	*21.9*	*8.5*	*4.1*
Sediment	A	*1.5*	*8.3*	*15.7*	*22.3*	*31.2*	*34.9*	*33.3*	*41.0*	*22.2*
	B	*2.5*	*8.4*	*19.4*	*18.2*	*31.5*	*38.9*	*35.5*	*40.6*	*18.7*
	Mean	*2.0*	*8.4*	*17.6*	*20.3*	*31.4*	*36.9*	*34.4*	*40.8*	*20.5*
Total system	A	*102.6*	*99.9*	*100.2*	*90.5*	*84.0*	*66.5*	*51.0*	*48.4*	*27.0*
	B	*101.8*	*98.6*	*98.8*	*91.8*	*83.4*	*73.9*	*61.4*	*50.0*	*22.0*
	Mean	*102.2*	*99.25*	*99.5*	*91.15*	*83.7*	*70.2*	*56.2*	*49.2*	*24.5*

DAT: Days after treatment
Values calculated during dossier preparation are given in *italics*

Table 7.2.2.3-134: Degradation of [^{14}C]AMPA in water of test system Unter Widdersheim quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	100.7	81.6	53.8	58.4	33.0	20.5	n.a.	n.a.	n.a.
	B	100.1	79.1	49.7	58.8	27.2	6.1	n.a.	2.6	n.a.
	Mean	100.4	80.4	51.8	58.6	30.1	13.3	-	1.3	-
About zero	A	n.d.	n.d.	3.7	1.0	n.d.	n.d.	n.a.	n.a.	n.a.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.a.
	Mean	-	-	1.9	0.5	-	-	-	-	-
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.a.	n.d.	n.a.
	Mean	-	-	-	-	-	0.2	-	-	-
About 0.9	A	n.d.	4.8	n.d.	7.3	6.1	9.6	n.a.	n.a.	n.a.
	B	n.d.	4.5	n.d.	6.3	n.d.	2.2	n.a.	3.9	n.a.
	Mean	-	4.7	-	6.8	3.1	5.9	-	2.0	-
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	100.7	86.4	52.9	58.5	35.1	20.2	n.a.	n.a.	n.a.
	B	100.1	83.5	47.0	57.5	24.4	6.0	n.a.	2.0	n.a.
	Mean	100.4	85.0	50.0	58.0	29.8	13.1	-	1.0	-

Table 7.2.2.3-134: Degradation of [^{14}C]AMPA in water of test system Unter Widdersheim quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

About zero	A	n.d.	n.d.	2.2	8.3	2.5	4.6	n.a.	n.a.	n.a.
	B	n.d.	n.d.	2.7	7.7	1.7	1.2	n.a.	1.8	n.a.
	Mean	-	-	2.5	8.0	2.1	2.9	-	0.9	
About 0.2	A	n.d.	n.d.	2.5	n.d.	1.4	5.3	n.a.	n.a.	n.a.
	B	n.d.	n.d.	n.d.	n.d.	1.1	1.4	n.a.	2.6	n.a.
	Mean	-	-	1.3	-	1.3	3.4	-	1.3	-

DAT: Days after treatment
n.d.: Not detectable (calculated detection limit of 0.3 % AR)
n.a.: Not analysed (below 5 % AR in the extract to be analysed)

Table 7.2.2.3-135: Degradation of [^{14}C]AMPA in sediment extracts of test system Unter Widdersheim quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

		DAT								
Compound	**Replicate**	**0**	**0.25**	**1**	**2**	**7**	**14**	**30**	**59**	**100**
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	1.3	14.4	40.2	24.1	38.5	39.1	40.0	33.6	28.3
	B	1.5	16.5	49.2	24.8	46.4	53.5	33.5	30.7	35.8
	Mean	1.4	15.5	44.7	24.5	42.5	46.3	36.8	32.2	32.1
About zero	A	n.d.	n.d.	n.d.	1.5	1.8	2.5	7.5	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	1.9	3.5	7.6	n.d.	n.d.
	Mean	-	-	-	0.8	1.9	3.0	7.6	-	-
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
About 0.8	A	n.d.	n.d.	n.d.	n.d.	5.1	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	6.5	n.d.	n.d.	n.d.	n.d.
	Mean	-	-	-	-	5.8	-	-	-	-
About 0.9	A	n.d.	n.d.	n.d.	5.3	5.9	13.0	10.5	12.5	10.0
	B	n.d.	n.d.	n.d.	5.1	5.0	17.3	10.4	10.4	10.1
	Mean	-	-	-	5.2	5.5	15.2	10.5	11.5	10.1
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	n.a.	14.0	38.8	30.2	40.0	37.2	38.2	34.2	24.9
	B	n.a.	15.9	47.0	29.1	44.5	54.7	33.6	28.7	34.1
	Mean	-	15.0	42.9	29.7	42.3	46.0	35.9	31.5	29.5
About zero	A	n.a.	0.4	1.4	0.7	6.1	9.0	13.4	6.7	8.7
	B	n.a.	0.6	2.2	0.9	6.3	7.7	11.4	5.9	8.4
	Mean	-	0.5	1.8	0.8	6.2	8.4	12.4	6.3	8.6
About 0.2	A	n.a.	n.d.	n.d.	n.d.	5.3	8.5	6.3	5.3	4.7
	B	n.a.	n.d.	n.d.	n.d.	9.0	12.0	6.4	6.5	3.4
	Mean	-	-	-	-	7.2	10.3	6.4	5.9	4.1

DAT: Days after treatment
n.d.: Not detectable (calculated detection limit of 0.3 % AR)
n.a.: Not analysed (below 5 % AR in the extract to be analysed)
[1] Reduced detection limit of 0.1 % AR

Table 7.2.2.3-136: Degradation of [^{14}C]AMPA in the total system of test system Unter Widdersheim quantified by two different TLC systems "SSl" and "SS2" (expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Rf-value "SS1"										
AMPA (Parent) about 0.8	A	102.0	96.0	94.0	82.5	71.5	59.6	40.0	33.6	28.3
	B	101.6	95.6	98.9	83.6	73.6	59.6	33.5	33.3	35.8
	Mean	101.8	95.8	96.5	83.1	72.6	59.6	36.8	33.5	32.1
About zero	A	n.d.	n.d.	3.7	2.5	1.8	2.5	7.5	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	1.9	3.5	7.6	n.d.	n.d.
	Mean	-	-	1.9	1.3	1.9	3.0	7.6	-	-
About 0.4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	n.d.	n.d.
	Mean	-	-	-	-	-	0.2	-	-	-
About 0.8	A	n.d.	n.d.	n.d.	n.d.	5.1	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	6.5	n.d.	n.d.	n.d.	n.d.
	Mean	-	-	-	-	5.8	-	-	-	-
About 0.9	A	n.d.	4.8	n.d.	12.6	12.0	22.6	10.5	12.5	10.0
	B	n.d.	4.5	n.d.	11.4	5.0	19.5	10.4	14.3	10.1
	Mean	-	4.7	-	12.0	8.5	21.1	10.5	13.4	10.1
Rf-value "SS2"										
AMPA (Parent) about 0.3	A	100.7	100.4	91.7	88.7	75.1	57.4	38.2	34.2	24.9
	B	100.1	99.4	94.0	86.6	68.9	60.7	33.6	30.7	34.1
	Mean	100.4	99.9	92.9	87.7	72.0	59.1	35.9	32.5	29.5
About zero	A	n.d.	0.4	3.6	9.0	8.6	13.6	13.4	6.7	8.7
	B	n.d.	0.6	4.9	8.6	8.0	8.9	11.4	7.7	8.4
	Mean	-	0.5	4.3	8.8	8.3	11.3	12.4	7.2	8.6
About 0.2	A	n.d.	n.d.	2.5	n.d.	6.7	13.8	6.3	5.3	4.7
	B	n.d.	n.d.	n.d.	n.d.	10.1	13.4	6.4	9.1	3.4
	Mean	-	-	-	-	8.4	13.6	6.4	7.2	4.1

DAT: Days after treatment
n.d.: Not detectable (calculated detection limit of 0.3 % AR)

Table 7.2.2.3-137: Amounts of [^{14}C]AMPA in water, sediment extracts and total system of test system Unter Widderheim (mean of both TLC systems, expressed in % AR)

Compound	Replicate	DAT								
		0	0.25	1	2	7	14	30	59	100
Water	A	*100.7*	*84.0*	*53.4*	*58.5*	*34.1*	*20.4*	*n.a.*	*n.a.*	*1.2*
	B	*100.1*	*81.3*	*48.4*	*58.2*	*25.8*	*6.1*	*n.a.*	*2.3*	*n.a.*
	Mean	*100.4*	*82.65*	*50.9*	*58.35*	*29.95*	*13.25*	*n.a.*	*2.3*	*1.2*
Sediment	A	*1.3*	*14.2*	*39.5*	*27.2*	*39.3*	*38.2*	*39.1*	*33.9*	*26.6*
	B	*1.5*	*16.2*	*48.1*	*27.0*	*45.5*	*54.1*	*33.6*	*29.7*	*35.0*
	Mean	*1.4*	*15.2*	*43.8*	*27.1*	*42.4*	*46.2*	*36.4*	*31.8*	*30.8*
Total system	A	*101.4*	*98.2*	*92.9*	*85.6*	*73.3*	*58.5*	*39.1*	*33.9*	*27.8*
	B	*100.9*	*97.5*	*96.5*	*85.1*	*71.3*	*60.2*	*33.6*	*32.0*	*35.0*
	Mean	*101.2*	*97.9*	*94.7*	*85.35*	*72.3*	*59.4*	*36.4*	*33.0*	*31.4*

DAT: Days after treatment
n.a.: Not analysed (below 5 % AR in the extract to be analysed)
*Values calculated during dossier preparation are given in *italics*

Table 7.2.2.3-138: Fractionation of day 100 post extracted sediment (in % AR)

	Bickenbach Rep 1	Bickenbach Rep 2	Unter Widdersheim Rep 1	Unter Widdersheim Rep 2
Combustion (residual radioactivity)	21.8	16.3	23.8	25.9
Pool [1]	8.5	6.9	7.7	8.5
fulvic acids	7.1	5.8	4.3	5.3
humic acids	0.7	0.5	3.0	2.5
∑ fulvic acids and humic acids	7.8	6.3	7.3	7.8
Humin [2]	13.3	9.4	16.1	17.4
∑ fulvic acids, humic acids and humin in % of residual radioactivity [3]	96.8	96.3	98.3	97.3

[1] Previous measurement of summed % AR of fulvic acids and humic acids (pool)
[2] Calculated: % AR combustion - % AR pool
[3] ((fulvic acids + humic acids + humin [in % AR])/residual radioactivity [% AR]) x 100 %

B. MASS BALANCE

The mean recoveries of applied radioactivity (AR) were 103.7 % (97.4 to 106.5 % AR) for the Bickenbach system and 102.1 % (97.7 to 105 % AR) for the Unter Widdersheim system.

C. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

In water at 20°C, the level of applied radioactivity declined very rapidly from 101.2 % at 0 DAT, 40.0 % at 14 DAT and to 10.2 % at 100 DAT in the Bickenbach water and from 100.4 % to 19.4 % and to 2.9 % in Unter Widdersheim water at the same time points.

This decline was associated with an increasing concentration in sediment extracts to 49.0 % AR (Bickenbach) and to 67.0 % AR (Unter Widdersheim) by 100 DAT.

Non-extractable sediment residues represented 19.1 % (Bickenbach) and 24.9 % (Unter Widdersheim) of applied radioactivity at 100 DAT. When the non extractable radioactivity at 100 DAT was further fractionated into humin, humic and fulvic acid fractions, the residual radioactivity was mainly associated with the humin fraction, accounting for, 13.3 % AR (Bickenbach) and 17.4 % AR (Unter Widdersheim). Radioactivity associated with the fulvic acid fraction amounted to 7.1 % AR (Bickenbach) and 5.3 % AR (Unter Widdersheim), and with the humic acid fraction it amounted to 0.7 % AR (Bickenbach) and 3.0 % AR (Unter Widdersheim).

D. VOLATILE RADIOACTIVITY

Significant mineralisation was observed with volatile radioactivity (identified as CO_2) representing 38.0 % AR (Bickenbach) and 29.1 % AR (Unter Widdersheim) at 100 DAT. Organic volatiles determined were ≤0.3 % AR for both systems at all sampling points. The barium precipitation test confirmed the identity of volatiles as carbon dioxide.

E. TRANSFORMATION OF THE TEST ITEM

The two TLC systems (SS1 and SS2) separated the water samples and sediment extracts into AMPA and either three (SS1) or two (SS2) radioactive metabolite zones. The AMPA results for the two TLC systems were in good agreement, indicating that AMPA was well separated in both systems. There was not a simple correlation between the radioactivity in the metabolite zones in the two systems, suggesting that there were at least four compounds present, some of which co-eluted.

The amounts of AMPA in the water (mean of both TLC systems) decreased from 0 DAT to 100 DAT from 101.2 to 4.1 % AR in system Bickenbach and from 100.4 to 1.2 % AR in system Unter Widdersheim.

The amounts of AMPA in the sediment of system Bickenbach (mean of both TLC systems) increased from 2.0 % AR at 0 DAT to 40.8 % AR at 59 DAT and decreased to 20.5 % AR at 100 DAT. The amounts of AMPA in the sediment of system Unter Widdersheim (mean of both TLC systems) increased from 1.4 % AR at 0 DAT to 46.2 % AR at 14 DAT and decreased to 30.8 % AR at 100 DAT.

The amounts of AMPA in the total system (mean of both TLC systems) decreased from 0 DAT to 100 DAT from 102.2 to 24.5 % AR in system Bickenbach and from 101.2 to 31.4 % AR in system Unter Widdersheim.

For both test systems, the unidentified radioactivity in the water phase remained below 10 % AR at all time points for both TLC systems. For the sediment extracts, the TLC system with the best separation ("SS2") showed radioactive zones containing up to 12 % AR. Since the test item was the metabolite AMPA and not the parent compound, glyphosate, no attempts were made to identify these breakdown products.

F. KINETICS

Degradation kinetics were updated according to latest guidance documents and can be found in [redacted] 2020, CA 7.2.2.3/001.

III. CONCLUSIONS

The major route of dissipation of AMPA is through partitioning to the sediment. Once in the sediment, AMPA degrades through degradation to metabolites, mineralisation, and formation of non-extractable residues.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is conducted consistent with the current guideline, showing minor deviations. Samples were collected from top 15 cm instead of recommended 5-10 cm. Furthermore, the air used for purging the systems was CO_2 free. The deviations are considered to not influence the overall outcome of the study.

The study is considered valid to evaluate the degradation of AMPA in water/sediment systems.

Assessment and conclusion by RMS:

Storage stability study

1. Information on the study

Data point:	CA 7.2.2.3/022
Report author	[redacted]
Report year	1989
Report title	Storage Stability of Glyphosate in Environmental Water
Report No	MSL-8626
Document No	R.D. No. 1005
Guidelines followed in study	Guideline 171-4
Deviations from current test guideline	No guideline available
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary
The storage stability of glyphosate and aminomethylphosphonic acid (AMPA) was investigated in environmental water under conditions of frozen storage for 24 months in maximum.

Environmental water samples were fortified with both glyphosate and AMPA at 0.5 ppm and stored at <-18 °C in plastic bottles. Duplicate samples were analysed after 0, 186, 313, 368, 551, and 734 days (0, 6, 10, 12, 18, and 24 months).

Average glyphosate residues, corrected for recovery in fortified control samples, ranged from 95.8 to 110.7 %. Average AMPA residues, corrected for recovery in fortified control samples, ranged from 96.2 to 108.8 %. These results confirm that both glyphosate and AMPA residues are stable in environmental water after two years in frozen storage.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Test item 1:
Identification: Glyphosate (batch no and purity not reported?)

Test item 2:
Identification: Aminomethylphosphonic acid (AMPA, batch no and purity not reported?)

2. Water:
Water samples were collected from a lake in the Busch Wildlife Area, St. Charles County, Missouri, USA. Samples were filtered through glass wool or filter paper and kept in refrigerated storage until fortification.

B. STUDY DESIGN

1. Experimental conditions
Water samples in Nalgene plastic bottles were fortified with both glyphosate and AMPA at 0.5 ppm and kept in frozen storage at <-18 °C until analysis.

2. Sampling
Duplicate samples were removed from frozen storage after 0, 186, 313, 368, 551, and 734 days (0, 6, 10, 12, 18, and 24 months). Stability and control samples were also analysed after 96 days (3 months) of frozen storage but as no fortified samples were measured, no results were reported.

3. Analytical procedures
To each sample 5 mL of concentrated hydrochloric acid were added and the solvent was evaporated to dryness under reduced pressure. The remainder is reconstituted in 2.9 mL of 5 mM KH_2PO_4 in 4 % methanol/deionized water at pH 2.1. 0.1 mL of 0.03 M disodium EDTA solution were added and the sample was filtered through a 0.45 µm filter. Samples were separated by HPLC using a cation-exchange column. Fluorescence detection was performed after a post-column reaction. Therefore, a calcium hypochlorite solution was introduced into the stream to oxidize glyphosate to a primary amine prior to fluorogenic derivatisation with o-phthaladehyde (OPA). OPA also reacts with AMPA and the two derivatised compounds were quantitated via a fluorometer at 455 nm after excitation at 340 nm.

II. RESULTS AND DISCUSSION

Recoveries for the fortified samples ranged from 81.5 % to 100.9 % for glyphosate and from 73.3 % to 96.1 % for AMPA. The average glyphosate residues, corrected for recoveries in fortified control samples, ranged from 95.8 to 110.7 %. Average AMPA residues, corrected for recovery in fortified control samples, ranged from 96.2 to 108.8 %. Detailed values can be found in the table below.

Table 7.2.2.3-139: Storage stability of glyphosate and AMPA at <-18 °C (mean values of two replicates)

Compound	Corrected recovery (%)					
	Days in storage					
	0	186	313	368	551	734
Glyphosate	*97.1*	101.1	95.8	110.5	101.9	110.7
AMPA	*99.2*	106.1	96.2	110.2	108.1	108.8

Values calculated during dossier preparation are given in *italics*

III. CONCLUSIONS

The data indicate that both glyphosate and AMPA residues are stable in environmental water after two years in frozen storage. At all analysis points, average glyphosate and AMPA residues were greater than 95 % of their original levels.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study confirmed glyphosate and AMPA to be stable in natural water for a period of 24 months.

The study is considered as supportive information.

Assessment and conclusion by RMS:

Relevant articles from literature search

1. Information on the study

Data point:	CA 7.2.2.3/023
Report author	Wang, S. *et al.*
Report year	2016
Report title	(Bio)degradation of glyphosate in water-sediment microcosms - A stable isotope co-labeling approach
Document No	DOI 10.1016/j.watres.2016.04.041 E-ISSN 1879-2448
Guidelines followed in study	OECD guideline 308
Deviations from current test guideline	From OECD 308_ - Exremely high application rate - Water/sediment systems may have received inputs of glyphosate or AMPA within the previous 4 years
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (water/sediment systems may have received inputs of glyphosate or AMPA within the previous 4 years; extremely high application rate)

2. Full summary

Glyphosate and its metabolite aminomethylphosphonic acid (AMPA) are frequently detected in water and sediments. Up to date, there are no comprehensive studies on the fate of glyphosate in water sediment microcosms according to OECD 308 guideline. Stable isotope co-labeled $^{13}C_3{}^{15}N$-glyphosate was used to determine the turnover mass balance, formation of metabolites, and formation of residues over a period of 80 days. In the water-sediment system, 56 % of the initial $^{13}C_3$-glyphosate equivalents was ultimately mineralised, whereas the mineralisation in the water system (without sediment) was low, reaching only 2 % of ^{13}C-glyphosate equivalents. This finding demonstrates the key role of sediments in its degradation. Glyphosate was detected below detection limit in the water compartment on day 40, but could still be detected in the sediments, ultimately reaching 5 % of $^{13}C_3{}^{15}N$-glyphosate equivalents. A rapid increase in $^{13}C_3{}^{15}N$-AMPA was noted after 10 days, and these transformation products ultimately constituted 26 % of the $^{13}C_3$-glyphosate equivalents and 79 % of the ^{15}N-glyphosate equivalents. In total, 10 % of the ^{13}C label and 12 % of the ^{15}N label were incorporated into amino acids, indicating no risk bearing biogenic residue formation from $^{13}C_3{}^{15}N$-glyphosate. Initially, glyphosate was biodegraded via the sarcosine pathway related to microbial growth, as shown by co-labeled $^{13}C_3{}^{15}N$-glycine and biogenic residue formation. Later, degradation via AMPA dominated under starvation conditions, as shown by the contents of ^{13}C-glycine. The presented data provide the first evidence of the speciation of the non-extractable residues as well as the utilization of glyphosate as a carbon and nitrogen source in the water-sediment system. This study also highlights the contribution of both the sarcosine and the AMPA degradation pathways under these conditions.

Materials and Methods

Chemicals
All the chemicals used were analytical or reagent grade and were obtained from the Carl Roth Company (Karlsruhe, Germany) if not specified otherwise. Resin for amino acid purification (Dowex 50W-X8, 50-100 mesh) was purchased from VWR/Merck (Darmstadt, Germany). Methanol and ammonium acetate for ultraperformance liquid chromatography-mass spectrometry (UPLC/MS) measurements were provided by Biosolve (Valkenswaard, Netherlands). Labeled $^{13}C_3{}^{15}N$-glyphosate was purchased from IsoSciences Company (Trevose, PA, USA). The isotopical enrichment of glyphosate was 99 % for ^{13}C and 98 % for ^{15}N; the chemical purity was 98 %.

Sediments and water
The sediments and associated water were collected from the Getel creek, Harz Mountains in Saxony-Anhalt, Germany. The catchment of this creek comprises agricultural lowlands with continuous crop rotation and pesticide application. It is thus a high risk area for exposure to pesticides. The sediments contained 38 % (±0.7 %) sand (>0.05 mm), 62 % (±0.7 %) silt + clay (<0.05 mm), 85 mg/g (±2 mg/g) total organic carbon and 15 mg/g (±1 mg/g) total nitrogen. The pH of the sediments and creek water was 7.1 and 8.8, respectively. The content of total organic carbon of the suspended matter in the creek water was 8 mg/L (±1 mg/L), and the content of total nitrogen was 3 mg/L (±0.6 mg/L). Neither glyphosate nor AMPA were detected in the sediments or creek water. Sediments and associated water were taken from the upper layer (up to 5 cm) of the Getel creek sediment. The sediments were separated from the water by filtration, wet sieved and gently homogenized.

Incubation experiment
Degradation experiments were conducted according to the OECD guideline 308 in biometer flasks to address the transformation in aquatic sediment systems. Six incubations were performed: 1) water-sediment without glyphosate (non-amended control), 2) water-sediment with unlabeled glyphosate (unlabeled control), 3) water-sediment with labeled glyphosate (biotic system), 4) water with unlabeled glyphosate (unlabeled control), 5) water with labeled glyphosate and 6) sterilized water sediment with labeled glyphosate (abiotic system). The two controls without glyphosate and unlabeled glyphosate were used to correct for the natural abundances of ^{13}C (~1.1 at %) and ^{15}N (~0.37 at %) in the sediment, and water systems without sediment were prepared to test the effect of sediment on the microbial degradation of glyphosate. Abiotic controls were incubated to distinguish between abiotic and biotic degradation of $^{13}C_3{}^{15}N$-glyphosate. In these controls, sediment and water were sterilized by autoclaving three times at 120°C for 20 min prior to incubation.

50 g (dw) of sediment and 90 mL of creek water containing either unlabeled or labeled glyphosate were added to glass bottles. The initial concentration of glyphosate was 50 mg/L in water and water-sediment systems, except in the blanks containing no glyphosate. This concentration is well above environmentally relevant levels, but it was required to obtain reliable isotopic enrichment results in the water-sediment systems given the limited sensitivity of $^{13}C/^{15}N$ isotope analytical methods and the high background due to natural abundance of the heavy isotopes in the controls. To assess the overall fate and turnover at lower concentrations that were closer to environmentally relevant concentrations, additional water-sediment experiments at 3 mg/L (minimum ^{13}C and ^{15}N label detection limit) were prepared. Incubation experiments were conducted in the dark and at constant temperature (20°C) for 80 days. The bottles were sampled after 0, 5, 10, 20, 40 and 80 days (abiotic, blank, water and 3 mg/L systems only after 80 days). At each sampling time, the respective systems were destructively sampled, and the water and sediments were separated by filtration and subjected to further analyses. The CO_2 evolved from the mineralised glyphosate was trapped in 2 M NaOH; the NaOH solution was exchanged at regular intervals. Because the pH of the water was >7.0, a certain amount of CO_2 originating from the mineralisation of glyphosate may partition into the water phase, which therefore has been analyzed in addition to the NaOH traps. Mineralisation in biotic and abiotic systems includes $^{13}CO_2$ in both the sodium hydroxide and water phases.

Chemical analyses
A general mass balance of the ^{13}C and ^{15}N labels in the systems was set up based on the contents and isotopic compositions of CO_2, the extractable glyphosate and its metabolites and either ^{13}C or ^{15}N in the

total NER. Proteins were hydrolyzed, and the amino acids (AA) were extracted and analyzed for their concentration and isotopic composition to estimate the extent that C and N from $^{13}C_3{}^{15}N$-glyphosate were incorporated into microbial biomass and ultimately into biogenic residues. Proteins are the main constituents of microbial biomass (50 % of cells); therefore, the quantification of biogenic residues formation was based on a factor of 2 for both ^{13}C and ^{15}N-amino acids (AA).

CO_2 measurements
The ^{13}C labeled CO_2 was quantified by measuring the total inorganic C in a 2 M NaOH solution on a total organic carbon analyzer. The isotopic composition of CO_2 (at % ^{13}C) was measured by GC-combustion-isotope ratio mass spectrometry (GC-C-irMS; Finnigan MAT252 Thermo Electron coupled to a Hewlett Packard 6890 GC) with a Porabond Q-HT Plot FS column (50 m - 0.32 m - 5 µm).

Extractable glyphosate and AMPA
Glyphosate and AMPA were extracted with borate buffer (40 mM, pH 9.2) from sediments and derivatized with 0.5 mL of fluorenylmethyloxycarbonyl (Fmoc). The water samples were directly derivatized with Fmoc in borate buffer. The concentrations of glyphosate and AMPA were determined by UPLC-MS i-Class system (Waters, Manchester, UK) with an Acquity UPLC HSS T3 column (1.7 µm, 2.1 x 100 mm; Waters, Milford, MA, USA). The temperatures of the column and the autosampler were set at 60°C and 4°C, respectively. The injection volume was 10 µL. The eluents were 5 mM NH_4 acetate (pH 8) in water (eluent A) and methanol (eluent B). The flow rate was set to 0.6 mL/min. The gradient program was as follows: 0-3 min 5 % B, 7-8 min 95 % B, 8.1-10 min 5 % B. The MS analysis was performed using a Xevo TQ-S mass spectrometer (Waters, Manchester, UK) equipped with an ESI source in negative ion mode working in multiple reaction monitoring (MRM) mode. A capillary voltage of 2 kV and a desolvation temperature of 600°C were used. The flow of the desolvation gas was set at 1000 L/h. Unlabeled glyphosate and AMPA were used for calibration and as internal standards for correction of possible matrix effects which may occur during the measurement of glyphosate and AMPA concentrations. Transitions, cone voltages, and collision energies were automatically tuned for the compounds: $^{13}C_3{}^{15}N$-glyphosate (m/z 172 / m/z 154, cone: 58 V, collision energy: 10 V; m/z 172 / m/z 63, cone: 58 V, collision energy: 16 V) and $^{13}C_3{}^{15}N$-AMPA (m/z 112 / m/z 63, cone: 58 V, collision energy: 16 V; m/z 112 / m/z 79, cone: 58 V, collision energy: 10 V). The detection limit (LOD) of glyphosate was determined at 20 µg/L, and the LOD for AMPA was 30 µg/L based on the signal-to-noise method (signal >3 S/N). For the entire procedure, including the extraction of the sediment samples, the detection limits were 0.608 mg/kg (glyphosate) and 0.912 mg/kg (AMPA). The recovery of glyphosate and AMPA was >98 %. The values of the coefficient of determination (R^2) for all calibration curves were greater than 0.99. The relative error of UPLC-MS measurements was <10 %.

Non-extractable residues (NER)
After the extraction of glyphosate and AMPA, the sediment sample containing unextracted ^{13}C and ^{15}N label as NER was air-dried. An aliquot of 4-5 mg was weighed and combusted using an elemental analyzer-combustion-isotope ratio mass spectrometer combination (EA-C-irMS; Euro EA 3000, Eurovector, Milano, Italy + Finnigan MAT 253, Thermo Electron, Bremen, Germany). Glyphosate-derived C and N were calculated as the excess ^{13}C and ^{15}N over the controls. The values of the coefficient of determination (R^2) for all calibration curves were greater than 0.99.

Amino acids (AA)
Amino acids were analyzed in the living microbial biomass AA fraction of sediment (bioAA) and in the total AA pool of the sediment fraction (tAA). Microbial biomass was extracted from the sediment with ion exchanger and sodium deoxycholate/polyethylenglycol solution. The sediment and microbial biomass pellets containing accordingly tAA and bioAA were hydrolyzed using 6 M HCl. Thereafter, the hydrolysate was purified over a cation exchange resin. The detailed extraction, purification and derivatization methods for bioAA and tAA were described previously. The identity and quantity of AA were measured using GC-MS, HP 6890 with a BPX-5 column. The isotopic composition of the respective AA (at % ^{13}C and at % ^{15}N) was determined by GC-C-irMS, Finnigan MAT 253 coupled to a Trace GC, with a BPX-5 column. The details on the analytical conditions for AA separation by GC-MS and GC-C-irMS are reported in Nowak *et al.* (2013). For quantification and identification of respective AA in samples, an external standard containing all detectable AA in the samples (alanine, glycine, threonine, valine, leucine, isoleucine, proline,

aspartate, glutamate, phenylalanine and lysine) was used. The internal standard L-norleucine was added to each sample before hydrolysis to estimate the losses in AA analyses. The recovery of all measured AA was >90 %, except from threonine (>80 %). The measured isotopic compositions were corrected for shifts due to derivatization.

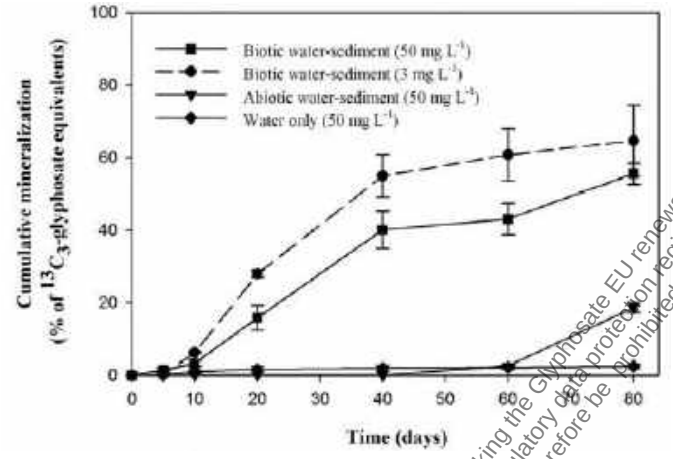
Data analyses and mass balance
All incubation experiments and chemical analyses were conducted in triplicate, and the data are presented as averages of three replicates. Mineralisation, extractable and non-extractable $^{13}C_3$ ^{15}N-glyphosate residues were quantified for each sampling date in order to set up the full carbon and nitrogen mass balance, and to determine the compound degradation kinetics. The contents of the $^{13}CO_2$, ^{13}C and ^{15}N-NER, ^{13}C and ^{15}N-AA (bioAA + tAA) were based on quantitation of the total concentration of the respective carbon or nitrogen pool and on analyzing the excess of ^{13}C (^{15}N) over the controls (non-amended without glyphosate and unlabeled containing unlabeled glyphosate) as described by Lerch *et al.* (2009). The results were expressed as a percentage of ^{13}C or ^{15}N label relative to the initial $^{13}C_3$-glyphosate equivalents or ^{15}N-glyphosate equivalents. The total uncertainty of the carbon pool in CO_2 and of the carbon and nitrogen pools in NER was <10 %, whereas the total uncertainty of the determination of at % ^{13}C and at % ^{13}N isotope signatures was <0.5 % for unlabeled samples, but <3 % for the labeled ones. The relative average error of the label excess (based on Gaussian error propagation) was <10 % for CO_2 and NER.

The total uncertainty of carbon and nitrogen pool in tAA and bioAA was <15 %. The total uncertainty on the determination of at % ^{13}C and at % ^{15}N isotope analysis was <0.5 % for unlabeled samples, but <1 % for labeled ones. The relative average error of the label excess (based on Gaussian error propagation) was <10 % for tAA and bioAA.

The recovery of the ^{13}C and ^{15}N labels expressed as a percentage of the initially applied isotope label equivalents ranged from 93 to 110 % for C and from 86 to 110 % for N. Incorporation of the ^{13}C and ^{15}N labels into the microbial biomass and thus the total content of biogenic residues formed during degradation of $^{13}C_3{}^{15}N$-glyphosate in the water-sediment system were estimated from ^{13}C -tAA and ^{15}N-tAA, considering that AA constituted approximately 50 % of the total C and total N in the biomass. The recovery of microbial biomass extraction is estimated at 40 %. The bioAA results are presented both as the original data and the recalculated values based on 40 % extraction efficiency, but interpretation of bioAA was based on the original data.

Results and Discussion
Mineralisation of $^{13}C_3$-glyphosate
Mineralisation of $^{13}C_3$-glyphosate in the biotic water-sediment system consisted of three periods (Figure 7.2.2.3-7): an initial short lag-phase from day 0 to day 10 characterized by low mineralisation rates (0.3 %/day), day 10-40 characterized by the highest mineralisation rate (1.2 %/day), and day 40-80 characterized by decreasing mineralisation rates to 0.4 %/day. At the end of incubation, a total of 56 % of the $^{13}C_3$-glyphosate had been mineralised. Abiotic processes played a minor role in the mineralisation of $^{13}C_3$-glyphosate (<20 %). The mineralisation rates of $^{13}C_3$-glyphosate in the water system (without sediment) were very low and increased slowly during the first ten days (~0.1 %/day). Thereafter, the mineralisation rate decreased and only 2 % of $^{13}C_3$-glyphosate equivalents were mineralised at the end, demonstrating the key role of sediments in the mineralisation of $^{13}C_3$-glyphosate. Mineralisation of $^{13}C_3$-glyphosate at 3 mg/L was slightly higher (65 % of $^{13}C_3$-glyphosate equivalents) than at 50 mg/L. The acclimation period at 50 mg/L was longer (10 days vs. 5 days for 3 mg/L). The mineralisation rate in the initial phase (0 – 10 days) was two-fold higher at 3 mg/L (0.6 %/day) than at 50 mg/L (0.3 %/day) and 1.3-fold higher in the second phase (10 – 40 days; 1.6 %/day compared to 1.2 %/day, respectively). In the third phase (40 – 80 days), the mineralisation rate was 2-fold lower at 3 mg/L (0.2 %/day) than at 50 mg/L (0.4 %/day).To date, there are no reports on the mineralisation of $^{13}C_3$-glyphosate in water-sediment systems. Although glyphosate was below the detection limit in the sediment and associated water used in the present experiments, prior exposure to this herbicide is very likely due to input from the agricultural area in the catchment, with major biodegradation occurring in the sediment phase. In contrast to the high $^{13}CO_2$ evolution from $^{13}C_3$-glyphosate equivalents, no or minimal mineralisation of ^{15}N-glyphosate was found in the present study because the total recovery of the ^{15}N label ranged from 86 to 110 %.

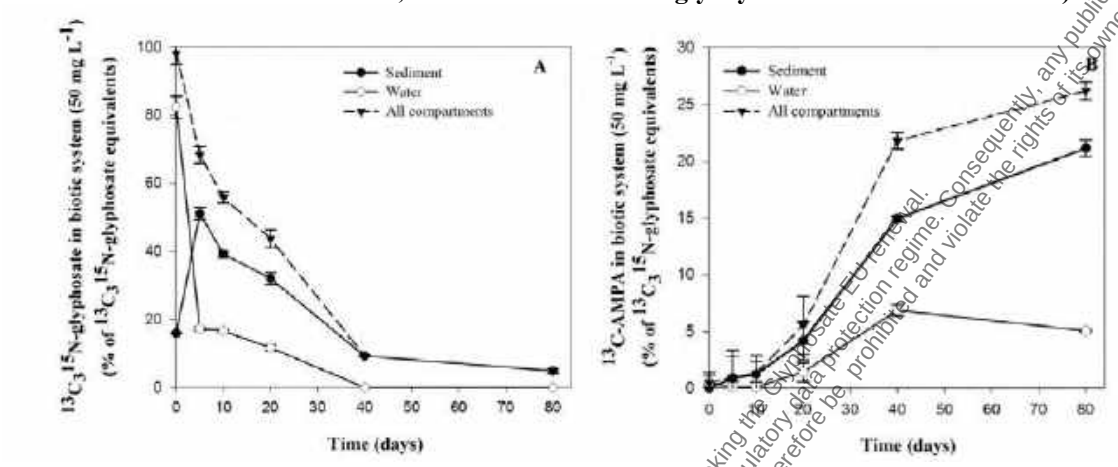
Figure 7.2.2.3-7: Cumulative mineralisation of $^{13}C_3$-glyphosate in water-sediment and water only systems over 80 days given as percentages of applied $^{13}C_3$-glyphosate equivalents



Turnover of $^{13}C_3{}^{15}N$-glyphosate

The content of $^{13}C_3{}^{15}N$-glyphosate in the biotic 50 mg/L water sediment system decreased rapidly until day 40 (Figure 7.2.2.3-8), indicating its low persistence reflected in its half-life (DT_{50}) of 15 days. In the water compartment, glyphosate dissipated rapidly during the first five days. Thereafter, elimination of this herbicide continued slowly until its ultimate removal by day 40. From day 40 onwards, $^{13}C_3{}^{15}N$-glyphosate was detected only in the sediment compartment although it was initially spiked in the water phase. This indicates that elimination from the water compartment was a combined process of sorption onto sediments and microbial transformation. A quick partitioning of glyphosate from the water compartment to the sediments had already been observed on day 0. At the initial sampling, which was performed 3 h after the addition of the glyphosate-spiked water to allow for particle sedimentation, 16 % of the initially added $^{13}C_3{}^{15}N$-glyphosate was already detected in the sediment phase. The high abundance of the silt + clay fraction (62 %), which is typically rich in oxides, of the sediments might explain the rapid elimination of glyphosate from the water by adsorption to the sediments. The turnover kinetics of $^{13}C_3{}^{15}N$-glyphosate in the sediment was much slower than in the water. A maximum amount of $^{13}C_3{}^{15}N$-glyphosate (51 % of the initially added $^{13}C_3{}^{15}N$ label) was detected in the sediments on day 5. Therefore, a potential risk by residual glyphosate in the sediment is given. Thereafter, elimination of $^{13}C_3{}^{15}N$-glyphosate from sediments was rapid (days 5-40), followed by a slower disappearance towards the end to ultimately result in 5 % of the initially added $^{13}C_3{}^{15}N$ label.

Figure 7.2.2.3-8: Distribution of the extracted $^{13}C_3{}^{15}N$-glyphosate (A) and $^{13}C1$-AMPA (B) in biotic water-sediment systems (50 mg/L) over 80 days expressed as the percentage of applied $^{13}C_3{}^{15}N$-glyphosate. (Please note: ^{13}C-AMPA only contains one labeled carbon atom; the second metabolite glyoxylate contains the other two)



The decrease in $^{13}C_3{}^{15}N$-glyphosate in both the water and sediment compartments during days 5-40 parallels the increasing mineralisation of glyphosate. At the same time, a large amount of the recovered $^{13}C_3{}^{15}N$-glyphosate from the water-sediment system was associated with the sediments (~74 %), whereas only ~25 % was dissolved in the water. Finally, when $^{13}C_3{}^{15}N$-glyphosate was detected only in the sediment compartment (40 – 80 days), mineralisation kinetics were slower indicating a limited bioavailability of glyphosate adsorbed onto sediment particles (Katagi, 2013). In the first ten days of the present experiments, only low contents of ^{13}C in AMPA (1 % of $^{13}C_3{}^{15}N$-glyphosate equivalents; Figure 7.2.2.3-8) and ^{15}N in AMPA (4 % of $^{13}C_3{}^{15}N$-glyphosate equivalents) were observed. Thereafter, (10-40 days), a rapid increase in the $^{13}C_3{}^{15}N$-AMPA contents was noted and was accompanied by the rapid degradation of $^{13}C_3{}^{15}N$-glyphosate in the water-sediment system with concomitant $^{13}CO_2$ formation. From day 40 onwards, when glyphosate partitioned into the sediment and its mineralisation rate decreased, the increase in the $^{13}C_3{}^{15}N$-AMPA contents slowed down. At the end of the experiment, ^{13}C in AMPA accounted for 26 % of the $^{13}C_3$-glyphosate equivalents. As only one of the three labeled ^{13}C atoms from the glyphosate, but all of the ^{15}N (one atom) is retained in AMPA (Figure 7.2.2.3-10) and the percentages are referred to the initial amount of labeled atoms (not molecules), the percentage of ^{15}N-AMPA was generally 3-fold higher than that of ^{13}C-AMPA and thus amounted to 79 % of the initially added ^{15}N-glyphosate. Similar to $^{13}C_3{}^{15}N$-glyphosate, the recovered $^{13}C_3{}^{15}N$-AMPA from the system was mostly associated to the sediment (70-90 %), whereas the residual (10-30 %) was dissolved in the water phase. In contrast to $^{13}C_3{}^{15}N$-glyphosate, $^{13}C_3{}^{15}N$-AMPA was more persistent; this was indicated by its continuous increase until the end of the experiment, indicating that $^{13}C_3{}^{15}N$-AMPAwas degraded more slowly than it was produced from glyphosate, as reported earlier (Mamy *et al.*,2005). Unfortunately, our data do not allow quantification of microbial AMPA degradation due to the simultaneous formation and degradation. Due to the continuing production of AMPA at a higher rate than degradation, a potential risk may be given by this metabolite. Compared to the biotic systems, the abiotic controls, water without sediment and biotic systems at 3 mg/L showed much lower formation of $^{13}C_3{}^{15}N$-AMPA from glyphosate.

Figure 7.2.2.3-9: Time dependent ^{13}C- (A) and ^{15}N-label (B) incorporation into tAA, bioAA and recalculated bioAA (40 % extraction efficiency) during microbial degradation of $^{13}C_3{}^{15}N$-glyphosate in biotic water-sediment system (50 mg/L) expressed as the percentage of applied $^{13}C_3{}^{15}N$-glyphosate equivalents

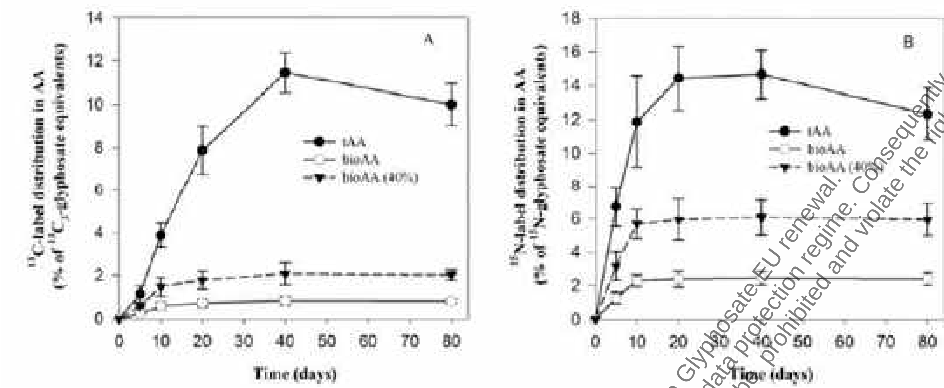
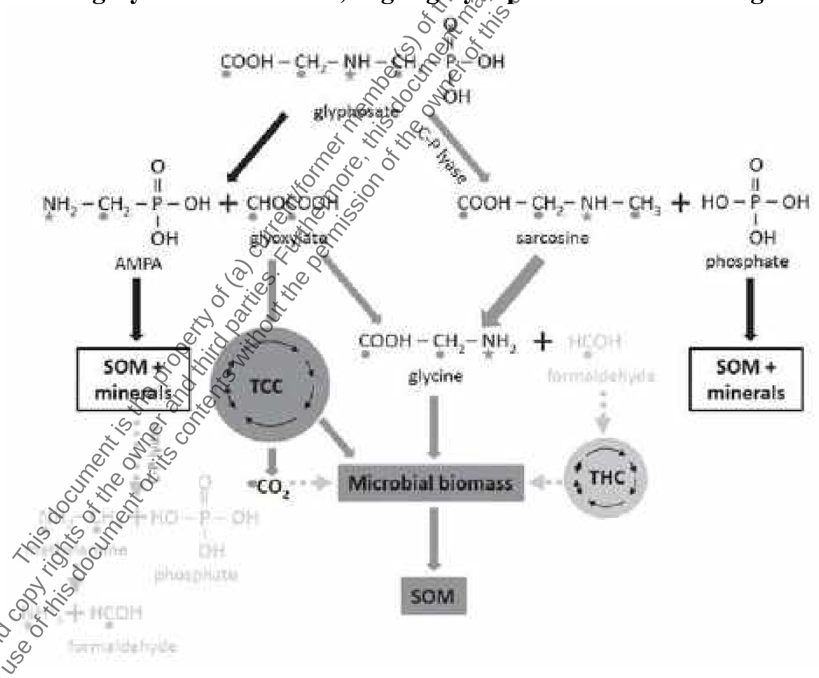


Figure 7.2.2.3-10: Pathways of microbial degradation of glyphosate through sarcosine and AMPA in biotic water-sediment system (50 mg/L). Dark grey arrows: biogenic residue formation; black arrows: xenobiotic NER formation; TCC: tricarboxylic acid cycle; THC: tetrahydrofolate cycle; grey circles = ^{13}C label; grey stars = ^{15}N label; Light grey = presumed further degradation



Incorporation of the ^{13}C and ^{15}N labels into AA and biogenic residues

The total AA pool of sediment (tAA) includes the AA in the living biomass and in the dead and decaying necromass. Neither ^{13}C nor ^{15}N enrichment in AA was detected in the suspended particles in either the water compartment of the abiotic sediment-water systems or the water systems. In the biotic water-sediment systems, ^{13}C label incorporation into the bioAA fraction was observed in the first sampling, and the contents of ^{13}C-bioAA increased rapidly to a maximum on day 40 (0.83 % of $^{13}C_3$-glyphosate equivalents; Figure 7.2.2.3-9). A continuous flux of ^{13}C--labeled AA from the living biomass to the non-living fraction OM was noted from day 5 onwards. ^{13}C-tAA initially increased sharply whereas from day 20 onwards, the ^{13}C-bioAA remained nearly constant, and approximately 92 % of the ^{13}C label in the tAA could be attributed

to the non-living OM. In contrast to the ^{13}C bioAA, the ^{13}C-tAA contents slightly decreased after 40 days. At the end of the experiment, the contents of ^{13}C in tAA reached 10 % of the initially added $^{13}C_3$-glyphosate. Considering a protein content of 50 % in bacterial cells (Nowak *et al.*, 2013), we arrive at a total of 20 % ^{13}C-biogenic residues at the end of the experiment. Similar to ^{13}CAA, incorporation of the ^{15}N label into bioAA and tAA was also observed starting from day 5 (Figure 7.2.2.3-9). In contrast to ^{13}C-bioAA, ^{15}N-bioAA contents plateaued on day 10 (2.38 % of ^{15}N-glyphosate equivalents). The incorporation of ^{15}N-bioAA into the non-living OM fraction was also similar to that of ^{13}C-bioAA, starting rapidly (on day 5), and approximately 81 % of the ^{15}N-tAA was stabilized in the non-living OM at the end. The rapid initial increase in ^{15}N-tAA continued until day 20 and then remained stable until day 40. Analogous to ^{13}C-tAA, ^{15}N-tAA decreased slightly towards the end.

^{15}N-tAA amounted to 12 % of the initially added ^{15}N-glyphosate at the end (similar to ^{13}C-tAA), and 24 %was observed for ^{15}N-biogenic residues based on a conversion factor of two for biomass in general. The dominant incorporation of both the ^{13}C and ^{15}N labels into the glycine was observed throughout the experiment and was most pronounced in the initial incubation period (see Table 7.2.2.3-140 and Table 7.2.2.3-141). Various AA were progressively enriched in both isotopes over time. In general, incorporation of ^{15}N proceeded faster than that of ^{13}C. In contrast to ^{13}C, the ^{15}N label disappeared from $^{13}C^{15}N$-glycine quickly and was distributed within different AA more rapidly than the ^{13}C label.

The results based on the ^{13}C- and ^{15}N-colabeling technique allowed comprehensive insight into the C and N fluxes from the colabeled $^{13}C^{15}N$ -glyphosate via microbial biomass to the non-living OM. Microorganisms assimilated the carbon and nitrogen from glyphosate to synthesize biomass compounds, as shown by the ^{13}C and ^{15}N-labeled bioAA. After death and cell lysis, their biomass constituents were progressively incorporated into the non-living OM fraction where they were stabilized and ultimately formed non-toxic biogenic residues.

Table 7.2.2.3-140 ^{13}C label distribution in diverse ^{13}C-bioAA (A) and ^{13}C-AA in the non-living SOM (B) during biodegradation of $^{13}C_3$-glyphosate in biotic water-sediment system (50 mg/L)

(A)

Incubation time (days)	^{13}C-bioAA (% of $^{13}C_3$-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.06 (±0.00)	0.07 (±0.00)	0.07 (±0.02)	0.07 (±0.00)	0.06 (±0.01)
Glycine	n.d.	**0.13** (±0.02)	**0.18** (±0.01)	0.14 (±0.01)	0.15 (±0.03)	**0.11↓** (±0.01)
Threonine	n.d.	n.d.	n.d.	0.07 (±0.00)	0.06 (±0.01)	0.06 (±0.02)
Valine	n.d.	n.d.	0.10 (±0.02)	0.10 (±0.01)	0.09 (±0.02)	0.09 (±0.00)
Leucine	n.d.	n.d.	n.d.	n.d.	0.14 (±0.00)	0.12 (±0.01)
Isoleucine	n.d.	n.d.	0.11 (±0.00)	0.14 (±0.00)	0.05 (±0.01)	0.04 (±0.02)
Proline	n.d.	n.d.	n.d.	0.05 (±0.03)	0.04 (±0.01)	0.09 (±0.00)
Aspartate[a]	n.d.	n.d.	0.02 (±0.00)	0.03 (±0.00)	0.04 (±0.02)	0.08 (±0.01)
Glutamate[b]	n.d.	n.d.	0.06 (±0.03)	0.07 (±0.03)	0.05 (±0.02)	0.05 (±0.00)
Phenylalanine	n.d.	n.d.	n.d.	n.d.	0.10 (±0.03)	0.07 (±0.01)
Lysine	n.d.	n.d.	0.04 (±0.01)	0.04 (±0.03)	0.04 (±0.06)	0.03 (±0.01)
Total	**n.d.**	**0.23 (± 0.03)**	**0.58 (± 0.07)**	**0.71 (± 0.13)**	**0.83 (± 0.21)**	**0.80 (± 0.10)**

(B)

Incubation time (days)	^{13}C-non-living AA (% of $^{13}C_3$-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.4 (±0.1)	0.6 (±0.1)	1.3 (±0.3)	1.4 (±0.1)	0.7 (±0.1)
Glycine	n.d.	**0.5** (±0.2)	**1.0** (±0.1)	1.0 (±0.1)	0.5 (±0.1)	0.6 (±0.2)
Threonine	n.d.	n.d.	n.d.	0.4 (±0.1)	0.1 (±0.1)	0.5 (±0.1)
Valine	n.d.	n.d.	n.d.	1.0 (±0.6)	1.1 (±0.1)	1.3 (±0.3)
Leucine	n.d.	n.d.	n.d.	n.d.	1.6 (±0.3)	1.2 (±0.1)
Isoleucine	n.d.	n.d.	n.d.	0.7 (±0.1)	1.1 (±0.1)	0.9 (±0.3)
Proline	n.d.	n.d.	0.5 (±0.1)	0.5 (±0.1)	0.5 (±0.1)	0.3 (±0.1)
Aspartate[a]	n.d.	n.d.	0.5 (±0.1)	0.5 (±0.1)	1.1 (±0.2)	0.8 (±0.1)
Glutamate[b]	n.d.	n.d.	0.7 (±0.1)	0.5 (±0.1)	1.1 (±0.1)	1.6 (±0.2)
Phenylalanine	n.d.	n.d.	n.d.	n.d.	1.0 (±0.3)	0.6 (±0.1)
Lysine	n.d.	n.d.	n.d.	1.3 (±0.1)	1.3 (±0.4)	0.9 (±0.3)
Total	**n.d.**	**0.9 (± 0.3)**	**3.3 (± 0.5)**	**7.2 (± 1.6)**	**10.8 (± 2.0)**	**9.4 (± 1.9)**

[a] Incl. Asparagine.
[b] Incl. Glutamine; n.d. - not detectable; values are presented as averages ± standard deviation; values printed in bold show characteristic values; arrows illustrates increases or decreases of the respective AA compared to the preceding sampling event.

Table 7.2.2.3-141 ^{15}N label distribution in diverse ^{15}N-bioAA (A) and ^{15}N-AA in the non-living SOM (B) during biodegradation of ^{15}N-glyphosate in biotic water-sediment system (50 mg/L)

(A)

Incubation time (days)	^{15}N-bioAA (% of ^{15}N-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.01 (±0.08)	0.09 (±0.1)	0.03 (±0.02)	0.15 (±0.02)	0.23↓ (±0.07)
Glycine	n.d.	**0.70** (±0.07)	**1.20**↑ (±0.01)	**1.20** (±0.09)	**0.80**↓ (±0.10)	**0.43**↓ (±0.07)
Threonine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valine	n.d.	0.38 (±0.07)	0.28 (±0.03)	0.28 (±0.02)	0.30 (±0.05)	0.28 (±0.02)
Leucine	n.d.	0.17 (±0.02)	0.17 (±0.04)	0.14 (±0.07)	0.40 (±0.09)	0.38 (±0.03)
Isoleucine	n.d.	n.d.	0.30 (±0.01)	0.28 (±0.01)	0.09 (±0.07)	0.13 (±0.06)
Proline	n.d.	n.d.	0.04 (±0.04)	0.04 (±0.13)	0.05 (±0.04)	0.22 (±0.05)
Aspartate[a]	n.d.	n.d.	n.d.	0.13 (±0.06)	0.10 (±0.09)	0.22 (±0.10)
Glutamate[b]	n.d.	n.d.	0.10 (±0.03)	0.19 (±0.07)	0.19 (±0.08)	0.20 (±0.02)
Phenylalanine	n.d.	n.d.	0.06 (±0.03)	0.08 (±0.02)	0.21 (±0.04)	0.19 (±0.02)
Lysine	n.d.	n.d.	0.04 (±0.03)	0.01 (±0.01)	0.10 (±0.05)	0.10 (±0.08)
Total	**n.d.**	**1.26 (±0.24)**	**2.28 (±0.32)**	**2.38 (±0.5)**	**2.43 (±0.63)**	**2.38 (±0.52)**

(B)

Incubation time (days)	^{15}N-non-living AA (% of ^{15}N-glyphosate equivalents applied)					
	0	5	10	20	40	80
Alanine	n.d.	0.2 (±0.1)	0.6 (±0.1)	0.4 (±0.1)	0.8 (±0.2)	0.2 (±0.1)
Glycine	n.d.	**1.1** (±0.1)	1.2 (±0.2)	1.2 (±0.1)	**0.8**↓ (±0.1)	**0.7**↓ (±0.1)
Threonine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valine	n.d.	0.8 (±0.1)	1.4 (±0.2)	1.5 (±0.3)	1.3 (±0.2)	1.0 (±0.2)
Leucine	n.d.	0.7 (±0.1)	1.2 (±0.8)	2.8 (±0.4)	1.8 (±0.1)	1.5 (±0.1)
Isoleucine	n.d.	n.d.	1.5 (±0.1)	1.2 (±0.2)	1.6 (±0.1)	1.4 (±0.3)
Proline	n.d.	n.d.	0.6 (±0.1)	0.8 (±0.1)	0.8 (±0.1)	0.4 (±0.1)
Aspartate[a]	n.d.	n.d.	0.1 (±0.2)	0.3 (±0.1)	1.0 (±0.1)	0.6 (±0.2)
Glutamate[b]	n.d.	**1.2** (±0.1)	1.0 (±0.1)	1.7 (±0.1)	1.4 (±0.1)	1.8 (±0.1)
Phenylalanine	n.d.	n.d.	0.7 (±0.1)	1.4 (±0.1)	1.4 (±0.1)	1.2 (±0.1)
Lysine	n.d.	n.d.	0.1 (±0.1)	1.6 (±0.1)	1.7 (±0.1)	0.9 (±0.1)
Total	**n.d.**	**4.0 (±0.5)**	**8.4 (±2.0)**	**12.1 (±1.8)**	**12.6 (±1.2)**	**9.7 (±1.4)**

[a] Incl. Asparagine.
[b] Incl. Glutamine; n.d. - not detectable; values are presented as averages ± standard deviation; values printed in bold show characteristic values; arrows illustrates increases or decreases of the respective AA compared to the preceding sampling event.

Indication of different (bio)degradation pathways of glyphosate in water-sediment systems
Based on the detailed glyphosate turnover mass balance and the patterns of ^{13}C and ^{15}N labeled AA over time, particularly of the dominant glycine, we could distinguish between two degradation pathways of this herbicide in water-sediment system. The dominance of co-labeled $^{13}C^{15}N$-glycine especially in the first sampling event indicates its formation via the sarcosine pathway (Borggaard and Gimsing, 2008; Singh and Walker, 2006; Figure 7.2.2.3-10). However, the occurrence of the sarcosine pathway in soil or sediment has not yet been proven (Borggaard and Gimsing, 2008; Singh andWalker, 2006). We could not detect sarcosine in our experiment, but this compound is rapidly oxidized to glycine and thus does not accumulate. The formed glycine is directly incorporated into microbial biomass, resulting in the observed occurrence of co-labeled $^{13}C^{15}N$-glycine in the living biomass AA. The negligible mineralisation (3 % of ^{13}C equivalents initially applied) with high simultaneous removal of $^{13}C_3{}^{15}N$ -glyphosate and the maximum contents of ^{13}C and ^{15}N glycine on day 10 also support the hypothesis that glyphosate is initially degraded via the sarcosine pathway. Hence, the sarcosine pathway was actually contributing at the beginning of glyphosate degradation, whereas the AMPA pathway dominated in the later degradation phase. A later decrease of co-labeled $^{13}C_3{}^{15}N$-glycine (10-20 days) was accompanied by a rapid increase in AMPA over time.

The risk potential of glyphosate residues in water-sediment systems
To date, there is no detailed information on the metabolic fate of glyphosate residues and their distribution in the water-sediment system. The present results provide detailed insight into the biodegradation processes of $^{13}C_3{}^{15}N$-glyphosate in the water-sediment system and into the transformation of this herbicide into AMPA, microbial biomass and NER. Since glyphosate is biodegraded and the NER are dominantly biogenic residues, the highest potential risk is provided by the significant concentrations of AMPA.

Non-extractable $^{13}C_3$-glyphosate residues were formed immediately (6 % of the initially added ^{13}C label, see Table 7.2.2.3-142). The NER contents increased until day 10 and then remained on a high level. From day 20 onwards, their contents decreased and ultimately reached 23 % of the $^{13}C_3$-glyphosate equivalents.

The chemical composition of the NER formed during degradation of glyphosate is not yet known, and their analyses are limited to quantification. In the present study, glyphosate was initially a source of xenobiotic ^{13}CNER formation that was dominant until day 10 (Figure 7.2.2.3-11). However, immobilized glyphosate in the NER was microbially degradable, as shown by the continuous decrease of xenobiotic NER over time, specifically of ^{13}C-xenobiotic NER. Microorganisms used the carbon and nitrogen from $^{13}C_3{}^{15}N$-glyphosate to synthesize their biomass compounds, as shown by the ^{13}C and ^{15}N incorporation into microbial AA, leading to biogenic residues in OM after cell death and lysis. Based on the ^{13}C-tAA content, 20 % of the ^{13}C-biogenic residues were formed and constituted the major fraction of ^{13}C-NER (87 %, Figure 7.2.2.3-11). These results agree with previous studies on biogenic residue formation during biodegradation of ^{13}C-labeled pesticides or pharmaceuticals.

Table 7.2.2.3-142 Mass balance of $^{13}C_3{}^{15}N$-glyphosate degradation in biotic and abiotic water-sediment systems and in water over 80 days (% of initially applied ^{13}C- and ^{15}N-label equivalents)

	% of initial ^{13}C					
	Time (days)	Mineralization	Glyphosate	AMPA	NER	Recovery
	0	n.d.	98 (± 1)	0.2 (±0)	6 (± 1)	104 (± 2)
Biotic	5	1 (± 1)	68 (± 2)	1 (± 1)	28 (± 1)	98 (± 4)
	10	3 (± 1)	56 (± 2)	1 (± 0)	33 (± 5)	93 (± 7)
	20	16 (± 0)	44 (± 2)	6 (± 1)	35 (± 1)	101 (± 5)
	40	40 (± 1)	9 (± 1)	22 (± 1)	31 (± 1)	102 (± 4)
	80	**56 (± 3)**	**5 (± 1)**	**26 (± 1)**	**23 (± 2)**	**110 (± 6)**
Abiotic	80	19 (± 1)	2 (± 1)	11 (± 1)	26 (± 0)	97 (± 3)
Only water	80	2 (± 1)	90 (± 2)	2 (± 0)	n.d.	94 (± 3)

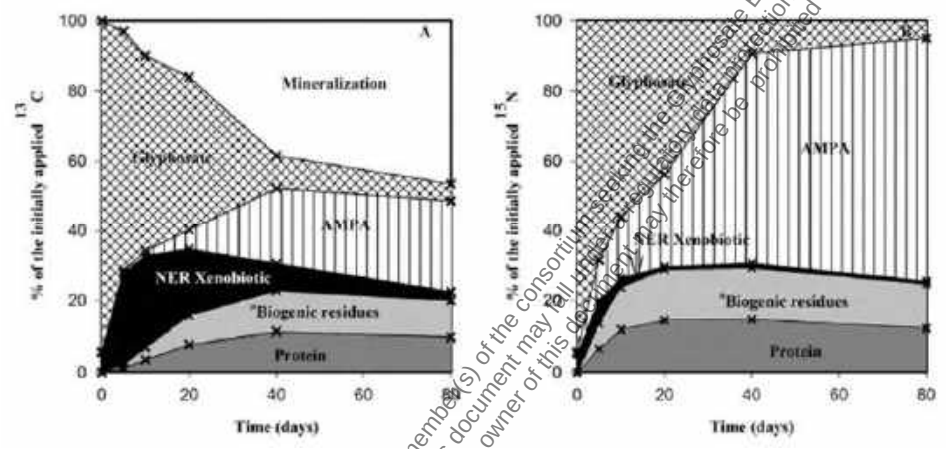
	% of initial ^{15}N				
	Time (days)	Glyphosate	AMPA	NER	Recovery
	0	98 (± 1)	1 (± 1)	5 (± 0)	**104 (± 2)**
Biotic	**5**	68 (± 2)	3 (± 1)	20 (± 2)	91 (± 6)
	10	56 (± 2)	4 (± 1)	26 (± 3)	86 (± 6)
	20	44 (± 2)	17 (± 3)	30 (± 1)	91 (± 6)
	40	9 (± 1)	65 (± 4)	31 (± 0)	105 (± 5)
	80	**5 (± 1)**	**79 (± 5)**	**26 (± 0)**	**110 (± 6)**
Abiotic	**80**	41 (± 2)	33 (± 1)	26 (± 3)	100 (± 6)
Only water	**80**	90 (± 2)	6 (± 1)	n.d.	96 (± 2)

n.d. = not detectable; values are shown as averages ± standard deviation

The kinetics of ^{15}N-NER formation showed a similar pattern to that of ^{13}C-NER and reached 26 % of the initially added ^{15}N-glyphosate. Analogous to the ^{13}C-biogenic residues, the ^{15}N-NER were primarily biogenic (Figure 7.2.2.3-11); at the end of the experiment, the ^{15}N-biogenic residues amounted to 24 % of ^{15}N-glyphosate equivalents and constituted 92 % of the ^{15}N-NER. In contrast to the ^{13}C-biogenic residues, the ^{15}N-biogenic residues were formed rapidly, which is in line with the metabolization and mineralisation of $^{13}C_3{}^{15}N$-glyphosate via the sarcosine pathway without N mineralisation in the initial degradation phase. The contents of extractable $^{13}C_3{}^{15}N$-glyphosate residues (31 % of the $^{13}C_3$-glyphosate equivalents and 84 % of the ^{15}N-glyphosate equivalents) comprised a large proportion of the ^{13}C and ^{15}N-isotope mass balance at the end, with AMPA accounting for almost all of these residues. The percentage of ^{15}N-AMPA was 3-fold higher than that of ^{13}C-AMPA because only one out of three ^{13}C atoms, but all ^{15}N atoms from the co-labeled glyphosate are transferred to AMPA during metabolization (see Figure 7.2.2.3-10). In the sediment-water

systems nearly all of the NER could be explained by biogenic residues bearing no potential risk. However, high contents of extracted AMPA were detected, which typically biodegrades slower than glyphosate. The detailed fate of AMPA needs to be investigated to assess the potential risks related to this degradation product of glyphosate. In contrast to previous studies in which biogenic residues remained constant, ^{13}C and ^{15}N biogenic residues from glyphosate slightly decreased towards the end of the experiment. Total hydrolysable ^{13}C- and ^{15}N-labeled AA decreased progressively after 69 days in sediments incubated with ^{13}C-glucose and ^{15}N-labeled ammonium, which is in agreement with the present study.

Figure 7.2.2.3-11: Detailed mass balance including biogenic residue formation (A) of $^{13}C_3$-glyphosate and (B) of ^{15}N-glyphosate in biotic water-sediment system (50 mg/L). a: Biogenic residues were calculated based on a conversion factor of 2 for proteins



Conclusions

This is the first detailed glyphosate turnover mass balance including NER speciation in water-sediment systems using stable isotope co-labeled tracers (^{13}C and ^{15}N).

Sediment plays a key role in the microbial degradation of glyphosate via both the sarcosine and AMPA pathway.

At the end, nearly all of the NER can be assigned to non-toxic biogenic residues after degradation of the parent compound.

Accumulation of main metabolite of glyphosate, AMPA, may because for environmental concern; therefore, an additional investigation of the fate of AMPA in water-sediment systems is needed.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article reports the results from a water-sediment dissipation experiment with ^{13}C-^{15}N-labelled-glyphosate, conducted according to OECD guideline 308. The methods and results are generally well described and conclusive. However, the water and associated sediment were taken from a German small water body located in agricultural lowlands with continuous crop rotation and pesticide application, which is considered a high risk area for exposure to pesticides. Thus, it cannot be excluded that the water/sediment system received inputs of glyphosate or AMPA within the previous 4 years, as is required in OECD 308 guideline.

In addition, the use of stable-isotope enriched glyphosate does not allow to differentiate between applied substance and existing background. This is also documented by the fact that no metabolites were detected other than AMPA which is known for its potential stability under such test conditions.

In the main experiment, the application rate was extremely high (50 mg/L, equivalent to an application rate of 150 kg/ha when assuming overspray of a 30 cm deep water body) while in OECD 308 guideline it is recommended that the test concentration should be close to application rate or environmental concentrations. Hence the exaggerated test concentration may affect the route and rate of degradation.

The article is seen as reliable with restrictions and therefore supportive information.

Assessment and conclusion by RMS:

CA 7.2.2.4 Irradiated water/sediment study

The route and rate of degradation of glyphosate in water and sediment were comprehensively studied in sections CA 7.2.1.1 to CA 7.2.2.3. Therefore, an irradiated water/sediment study is not required and was not conducted.

CA 7.2.3 Degradation in the Saturated Zone

A study on degradation in the saturated zone is not required and was not conducted.

CA 7.3 Fate and Behaviour in Air

CA 7.3.1 Route and rate of degradation in air

The Henry´s constant of glyphosate is $<2.21 \times 10^{-8}$ Pa m^3/mol and its vapour pressure is 1.31 x 10^{-5} Pa (25 °C).

One study is available which is considered valid to address the atmospheric half-live of glyphosate ([redacted] 2020, CA 7.3.1/001). In addition, one study provides supportive information ([redacted] 2012, CA 7.3.1/002). Two valid studies on volatilisation of glyphosate and glyphosate-trimesium are available ([redacted] 1996, CA 7.3.1/004; [redacted] 1992, CA 7.3.1/007) supported by two further studies ([redacted] 1997, CA 7.3.1/003; [redacted] 1993, CA 7.3.1/006). For studies performed with glyphosate-trimesium only the results for the glyphosate (PMG) anion are considered for evaluation and further assessment.

Glyphosate acid degrades very rapidly in air with an estimated half-life of 0.135 days (1.625 hours). The supportive studies estimated the same half-life for glyphosate in air. For the salts of glyphosate, estimated atmospheric half-lives were similar.

No volatilisation of glyphosate from plants and soil was observed after the application of glyphosate in laboratory and field experiments.

Glyphosate can be classified as not volatile based on its Henry's law constant and on volatilisation experiments from soil and plants with no significant rates. Due to no significant UV-absorption, direct photolysis in air is not relevant. In case reaching the atmosphere, glyphosate will rapidly be removed by photochemical oxidative degradation.

In the scientific literature review for glyphosate (2010-2019), one article was identified to provide further information relevant to the data point (Bento *et al.*, 2017, CA 7.3.1/008). In a laboratory experiment with a small-scale wind tunnel, the distribution of glyphosate and AMPA in different particle size fractions of a loess soil after artificial wind erosion was analysed. Glyphosate and AMPA concentrations increased with decreasing particle size and were mainly correlated with contents of clay and organic matter of the particles. The results show that glyphosate and AMPA can be found in wind-eroded particles when present in dry top soil (soil moisture ≤2 %) in windy situations (at least 6.5 m/s at ground level). Further conclusions on implications under field conditions are limited due to the artificial experimental conditions. The potential environmental exposure of organisms to entries of glyphosate and AMPA via wind-eroded sediment is considered to be limited and sufficiently covered by worst-case calculations of predicted environmental concentrations in soil, surface water and sediment.

Table 7.3.1-1: Studies on route and rate of degradation in air

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.3.1/001	[redacted] 2020	Atmospheric half-life calculation	Glyphosate	Valid	Calculated for glyphosate acid
CA 7.3.1/002	[redacted] 2012	Atmospheric half-life calculation	Glyphosate	Supportive	Calculated for glyphosate acid as well as for salts
CA 7.3.1/003	[redacted] s, 1997	Volatilisation	Glyphosate	Supportive	
CA 7.3.1/004	[redacted] 1996	Volatilisation	Glyphosate	Valid	Analytical phase report to [redacted] 1995
CA 7.3.1/005	[redacted] 1995	Volatilisation	Glyphosate 360 SL formulation	Valid	Field phase report related to [redacted] 1996
CA 7.3.1/006	[redacted] 1993	Volatilisation	Glyphosate 360 SL formulation	Supportive	Report not available
CA 7.3.1/007	[redacted] 1992	Volatilisation	Glyphosate-trimesium	Valid	

Table 7.3.1-2: Behaviour in air – relevant articles from literature search

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 7.3.1/008	Bento *et al.*, 2017	Wind tunnel experiment	Glyphosate, AMPA	Reliable	

Studies on estimation of atmospheric half-life

1. Information on the study

Data point:	CA 7.3.1/001
Report author	[redacted]
Report year	2020
Report title	Glyphosate: Calculation of the Chemical Half-Life in the Troposphere
Report No	110054-016
Document No	
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, not applicable for this study type
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The half-life in air of glyphosate was estimated according to structure-activity relationship (SAR) methods developed by Atkinson *et al.*

The half-life time ($t_{1/2}$) of glyphosate was estimated with 0.135 days (1.625 hours) assuming the typical OH radical concentration averaged over 12 hours (1.5 x 10^6 radicals/cm³). This concentration during a 12 hour day is regarded as a typical OH radical concentration during daylight hours.

I. METHODS

The half-life of glyphosate in air was estimated according to structure-activity relationship (SAR) methods developed by Atkinson *et al.* The approach of Atkinson *et al.* was based on a comprehensive set of experimental data to result in a quantitative structure-üactivity relationship (QSAR) mathematic model that allows for estimation by calculation, starting from the molecular structure of a compound. The calculation procedure has been transferred into the personal computer program "Atmospheric Oxidation Program" (AOP) by Meylan & Howard. The version AOPWIN™ 1.92a (U.S. EPA, 2008) was used for the calculations being part of the EPI Suite™ set of programs.

Considering the chemical structure of glyphosate, it can be concluded that reactions with photochemical produced hydroxyl radicals will mainly determine its degradation rate ($K_{total,\ indirect\ photoreaction} \approx k_{OH}$) in the air. No significant ozone reaction is expected and therefore not included in the assessment. The diurnally

and annually averaged 12-h daylight hydroxyl radical concentration of 1.5×10^6 molecules (radicals)/cm^3 was used.

II. RESULTS AND DISCUSSION

The overall reaction rate of glyphosate with hydroxyl radicals is estimated to be The overall reaction rate of glyphosate with hydroxyl radicals is estimated to be 79.008 x 10^{12} cm^3 / (molecule x s). This rate is derived mainly from incremental reactions like hydrogen abstraction (15.2009 x 10^{-12} cm^3 / (molecule x s), value estimated) and reactions to OH groups (assumed value of 63.8000 x 10^{-12} cm^3 / (molecule x s)).

Based on the overall hydroxyl radical reaction rate constant in combination with the concentration of these radicals in the atmosphere (*i.e.* 1.5 x 10^6 OH radicals/cm^3) the half-life of f glyphosate in air is derived to Based on the overall hydroxyl radical reaction rate constant in combination with the concentration of these radicals in the atmosphere (*i.e.* 1.5 x 10^6 OH radicals/cm^3). This estimate should be regarded as worst-case assumption as the approach does not consider the contribution of any other reactive species than hydroxyl radicals to the overall atmospheric degradation of glyphosate in air.

III. CONCLUSIONS

The active substance glyphosate is considered to be susceptible to reactions with hydroxyl radicals which contribute to the overall degradation of the substance in the atmosphere. An attack by hydroxyl radicals should result in the formation of multiple primary radicals. Their formation may be followed by secondary oxidation products that can be eliminated from the atmosphere by wet and/or dry deposition.

The half-life of glyphosate in air was estimated with 0.135 days (1.625 hours).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is considered valid to address the data point. The half-life of glyphosate in air was estimated with 0.135 days (1.625 hours).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.3.1/002
Report author	[redacted]
Report year	2012
Report title	Atmospheric Oxidation of Glyphosate Salts - Atkinson Calculation
Report No	MSL0024050
Document No	
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The rate constants for the atmospheric gas phase reaction between photochemically produced hydroxylradicals with several glyphosate salts were calculated based on the computer modelling programAOPWIN™ (Atmospheric Oxidation Program for Microsoft Windows). The model estimated an atmospheric half-life of 1.380 hours (0.115 days based on 12 hour days) for glyphosate-isopropylamine salt. The corresponding atmospheric half-lives for glyphosate potassium salt and glyphosate ammonium salt were both estimated as 1.719 hours (0.143 days based on 12 hour days). The model estimated the atmospheric half-life to be 1.663 hours (0.139 days based on 12 hour days) for glyphosate dimethylamine salt.

I. MATERIAL AND METHODS

The reaction of glyphosate acid in the atmosphere with hydroxyl radicals has been estimated using the method of Atkinson.

For the calculation, the Atmospheric Oxidation Program AOPWIN, version 1.92 was used. This is a computer programme that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. It also estimates the gas-phase reaction between ozone and olefinic/acetylinic compunds. The rate constants estimated by the program are used to calculate an atmospheric half-life for the organic compound based upon average atmospheric concentrations of hydroxyl radicals and ozone.

II. RESULTS AND DISCUSSION

Results for the different glyphosate salts are presented in the table below.

Table 7.3.1-3: Results of Atkinson calculation

Compound	Half-life (hours)[1]	Half-life (days)	Overall Rate Constant (cm^3/molecule/sec)
Glyphosate Free Acid[2]	1.6	0.135	79.0×10^{-12}
Glyphosate Isopropylamine Salt	1.380	0.115	93.0×10^{-12}
Glyphosate Potassium Salt	1.719	0.143	74.7×10^{-12}
Glyphosate Ammonium Salt	1.719	0.143	74.7×10^{-12}
Glyphosate Dimethyamine Salt	1.663	0.139	77.2×10^{-12}

[1] Tropospheric half-life is based on OH concentrations of 1.5×10^6 OH radicals/cm^3 and 12 hours day.
[2] Values from the Glyphosate Monograph list of endpoints as stipulated in SANCO 651l/VI/99-final.

III. CONCLUSIONS

The computer program AOPWIN has estimated the overall rate constant for the gas-phase reaction between hydroxyl radicals (OH) and three different salts of glyphosate to range from 74.7×10^{-12} to 93.0×10^{-12} cm^3/molecule/sec. The atmospheric half-life of glyphosate salts is estimated to be in the range of 1.380 to 1.719 hours (0.115 to 0.143 days) as a result of gas-phase reactions between glyphosate salt and photochemically produced atmospheric hydroxyl radicals. Since glyphosate contains no olefinic or acetylenic sites, no reaction with atmospheric ozone was estimated.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The photochemical oxidative decomposition of glyphosate in the atmosphere has been assessed via the method described by Atkinson, resulting in half-lives in a range of 1.380 to 1.719 hours for several glyphosate salts. The assessment is considered supportive.

Assessment and conclusion by RMS:

Studies on volatilisation from soil and plants

1. Information on the study

Data point:	CA 7.3.1/003
Report author	[redacted]
Report year	1997
Report title	Determination of the rate of volatilization of glyphosate from soil and plant surface (french beans)
Report No	191071
Document No	
Guidelines followed in study	BBA Guideline Part IV, 6-1
Deviations from current test guideline	No current guideline in force - for soil indirect method with recovery following combustion is not quantitative
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive for plant experiment Invalid for soil experiment
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The volatility of glyphosate from soil and plant surface was determined. Therefore, glyphosate was applied as a formulated product on soil and plant surface under standard conditions in an apparatus allowing an air flow of 1.1 to 1.3 m/s to pass over test soil and plants and at an application rate of 4.32 kg/ha (12 L of the 360 g/L formulation/ha). Quantification was performed using ^{14}C-labelled glyphosate, which was added to the formulated product before application (specific activity 2.00 Gbq/mmol). Treated soil and plant surface were exposed to air with a flowrate of > 1 m/s at room temperature for 24 hours. Samples were taken at t=0, 3, 6 and after 24 hours. The amount of glyphosate left on the surface was quantified by liquid scintillation counting (LSC). From this the amount which was evaporated was calculated.

After 24 hours, 82 ± 6 % of the glyphosate relative to the amount determined at t=0 was recovered from the soil samples. The amount of test substance in the soil samples relative to applied varied from 68 to 96 %. Based on these results, it is concluded that less than 20 % of the test substance evaporates from the soil samples within 24 hours under the conditions of the test. The recovery of the [^{14}C]glyphosate after combustion of the soil samples varies. Directly after application recoveries of 97 % were found. However, if the soil sample is combusted after storage, the recovery is not quantitative anymore. In one experiment,

the recovery after a storage period of three days was only 80 %. A possible explanation for the incomplete combustion after storage is very strong binding of glyphosate to soil, even under the combustion conditions.

After 24 hours, 103 ± 1 % of the glyphosate relative to the amount determined at t=0 was recovered from the plant surface. The amount of test substance on the plant samples relative to applied (nominal value) varied from 89 to 96 %. Based on these results it is concluded that only up to 11 % of the test substance evaporates from the plant leaves within 24 hours under the conditions of the test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled

Identification:	[^{14}C]glyphosate (NOTOX substance 63711)
Lot No.:	25A
Specific activity:	11.07 MBq/mg
Radiochemical purity:	98.3 %
Chemical purity:	not indicated

Unlabelled

Identification:	formulation glyphosate 360 g/L (NOTOX substance 68679)
Lot No.:	907116
Composition:	360 g/L

Spraying solutions of different concentrations were prepared by mixing [^{14}C]glyphosate with non-radiolabelled formulation glyphosate 360 g/L.

2. Soil:

The study was performed with LUFA Speyer 2.1 standard soil. After receipt, the soil was stored at NOTOX in the open air, in open containers. Before use, the soil was sieved through a 2 mm analytical sieve. Before the start of the test, the soil was adjusted to approximately 60 % of the maximum water capacity. Soil properties are given in the table below.

Table 7.3.1-4: Soil properties of Speyer 2.1 (slight humic sand)

Location	RLP, RheinzabernTeufelskanzel
Horizon (cm)	20
Charge number	F12095
Organic carbon content (%)	0.62
Organic matter content (%) [1]	1.07
Particles < 20 µm (%)	6.5
pH	5.9
Maximum water capacity (%)	30.6

[1] Calculated from organic carbon according to OM = OC / 0.58

3. Plant:

French beans were cultivated in soil at approximately 22 °C and 16 hours simulated daylight each day. Leaves with an area of at least 30 cm² are used for the volatility test.

B. STUDY DESIGN

Several tests with soil and one test with leaves were carried out, an overview is given in the table below.

Table 7.3.1-5: Overview of test conditions

	Test 1	Test 2	Test 3	Test 4
Matrix	Soil	Soil	Soil	Leaves
Concentration in spraying solution (g/L)	21.6	21.6 1.1 348	3.66	21.6
Application rate (kg a.s./ha)	4.32	0.2 4 70	Not indicated	4.32
Temperature (°C)	19.5 ± 1.3	20.1 ± 1.3	Not indicated	20.8 ± 3.0
Min/max Temperature (°C)	16.8 21.6	15.0 22.0	Not indicated	14.8 24.9
Rel. humidity (%)	35.2 ± 5.0	20.0 ± 3.9	Not indicated	42.8 ± 2.8
Mmin/max rel. humidity (%)	25.7 42.5	16.2 34.4	Not indicated	31.2 51.3
Air flow (m/s)	1.2-1.3	1.2	Not indicated	1.1-1.2

1. Experimental conditions
The tests were conducted in a rectangular box. This was filled with either the soil samples or the leaves and a constant air flow was set. Samples (except for t=0) were kept in this apparatus allowing an air flow of 1.1-1.3 m/s to pass over the test soil or plants. The soil or plant samples, with the exception of the t = 0 samples, were transferred into the experimental set up.

2. Sampling
Samples were taken 1 hour, 3 hours, 6 hours and 24 hours after application. Samples t = 0 were extracted immediately after application and temperature, humidity and air flow were logged.

3. Analytical procedure
Soil samples were extracted and subsequently submitted to LSC in order to determine non-extractable residues or directly combusted and analysed via LSC. Plant leaf samples were not extracted but analysed directly via LSC. For direct LSC analysis, triplicate soil subsamples of 2 g or whole plant leaves were combusted using an oxidiser. The resulting ^{14}C-CO_2 was trapped and analysed using LSC.

The results of the first test showed that the amount of glyphosate in the soil was > 80 % relative to t = 0 at each time point. The amount of glyphosate relative to the applied amount, however, was slightly below 80 % (74 %) at t=24 hours. For this reason, the volatilisation over 24 hours was further investigated at different application rates (please refer to Table 7.3.1-5) in the second test. Samples were either extracted prior to combustion or combusted directly without extraction.

Based on the results of test 1, it was suspected that the combustion is not completely quantitative for glyphosate. In order to confirm this, the third test was carried out with all samples combusted directly.

The radiochemical purity was determined before start and after finalisation of the experiments by HPLC method as 96.7 % and 97.1 %, respectively. LOD and LOQ were not given.

II. RESULTS AND DISCUSSION

A. DATA
Results of the determination of recovery on soils and plants are presented in Table 7.3.1-6 to Table 7.3.1-9.

Table 7.3.1-6: Recovery from soil, first test

t (h)	Recovery after extraction (% AR)	Recovery after combustion (% AR)	Total recovery (% AR)	Average recovery ± SD (% AR)	Average recovery ± SD relative to t=0
0	67.4	20.1	87.5	89 ± 4.5	100
0	62.7	21.8	84.5		
0	77.8	17.3	95.1		
0	72.5	17.1	89.6		
1	74.0	17.7	91.7	91 ± 0.5	102 ± 0.6
1	74.0	17.0	91.0		
3	72.5	14.6	87.1	87 ± 0.5	97 ± 0.5
3	73.4	13.0	86.4		
6	73.6	22.4	96.0	94 ± 3.3	105 ± 3.6
6	72.5	18.9	91.4		
24	61.7	10.6	72.3	74 ± 5.4	82 ± 6.0
24	59.6	8.8	68.4		
24	58.2	14.1	72.3		
24	64.1	17.0/16.6[1]	81.1		

[1] In order to check if the low recovery was due to non-reproducible combustion, the combustion of triplicate subsamples of this sample was repeated. The result was almost the same as the first combustion, confirming a reproducible combustion.

Table 7.3.1-7: Recovery from soil, second test

t (h)	Application rate (kg a.s./ha)	Recovery after extraction (% AR)	Recovery after combustion (% AR)
0	0.2	78.1	-
0		77.7	-
24		-	85.3
24		-	86.8
24		-	81.2
24		-	81.3
0	4	87.7	-
0		86.4	-
24			89.9
24			65.9/72.1[1]
0	70	96.6	-
0		94.3	-
24		-	135.0
24		-	127.0
24		-	153.0
24		-	128.0

[1] In order to check if the low recovery was due to inhomogeneity of the sample, the entire sample was combusted in portions of ca. 2 g. The result is similar the first combustion, indicating that the sample was homogeneous.

Table 7.3.1-8: Results volatility from soil, third test

t (h)	Recovery of test substance (% AR)	Recovery of test substance (% AR)
0	95.5	96.8
0	96.4	
0	96.7	
0	98.4	
72	86.3	80.2
72	89.5	
72	69.7	
72	75.1	

Table 7.3.1-9: Recovery from plant leaves

t (h)	Recovery after combustion (% AR)	Average recovery ± SD (% AR)	Recovery ± SD relative to t=0
0	90.7	92 ± 2.5	100
0	94.6		
0	89.6		
0	94.1		
1	89.2	91 ± 2.3	98 ± 2.5
1	92.4		
3	90.7	92 ± 1.7	100 ± 1.8
3	93.1		
6	91.0	92 ± 0.8	99 ± 0.8
6	92.1		
24	94.2	95 ± 0.9	103 ± 0.9
24	96.1		
24	95.3		
24	95.9		

B. EXTRACTABLE AND NON-EXTRACTABLE RESIDUES

At the first soil test after 24 hours, 82 ± 6 % relative to t = 0 was recovered from the soil samples. The amount of test substance in the soil samples relative to applied varied from 68 to 96 %. The relatively low recovery, even at t = 0 hours can be explained by the fact that the samples were first extracted, then stored for one day, and then combusted.

The results of the second test confirm the results of the first test. At both 0.2 and 4 kg a.s./ha application rate, the amount recovered after 24 hours relative to applied is around or slightly below 80 %. At the application rate of 70 kg a.s./ha recoveries of 127-153 % are found after 24 hours. Apparently, a mistake was made during preparation or application of this formulation (which is rather viscous due to the high concentration). Therefore, these results were not taken into account. The relatively low recovery, at t = 0 h can be explained by the fact that the t = 0 hours samples were only extracted and not combusted. Apparently, the recovery of the extraction is not quantitative. This is also supported by the data of the first soil test. In general, combustion leads to better recovery of the test item than extraction which shows these two methods are not directly comparable.

In the third soil test, recoveries decreased from 96.8 to 80.2 % after 3 days. From these results it can be concluded that glyphosate can be recovered from soil by combustion almost quantitatively directly after application However, the recovery after a period of three days was only 80 %. A possible explanation for the incomplete combustion after storage is a very strong binding of glyphosate to soil, even under the combustion conditions.

After 24 hours, 103 ± 1 % relative to t = 0 was recovered from the plant samples. The amount of test substance on the plant samples relative to applied varied from 89 to 96 %. Based on these results, it is concluded that less than 2 % of the test substance evaporates from the plant leaves within 24 hours under the conditions of the test.

III. CONCLUSIONS

After 24 hours, 82 ± 6 % of the glyphosate relative to the amount determined at t = 0 was recovered from the soil samples. The amount of test substance in the soil samples relative to applied varied from 68 to 96 %. The recovery of the [^{14}C]glyphosate after combustion of the soil samples varies and is better than following extraction. Directly after application recoveries of 97 % were found. However, if the soil sample is combusted after storage, the recovery is not quantitative anymore. In a third experiment, the recovery after a storage period of three days was only 80 %. A possible explanation for the incomplete combustion after storage is very strong binding of glyphosate to soil, even under the combustion conditions. Overall, based on these results, it is concluded that less than 20 % of the test substance evaporates from the soil samples within 24 hours under the conditions of the test.

After 24 hours, 103 ± 1 % of the glyphosate relative to the amount determined at t=0 was recovered from the plant surface. The amount of test substance on the plant samples relative to applied (nominal value) varied from 89 to 96 %. Based on these results it is concluded that less than 20 % of the test substance evaporates from the plant leaves within 24 hours under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was conducted in accordance with the guideline relevant at that time.
The methodology is to measure the loss of glyphosate from soil and plants indirectly through extraction/combustion. For the two types of experiments, only the recovery relative to time zero is considered applicable as the recoveries relative to nominal application rate were already below 100 % at time zero for all relevant experiments.

For the plant experiment, after 24 hours complete recovery was achieved, which allows to conclude that volatilisation of glyphosate from plants was negligible. For the soil experiment, recovery after 24 hours was 82 %, but bacause the report indicated that recovery from extraction and combustion was not quantitative, and no measurements of volatiles were conducted, it could not be demonstrated that the difference in recovery was caused by volatilised glyphosate.

In conclusion, the results from the plant experiment are considered supporting information while the results from the soil experiment are considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.3.1/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate: Determination of volatilisation - Field study
Report No	PR94/032
Document No	
Guidelines followed in study	BBA Guideline, part IV, 6-1 "Analysenmethoden zur Bestimmung von Pflanzenschutzmittelrückständen in der Luft"; Nachrichtenblatt Deutscher Pflanzenschutzdienst, 46, 1994, 60-61
Deviations from current test guideline	No current guideline in force
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

Data point:	CA 7.3.1/005
Report author	[redacted]
Report year	1995
Report title	Final report – About testing volatilization behavior of TAIFUN forte in bush beans under field conditions
Report No	AGR/RV-95/FSG
Document No	
Guidelines followed in study	Guidelines on Producing Pesticides Residue Data from Supervised Trials FAO Rome IVA-Guidelines for residue tests, Section IA and IB, 2nd edition BBA Guidelines Section IV/3-3 BBA Guidelines Section IV/6-1
Deviations from current test guideline	No current guideline in force
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The volatilisation of glyphosate (isopropylamine salt) from bush beans after the application of the formulation Taifun forte was investigated in a field study. The study was performed in August 1995. Taifun forte was applied once at a rate of 5 L/ha under normal agricultural practice conditions. Weather data were recorded continuously during the whole experiment in two heights (0.5 and 1.5 m) above the plants. Air samples were collected at certain time intervals. Furthermore 24-hour samples (cumulative samples) were collected. For air sampling the same heights were chosen as for recording the weather data. Additionally

also plant samples were analysed. No glyphosate was measurable in the air samples after the application of Taifun forte in this field study. Neither in the first 2-hour samples nor in the cumulative 24-hour samples glyphosate was determined.

The concentration in the measured plant samples is constant within the first 4 hours after application. Then the concentration in the plants decreases rapidly. Obviously this decrease is due to uptake and / or metabolism in the plants. In the case of glyphosate no conclusion can be drawn from the plant measurements (indirect method), because glyphosate in plants is not stable within the time scale of the test. Only the measurements of air samples (direct method) can be taken to receive results.

No volatilisation of glyphosate was observed after the application of Taifun forte in this field study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine) as isopropylamine salt
Tested formulation:	TAIFUN forte
Lot No.:	0395040
Nominal concentration:	360 g/L glyphosate

B. STUDY DESIGN

1. Test sites

The test field site was located in Germany. The test substance Taifun forte was applied once only at growth stage ES 75-77 of the bush beans. The field size was 1600 m^2. The test substance was applied to bush beans which had a height of approximately 0.5 m. The bush beans did cover the field tightly. Weather data were logged by a mobile weather station directly placed in the field. Characteristics of the trial location are summarised in the table below.

Table 7.3.1-10: Trial location

Location	47574 Goch, Germany
Soil type	Sandy loam
pH	6.5
OC (%)	1.9
OM (%)[1]	3.3.
Depth of topsoil (m)	0.3

[1] Calculated from organic carbon according to OM = OC / 0.58

2. Application

Application was performed on 9 August 1995 with an application rate of 5.0 L/ha corresponding to 1794.58 g a.i./ha with a hardy trailed sprayer.

3. Sampling

Residue plant specimens were taken from treated plots before application, directly after treatment and at the time intervals 1, 2, 4, 8 and 24 hours after application. Air samples were collected in the middle of the field in two heights of 0.5 and 1.5 m above the plants.

4. Specimen handling and preparation

The specimens were frozen within 15 min after taking at a temperature ≤ 20 °C and transported in thermos containers to the test facility. Before analysis the samples were crushed and homogenised.

5. Analytical methods

Air samples:
The air sample was sucked through a gas washing bottle which is filled with 50 mL water serving as adsorbent. After the enrichment, internal standard was added. Furthermore the sample was acidified with phosphoric acid evaporated to dryness. Then derivatisation was performed using trifluoroacetic anhydrid and trifluoroethanol. The sample was cleaned using HPLC-clean-up. The HPLC fraction was diluted with water and concentrated using a RP 18-cartridge. Finally the sample was eluted from the cartridge using acetic ester. The determination of the substance was performed using GC-MS.

For method validation recovery tests were performed. Mean recovery of all performed recovery experiments was 89 % (± 13.6 %). The limit of quantification (LOQ) was set at 20 ng/sample which corresponds to about double the limit of detection (LOD) of 10 ng/sample.

Plant samples:
The plant specimens were extracted with water under addition of hydrochloric acid. The extract was filtered and brought to a defined volume. Isotope marked standards were added to an aliquot of the extract. The extract first was cleaned by means of charcoal, following a clean up step using an anion exchanger. The eluate of the ion exchanger was derivatised with trifluoroacetic anhydrid and trifluoroethanol. Finally the derivatised sample was cleaned by liquid / liquid partition. Quantitative determination was performed by GC-ECD.

The analytical method was validated by suitable fortification experiments. The fortification experiments performed at levels of 10 mg/kg and 200 mg/kg and the overall mean recovery of glyphosate was found to be 117 % (± 9 6 %). The limit of determination was set at 10 mg/kg corresponding to the limit of detection (LOD) of 0.1 mg/kg.

II. RESULTS AND DISCUSSION

A. DATA
Results of air and plant samples are summarised in Table 7.3.1-11 to Table 7.3.1-12.

Table 7.3.1-11: Results of air analysis

Sample (h) / height (m)	Determined value (ng/sample)
0 / 0.5	< 10
0 / 1.5	< 10
Sample 1[1] / 0.5	< 10
Sample 1[1] / 1.5	< 10
24 / 0.5	< 10
24 / 0.5	< 10
24 / 1.5	< 10
24 / 1.5	< 10

[1] First samples taken after application

Table 7.3.1-12: Results of plant analysis

Sample (h)	Determined value mg/kg
-1	< 1
0	363
1	351
2	329
4	348
8	272
24	174

B. CHARACTERISATION OF RESIDUES

No glyphosate was measurable in the air samples after the application of Taifun forte to the bush beans. Neither in the first 2 hour samples nor in the cumulative 24 hour samples glyphosate was determined.

The concentration in the measured plant samples is constant within the first 4 hours after application. Then the concentration in the plants decreases rapidly. Obviously this decrease is due to uptake and / or metabolism in the plants, in the case of glyphosate no conclusion can be drawn from the plant measurements (indirect method), because glyphosate in plants is not stable within the time scale of the test. Only the measurements of air samples (direct method) can be taken to receive results on volatilisation effects.

III. CONCLUSIONS

No volatilisation of glyphosate was observed after the application of Taifun forte in field conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is considered valid to conclude on no volatilisation of glyphosate in the field.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.3.1/006
Report author	[redacted]
Report year	1993
Report title	Determination of the volatilization of Glyphosate 360 SL from soil and plants
Report No	BE-EA-149-92-01-VOL-1
Document No	
Guidelines followed in study	Richtlinie Teil IV, 6-1 Biologische Bundesanstalt für Land- und Forstwirtschaft der Bundesrepublik Deutschland, "Prufung des Verfluchtigungsverhaltens und des Verbleibs von Pflanzenschutzmitteln in der Luft",
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No information available
Short description of study design and observations:	Volatilization of the formulation Glyphosate 360 Sl from soil and French beans (Phaseolus vulgaris var. nanus) was determined in an open test chamber for 24 h in a temperature controlled water bath. Soil Experiment: Soil characteristics (standard soil Speyer 2.1): Soil type: sand Clay: 3.5 % Silt: 9.1 % Sand: 87.4 % Organic carbon: 0.7 % pH: 5.9 (medium not stated)

Test system: 125 mL crystallizing glass dish; filled with soil adjusted to 60 % of the maximum water capacity using demineralized water
Application: ^{14}C-glyphosate as formulated solution (Glyfos); application at a field rate of 3.6 kg as/ha in 400 L/ha water to soil surface
CO_2: none
Organic volatiles: none
Sampling: immediately before and after treatment and after 1,3,6 and 24 hours
Work up: extraction of soil with sodium hydroxide solution
Analysis of radioactivity:
Soil extract: LSC
Soil residues: combustion/LSC

Study conditions monitored during study: air temperature, Soil temperature (20 +/- 2 °C), relative air humidity (30-50 %), soil moisture (60 +/- 20 % of the maximum water holding capacity during the test) and velocity of the wind speed above the volatilization surface (1 m/sec)

Plant Experiment:
Application: ^{14}C-glyphosate as formulated solution (Glyfos); application at a field rate of 3.6 kg as/ha in 400 L/ha water to surface of French beans
CO_2: none
Organic volatiles: none
Sampling: immediately before and after treatment and after 1,3,6 and 24 hours
Work up: plant samples were stored in water
Analysis of radioactivity:
Water: LSC
Plant residues: combustion/LSC

For volatilisation from plants the surface of French beans was treated with Glyphosate 360 Sl. Plants were maintained with an air temperature of 15 and 24 °C and humidity between 43 and 65 % with a photoperiod of 14 hours of light an 10 hours of dark. Water was added as needed for optimum plant growth.

Short description of results:

Soil Experiment:
Total recovery of radioactivity: 94.7-98.1 %
Radioactivity recovered from soil:
0 DAT: 95.3 % AR
1 DAT: 98.1 % AR
3 DAT: 94.7 % AR
6 DAT: 95.7 % AR
24 DAT: 95.4 % AR

Plant Experiment:
Recovery of radioactivity: 96.5-102.5 %
Radioactivity recovered from plant:
0 DAT: 98.6 % AR
1 DAT: 98.2 % AR

	3 DAT: 97.8 % AR 6 DAT: 96.5 % AR 24 DAT: 102.5 % AR No significant differences were seen for both the soil and plants when measured at the beginning and after the 24 hours exposure period. Thus, no volatilisation from soil and French bean was observed at room temperature within 24 hours.
Reasons why the study is not considered relevant/reliable or not considered as key study:	The study was accepted in the glyphosate Monograph (2000). As the notifier has no access to the study report, the summary was compiled from information available in the Monograph. The results are conclusive, therefore, the study is considered supportive.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 7.3.1/007
Report author	[redacted]
Report year	1992
Report title	Glyphosate-trimesium: Volatilization from soil and leaf surfaces
Report No	RJ1237B
Document No	
Guidelines followed in study	None
Deviations from current test guideline	No current guideline in force
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary

[^{14}C]glyphosate-trimesium, separately radiolabelled in the anionic and cationic positions, was applied to soil (Speyer 2.1) and leaf (Dwarf French Bean) surfaces. Application rates were 3626 g a.s./ha for the anionic soil study and 2836 g a.s./ha for the anionic leaf study. Information on the tests with the label on the cationic position refers to trimesium which is not relevant for current submission.

The treated soil pots were maintained in a constant air stream of > 1 m/s for 24 hours and individual plots analysed at regular intervals (0, 1, 3, 5.5, and 24 hours after application). The treated plants were maintained in a constant air stream of >2 m/s for 24 hours and samples analysed at regular intervals. The air temperature and relative humidity were monitored throughout the 24 hour period of each experiment. The degree of volatilisation at each time point was determined by calculation of the difference in residual activity from the activity in the zero time samples.

For glyphosate (PMG) in soil and leaf, the final radioactive recoveries were 94.2 % and 104.1 % of the applied radioactivity after 24 hours, respectively.

The results obtained in the study showed that glyphosate did not significantly volatilise from either soil or leaf surfaces (i.e. <10 % volatilisation after 24 hours).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Radiolabelled

Identification:	[^{14}C]glyphosate-trimesium (labelled in the anionic position) (PMG)
Lot No.:	91-J19
Specific activity:	2.070 GBq/mmol
Radiochemical purity:	98.3 % determined before start

Identification:	[^{14}C]glyphosate-trimesium (labelled in the cationic position) (TMS)
Lot No.:	91-70
Specific activity:	2.020 GBq/mmol
Radiochemical purity:	92.2 % determined before start

Unlabelled

Identification:	N-phosphonomethylglycine trimethylsulphonium salt
Lot No.:	ICIA0224

2. Soil:

The soil was received from LUFA (type Speyer 2.1) on 22 May 1995. After receipt, the soil was stored in the designated plots. Before use, the soil was sieved through a 2 mm analytical sieve. Before the start of the test, the soil was adjusted to approximately 60 % of the maximum water capacity. Soil properties are not indicated.

3. Plant:

Dwarf French bean leaves from plants at the flowering/first fruit stage were used, obtained from ICI Agrochemicals.

B. STUDY DESIGN

1. Experimental conditions

Soil Experiment

Ten treated soil pots were placed at the edge of a fume cupboard. The sash was adjusted such that the air flow over the soil surfaces was > 1 m/s. To maintain the moisture content of the soil, deionised water was pumped continuously into the soil pots using a peristaltic pump. Variations in the moisture content observed at each sampling interval were counteracted by changing the pumping rate. The moisture content of the soil samples was determined at each sampling interval. At sampling the soil pots were weighed. This weight

was then compared with the initial weight (at 60 % MWHC) and the moisture content was recalculated as a percentage of its MWHC.

Plant Experiment
Ten to twelve leaves, from plants in the same pot, were treated as above. The remainder of the leaves were removed and discarded. The plants were then transferred to a glasshouse where they were placed in front of an electric fan, the position of which (relative to the plants) was adjusted to deliver a wind speed ≥ 2 m/s around the plants.

The final application rates for the glyphosate labelled [^{14}C]glyphosate-trimesium were 3626 g a.s./ha for the soil study and 2836 g a.s./ha for the leaf study.

2. Sampling
Duplicate soil pots or individual leaves were removed at 0, 1, 3, 5.5 and 24 hours from the initiation of the air flow, for quantification. Samples t=0 were taken before the air flow was applied and temperature, relative humidity and air flow were logged.

3. Analytical procedure
The soil was quantitatively transferred into glass jars. The soil was then ultrasonicated with ca. 150 mL of acetonitrile for ca. 20 minutes. The extract was then separated from the debris by filtration under vacuum.

The individual leaves were macerated in the presence of ca. 50 mL of acetonitrile. The extract was separated by filtration under vacuum.

The amounts of radioactivity contained in extract and debris were measured using liquid scintillating counting (LSC and sample oxidation/LSC, respectively. LOD and LOQ were not indicated.

II. RESULTS AND DISCUSSION

A. DATA
Results of the determination of the volatility from soils and plants are presented in the following tables.

Table 7.3.1-13: Test concentrations and radioactivity measurements for the anion labelled soil volatility study

Time Interval (h)	Air Speed (m/s)	MWHC (%)	Activity Extracted (Bq)	Activity bound (Bq)	Total activity (Bq)	Mean activity (Bq)	% of 0 h samples
0 **0**	-[1]	-[2]	0.0 0.0	5440.3 5288.0[3]	5440.3 5288.0	5364.2	100.0
1 **1**	1.2	58.9 58.9	0.0 0.0	5214.9 5363.9	5214.9 5363.9	5289.4	98.6
1 **1**	1.3	57.9 58.0	0.0 0.0	4730.3 5058.5	4730.3 5058.5	4894.4	91.2
5.5 **5.5**	1.3	56.9 57.3	0.0 0.0	5022.8 5506.6	5022.8 5506.6	5264.7	98.1
24 **24**	1.3	56.1 56.2	0.2[4] 0.0	4873.1 5227.6	4873.3 5227.6	5050.5	94.2

[1] The 0 h samples were taken before the air flow was applied to the soil pots.
[2] The 0 h moisture content was taken to be 60 % MWHC, as prepared.
[3] This figure represents half of the activity recovered as this soil pot was treated twice.
[4] Value was corrected by the sponsor

Table 7.3.1-14: Test concentrations and radioactivity measurements for the anion labelled leaves volatility study

Time Interval (h)	Air Speed (m/s)	Activity Extracted (Bq)	Activity bound (Bq)	Total activity (Bq)	Mean activity (Bq)	% of 0 h samples
0 **0**	-[1]	0.0 0.0	4217.9 4196.0	4217.9 4196.0	4207	100.0
1 **1**	2.3	0.0 0.0	3926.4 4493.3	3926.4 4493.3	4210.1	100.1
1 **1**	2.6	0.0 0.0	4588.7 3651.9	4588.7 3651.9	4120.3	97.9
5.5 **5.5**	2.4	0.0 0.0	3911.1 4282.1	3911.1 4282.1	4096.6	97.4
24 **24**	2.2	0.0 0.0	4167.1 4589.0	4167.1 4589.0	4378.1	104.1

[1] The 0 h samples were taken before the air flow was applied to the leaves.

B. EXTRACTABLE AND NON_EXTRACTABLE RESIDUES

After 24 hours, 94.2 % relative to t = 0 were recovered from the soils treated with glyphosate labelled test material. After 24 hours 104.1 % relative to t = 0 were recovered from leaves treated with test material labelled in the glyphosate part. Based on these results, it is concluded that no significant amounts of the test substance evaporate from the soil and plant leaves within 24 hours under the conditions of the test.

III. CONCLUSIONS

The results obtained in the study show that glyphosate does not volatilise from soil or leaf surfaces to any significant extent (i.e. < 10 % volatilisation after 24 hours). The indirect method and variability in recoveries does not allow to exactly quantify volatilisation.

For the glyphosate soil and leaf studies, the final radioactive recoveries were 94.2 % and 104.1 % of the applied radioactivity after 24 hours, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Although no specific guideline is followed in the study, methods and results are sufficiently described and conclusive. Therefore, the study is considered as supportive information. The results obtained in the study show that glyphosate does not volatilise from soil or leaf surfaces to any significant extent (i.e. <10 % volatilisation after 24 hours).

Assessment and conclusion by RMS:

Relevant articles from literature search

1. Information on the study

Data point:	CA 7.3.1/008
Report author	Bento, C.P.M. *et al.*
Report year	2017
Report title	Glyphosate and AMPA distribution in wind-eroded sediment derived from loess soil
Report No	DOI 10.1016/j.envpol.2016.11.033 E-ISSN 1873-6424
Document No	
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Glyphosate is one of the most used herbicides in agricultural lands worldwide. Wind-eroded sediment and dust, as an environmental transport pathway of glyphosate and of its main metabolite aminomethylphosphonic acid (AMPA), can result in environmental- and human exposure far beyond the agricultural areas where it has been applied. Therefore, special attention is required to the airborne transport of glyphosate and AMPA. In this study, the behaviour of glyphosate and AMPA in wind-eroded sediment was investigated by measuring their content in different size fractions (median diameters between 715 and 8 μm) of a loess soil, during a period of 28 days after glyphosate application. Granulometrical extraction was done using a wind tunnel and a Soil Fine Particle Extractor. Extractions were conducted on days 0, 3, 7, 14, 21 and 28 after glyphosate application. Results indicated that glyphosate and AMPA contents were significantly higher in the finest particle fractions (median diameters between 8 and 18 μm), and lowered significantly with the increase in particle size. However, their content remained constant when aggregates were present in the sample. Glyphosate and AMPA contents correlated positively with clay, organic matter, and silt content. The dissipation of glyphosate over time was very low, which was most probably due to the low soil moisture content of the sediment. Consequently, the formation of AMPA was also very low. The low dissipation of glyphosate in our study indicates that the risk of glyphosate transport in dry sediment to off-target areas by wind can be very high. The highest glyphosate and AMPA contents were found in the smallest soil fractions (PM10 and less), which are easily inhaled and, therefore, contribute to human exposure.

Materials & Methods

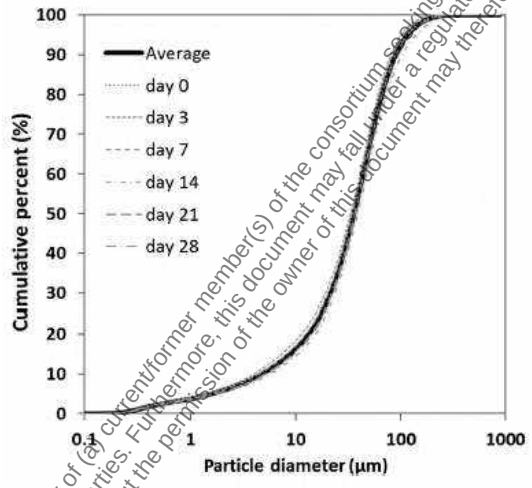
Soil

We used the topsoil of a silty loam loess soil from Huldenberg, Belgium. The soil was air-dried and then sieved through a 1-mm sieve. It was tested for glyphosate and AMPA residues and found free of glyphosate and AMPA. The main soil properties of the sieved soil are shown in the table below. Figure 7.3.1-1 shows the grain size distribution of the soil after disintegration of all aggregates.

Table 7.3.1-15: Soil properties of the loess soil used in this study

Parameters	Value
Particle size distribution:	
<2 µm (clay) (%)	10
2–50 µm (silt) (%)	79
>50 µm (sand) (%)	11
pH $CaCl_2$	5.8
Organic matter (OM) (%)	3.2
Particle density (g cm^{-3})	2.5
N total (g kg^{-1})	1.7
P available (mg kg^{-1})	0.4
K available (mg kg^{-1})	209
Mg available (mg kg^{-1})	121
Na available (mg kg^{-1})	10
C/N ratio	9

Figure 7.3.1-1: Particle size distribution of the start soil after disintegration of all aggregates



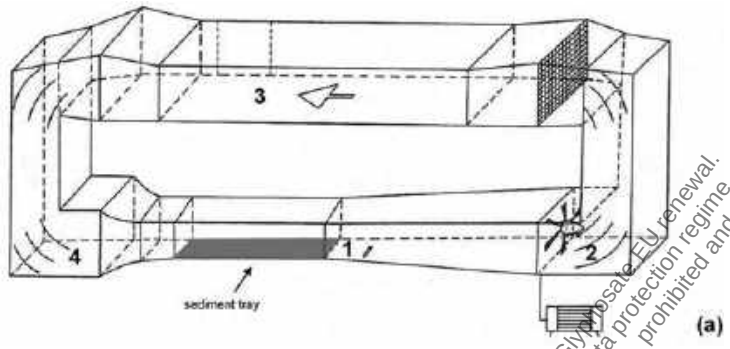
Glyphosate preparation and application in the soil
Preparation of glyphosate solution
Glyphosate solution was prepared by diluting 980 mL of CLINIC®, a glyphosate-based herbicide that contains 360 g/L of glyphosate, in Millipore water to achieve a final stock solution of 0.42 g/L. A concentration of glyphosate in soil of 8.4 mg/kg was used in this study, which corresponds to an application rate of 1.26 kg a.i./ha (typically applied in agricultural fields), assuming a soil depth of 1 cm and a bulk density of 1.5 g/cm^3.

Application in soil
A plastic sheet was put on the ground and an approximately 5-cm thin layer of the air-dried and sieved soil (42 kg) was spread on it. The soil was then sprayed with the prepared glyphosate solution. During the application the soil was thoroughly mixed with a rake. The soil was then stored in a plastic bag at room temperature (22 ºC) and dark conditions. A small portion of the soil was collected after glyphosate application and oven-dried (105 ºC) for 24 h to determine the initial soil moisture content, which was found to be 5.4 % (w/w).

Facilities and instrumentation
The experiment was carried out in the facilities of the Geography and Tourism Research Group of the Katholieke Universiteit Leuven, Belgium. A closed-return wind tunnel was used. The tunnel has two test sections, both of which were used in this study. The dimensions of the large test section are 760 cm (length) × 120 cm (width) × 60 cm (height), and those of the small test section are 150 cm (length) × 35 cm (width)

× 30 cm (height). A detailed description of the wind tunnel can be found in the technical report by Goossens and Offer (1988).

Figure 7.3.1-2: Leuven wind tunnel, with locations of the sampling sites 1 to 4



Apart from the wind tunnel, a modified version of the Soil Fine Particle Extractor developed in a previous study by Goossens (2012) was used. This instrument draws up the sediment, previously spread on a table, with a plastic hose attached to a BASE 440 three-engine vacuum cleaner connected to a cyclone dust separator (RIBO, Villanova, Italy). The hose is 300 cm long and 4 cm in diameter; the separator is 70 cm high and 40 cm in diameter. Coarse particles settle in the separator and are thus removed from the sample. Separation is accomplished by the circular motion of the particles and enhanced by selective gravitational settling. Some of the smallest particles remain suspended in the separator. After initial separation in the separator, the dust enters a tube 139 cm long and 16 cm in diameter, which operates as an elutriator. Dust is then accelerated through a small pipe 36 cm long and 7.6 cm in diameter and hits an impactor (diameter: 8.7 cm) installed near the bottom of a settling chamber. Only the finest particles will suspend in the chamber. These particles then enter a 200-cm long plastic tube. Further granulometrical separation is performed in this tube, which operates as a second elutriator. Particles then enter the vacuum cleaner and settle in a 50-L deposition chamber, where they can be collected. Three 1200-W engines that generate a suction rate up to 510 m^3/h and create an under pressure of 2200-mm H_2O power the instrument. For this study, only one engine (170 m^3/h) was used.

Experimental design

To perform each experimental run, a total of 8 kg of pre-treated soil (enough to fill the sediment tray in the wind tunnel) was taken one day before each experimental run. The soil was then oven-dried at 37.5°C for 24 h to ensure a soil moisture 2 % (the highest soil moisture allowed to guarantee wind erosion; see Nourzadeh *et al.* (2013)). Soil samples (in duplicate) were always taken before and after the drying process to control for any effect on glyphosate decay and AMPA formation/decay. The oven-dried soil was then subjected to wind erosion in the wind tunnel. In the small test section, a tray 150 cm long x 35 cm wide x 2 cm deep was installed. The upwind 75 cm were filled with a piece of wood; the downwind 75 cm were covered with a thin sheet of plastic (to avoid direct contact between the glyphosate-treated soil and the metal of the tray). The oven-dried soil was then put into the tray. Its surface was carefully flattened using a slat. The wind tunnel was then closed and turned on to allow the soil sediment to erode until the entire tray was empty. We used a free-stream wind speed of 10.0 m/s, which was well above the deflation threshold of the sediment used (6.5 m/s according to visual observations made before the test). It took approximately 1 h until the tray was empty. After each run, sediment samples (in triplicate) were collected (≥2 g for most of the samples; and always ≥1 g) at four different places in the wind tunnel using a clean brush. The distances from the trailing edge of the tray were as follows: sample 1: 10 cm; sample 2: 480 cm, sample 3: 1290 cm, and sample 4: 1865 cm. Due to aeolian selection, the samples become finer as they are taken further from the source. Because of the restricted length of the wind tunnel, sample 4 was the finest sample that could be obtained with the wind tunnel technique. To collect even finer samples, the Soil Fine Particle Extractor was used and three more samples were collected. After each wind tunnel run, the tunnel was first thoroughly cleaned with the vacuum cleaner. A sample (sample 7) was then taken from the deposition chamber of the vacuum cleaner, which at this stage was directly connected to the cyclone

separator. The sediment in the separator was then mixed with the remaining dust in the deposition chamber and put on a clean table. After assembling the entire Soil Fine Particle Extractor, the sediment on the table was sucked up and samples 5 and 6 were collected just downwind from the cyclone separator (sample 5) and in the deposition chamber of the vacuum cleaner (sample 6). All experimental runs (wind tunnel + Soil Fine Particle Extractor) and collection of samples were conducted on days 0, 3, 7, 14, 21 and 28 after glyphosate application. All samples were stored in plastic tubes and frozen at -18°C until glyphosate and AMPA analysis.

Particle size distribution and organic matter content
To analyse the particle size distribution of samples 2 to 7, a Malvern Mastersizer S laser particle size analyser (Malvern Ltd, Malvern, UK) was used. Sample 1, which exclusively consisted of large aggregates, was analysed optically with a microscope. For the latter sample, a subsample from the main sample and measured the nominal diameter of all aggregates was collected. Using these data, the aggregate size distribution of the sample could be determined. To get an idea of the internal particle size distribution of the large aggregates themselves, also several of these aggregates were collected, carefully crushed and dispersed, and then analysed with the Mastersizer instrument. The OM content was estimated by oxidation at 600ºC and detected by close infra-red using a SC-144DR equipment (LECO Corporation, St Joseph, MI, USA). When there was insufficient sample for analysis, the triplicates were mixed together.

Glyphosate and AMPA content
Glyphosate and AMPA contents in the samples were analysed as described by Bento *et al.* (2016). Briefly, glyphosate and AMPA were extracted from 1 g of soil or wind-eroded sediment with 5 mL of 0.6 M KOH (potassium hydroxide, p.a. 85 %). After shaking and centrifuging the samples, 1 mL of the supernatant was transferred to a 10-mL plastic tube. Isotopically labelled glyphosate and AMPA were added at this point and then a derivatisation step was carried out with FMOC to improve retention and MS/MS detection as described by Bento *et al.* (2016). Solvent standards with isotopically labelled internal standards were prepared together with all the samples for each batch of samples, and derivatized the same way. Glyphosate and AMPA contents were then determined by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using an XBridge™ Shield RP C18 column 100 mm x 2.1 mm i.d. (Aquity UPLC I-Class coupled to a Micromass Ultima triple-quadrople MS, Waters, The Netherlands). Chemicals used, mobile phases and instrumentation conditions of the HPLC-MS/MS were as described by Yang *et al.* (2015b) and Bento *et al.* (2016). With each batch of samples, two blank soil samples of the loess soil used in this study were fortified at 0.5 mg/kg and added as quality control (QC) samples. To ensure the quality of the analysis when processing real samples, the fortified samples were analysed twice, at the beginning and at the end of each batch. The quantification of the sample batch was considered satisfactory when the QC recoveries were between 70 and 120 %. A detailed description of the method validation and quality control can be found in Bento *et al.* (2016).

Statistical analysis
All statistical analyses were performed in SPSS 22, and the graphs in Figure 7.3.1-5 were produced in SigmaPlot 10.0. A one-way ANOVA to ln-transformed data followed by Dunett T3 post-hoc tests was performed to test for significant ($p < 0.05$) differences in clay, silt or organic matter (OM) content between extracted size fractions of the wind-eroded sediment. Besides, a power function was applied to the non-aggregated samples (sample 3-7) to test the correlation between the clay or OM content and the particle size of the samples. To test for significant differences of glyphosate or AMPA residues between extracted size fractions of the wind-eroded sediment, an analysis of covariance (ANCOVA) to ln-transformed data followed by Bonferroni tests was performed ($p < 0.05$). The assumption of homogeneity of regression slopes was not violated. Moreover, a categorical principal components analysis (non-linear PCA) was performed to determine the relationship between sediment properties (clay, silt, OM) and glyphosate or AMPA content in the wind-eroded sediment. The loading of a given variable was considered meaningful if its absolute value was ≥0.40 for a given component. Besides, a Pearson correlation was computed to assess the relationship between glyphosate or AMPA contents and clay, silt or OM. A reconstruction of the distribution of glyphosate in the original soil in the sediment tray before the start of each wind tunnel experiment was also performed. This was done by considering the glyphosate content for a large number of narrow grain size classes, which could be estimated by applying an exponential regression analysis to the data (only the samples without aggregates, i.e., samples 3-7).

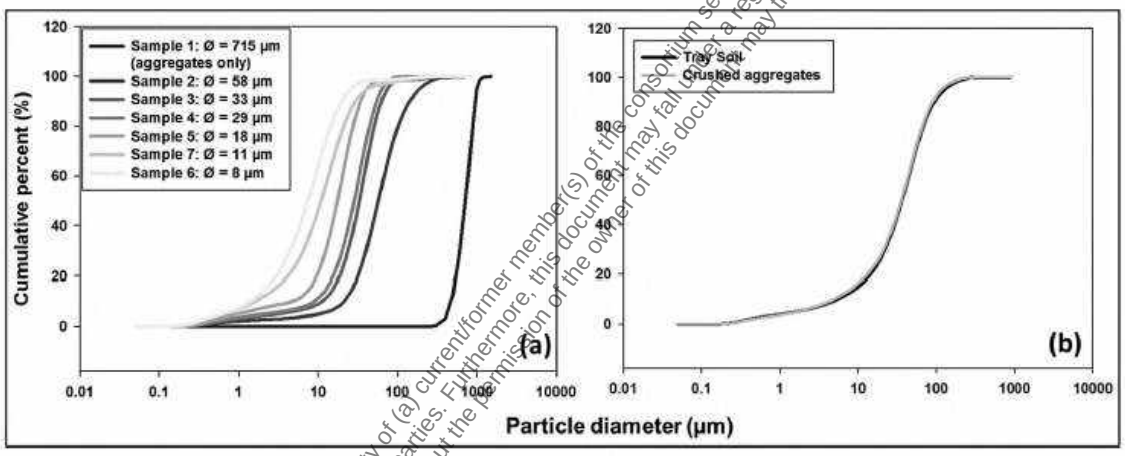
Results & Discussion

Physico-chemical composition of the wind-eroded sediment

Particle size distribution

The particle size distribution of the different extracted fractions of the wind-eroded sediment is shown in Figure 7.3.1-3. Sample 1 was composed of large, macroscopic aggregates only. Sample 2 consisted of individual grains and micro-aggregates, mixed with a few macroscopic aggregates. Samples 3-7 only contained individual grains with some small micro-aggregates (as verified under the microscope) and were mostly composed of particles ≤100 µm in diameter. More than 96 % of the particles of samples 5-7 were ≤50 µm in diameter. The median diameters of the samples were: 715 ± 69 µm (sample 1), 58 ± 2 µm (sample 2), 33 ± 1 µm (sample 3), 29 ± 1 µm (sample 4), 18 ± 1 µm (sample 5), 8 ± 1 µm (sample 6) and 11 ± 3 µm (sample 7). These median diameters are further used as reference codes in the data analysis presented here.

Figure 7.3.1-3: Particle size distribution of (a) the different extracted fractions of wind-eroded sediment; (b) the crushed aggregates and the original sediment in the sample tray. Ø = median diameter

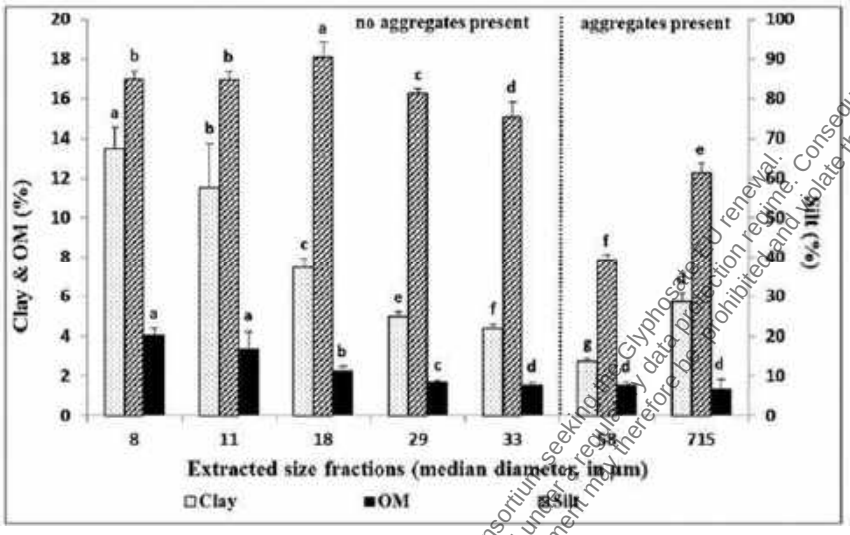


Crushing of the macroscopic aggregates (sample 1) and analysing their grain size distribution showed that the aggregates are perfect compositions of the original tray sediment (Figure 7.3.1-3), with a median particle diameter of 36 ± 2 µm for both the aggregates and the original tray soil.

Clay, silt and OM content

The clay (<2 µm), silt (2-50 µm) and OM content of the different extracted fractions of the wind-eroded sediment are shown in Figure 7.3.1-4. The clay content was significantly higher for the finest extracted size fraction (median diameter of 8 µm) and lowered significantly with increasing particle size (Figure 7.3.1-4), except for the samples with a 715-µm median diameter which consisted exclusively of macroscopic aggregates. A strong negative correlation was also observed between the clay content and the particle size of the non-aggregated samples (median diameters between 8 and 33 µm; Clay (%) = 67.7 $MDES^{-0.78}$, R^2 = 0.99; MDES = median diameter of the extracted sample). Likewise, the OM content was highest for the finest extracted fractions (samples with median diameter of 8 and 11 µm) and lowered significantly with increasing particle size (Figure 7.3.1-4). Nevertheless, this decrease in OM was no longer significant after a particle size ≥33 µm. A strong negative correlation was also observed between the OM content and the particle size of the non-aggregated samples (OM (%) = 13.1 $MDES^{-0.61}$, R^2 = 0.90). All samples were mostly composed of silt (Figure 7.3.1-4). The silt content decreased as the samples became coarser, but to a lower extent compared to clay and OM. In the aggregated samples (median diameters of 58 and 715 µm), the silt content was significantly lower than in the non-aggregated samples.

Figure 7.3.1-4: Clay, silt and organic matter (OM) content of the extracted size fractions. The 715-µm samples consist exclusively of large aggregates. Different lowercase letters within the same type of bars mean significant differences in silt or clay or OM between extracted size fractions (p <0.05)



Glyphosate and AMPA content in the wind-eroded sediment

Relationship between glyphosate or AMPA and particle size

Glyphosate content (Figure 7.3.1-5) varied between 5.5 and 16 µg/g, with a significantly higher content in the finest extracted fractions (median diameters from 8 to 18 µm). AMPA content, on the other hand, was rather low, varying between 0.07 and 0.7 µg/g. Here too, AMPA content was significantly higher in the finest extracted fractions. In Figure 7.3.1-6, the relationship between glyphosate (or AMPA) content and particle size of the wind-eroded sediment is better shown. Here, it is clearly visible that glyphosate and AMPA contents were highest in the finest samples (median diameter: 8 µm) and became lower with increasing particle size until ≈33 µm (Figure 7.3.1-6). Note that this does not necessarily mean that the highest amounts of glyphosate and AMPA in a sample occur in the finest fractions of that sample: the mass of coarse grains is much higher than that of fine grains, so even when the concentration is higher in the fine fractions it is possible that the coarse fractions contain more glyphosate and AMPA in weight. A larger spread was observed for AMPA than for glyphosate (Figure 7.3.1-6). However, this larger spread is not meaningful since it just reflects the increase of AMPA content in the course of time (see Figure 7.3.1-5). For the individual days, the lower AMPA content with increasing particle size became better visible. It also became stronger over time. The effect of the presence of macroscopic aggregates in a sample was also very prominent (Figure 7.3.1-6). Once macroscopic aggregates were present (samples with median diameters of 58 and 715 µm), glyphosate and AMPA contents remained constant regardless of how numerous or how large the aggregates were. This seems to be related with the fact that the aggregates are perfect compositions of the original soil in the sediment tray (Figure 7.3.1-3) regardless of their size. Because, in an aggregate, the largest mass is represented by the coarsest grains, glyphosate and AMPA contents will be rather low, approaching the concentration in the coarsest individual grains, albeit a little higher because of the presence of a higher percentage of fine particles in the aggregates. When comparing the glyphosate content in the different sediment fractions with its content in the parent soil, it was, on average, 1.4 times higher in the finest fractions of the wind-eroded sediment (median diameters between 8 and 18 µm) than in the parent soil. In contrast, the coarsest fractions (median diameters between 29 and 58 µm) had glyphosate contents that were, on average, 1.2 times lower than that in the parent soil. Only the samples entirely composed of macroscopic aggregates (median diameter of 715 µm) matched the glyphosate content of the parent soil, confirming once again that the large aggregates are perfect compositions of the original soil in the sediment tray. Clymo *et al.* (2005) also reported a much higher concentration of the herbicide pendimethalin in the PM2.5 fraction when compared to their field soil, but not for the herbicide metolachlor. According to these

authors, pendimethalin is less volatile than metolachlor and therefore, the former has a higher affinity to the particle phase while the latter has a higher affinity to the gas phase. Glyphosate is also non-volatile and tends to strongly adsorb to soil particles; therefore its preference to the particle phase is also expected.

Figure 7.3.1-5: **Glyphosate (a) and AMPA (b) content in the different extracted size fractions of the wind-eroded sediment during the 28 days after glyphosate application, and respective trend lines. Note the different vertical scales between (a) and (b). To the right of the legends, different lowercase letters mean significant differences in glyphosate (a) and AMPA (b) content between extracted size fractions, using an ANCOVA followed by Bonferroni tests ($p < 0.05$)**

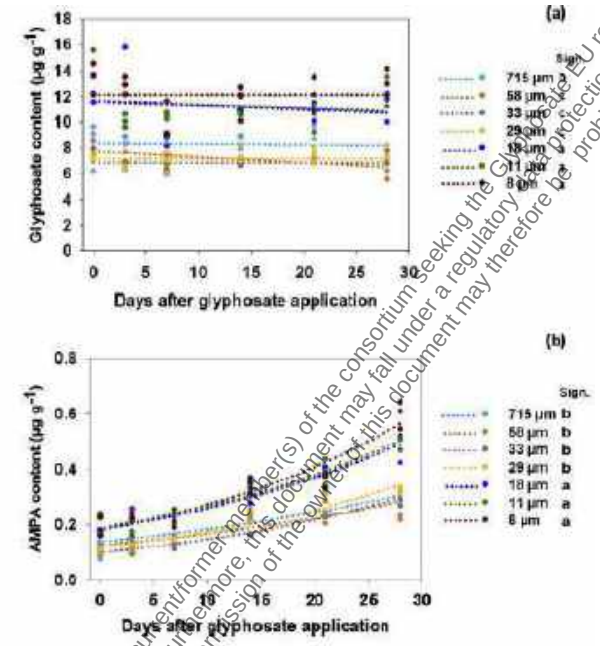
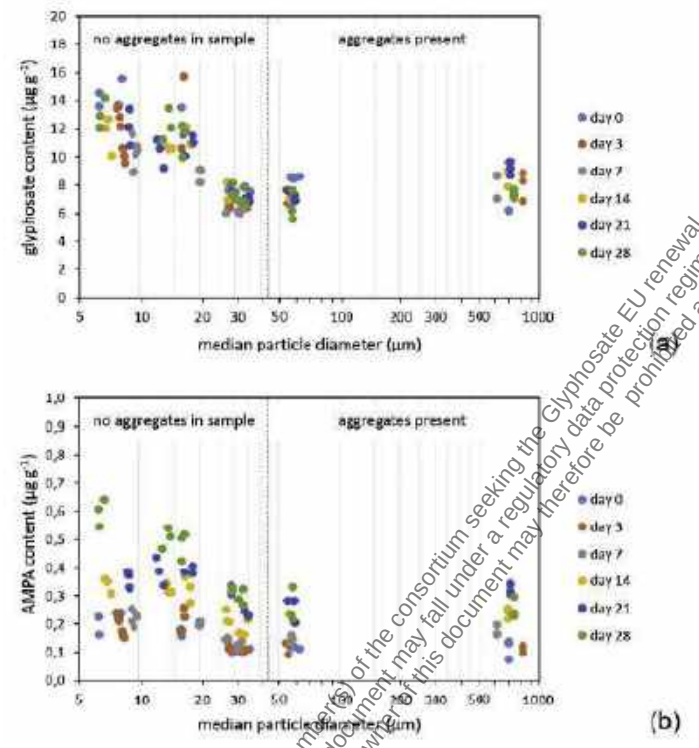


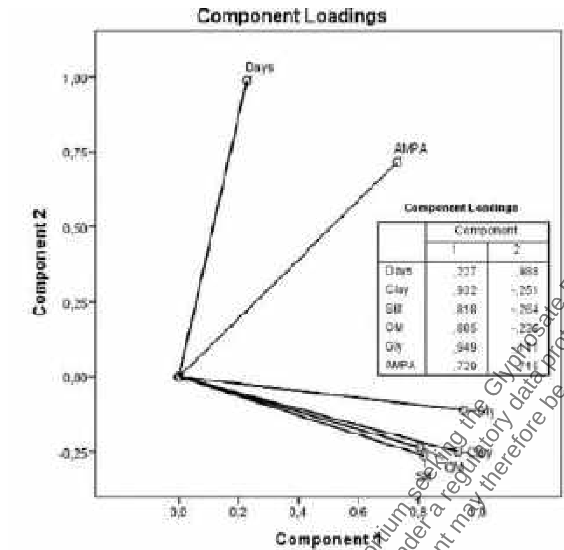
Figure 7.3.1-6: Relationship between (a) glyphosate content and particle size, (b) AMPA content and particle size



Relationship between glyphosate or AMPA and clay, silt and OM

Figure 7.3.1-7 shows the results of the categorical principal components analysis performed to determine the relationship between the studied sediment properties (clay, silt and OM) and glyphosate and AMPA content. The proportion of variance accounted-for by the first component is 61.1 %, whereas the second component accounts for 28.1 %. Thus, the two components together account for a considerable proportion (89.2 %) of the variance. All sediment properties analysed in this study loaded in the first component together with glyphosate and AMPA, whereas only the duration of the experiment (days) loaded in the second component together with AMPA (Figure 7.3.1-7). The studied sediment properties do, therefore, play a major role in adsorbing glyphosate and AMPA. The duration of the experiment, on the other hand, was only meaningful for AMPA.

Figure 7.3.1-7: Categorical principal components analysis (non-linear PCA). Gly = glyphosate; OM = organic matter



The order to which glyphosate and AMPA contents in the wind-eroded sediment are influenced by the studied sediment properties is as follows: clay >OM >silt (Figure 7.3.1-7). Glyphosate content correlates significantly and positively to the clay content ($R^2 = 0.63$, $p < 0.01$). For coarser soil fractions, such as silt, the relationship with glyphosate content is considerably less expressed ($R^2 = 0.27$) but still significant ($p < 0.01$). Significantly positive correlations were also observed between AMPA content and clay ($R^2 = 0.16$, $p < 0.01$), and AMPA content and silt ($R^2 = 0.10$, $p < 0.01$). Organic matter also appears as a strong factor influencing glyphosate adsorption to wind-eroded sediment: glyphosate content correlates significantly and positively to the OM content ($R^2 = 0.49$, $p < 0.01$). However, one should realize that a positive correlation between glyphosate content and OM would be observed anyway because both are a function of particle size (both are higher for smaller particles, see Figure 7.3.1-4). Therefore, the effect of OM on glyphosate adsorption cannot be confirmed with certainty. In summary, these results show that the highest concentrations of glyphosate and AMPA in the finest fractions are related to the higher clay and OM content in these same fractions, although the role of silt cannot be ignored. Sprankle *et al.* (1975) also reported that glyphosate was readily adsorbed to clay and OM, and that less glyphosate was adsorbed by a sandy loam soil than by a clayey loam soil.

Glyphosate and AMPA content through time and consequences for their airborne off-site transport with dust

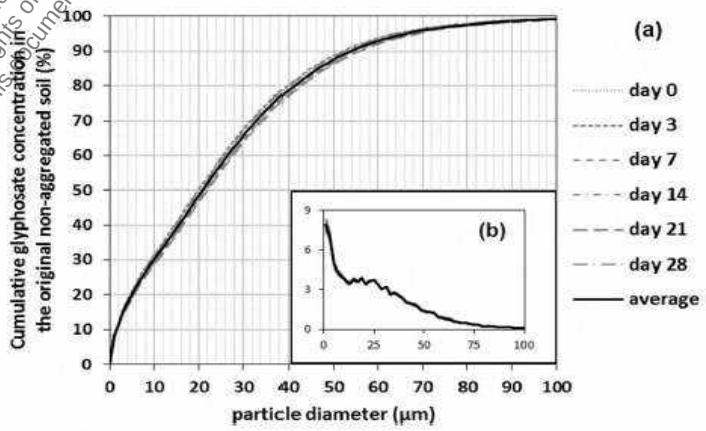
The fact that glyphosate and AMPA contents are highest in the fine fractions of the soil has important consequences for the airborne off-site transport of these compounds, because particles <20 µm have the capacity of being transported in long-term suspension. This can easily be shown by calculating the aeolian threshold for long-term suspension, which, according to the model of Pye and Tsoar (1990), is $u_\infty/u^* < 0.1$, where u_∞ is the terminal fall velocity and u^* the friction velocity. Using this criterion, 20-µm particles are already transported in long-term suspension when $u^* < 0.3$ m/s. Assuming a roughness length z_0 of 3-10 cm (typical value for agricultural areas, depending on the type of crop, see Ramli *et al.* (2009)), this corresponds to a 10-m height wind speed of 3.5-4.4 m/s, which are very typical values for many inland agricultural areas. For 10-µm particles, the critical wind speed is much lower: only 1.2-1.4 m/s (at 10 m height). At these wind speeds, particles are able to travel tens to even several hundreds of km before they settle back to the Earth's surface. During the 4-week experiment, nearly no glyphosate decay took place (Figure 7.3.1-5). Consequently, the formation of AMPA was very slow and remained low during the experimental period. Glyphosate and AMPA decay mostly by microbial activity (Bento *et al.*, 2016; Gimsing *et al.*, 2004; Nomura and Hilton, 1977), and for the latter a minimum soil moisture is required (Bento *et al.*, 2016;

Schroll *et al.*, 2006). In our study, the soil moisture content during storage, after applying glyphosate but before the 24-h drying process prior to each wind tunnel test, was 5.4 %. This soil moisture content revealed to be very low to allow for soil microbial activity and consequent glyphosate decay. Very important in this context is that wind erosion of fine, dusty particles only occurs when the topsoil (and, therefore, also the particles themselves) is sufficiently dry. Nourzadeh *et al.* (2013) tested several types of loamy soils using a field wind tunnel and found that the maximum moisture content to allow wind erosion of these soils was only 2 %, well below the limit for a substantial decay of glyphosate. Besides wind erosion, for many silty soils tillage erosion is a second (and in many cases even more important) mechanism for emission of fine particulates.

For tillage-emitted particles the probability for off-site transport is also highest when the particles are dry. Since the decay of glyphosate in our study occurred already extremely slowly for a soil moisture content of 5.4 %, its decay would be nearly inexistent for such dry wind-eroded sediment. Therefore, if glyphosate is applied during a dry period and emission of fine particles happens thereafter (either by wind erosion if the soil cover is still small, or by tillage activities if there is already some cover), then the potential for airborne glyphosate transport to off-site areas is considerable.

Potential contribution of glyphosate and/or AMPA contaminated airborne dust to human exposure
Figure 7.3.1-8 shows the reconstruction of the distribution of glyphosate in the original non-aggregated soil in the sediment tray before the start of each wind tunnel experiment. As expected, the glyphosate distribution was nearly identical for the six experimental runs, and it was predominantly concentrated in the finest fractions. On average for the six experimental runs, 13 % of the glyphosate in the original soil was concentrated in the PM2.5 fraction (particles <2.5 µm), 15 % in the PM4 fraction, and 28 % in the PM10 fraction. It is currently unknown whether the distribution of glyphosate in Figure 7.3.1-8 also applies to the macroscopic aggregates, but because the aggregates are almost perfect compositions of the original soil in the sediment tray (see Figure 7.3.1-3) the distribution of glyphosate within the aggregates is probably not far off from that shown in Figure 7.3.1-8. For AMPA, 14 % was concentrated in the PM2.5 fraction, 15 % in the PM4 fraction, and 29 % in the PM10 fraction. These results reconfirm that glyphosate and AMPA are considerably susceptible to be transported with airborne dust. After having accomplished their airborne transport trajectory, the glyphosate and/or AMPA containing soil particles will settle to the ground, thereby contaminating the deposition area. When the deposition is induced by rainfall and the particles and the soil become wet, glyphosate and/or AMPA will most probably further decay. When dry deposition occurs and the conditions remain dry for a while, glyphosate may remain in the deposited sediment until the soil becomes wet and the soil microorganisms active.

Figure 7.3.1-8: Calculated cumulative (a) and non-cumulative (b) distribution of glyphosate in the original soil (after destruction of the aggregates) for the six experimental days.



Conclusion
The study indicates that glyphosate and AMPA contents are highest in sediment particles <10 µm (PM10), and that their content diminishes with increasing particle size. The risk of off-site airborne transport of glyphosate and AMPA with dust is, therefore, very high. Because glyphosate and AMPA hardly decay under dry conditions of the soil, this risk is intensified if glyphosate is applied in arid and semi-arid areas or during long periods of draught. If glyphosate and AMPA contaminated PM10 fractions of soil are emitted to the atmosphere, they may be inhaled by humans and animals. This contributes to the risk of human and animal exposure and, therefore, more attention should be paid to this route of exposure in environmental and human health risk assessment studies. Moreover, glyphosate applications during dry periods in regions susceptible to wind erosion should be avoided.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the glyphosate and AMPA distribution in wind-eroded sediment derived from a laboratory wind tunnel experiment with loess soil. The distribution of the substances in different particle size fractions is evaluated. Correlations to different soil parameters are presented. Methods and results are sufficiently described.
The article was seen as reliable.

Assessment and conclusion by RMS:

CA 7.3.2 Transport via air

Based on a Henry´s constant of $<2.21 \times 10^{-8}$ Pa m³/mol and a vapour pressure of 1.31×10^{-5} Pa (25 °C) glyphosate is not expected to volatilise in significant amounts.

Any glyphosate that might enter the atmosphere would not be subject to gas phase transport over large distances, due to rapid indirect photochemical degradation; DT_{50air} =1.6 hours for hydroxyl radical reaction.

Therefore, an experimental study on deposition following volatilisation is not required and was not conducted.

CA 7.3.3 Local and global effects

Due to the negligible volatilisation potential and the fast degradation of glyphosate in air, no significant local and global effects from atmospheric transport of glyphosate are expected.

CA 7.4 Definition of the Residue

CA 7.4.1 Definition of the residue for risk assessment

The proposed residue definitions relevant for risk assessment are the following:

Table 7.4.1-1: Definition of the residue for risk assessment

Compartment	Residue Definition
Soil	Glyphosate
	AMPA
Groundwater	Glyphosate
	AMPA
Surface water	Glyphosate
	AMPA
	HMPA
Sediment	Glyphosate
	AMPA
Air	Glyphosate

CA 7.4.2 Definition of the residue for monitoring

The residue definition for monitoring for compartments soil, water and air is glyhosate only.

CA 7.5 Monitoring Data

For the current approval renewal, there are 10 new applicant studies, 7 existing applicant studies and 75 published peer-reviewed papers (considered reliable) covering the monitoring of glyphosate and its principal metabolite AMPA in soil, groundwater, surface water, transitional water, sediment, drinking water, air and water treatment. Given the number of compartments and the fact that some studies and articles contain data relevant to more than one compartment a matrix summarising all studies and articles presented in each compartment sub-chapter is provided in Table 7.5-3.

For soil, groundwater, surface water, transitional water, drinking water and sediment monitoring, there are two new applicant studies; [redacted] (2020, CA 7.5/001) which describes the collection process of public monitoring data (from regional/national environment agencies) for European countries for glyphosate, AMPA and HMPA and the report by [redacted] (2020, CA 7.5/002) which assesses the data collected by [redacted] (2020, CA 7.5/001). These two recent studies were designed to be the more comprehensive than previous studies by considering additional metabolites, compartments and time periods.

In addition:
For soil monitoring there are a further five published peer-reviewed papers presented.

For groundwater monitoring there are three new applicant studies, four existing studies and twelve published peer-reviewed papers presented.

For surface water monitoring there are seven new applicant studies and forty seven published peer-reviewed papers presented.

For transitional water monitoring there are two new applicant studies.

For drinking water monitoring there is one new applicant study, two existing applicant studies and two published peer-reviewed papers presented.

For sediment monitoring there is one existing applicant study and eight published peer-reviewed papers presented.

For air monitoring, there are no applicant studies. Three published peer-reviewed papers are presented.

For low-chemical water treatment processes, in addition to [redacted]. (2020, CA 7.5/002), there are two existing studies and nine published peer-reviewed papers presented.

For water treatment chemical processes, in addition to [redacted] (2020, CA 7.5/002), there is one existing applicant study and nine published peer-reviewed papers presented.

Headline Results
Concentrations of glyphosate (GLY), AMPA and HMPA arising from public monitoring datasets have been collected from regional/national environment agencies as well as published peer reviewed publications from literature searches and rated as potentially relevant/reliable are reported in this section. This data collection and analysis is extremely comprehensive as it considers a range of environmental compartments and for a number of these compartments evaluates a very large dataset that allows firm conclusions to be drawn.

The studies and publications assessed cover a number of different spatial extents ranging from pan-EU and country, to regional/provincial, and even specific locations/fields. Similarly, they cover a range of temporal scales ranging from a single sampling occasion to multi-monthly and annual sampling schemes. Assessment of rates of compliance with regulatory acceptable concentrations (RAC) and thresholds requires the dataset to be large enough to capture a range of agronomic, geographical, pedoclimatic and hydro(geo)logical situations. A larger sized dataset also ensures that there is good temporal coverage allowing assessment of the state of a compartment in different seasons and hydrological regimes, ranging from, for example,

summer low flows to runoff events. The dataset compiled by [redacted] (2020, CA 7.5/001) comprising 'raw monitoring data from national authorities' and 'aggregated monitoring data from reports published by national authorities' and analysed by [redacted] (2020, CA 7.5/002) best meets this criterium and in addition [redacted] (2020, CA 7.5/002) considers key RACs and thresholds to facilitate analysis within the context of the Water Framework Directive (2000/60/EC) and associated Groundwater Directive (2006/118/EC), Drinking Water Directive (1998/83/EC) and Priority Substances Directive (2008/105/EC28) in addition to the Plant Protection Products Directive (1107/2009/EC).

Table 7.5-1: Summary of minimum reported rates of compliance with regulatory acceptable concentrations (RAC) for measured concentrations of glyphosate (GLY) and AMPA in each environmental compartment

Compartment	Dataset Size	GLY		AMPA	
		RAC[1] (µg/L)	Compliance (%)	RAC[1]/ Threshold (µg/L)	Compliance (%)
Soil	Small	94.6 mg/kg	100	26.4 mg/kg	100
Groundwater	Very Large	0.1	99.38	10.0[2]	99.998
Surface Water	Very Large	400	99.994	1200	99.999
Transitional Water	Very Small	400	100	1200	100
Drinking Water	Large/Very Large	0.1	99.84	0.1[3] 10.0[2]	99.78 100
Sediment	Small/Medium	NA	-	NA	-
Air	Very Small	NA	-	NA	-

NA: Not applicable
[1] Regulatory acceptable concentration
[2] RAC for non-relevant metabolite
[3] Threshold value chosen to allow statistical comparisons only

The rates of compliance with different RACs and thresholds are provided in Table 7.5-1 and maximum reported concentrations in each compartment are summarised in Table 7.5-2. The rates of compliance with key RACs and thresholds for both GLY and AMPA are high (>99.4 % of samples) and in some cases absolute (100 %) with little or no exceedances reported. In all cases, exploration of exceedances indicates that these are sporadic and non-systematic both spatially and temporally. These rates of compliance are high despite many of the small number of exceedances likely being erroneous or anomalous values (in the case of AMPA, also despite this compound being formed from other compounds like detergents which are emitted from point sources).

Table 7.5-2: Summary of reported maximum concentrations of glyphosate (GLY) and AMPA in each environmental compartment

Compound	Maximum Concentration (µg/L unless stated)	
Compartment	GLY	AMPA
Soil	2.05 mg/kg	1.92 mg/kg
Groundwater	1005 39.2[1]	19.0
Surface Water	91600 57.0[1]	230000 224.4[1]
Transitional Water	1.2	0.9
Drinking Water	0.92	3.0
Sediment	2.84 mg/kg <4.0	9.56 mg/kg <4.0
Air	1.04 ng/m^3	<0.28 ng/m^3

[1] Maximum excluding outliers

At first glance several maximum concentrations that exceed RACs or maximum allowable concentration environmental quality standards are reported. However, in large datasets comprising many hundreds of thousands of analytical results a small number of anomalous values are likely and may occur at several points during the sampling, storage and analytical process. A transparent and precautionary data analysis approach retained and contextualised these values, for example, by demonstrating them to be statistical outliers or determining the percentile of the RAC in the distribution of concentration values to demonstrate this is much higher than the 99th percentile value. These observations are supported by publications which demonstrate lower concentrations in point pollution sources or transport pathways, like surface runoff, prior to entering environmental compartments where dilution would be expected, or they demonstrate deficiencies in monitoring networks/data and why caution should be shown when interpreting third party datasets.

It is acknowledged that some exceedances in some compartments may be real and that the monitoring data highlights elevated detection rates in some locations, for example, shallow groundwater in parts of southern Spain. Case studies presented in [redacted] [redacted] (2020, CA 7.5/002) investigating situations where public monitoring suggested elevated rates of detection demonstrate that local factors like open hand dug wells may influence detections of GLY and AMPA in groundwater, and that localised investigations to understand the situation better with a view to adapting local practice through targeted stewardship programs or defining drinking water protection zones around drinking water wells is the most appropriate means of addressing these situations where they arise.

Headline Conclusions

The data presented in this section demonstrate that the environmental concentrations typically encountered in all environmental compartments, likely associated with typical agricultural and urban usage, do not pose a risk for biota and ecosystems and also not to human health *via* drinking water. Safe use is demonstrated for the overwhelming majority of use environments in Europe following the use of GLY products according to the label.

All new applicant studies, existing applicant studies and published peer-reviewed papers that are considered in this monitoring chapter are listed in Table 7.5-3. For each tier summary, the full study summary is cited in the first relevant subchapter, in subsequent relevant subchapters only the study information and conclusion are cited. Except in the case of [redacted] (2020, CA 7.5/001) and [redacted] [redacted] (2020, CA 7.5/002) where a full study summary is cited in all relevant subchapters as each study summary is specific to that subchapter. In addition, Table 7.5-3 shows the relevant subchapter/s for which each study/literature article addresses the fate of glyphosate and or AMPA.

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
A. SOIL											
APPLICANT STUDIES											
New studies/assessments											
CA 7.5/001	[redacted] 2020	Collection of public monitoring data for European countries	Glyphosate AMPA HMPA	Valid	▨	▨	▨	▨	▨		
CA 7.5/002	[redacted] [redacted] [redacted] 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Valid	▨	▨	▨	▨	▨		▨

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters: A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
Existing studies/assessments											
There are no existing applicant monitoring data or studies covering soil.											
RELEVANT LITERATURE ARTICLES											
CA 7.5/003	Karanasios, E. *et al.*, 2018	Monitoring of glyphosate and AMPA in soil samples from two olive cultivation areas in Greece	Glyphosate AMPA	Reliable	■						
CA 7.5/004	Silva *et al.*, 2018	Distribution in European agricultural topsoils	Glyphosate AMPA	Reliable with restrictions	■						
CA 7.5/005	Napoli, M. *et al.*, 2016	A runoff experiment in a vineyard in Italy	Glyphosate AMPA	Reliable	■		■		■		
CA 7.5/006	Székács, A. *et al.*, 2014	Monitoring and biological evaluation of surface water and soil micropollutants in Hungary	Glyphosate	Reliable with restrictions	■						
CA 7.5/007	Daouk, S. *et al.*, 2013b	The role of infiltration and surface runoff	Glyphosate AMPA	Reliable with restrictions	■		■				
B. WATER											
B.1 GROUNDWATER											
APPLICANT STUDIES											
New studies/assessments											
CA 7.5/001	[redacted] 2020	Collection of public monitoring data for European countries	Glyphosate AMPA HMPA	Valid	■	■	■	■	■		
CA 7.5/002	[redacted] 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Valid	■	■	■	■	■		■
CA 7.5/008	[redacted] 2019a	Groundwater and surface water monitoring study in France	Glyphosate AMPA	Valid		■	■				
CA 7.5/009	[redacted] 2016	Groundwater and surface water monitoring study in France	Glyphosate AMPA	Valid		■	■				
CA 7.5/010	[redacted] 2016	Groundwater and surface water monitoring study in Europe	Glyphosate AMPA	Valid		■	■				

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters: A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
Existing studies/assessments											
CA 7.5/011	Anonymous, 2012	Groundwater monitoring in France	Glyphosate AMPA	Valid							
CA 7.5/012	Anonymous, 2012	Translation of CA 7.5/011	See above	See above							
CA 7.5/013	[redacted] 2012	Groundwater and surface water monitoring study in Europe	Glyphosate AMPA	Valid							
CA 7.5/014	[redacted] 2006	Clarification of well-related findings in groundwater	Glyphosate AMPA	Valid							
CA 7.5/015	[redacted] 2005	Investigation of reported borehole contamination in Sweden	Glyphosate	Valid							
RELEVANT LITERATURE ARTICLES											
CA 7.5/016	Rosenbom, A. *et al.*, 2019	The Danish Pesticide Leaching Assessment Programme	Glyphosate AMPA	Reliable							
CA 7.5/017	Poiger, T. *et al.*, 2017	Simplified procedure for determination in water samples	Glyphosate AMPA	Reliable							
CA 7.5/018	Di Guardo, A., Finizio, A., 2016	A moni-modelling approach to manage groundwater risk	Glyphosate	Reliable with restrictions							
CA 7.5/019	Rosenbom, A. *et al.*, 2015	The Danish Pesticide Leaching Assessment Programme	Glyphosate AMPA	Reliable							
CA 7.5/020	McManus, S. *et al.*, 2014	Groundwater monitoring study in Ireland	Glyphosate	Reliable							
CA 7.5/021	Norgaard, T. *et al.*, 2014	Leaching from an agricultural field	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/022	Martin, J. *et al.*, 2013	Review of 10 year monitoring of herbicides and water pollution in Reunion Island	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/023	Martin, J. *et al.*, 2013	Translation of CA 7.5/022	See above	See above							
CA 7.5/024	Mörtl, M. *et al.*, 2013	A monitoring study with an immunoassay analytical method	Glyphosate	Reliable							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
CA 7.5/025	Sanchis, J. *et al.*, 2012a	Analysis of groundwater samples by immunoassay and mass spectrometry	Glyphosate	Reliable							
CA 7.5/026	Sanchis, J. *et al.*, 2012b	Erratum to Sanchis, J. *et al.*, 2012a	Glyphosate	Reliable							
CA 7.5/027	Bruchet, A. *et al.*, 2011	Monitoring experiment in France	Glyphosate AMPA	Reliable							
CA 7.5/028	[redacted] 2011	Investigation of potential groundwater contamination in Lombardia region (North Italy)	Glyphosate	Reliable							
CA 7.5/029	[redacted] 2010	Groundwater monitoring in The Netherlands	Glyphosate AMPA	Reliable							
CA 7.5/030	[redacted] 2010	Translation of CA 7.5/029	See above	See above							
B.2a SURFACE WATER											
APPLICANT STUDIES											
New studies/assessments											
CA 7.5/001	[redacted] 2020	Collection of public monitoring data for European countries	Glyphosate AMPA HMPA	Valid							
CA 7.5/002	[redacted] 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Valid							
CA 7.5/031	[redacted] 2019	Mitigating glyphosate levels in surface waters	Glyphosate	Valid							
CA 7.5/008	[redacted] 2019a	Groundwater and surface water monitoring study in France	Glyphosate AMPA	Valid							
CA 7.5/032	[redacted] 2019b	Surface water monitoring study in France	Glyphosate AMPA	Valid							
CA 7.5/033	[redacted] 2018a	Monitoring data with respect to drained river areas and land use in France	Glyphosate	Valid							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
CA 7.5/034	[redacted] 2018b	Monitoring data with respect to drained river areas and land use in France	AMPA	Valid							
CA 7.5/009	[redacted] 2016	Groundwater and surface water monitoring study in France	Glyphosate AMPA	Valid							
CA 7.5/010	[redacted] 2016	Survey of groundwaters and surface waters in Europe	Glyphosate AMPA	Valid							
Existing studies/assessments											
CA 7.5/013	[redacted] 2012	Groundwater and surface water monitoring study in Europe	Glyphosate AMPA	Valid							
CA 7.5/035	[redacted] B., 1972	Run-off from inclined soil beds	Glyphosate	Invalid							
RELEVANT LITERATURE ARTICLES											
CA 7.5/036	Di Guardo, A., Finizio, A., 2018	Identifying surface waters at risk using pesticide monitoring data	Glyphosate	Reliable with restrictions							
CA 7.5/037	Huntscha, S. *et al.*, 2018	Seasonal dynamics in Lake Greifensee, Switzerland	Glyphosate AMPA	Reliable							
CA 7.5/038	Masiol, M. *et al.*, 2018	Herbicides in river water across northeastern Italy	Glyphosate AMPA	Reliable							
CA 7.5/039	Dairon, R. *et al.*, 2017	Long term impact of reduced tillage on water and pesticide flow in a drained context	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/040	Defrancq, M. *et al.*, 2017	High frequency monitoring of pesticides in runoff water	Glyphosate AMPA	Reliable							
CA 7.5/041	Lerch, R.N. *et al.*, 2017	Vegetative buffer strips for reducing herbicide transport in runoff	Glyphosate	Reliable with restrictions							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
CA 7.5/042	Mottes, C. *et al.*, 2017	Monitoring of glyphosate in a horticultural catchment in Martinique, French West India (part of the EU).	Glyphosate AMPA	Reliable							
CA 7.5/017	Poiger T. *et al.*, 2017	Simplified procedure for determination in water samples	Glyphosate AMPA	Reliable							
CA 7.5/043	Reoyo-Prats, B. *et al.*, 2017	Multicontamination phenomena in Mediterranean coastal watercourses (Têt River, France)	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/044	Desmet, N. *et al.*, 2016	A hybrid monitoring and modelling approach in large river catchments	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/045	Larsbo, M. *et al.*, 2016	Surface runoff of pesticides from a clay loam field in Sweden	Glyphosate AMPA	Reliable							
CA 7.5/005	Napoli, M. *et al.*, 2016	A runoff experiment in a vineyard in Italy	Glyphosate AMPA	Reliable							
CA 7.5/046	Schreiner, V. *et al.*, 2016	Monitoring results of pesticides in some EU Member States and the USA	Glyphosate	Reliable							
CA 7.5/047	Stenrød, M., 2015	Long-term trends of pesticides in Norwegian agricultural streams and potential future challenges in northern climate	Glyphosate	Reliable with restrictions							
CA 7.5/048	Szekács, A. *et al.*, 2015	Monitoring results for pesticide residues in surface and groundwater in Hungary	Glyphosate	Reliable with restrictions							
CA 7.5/049	Tang, T. *et al.*, 2015	Quantification and characterization of glyphosate use and loss in a residential area	Glyphosate AMPA	Reliable							
CA 7.5/050	Gasperi, J. *et al.*, 2014	Micropollutants in urban stormwater in three French sites	Glyphosate AMPA	Reliable with restrictions							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DW/FT
CA 7.5/051	Maillard, E., Imfeld, G., 2014	Pesticide loss and input in a stormwater wetland	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/021	Norgaard, T. *et al.*, 2014	Leaching from an agricultural field	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/052	Ramwell, C. *et al.*, 2014	Contribution of household herbicide usage in surface water drains	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/006	Székács, A. *et al.*, 2014	Monitoring and biological evaluation of surface water and soil micropollutants in Hungary	Glyphosate	Reliable with restrictions							
CA 7.5/053	Daouk, S. *et al*, 2013a	Validation of an analytical method in different water matrices	Glyphosate AMPA	Reliable							
CA 7.5/007	Daouk, S. *et al.*, 2013b	The role of infiltration and surface runoff	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/054	Houtman, C. *et al.*, 2013	Monitoring in the river Meuse in the Netherlands	Glyphosate AMPA	Reliable							
CA 7.5/055	Imfeld, G. *et al.*, 2013	Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/022	Martin, J. *et al.*, 2013	Review of 10 year monitoring of herbicides and water pollution in Reunion Island	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/024	Mörtl, M. *et al.*, 2013	A monitoring study with an immunoassay analytical method	Glyphosate	Reliable							
CA 7.5/056	Vialle, C. *et al.*, 2013	Pesticides in roof runoff in rural and suburban sites	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/057	Botta, F. *et al.*, 2012	Application and validation of a programme to reduce surface water contamination	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/058	Coupe, R. *et al.*, 2012	Fate and transport in agricultural surface waters	Glyphosate AMPA	Reliable with restrictions							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
CA 7.5/059	Petersen, J. *et al.*, 2012	Sampling of herbicides in streams during flood events	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/060	Zgheib, S. *et al.*, 2012	Priority pollutants in urban stormwater	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/061	Birch, H. *et al.*, 2011	Micropollutants in stormwater runoff and combined sewer overflow in Denmark	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/027	Bruchet A. *et al.*, 2011	Monitoring experiment in France	Glyphosate AMPA	Reliable							
CA 7.5/062	Lamprea, K., Ruban, V., 2011	Pollutant concentrations in stormwater and wastewater in France	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/063	Litz, N.T. *et al.*, 2011	Comparative studies on retardation and reduction during subsurface passage	Glyphosate AMPA	Reliable							
CA 7.5/064	Maillard, E. *et al.*, 2011	Removal of pesticide mixtures in a stormwater wetland	Glyphosate AMPA	Reliable							
CA 7.5/065	Meyer, B. *et al.*, 2011	Concentrations of dissolved herbicides and pharmaceuticals in a small river in Luxembourg	Glyphosate AMPA	Reliable							
CA 7.5/066	Busetto, M. *et al.*, 2010	Survey in waterways from the Lombardy region	Glyphosate AMPA	Reliable							
CA 7.5/067	Busetto, M. *et al.*, 2010	Translation of CA 7.5/066	See above	See above							
CA 7.5/068	Gregoire, C. *et al.*, 2010	Use and fate of 17 pesticides applied on a vineyard catchment	Glyphosate AMPA	Reliable							
CA 7.5/069	Hanke, I. *et al.*, 2010	Relevance of urban glyphosate use for surface water quality	Glyphosate AMPA	Reliable							
CA 7.5/070	Botta, F. *et al.*, 2009	Transfer to surface waters through sewerage systems	Glyphosate AMPA	Reliable with restrictions							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
CA 7.5/071	Ghanem, A., *et al.*, 2007	Concentrations and specific loads in French urban sewage sludge	Glyphosate	Reliable with restrictions							
CA 7.5/072	Peschka, M. *et al.*, 2006	Trends in pesticide transport into the River Rhine	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/073	Augustin, B., 2003	Urban sources of pesticide contamination of surface water	Glyphosate	Reliable with restrictions							
B.2b TRANSITIONAL WATER											
APPLICANT STUDIES											
New studies/assessments											
CA 7.5/001	[redacted] 2020	Collection of public monitoring data for European countries	Glyphosate AMPA HMPA	Valid							
CA 7.5/002	[redacted] 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Valid							
Existing studies/assessments											
There are no existing applicant monitoring data or studies covering transitional waters.											
RELEVANT LITERATURE ARTICLES											
There are no existing relevant literature articles coveringon transitional waters.											
B.3 DRINKING WATER											
APPLICANT STUDIES											
New studies/assessments											
CA 7.5/001	[redacted] 2020	Collection of public monitoring data for European countries	Glyphosate AMPA HMPA	Valid							
CA 7.5/002	[redacted] 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Valid							
CA 7.5/074	[redacted] 2015	Survey in drinking water in Europe – 2015	Glyphosate AMPA	Valid							
Existing studies/assessments											
CA 7.5/075	[redacted] 2008	Review of drinking water in selected European countries	Glyphosate AMPA	Valid							
CA 7.5/076	[redacted] 1997	Drinking water monitoring	Glyphosate	Invalid							
RELEVANT LITERATURE ARTICLES											
CA 7.5/077	Malaguerra, F. *et al.*, 2012	Pesticides in water supply wells in Zealand, Denmark	Glyphosate AMPA	Reliable with restrictions							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
CA 7.5/027	Bruchet, A. *et al.*, 2011	Monitoring experiment in France	Glyphosate AMPA	Reliable							
C. SEDIMENT											
APPLICANT STUDIES											
New studies/assessments											
CA 7.5/001	[redacted] 2020	Collection of public monitoring data for European countries	Glyphosate AMPA HMPA	Valid							
CA 7.5/002	[redacted] 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Valid							
Existing studies/assessments											
CA 7.5/035	[redacted] 1972	Run-off from inclined soil beds	Glyphosate	Invalid							
RELEVANT LITERATURE ARTICLES											
CA 7.5/041	Lerch, R.N. *et al.*, 2017	Vegetative buffer strips for reducing herbicide transport in runoff	Glyphosate	Reliable with restrictions							
CA 7.5/005	Napoli, M. *et al.*, 2016	A runoff experiment in a vineyard in Italy	Glyphosate AMPA	Reliable							
CA 7.5/051	Maillard, E., Imfeld, G., 2014	Pesticide loss and input in a stormwater wetland	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/078	Sabatier, P. *et al.*, 2014	Relationships among pesticide applications, mobility and soil erosion in a vineyard	Glyphosate AMPA	Reliable							
CA 7.5/055	Imfeld, G. *et al.*, 2013	Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/060	Zgheib, S. *et al.*, 2012	Priority pollutants in urban stormwater	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/064	Maillard, E. *et al.*, 2011	Removal of pesticide mixtures in a stormwater wetland	Glyphosate AMPA	Reliable							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
D. AIR											
APPLICANT STUDIES											
New studies/assessments											
There is no new applicant monitoring data or studies covering air.											
Existing studies/assessments											
There is no existing applicant monitoring data or studies covering air.											
RELEVANT LITERATURE ARTICLES											
CA 7.5/079	Ravier, S. *et al.*, 2019	Monitoring study in air in France	Glyphosate AMPA	Reliable							
CA 7.5/050	Gasperi, J. *et al.*, 2014	Micropollutants in urban stormwater in three French sites	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/056	Vialle, C. *et al.*, 2013	Pesticides in roof runoff in rural and suburban sites	Glyphosate AMPA	Reliable with restrictions							
E. DRINKING WATER TREATMENT											
E.1 LOW-CHEMICAL TREATMENT AND BANK FILTRATION											
APPLICANT STUDIES											
New studies/assessments											
CA 7.5/002	[redacted] 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Valid							
Existing studies/assessments											
CA 7.5/080	[redacted] 2012	Review of sustainable water treatment	Glyphosate AMPA	Valid							
CA 7.5/081	[redacted] 2010	Removal of glyphosate and AMPA by water treatment	Glyphosate AMPA	Valid							
RELEVANT LITERATURE ARTICLES											
CA 7.5/082	Hamann, E. *et al.*, 2016	The fate of organic micropollutants during long-term/long-distance river bank filtration	Glyphosate AMPA	Reliable							
CA 7.5/083	Hedegaard, M., Albrechtsen, H., 2014	Microbial pesticide removal in rapid sand filters for drinking water treatment – Potential and kinetics	Glyphosate	Reliable with restrictions							
CA 7.5/084	Jönsson J. *et al.*, 2013	Removal and degradation of glyphosate in water treatment	Glyphosate AMPA	Reliable							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
CA 7.5/085	Malaguerra, F. *et al.*, 2013	Computation model simulation for the contamination of drinking water wells	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/086	Ruel, S. *et al.*, 2012	Occurrence and fate of relevant substances in wastewater treatment plants	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/027	Bruchet A. *et al.*, 2011	Monitoring experiment in France	Glyphosate AMPA	Reliable							
CA 7.5/063	Litz, N.T. *et al.*, 2011	Comparative studies on retardation and reduction during subsurface passage	Glyphosate AMPA	Reliable							
CA 7.5/087	Ruel, S. *et al.*, 2011	Evaluation of removal of 100 micropollutants through wastewater treatment processes	Glyphosate AMPA	Reliable							
CA 7.5/088	Schoonenberg Kegel, F.S., 2010	Removal of 47 micropollutants from river bank filtrate	Glyphosate	Reliable with restrictions							
CA 7.5/072	Peschka, M. *et al.*, 2006	Trends in pesticide transport into the River Rhine	Glyphosate AMPA	Reliable with restrictions							
E2 CHEMICAL WATER TREATMENT											
APPLICANT STUDIES											
New studies/assessments											
CA 7.5/002	[redacted] 2020	European public monitoring data assessment and interpretation	Glyphosate AMPA	Valid							
Existing studies/assessments											
CA 7.5/081	[redacted] 2010	Removal of glyphosate and AMPA by water treatment	Glyphosate AMPA	Valid							
RELEVANT LITERATURE ARTICLES											
CA 7.5/084	Jönsson J. *et al.*, 2013	Removal and degradation of glyphosate in water treatment	Glyphosate AMPA	Reliable							
CA 7.5/087	Ruel, S. *et al.*, 2011	Evaluation of removal of 100 micropollutants through wastewater treatment processes	Glyphosate AMPA	Reliable							

Table 7.5-3: List of all monitoring studies and literature articles summarized

Data point	Study (Author, year)	Study type	Substance(s)	Status	Relevant subchapters						
					A. SOIL	B1. GW	B2. SW & TW	B3. DW	C. SED	D. AIR	E. DWT
CA 7.5/089	Shen, Y. *et al.*, 2011	Ozonation	Glyphosate	Reliable							
CA 7.5/090	Shen, Y. *et al.*, 2011	Translation of CA 7.5/089	See above	See above							
CA 7.5/091	Assalin, M. *et al.*, 2010	Degradation by several oxidative chemical processes	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/092	Boucherie, C. *et al.*, 2010	Ozone and GAC filtration synergy	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/093	Manassero, A. *et al.*, 2010	Degradation in water employing the H_2O_2/UVC process	Glyphosate	Reliable							
CA 7.5/094	Brosillon, S. *et al.*, 2006	Chlorination kinetics of glyphosate and its by-products	Glyphosate AMPA	Reliable with restrictions							
CA 7.5/095	Mehrsheikh, A. *et al.*, 2006	Investigation of the mechanism of chlorination of glyphosate	Glyphosate	Reliable							
CA 7.5/096	Klinger, J. *et al.*,2008	Formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra	Glyphosate AMPA	Reliable with restrictions							

GW: Groundwater; SW: Surface water; TW: Transitional water; DW: Drinking water; SED: Sediment; DWT: Drinking water treatment

: Reference cited in subchapter

: Reference not cited in subchapter

Table 7.5-4: List of articles cited and submitted without summary

Data point	Study (Author, year)	Study type	Substance(s)	Status	Remark
CA 7.5/097	Gillefalk, M. *et al.*, 2018	Conceptual review of bank filtration processes	Not applicable	Not applicable	Cited in [redacted] 2020, CA 7.5/002
CA 7.5/098	Van der Hoek, J.P. *et al.*, 2014	Review of drinking water treatment technologies	Not applicable	Not applicable	Cited in [redacted] 2020, CA 7.5/002

A. Soil

Concentrations of glyphosate (GLY), AMPA and HMPA in soil arising from public monitoring datasets have been collected from regional/national environment agencies as well as published peer reviewed publications from literature searches and rated as potentially relevant/reliable are reported in this section.

There are two new applicant studies on soil. [redacted] (2020, CA 7.5/001) describes the collection process of public monitoring data (from regional/national environment agencies) for European countries for the compartment soil (as well as water, sediment and air) for glyphosate, AMPA and HMPA. [redacted] (2020, CA 7.5/002) assesses the data collected by [redacted] (2020, CA 7.5/001). These two recent studies were designed to be the more comprehensive than previous studies by considering additional metabolites, compartments and time periods. This subchapter only includes the results of [redacted] (2020, CA 7.5/002) relevant to soil.

Five published peer-reviewed papers are also reported in this section. These papers report concentrations that are partly not directly comparable with the soil compartment that is typically risk assessed as part of the approval process, e.g. concentrations in soil pore water. They were identified in the formal literature search conducted for the current submission and cover a wide range of use settings, predominantly agricultural, including rotational and permanent crops.

Karanasios *et al*. (2018, CA 7.5/003) reports monitoring data for glyphosate and AMPA in Greek agricultural soils associated with olive production, while Napoli *et al*. (2016, CA 7.5/005) describes a runoff experiment with glyphosate in a vineyard in Italy, where soil residues after 12 months were additionally assessed. Daouk (2013b, CA 7.5/007) assesses glyphosate and AMPA in soil after application of the parent to a vineyard soil in Switzerland. Silva *et al*. (2018, CA 7.5/004) describes the result from a field study to measure the distribution of glyphosate and AMPA in European topsoils. Székács *et al.* (2014, CA 7.5/006) reports measurements of glyphosate in soils in Hungary from agricultural and industrial settings.

The monitoring data presented in this section that are suitable for use in assessing the state of the soil environmental compartment and evaluating potential impacts on biota are tabulated below to facilitate comparison with the regulatory acceptable concentrations (RAC).

Table 7.5-5: Summary of reported maximum concentrations of glyphosate (GLY) and AMPA in soil

Reference	Use Setting	Maximum Concentration (mg/kg unless indicated)	
		GLY	AMPA
[redacted] 2020, CA 7.5/001	Various incl. rotational and permanent crops	NA	NA
[redacted] 2020, CA 7.5/002	Various incl. rotational and permanent crops	2.05[1]	1.92[1]
Karanasios, E. *et al.* 2018, CA 7.5/003	Olive	0.35	0.65
Silva, V. *et al.* 2018, CA 7.5/004	Various incl. rotational and permanent crops	2.05	1.92
Napoli, M. *et al.* 2016, CA 7.5/005	Vineyards	<LOD	0.065 □ 0.006
Székács, A. *et al.* 2014, CA 7.5/006	Agricultural (unspecified); Industrial	0.56 ± 0.26	NA
Daouk, S., *et al.* 2013b, CA 7.5/007	Vineyard soil pore water (40cm depth)	<14 µg/L	<8 µg/L

[1] Silva *et al*. 2018 was included in this study as an aggregated report
NA – Not applicable

All reported soil concentrations are well below the RACs of 94.6 mg/kg for glyphosate (GLY) and 26.4 mg/kg for AMPA. As such, the available data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in the soil compartment.

Applicant studies

New studies/assessments

1. Information on the study

Data point:	CA 7.5/001
Report author	[redacted]
Report year	2020
Report title	Collection of public monitoring data for European countries for the compartments soil, water, sediment and air for Glyphosate, AMPA and HMPA
Document No	110057-1
Guidelines followed in study	Methodology is based on the Groundwater Monitoring guideline document (Gimsing *et al.*, 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations') Minimum quality criteria of monitoring data described by the FOCUS Ground Water Work Group chapter 9.5 (European Commission, 2014)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
The report provides information about the outcome of a search for readily accessible and available monitoring data in European countries at a regional/national level for the time period 1995-2019. The main focus was on the time period 2012-2019 while earlier years are already covered by existing data. The search included raw data, requested from regional/national authorities or downloadable from their websites, as well as aggregated data extracted from reports compiled by authorities.

Data from 14 European countries were considered: Austria, Belgium, Denmark, France, Germany, Hungary, Ireland, Italy, The Netherlands, Poland, Romania, Spain, Sweden and the United Kingdom. The countries represent the major markets of products containing glyphosate sold in the EU. The data compilation included the active substance glyphosate and its metabolites AMPA and HMPA, in the soil, groundwater, surface water, tidal water, drinking water, sediment and air environmental compartments.

As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities and other bodies of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were actually not included in any of the monitoring programs.

Soil Compartment Conclusion
There were hardly any official programs in place targeting monitoring of glyphosate or its metabolites residues in soil. Raw data for glyphosate and AMPA were available for the German federal state of Brandenburg. Aggregated monitoring data at the EU level for soil were obtained in the form of a research article.

I. MATERIALS AND METHODS

The general methodology of data collection of public monitoring data and minimum quality criteria is based on existing guideline documents for groundwater monitoring programs. The underlying principles have been applied to all environmental compartments, especially where no specific guidance is at hand. Data search, acquisition and processing approaches are described below. The same approach was applied for each country, compartment and substance. Country specific adaptations to the general procedure were made in order to generate a harmonized database. The data collected for this report refers to third party organization data regarding all environmental compartments (SOIL, GW, SW, TD, DW, SD, AIR) and was further differentiated into the two different data types, i.e. raw data and aggregated data. Aggregated data refers to information provided in publicly available reports, e.g. from environmental agencies or research institutes. Such reports might hold only summary information on substance findings over space and time and may intersect with the raw data. Raw data refers to mid to long term time series of data that are provided on request by e-mail or by database from governmental authorities and are therefore recognized as official monitoring data. These datasets hold the information of sampling values, quality information (sampling, treatment, limit of detection - LOD, limit of quantification - LOQ) as well as information of location and time of sampling.

The following data source types were taken into account in order to collect monitoring data:

- E-mail requests: a general e-mail was sent to the national responsible authorities with regard to the required information.

- Governmental webpages: the official webpages of the national responsible authorities were searched for information regarding available reports and datasets.

- Public online databases: available data from online databases were downloaded as provided by the webpages of governmental authorities and other institutions.

- Professional contacts: information indicated by experts in frequent professional contact to governmental authorities and other institutions were considered in order to complement data sources and datasets.

The data search resulted in a very heterogeneous collection of tabular data and reports in different formats and structure. Data were processed into a harmonized tabular format by selecting relevant information and adapting data organisation. In general, the complete datasets were included in the final harmonized database as provided by the authorities, but obvious duplicates were deleted. In general, all entries for the digital database were checked for consistency and plausibility. For the raw data it was assumed that information was already subjected to critical scrutiny by the respective organization. For the aggregated data the same assumption was made with quality assurance of the data (mostly summaries) being the responsibility of the authors of the respective reports.

II. RESULTS AND DISCUSSION

The final data collection of raw data and aggregated data is summarised for each compartment and each country in Table 7.5-6.

Soil
Raw monitoring data from national authorities for soil were provided by the regional authorities of Brandenburg. Aggregated monitoring data at the EU level for soil were obtained in the form of a research article.

Table 7.5-6: Overview of public monitoring data availability of raw data (R) and aggregated data (A)

Country	Soil	Water				Sediment	Air
		Ground	Surface	Tidal	Drinking		
Austria	-	R, A	R, A	-	A	-	-
Belgium	-	R	R	-	A (Flanders)	-	-
Denmark	-	R, A	A	-	A	-	-
France	-	R	R	-	A	R	-
Germany	R (Brandenburg)	R, A	R, A	R	R (Schleswig-Holstein), A	-	-
Hungary	-	A (one research article)	A (one research article)		-	-	-
Ireland	-	R, A	R, A	-	R, A	-	-
Italy	-	R (Lombardia), A	R, A	-	-	-	-
The Netherlands	-	R, A	R, A	-	R	-	-
Poland	Confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Romania	Confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Spain	-	R, A	R, A	-	A	-	-
Sweden	-	R, A	R	-	R, A	R	-
UK England	-	R	R	R	A	-	-
UK Northern Ireland	-	R	-	-	-	-	-
UK Scotland	-	-	R	-	-	-	-
UK Wales	-	-	R	-	A	-	-

R raw data available; A aggregated data from reports available; - no raw or aggregated data available

III. CONCLUSIONS

The collection of public monitoring data for glyphosate, AMPA and HMPA in soil, groundwater, surface water, drinking water, tide water, sediment and air resulted in a comprehensive database of 'raw monitoring data from national authorities' and 'aggregated monitoring data from reports published by national authorities'. As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities of all other countries provided raw data or aggregated data for at

least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were actually not included in any of the monitoring programs.

There were hardly any official programs in place targeting monitoring of glyphosate or its metabolites residues in soil. Raw data for glyphosate and AMPA were available for the German federal state of Brandenburg. Aggregated monitoring data at the EU level for soil were obtained in the form of a research article.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes the collection process of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA
The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Document No	-
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al.*, 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
The report provides information about the outcome of an analysis of public monitoring data comprising environmental concentrations of glyphosate (GLY) and its primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA) collated from readily available public monitoring databases held by national/regional environment agencies. This data collection and analysis was designed to expand previous reviews to include other compartments and supplement them for surface

water, groundwater and drinking water. Public monitoring data from the following Member States (MS) were assessed for the water, sediment and soil compartments: Austria (AT), Belgium (BE), Denmark (DK), France (FR), Germany (DE), Ireland (IE), Italy (IT), Netherlands (NL), Spain (ES), Sweden (SE) and the United Kingdom (UK). Three MS, namely Poland (PL), Hungary (HU), and Romania (RO) confirmed that they do not conduct analyses for GLY, AMPA and HMPA in any environmental compartment. No data for HMPA was identified for any MS or compartment. Note that at the time the study was started the UK was a Member State and is referred to as a Member State throughout the report.

Analyses of the large spatial and temporal dataset of measured concentrations occurring in several environmental compartments, namely surface water, groundwater, drinking water, tidal water, sediment and soil, were conducted to assess their state. This analysis not only sought to assess the state of the environmental compartment but also to consider the potential impacts this might have on biota, ecosystems and human health by using regulatory endpoints and thresholds from a range of European (EU) Directives. These included the Water Framework Directive (Directive 2000/60/EC) and associated Groundwater (2006/118/EC), Drinking Water (1998/83/EC) and Priority Substances (2008/105/EC28) Directives in addition to the Plant Protection Products Directive (1107/2009/EC).

Soil
A small number (57 samples from 29 sites) of GLY and AMPA analyses from agricultural soils were collected and analysed. These were from a single MS, namely DE, in the Bundesland of Brandenburg. The data were assessed against the soil regulatory acceptable concentration (RAC) of 94.6 mg/kg for GLY and 26.38 mg/kg for AMPA.

Compliance was 100 % with no exceedances of the RAC indicated by the data for both GLY and AMPA. The maximum measured concentrations of 0.25 mg/kg for GLY and 0.975 mg/kg for AMPA are well below the RAC. These are comparable with data from a much larger published pan-European dataset where the maximum measured concentrations were 2.05 mg/kg for GLY and 1.92 mg/kg for AMPA, which are also well below the RAC.

Soil Compartment Conclusions
While limited in number, spatial and temporal scope, the available soil data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in this environmental compartment.

I. MATERIALS AND METHODS

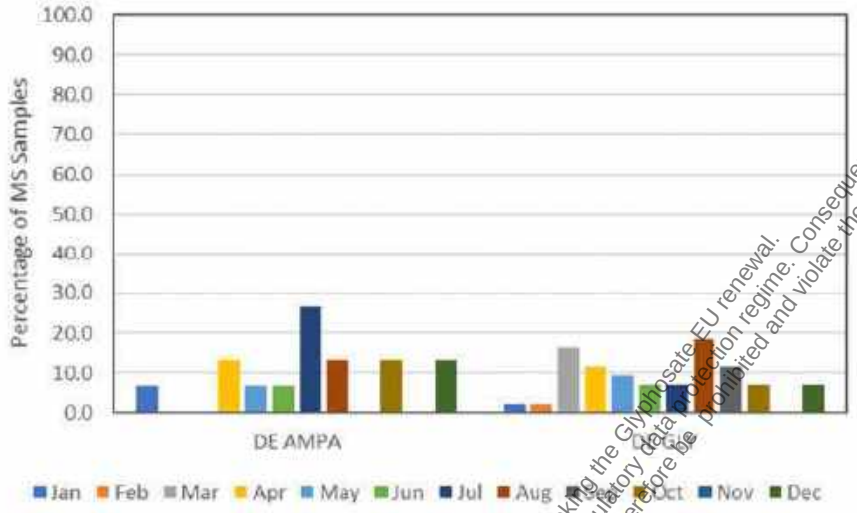
The dataset analysed comprised individual sediment analysis records as well as existing aggregated analyses extracted from reports sourced from regional/national environment agencies (see [redacted] 2020, CA 7.5/001). The approach taken for the data processing encompassed a precautionary approach that preserved samples in the analysis where there was any doubt regarding their reliability. As such no soil records were excluded from the analysis. Similarly, no attempt to remove outliers was undertaken. Analysis and assessment of the data against thresholds was undertaken in Excel and was evaluated against the following thresholds and endpoints:

- Ecotoxicological endpoint: Regulatory acceptable concentration (RAC) of 94.6 mg/kg for GLY and 26.38 mg/kg for AMPA.

II. RESULTS AND DISCUSSION

The data analysed was very limited (57 samples) and as such is biased both spatially and temporally. While it is not stated which kinds of landuse were sampled, visual assessment of monitoring locations in GIS suggest that the samples were largely of arable agricultural land. All of the data comes from a DE dataset which comprises 29 sites located in the Bundesland of Brandenburg. This dataset covers 9 years spanning the period 2008 – 2018. Monthly sampling effort for both GLY and AMPA appears to be variable (see Figure 7.5-1).

Figure 7.5-1: Bar chart of soil monthly glyphosate (GLY) and AMPA sampling effort



Analysis of the GLY soil dataset indicates that GLY is quantified in ~30 % of samples (see Table 7.5-7), albeit the number of samples is quite limited at 43. Compliance was 100 % given no analyses exceeded the RAC or came close to doing so with the maximum measured concentration being 0.25 mg/kg. Comparison with the larger aggregated report dataset suggests agreement (see Silva *et al*., 2018). These published aggregated results stem from data that are well distributed across the EU and sample a range of different cropping systems, however, they are temporally limited with 300 of the samples being collected as part of the LUCAS topsoil project between April and October of 2015 and 17 samples from three independent vineyards in north-central Portugal taken in September 2015. Aggregated data from EU MS reports are presented in Table 7.5-8 which suggest GLY is quantified in ~21 % of 317 soil samples, however none exceed the RAC, or come close to doing so, with the maximum concentration being 2.05 mg/kg associated with permanent crops (vineyards) in central Portugal.

Analysis of the AMPA soil dataset indicates that AMPA is quantified in ~86 % of samples (see Table 7.5-7), albeit the number of samples is very limited at 14. Compliance was 100 % given no analyses exceeded the RAC or come close to doing so with the maximum measured concentration being 0.975 mg/kg. Aggregated data from a report are presented in Table 7.5-8 which suggests AMPA is quantified in ~42 % of 317 soil samples, however none exceed the RAC, or come close to doing so, with the maximum concentration being 1.92 mg/kg associated with permanent crops in central Portugal.

Table 7.5-7: Summary results of glyphosate (GLY) and AMPA analyses in soil

Substance	Number of Sites	Number of Samples	Years	LOQ	Detected >LOQ		Detected >RAC		Measured Concentration (mg/kg)
				Mean (min - max)	Samples	%	Samples	%	Median (min - max)
AMPA	13	14	2011 - 2018	0.1 mg/kg (0.1 - 0.1)	12	85.7	0	0	0.059 mg/kg (0.029 - 0.975)
GLY	29	43	2008 - 2018	0.025 mg/kg (0.001 - 0.5)	13	30.2	0	0	0.0125 mg/kg (0.001 - 0.25)

Table 7.5-8: Summary of soil monitoring data aggregated in reports for glyphosate (GLY) and AMPA in soil

MS	Substance	Number of reports identified	Reports with data relating to threshold						Maximum value (mg/kg)
			Number of reports	Date range	Number of samples	Threshold LOQ (mg/kg)	Samples above threshold	% samples above threshold	
EU	AMPA	1	1	2015	317	0.05	133	42	1.92
	GLY	1	1	2015	317	0.05	67	21	2.05

III. CONCLUSIONS

Compliance for the soil compartment was 100 % with no exceedances of the RAC indicated by the data for both GLY and AMPA. The maximum measured concentrations of 0.25 mg/kg for GLY and 0.975 mg/kg for AMPA are well below the RAC. These are comparable with data from a much larger published pan-European dataset where the maximum measured concentrations were 2.05 mg/kg for GLY and 1.92 mg/kg for AMPA, which are also well below the RAC. While limited in number, spatial and temporal scope the available soil data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in this environmental compartment.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes the analysis of public monitoring data for European countries for the compartment soil, water and sediment for Glyphosate and AMPA. Maximum measured soil concentrations were 2.05 mg/kg for GLY and 1.92 mg/kg for AMPA. The available soil data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in the soil compartment.
The study is considered valid.

Assessment and conclusion by RMS:

Existing studies/assessments

There is no existing applicant monitoring data on soil.

Relevant literature articles

1. Information on the study

Data point:	CA 7.5/003
Report author	Karanasios, E. *et al.*
Report year	2018
Report title	Monitoring of glyphosate and AMPA in soil samples from two olive cultivation areas in Greece: aspects related to spray operators activities
Document No	Environ Monit Assess (2018) 190: 361
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

The persistence of glyphosate and its primary metabolite AMPA (aminomethylphosphonic acid) was monitored in two areas in Southern Greece (Peza, Crete and Chora Trifilias, Peloponnese) with a known history of glyphosate use, and the levels of residues were linked to spray operators' activities in the respective areas. A total of 170 samples were collected and analysed from both areas during a 3-year monitoring study. A new method (Impact Assessment Procedure - IAP) designed to assess potential impacts to the environment caused by growers' activities, was utilised in the explanation of the results. The level of residues was compared to the predicted environmental concentrations in soil. The ratio of the measured concentrations to the predicted environmental concentrations (MCs/PECs) was >1 in Chora the first 2 years of sampling and <1 in the third year, whilst the MCs/PECs ratio was <1 in Peza, throughout the whole monitoring period. The compliance to the instructions for best handling practices, which operators received during the monitoring period, was reflected in the amount of residues and the MCs/PECs ratio in the second and especially the third sampling year. Differences in the level of residues between areas as well as sampling sites of the same area were identified. AMPA persisted longer than the parent compound glyphosate in both areas.

Materials and methods

Field sites

Sampling was carried out between 2012 and 2014 in two typical olive-growing areas of Southern Greece (Peza, Crete and Chora Trifilias, Peloponnese). The first year of the monitoring program, sampling was carried out at each site in order to quantify the background pollution levels. A total of 51 sites were selected in Peza, 16 of which did not receive any glyphosate during the 3-year sampling period. Further, soil from 27 sites from conventional farms (6 of which were not treated with glyphosate) and 13 sites from organic farms in Chora Trifilias were collected and analysed. The selection of the study sites was based on the following criteria: (i) the spatial distribution within the studied areas and the landscape variability, (ii) the soil texture and properties and (iii) the farming practices/production schemes. Soil types varied between target areas and within sampling sites of the same area (Table 7.5-9). The physiochemical characteristics of soils are presented in Table 7.5-10.

Soil samples

Samples for residual analysis were taken from the 0 to 30-cm topsoil layer using a soil sampler. At least four soil sub-samples were collected per plot and pooled to obtain a representative sample for each site. Each soil sample consisted of 1 kg stored in labelled clean plastic bags and sent for analysis to the Laboratory of Chemical Control of Pesticides of Benaki Phytopathological Institute in portable

containments under low temperature conditions and constant darkness. For practical reasons, sampling was carried out at variable dates after application of glyphosate (Table 7.5-11).

Table 7.5-9: Characterisation of soil in sampling sites in Chora and Peza

	Number of sites at each soil type	
	Chora[a]	Peza
Clay	2	23
Clay-loam	11	16
Loam	7	6
Sandy-loam	5	2
Sandy-clay	0	3
Sandy-clay-loam	9	1
Silty-clay-loam	5	0

[a] Not determined for two soils

Table 7.5-10: Physicochemical characteristics of soils

	No of samples	Organic carbon	Clay	CEC	pH	*P* (mg kg^{-1})	*N* (mg kg^{-1})
Peza	51	1.57 (0.6–3.02)	37.1 (16.8–61.6)	20.8 (6.8–41.8)	7.6 (7.2–7.9)	11.64 (0.94–62.6)	16.37 (6.6–77)
Chora	39[a]	1.42 (0.7–2.7)	28.9 (10.4–42.4)	17.3 (7.8–23.9)	7.2 (4.7–7.8)	20.62 (1.10–162.9)	14.68 (4.2–66.7)

CEC cation exchange capacity, *P* P Olsen

[a] Data for two sites are not available

Table 7.5-11: Application of glyphosate in the two target areas

	Date of application			Application rate (g glyphosate ha^{-1})			Interval between application and sampling (days)		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
Peza	5 Mar–22 Apr	10 Feb–25 Apr	15 Mar–5 Apr	973–4186	900–4003	1362–4153	13–23	47–134	15–21
Chora	27 Mar–30 Apr[a]	28 Feb–26 Apr	5 Mar–5 May	216–3750	83.9–3428	360–3333	44–107	58–276	33–137

[a] Complementary applications in three sites during July–August at rates between 100 and 240 g a.i./ha

Glyphosate applications

Glyphosate was applied once after the onset of rainfalls in both regions between mid-February and early May, after weeds had emerged or were actively growing at the time of spraying, except for three soil sampling sites in Chora where a complementary application of glyphosate, at a much lower dose, was carried out in the middle of summer (Table 7.5-11).

Application rate of glyphosate varied between and within areas due to differences in the target weeds and local practicalities. Weed management differed among olive groves and depended on weed species present, parcel's soil type, application of irrigation and various other factors related to the farming system applied. At least two weed surveys per year (late winter and end of spring) were conducted by agronomists and included identification of the weed species and determination of weed density. These surveys were the basis for weed management advices provided by agronomists to operators related to herbicide choice and practices on rational handling, spraying and herbicides remnants management. In few olive groves, combinations and/or sequential applications of herbicides were required to provide effective weed control.

Analytical standards
High purity analytical standards of glyphosate (98 %) and aminomethyl phosphonic acid (AMPA) (99.8 %) were purchased from ChemService (USA). Analytical standards of glyphosate-FMOC (97 %) and aminomethyl phosphonic acid-FMOC (97.5 %) were obtained from Dr. Ehrenstorfer. Individual stock solutions of glyphosate and AMPA were prepared by gravimetric weighing of high purity standards at concentrations of approximately 1000 mg L^{-1} in water (HPLC grade).Working solutions of individual compounds, their mixtures and spiked samples were prepared at different concentration levels, by appropriate dilutions of the stock solutions in water. Glyphosate and AMPA mixture working solutions were used for the estimation of recovery. Individual stock solutions of glyphosate-FMOC and AMPA-FMOC were prepared by gravimetric weighing of the high purity analytical standards at concentrations 492.76 and 970 µg/mL respectively, in an appropriate mixture of water:methanol (75:25) (HPLC grade). Working solutions of their mixtures were prepared in methanol at the concentrations of 0.01, 0.05, 0.1, 0.5 and 1 µg/mL and were used for establishing the linearity of the chromatographic system. All the standard and working solutions were stored in amber nonsilanized glasses at 0-1°C in dark. Before each use, the standard solutions were equilibrated at room temperature and weighed to check for evaporation losses.

Solvents and reagents
Analytical reagent-grade sodium tetraborate decahydrate of 100 % purity and 9-fluorenylmethyl-chloroformate (FMOC-Cl) of 98 % purity were obtained form LACHNER (Czech Republic) and ACROS ORGANICS respectively. Reagent grade hydrochloric acid and potassium hydroxide (KOH) was purchased from Panreac Quimica S.A. (Spain), and ammonium acetate from (NH_4Ac) of 98 % purity was obtained from Merck (Germany). Hydrochloric acid 11.65 N, LC-MS grade water and acetonitrile and HPLC water used in this study were supplied by Fisher Scientific (UK). Solution of 5 % borate buffer at approximately pH 9 in water of HPLC grade and solution containing 12,000 mg/L of FMOC-Cl in acetonitrile were used for the derivatization step of the samples. Argon (Ar), used as collisioninduced gas (CID gas) in the triple quadrupole, was obtained from Air Liquid (Greece).

Sample preparation and extraction method
Sample preparation was based on the method proposed by Ibanez *et al.* (2005) with minor modifications as described below. Soil samples were air dried at room temperature in the dark, sieved through 2-mm sieve and frozen at - 40°C till extraction. Soil samples were allowed to reach ambient temperature and after thorough mixing of the sample, a subsample of 5 g (± 0.1) was transferred to a centrifuge tube (50 mL) with 10 mL of 0.6 M KOH, shaken mechanically in a horizontal shaker for 30 min and then centrifuged at 3000 rpm for 30 min. The alkaline supernatant was separated and neutralised by adding drops of 6 N and 0.6 N HCl until approximately pH 7.0. After that, the neutralised supernatant was tenfold diluted with water of HPLC grade. The next step concerns the derivatisation step in which 2 mL of the tenfold diluted supernatant was pipetted into a glass tube together with 120 µL HPLC water, 120 µL of borate buffer (pH 9) and 120 µL of FMOC-Cl reagent (12,000 mg/L). The tube was swirled and left overnight at room temperature, and then the samples were acidified with hydrochloric acid until pH 1.5, filtered through 0.45 µm syringe filter and injected directly to LC-ESI-MS/MS system. It should be mentioned that the tenfold dilution of soil samples with water was assayed as a simple and fast way to minimize matrix interferences.

Instrumental
The high-performance liquid chromatograph used for the separation glyphosate and AMPA was a Varian (USA) system (working pressure maximum 400 bar), composed of two Prostar pumps (VARIAN, Prostar 210), a vacuum degasser (Metachem Technologies Inc), an autosampler (Varian, Prostar 420) with a 10-µL sample loop and a column oven (Varian, Prostar 510). The analytical column employed was a reversed phase C18 of 50 mm × 2 mm × 5 µm particle size (Agilent Zorbax Eclipse Plus). The mobile phases, A and B, consisted of water 5 mM acetic acid/ammonium acetate adjusted at pH 4.6 and acetonitrile at a ratio 10:90 respectively. The flow rate was set at 0.2 mL/min and the column gradient program consisted of 90 vol. % of A and 10 vol. % of B where it remained for 5.06 min. Next, at 5.1 min, it was reversed to 10 vol. % of A and 90 vol. % of B where it remained for 10 min. At 10.01 min, the gradient was returned to the initial conditions (90 vol. % A) where it maintained up to the end of the analysis at 20 min. After the 20 min run time, the column was re-equilibrated for 10 min at the initial mobile phase composition. The

column temperature was maintained at 30°C during all runs and the injection volume was 5 µL. In order to avoid carry over, the autosampler was purged with a mixture methanol/water (50:50 v/v) before sample injection.

The triple quadrupole system used was a Varian 1200 L (VARIAN, USA) Quadrupole MS-MS spectrometer fitted with an electrospray ionisation (ESI) interface. The ESI-MS interface was operated in the positive ion detection mode. The ESI source conditions were capillary voltage, 5000 V in positive-ion (PI) mode; drying gas temperature, 300°C; nebuliser gas pressure, 45 psi (both nebuliser and drying gas were high purity nitrogen, produced by a high purity generator) and electron multiplier voltage, 1600 V. MS/MS experiments were carried out with Argon (purity 99.9 %) at pressure of approximately 1.5 mTorr in the collision cell. Cone voltage and collision energy values optimised for each of the two compounds selected, were used. For selected ion monitoring (SIM) experiments, both Q1 and Q3 were set at fixed m/z values. For each analyte, the most abundant and characteristic fragment ion was chosen for quantization and two fragment ions selected for confirmation (Table 7.5-12). Dwell times of 0.1 ms were set. For instrument control, data acquisition and processing, the Varian MS Workstation software version 6.8 was used. The selected ion monitoring (SIM) mode was applied, and the selected characteristic ions are presented at Table 7.5-12. The transition of the most abundant product ion was used for quantitation and the second one in abundance for identification. The first step involved selection of the precursor ion for each compound.

Table 7.5-12: Mass spectrometry parameters for glyphosate and AMPA

Mass spectrometry and chromatography parameters	Glyphosate-FMOC	AMPA-FMOC
Quantification transition (m/z)	392 → 88.1	334 → 179.1
Capillary voltage (V)	60	60
Collision energy (eV)	20	15
Qualifier transition (m/z)	392 → 214.1	334 → 112.1
Capillary voltage (V)	50	60
Collision energy 2 (eV)	10	10
Rt (min)	7.8	8.2

For both compounds, glyphosate and AMPA and in the positive-ion electrospray full scan spectrum, the protonated derivatized molecule [M + H] + was recorded at m/z 392 and 334, respectively. In the case of glyphosate, the MS/MS spectra showed two abundant fragments at m/z 214 and 88, whereas in the case of AMPA the respective abundant fragments were at m/z 112 and 179.

Validation study
The method has been fully validated following the European Union SANCO guidelines. The precision (repeatability, in terms of % RSD) and the accuracy (percentage recoveries) of the method were estimated by recovery experiments in soil which was free of glyphosate and AMPA at three fortification levels.

Linearity
Linearity for glyphosate and AMPA was evaluated using calibration curves at five concentration levels covering concentrations at three orders of magnitude: 0.01 - 1 µg/g, based on the linear regression and squares correlation coefficients, R^2. Regression analysis exhibited an excellent relationship, as correlation coefficients (R^2) were 0.9987 for AMPA and 0.9978 for glyphosate.

Precision
The repeatability of the method was determined at the concentration level of 0.05 µg/g dry weight, by the analysis of five spiked matrix extracts (n = 5). The calculated RSDs ranged between 5 and 15 %. Inter-day RSDs were calculated for 5 days and varied between 7 and 19 %. According to "Guidance document on

pesticide residue analytical methods", these results were considered to be acceptable and demonstrated a satisfactory repeatability of the method and therefore its effectiveness for quantitative purposes. The accuracy of the method was verified by measuring from spiked blank samples at three concentration levels, i.e. at 0.01, 0.05 and 0.5 µg/g dry weight. All experiments were performed five times, and the relative standard deviation (RSD %) was calculated, and the values obtained were used for the estimation of the precision of the extraction method.

Recovery and limit of quantitation

The accuracy of the method was verified by measuring recoveries form spiked blank samples at three concentrations levels, i.e. at 0.01, 0.05 and 0.5 µg/g dry weight. All experiments were performed five times, and the relative standard deviation (RSD) was calculated. Recovery ranged between 89.6 and 118.8 % for glyphosate and between 67.9 and 94.6 % for AMPA whereas the RSD was 15.35 % for glyphosate and 11.9 % for AMPA in all cases.

The validated LOQs were defined as the lowest validated spike level (expressed in µg/g dry weight) for which a recovery in the 70-120 % range could be obtained, with a corresponding RSD ≤20 %, according to the EU SANCO document on validation and QC procedures. Based on the EU SANCO, the validated LOQs were defined as the lowest calibrated spiked level and were 0.01 µg/g soil dry weight for both compounds. Recoveries for the studied compounds were in the range 75.62-113.65 %, thus, the concentration of pesticides in soil samples was not corrected for recovery.

A soil sample free from glyphosate and AMPA residues was used for recovery experiments. The specific sample was previously analysed to ensure that it did not contain the studied compounds and was used as blank soil sample. This blank soil sample used for the estimation of recovery was treated as follows: 10 g of the sample (blank soil sample) was placed in a centrifuge tube (50 mL) along with 1 mL of the standard mixture of the desired pesticide concentration in water. It was homogenised by mechanical shaking for 60 min for better analyte distribution, and the bulk of the solvent was left to evaporate at ambient temperature and controlled by weight. This is a procedure able to mimic weathered residues. Then, spiked samples were extracted in the same way as described in the sample preparation and extraction method.

Predicted environmental concentration

The concentration of glyphosate and AMPA in soil was estimated with the soil persistence model of the Soil Modelling Work group of FOCUS:

$$PEC_s(t_0) = \frac{A \times (1 - f_{int})}{100 \times \text{depth} \times \text{bd}} \quad (1)$$

$$PEC_s(\text{after n applications}) = PEC_s(t_0) \times \frac{1 - e^{-nki}}{1 - e^{-ki}} \quad (2)$$

$$k = \frac{\ln 2}{DT_{50}} \quad (3)$$

where A is the application dose (g/ha); f_{int} is the fraction intercepted by crop canopy; depth is the mixing depth (cm) and bd is the dry soil bunk density (g/cm^3); k is the dissipation rate constant and DT_{50} the time for disappearance of half the chemical. The following assumptions were made: the f_{int} was set to 0, the mixing depth to 15 cm, the DT_{50} of glyphosate and AMPA were 8.2 and 137.2 days, respectively (geomean of available EU data); and the formation factor of AMPA was set to 27.5 %.

Scoring of environmental impact-IAP method

The results of the IAP (Impact Assessment Procedure) method (under publication), which was implemented in the two target areas (Chora and Peza) in the context of the LIFE09 ENV/GR/000302 SAGE10 project, were used to explain the results from the soil monitoring studies. According to the IAP concept, each impact is expressed as a combination of three elements (called in IAP *Triplet*): *Aspect* (growers' activities)-*Impact*-*Compartment* (soil, water, humans, biodiversity). Several parameters were utilised for

the assessment of the environmental impacts in the two target areas. Parameters, which can be related to the farmers' practices and choices or to the resilience of the environment to contamination, were recorded and weighted. Each of the 200 olive-groves which were randomly selected in each area received a score, was based on data collected by agronomists. Data for the value or class of parameters were collected annually for three consecutive years (starting 1 year prior to the initiation of the monitoring). The score of each triplet was normalised to a 0-1 scale, where 0 represents the absence of expected impact and 1 the possibility of significant impact. The four triplets which are related to point source pollution with pesticides are the handling of wastewater loads from pesticide use (emptying, filling and cleaning of equipment), the management of empty containers, the transport and the storage of agrochemicals. In each of these triplets, the impact was pollution and the compartment was the abiotic environment. Groves under organic farming system or groves where chemicals were not used for weed control were excluded.

Results

Monitoring of glyphosate and AMPA residues in conventional olive farms of Chora and Peza with long history of glyphosate use

The analysis of the soil residues was restricted to glyphosate which was extensively used in both studied areas. Its major metabolite AMPA was also determined in all analysed soil samples. The analyses results for glyphosate and AMPA in soil samples during the three sampling years (2012-2014) are given in Table 7.5-13 and Table 7.5-14. For practical reasons (workload, distance between parcels, number of sampling sites etc), sampling was conducted at various intervals after glyphosate application as presented in Table 7.5-11, thus, the side-by-side comparison of the residue levels between years and sampling sites is not possible. In order to compare the level of glyphosate and AMPA residues in different sites, the measured concentration in soil (MCs) was associated with the estimated PECs which corresponds to the time of sampling, using the initially applied dose and the theoretical dissipation rate constants for glyphosate and AMPA (MCs/PECs ratio; Figure 7.5-2).

Glyphosate and AMPA concentrations from soil samples collected in Peza ranged from <LOQ to 240 µg/kg and from <LOQ to 100 µg/kg, respectively. Glyphosate residues exceeding the LOQ were determined in 6 out of 35 glyphosate-treated sites. Concentrations of glyphosate and AMPA were generally far lower than the theoretically estimated levels (PECs) except for three sampling occasions (Table 7.5-13), which suggest that glyphosate was rapidly degraded in this area. The MCs/PECs ratio was 0.35 in the first sampling event and reduced further at subsequent samplings (Figure 7.5-2). The decrease of the MCs/PECs ratio can be linked to the reduction of glyphosate losses from improper pre- and post-application handling as suggested by the improvement of the triplet score through the application of IAP Method. The targeted training that operators received led to the lowering of the mean score for two of the four examined aspects (remnant handling and transport) (Table 7.5-15). The change in score was significant in groves which received the highest score in the baseline year (score class 0.3-0.4). The reduction of transport distance, the selection of zero-slope spots for handling and disposal of spay leftovers and the frequency of use of spraying equipment are the associated parameters which were refined in the 2012-2013 period. The adoption of environmentally sound practices was mirrored in the slight improvement of specific indicators: the proportion of operators which accurately performed the triple rinsing of empty containers (increase from 55 % in the baseline year to 63 % in 2013, data not shown) and the proportion of spraying equipment without visible leakages (increased by 9.5 % in the same period). It is noticeable that glyphosate remained one of the prevalent weed control practices in the area as the total glyphosate load was reduced by only 9.9 % between 2011 and 2013 in the area.

Based on the results of the first sampling year in Peza, an estimation of the rate of degradation of glyphosate was done, assuming that residue decline follows simple first-order kinetics. Residues of glyphosate reached the limit of quantification (LOQ) within 2-3 weeks after application in 15 out of 18 samples in 2012. In the three remaining sites from the 2012 sampling, some glyphosate residues were traced (31-240 µg/kg). If first order degradation is assumed, the estimated half-lives for AMPA in these three soils could be approximated to range from 3.6 to 5.7 days. Thus, DT_{50} can be anticipated to be close to the lowest recorded values for this active substance. The absence of substantial residual amounts of glyphosate and AMPA indicates that built-up of residues after repeated use of glyphosate products is not expected in this area.

The concentration of glyphosate and AMPA in soil received from Chora ranged from <LOQ to 350 µg/kg and <LOQ to 650 µg/kg, respectively. The variation in the level of residues between sites may be explained by differences in the application rates, the frequency of application events and the interval between last application and sampling. The analysed concentrations of glyphosate and AMPA in Chora exceeded the theoretically estimated values in a number of sites, especially at the first sampling year. The maximum measured concentration of AMPA is soil (650 µg/kg) is, however, lower that the theoretical worst-case plateau concentration of AMPA in permanent crops (4140 µg/kg) after 10 years of continuous glyphosate applications. The proportion of sampling sites with residues exceeding the theoretically estimated values was reduced from 88 % in 2012 to 36 % in 2013 and 21 % in 2014. To be noted that residual AMPA residues from applications before 2012 were not included (the baseline concentration of AMPA was not set). Various sources of contamination linked to the handling of pesticide equipment and the management of the application leftovers are possible to have contributed to the exceeding of the theoretical values.

The results of a survey in Chora showed a perceivable improvement in mean score of triplets and more specifically for aspects linked to point source contamination of soil with pesticides in 2012 and especially 2013 compared to 2011 (Table 7.5-15). The adoption of environmentally friendlier aptitude at the second and especially the third year of monitoring is mirrored in the steep decrease of MCs/PECs values in Chora between 2013 and 2014 (Figure 7.5-2). The mean MCs/PECs ratio was 6.95 in the first sampling year and reduced to <1 in the third year. The aspects which were improved are the handling of remnants from application, the safe transport of pesticide loads and the management of obsolete containers. On the contrary, the safety of storage practices was not practically improved in the monitoring period. The improvement of the environment impact score between 2011 and 2013 is directly linked to the training which operators received by experts during the same period in the context of the program SAGE10. Further scrutiny of the parameters linked to the triplet score revealed that the most significant contributing factors to the year-by-year decrease of the score in the area are the quantity of pesticides in transport and the transport distance, the lowering of the distance between handling areas and surface water bodies and the improvement in the frequency of the visual examination and calibration of spraying equipment before use. Further, in-site inspections and interviews revealed a shift to environmentally friendlier practices. The number of operators which are considered to accurately perform the triple-rinsing increased from 57 to 64 %, and the proportion of spraying equipment without visible leakages increased from 60 to 64 % in the same period (data not presented). Other contributing factor is the reduction of total glyphosate load in the catchment between 2011 and 2013. The total amount of glyphosate was reduced by 61.2 %, due to the gradual shifting to other chemical solutions (oxyfluorfen, glufosinate-ammonium) as part of the Conyza spp. resistance management.

The mean level of AMPA residues in Chora for all sampling years was higher compared to Peza despite the fact that the mean application dose was higher in Peza, and the interval between application and sampling was narrower (Table 7.5-11). Differences were more striking in the first year of application. Variances in residue levels may reflect differences in pesticide residue management or the dissipation potential of soils. Further, differences in the application technique may have influenced the residual amount of glyphosate in soil. In a significant proportion of olive groves in the Peza Region (46.9-63.5 %, depending on the year), glyphosate is carried out as spot application, whilst most spray operations in Chora are usually performed by broadcast spraying (73.0-99.1 % of groves in the 2001-2003 period). Further, the two regions belong to different climatic zones: Chora has a subhumid climate whilst Peza belongs to the semi-arid zone, which may affect the dissipation potential. Compared to Chora, a more favourable environmental profile was observed in Peza as regards the handling of pesticide leftovers and the management of empty containers. On the contrary, a lower mean score was recorded for Chora as regards storage, irrespectively of the year and transport in 2012-2013. However, it should be noted that the initial mean score was generally low in both areas as only a few triplets received a score of higher than 0.3.

Only slight differences in the physicochemical characteristics of soils in the two sites were seen, except for Olsen-P content. The presence of phosphate in soil has been reported to compete with glyphosate and AMPA for sorption sites and thus can affect the bioavailability of the both substances as well as stimulate the glyphosate degradation. However, due to the lack of relevant data, it is not possible to correlate the higher levels of P in the Chora region with the presence of naturally occurred phosphates and/or phosphate fertilisation. Despite the significant number of samples taken for analysis, correlation analysis performed

did not reveal association between detected residue levels and pH, soil type or any of the physicochemical soil properties probably due to the fact that the interval between application and sampling differed significantly between sites.

Monitoring of glyphosate and AMPA in organic farms in Chora and conventional farms in Chora and Peza where glyphosate is not used

A number of soil samples were collected from certified organic farms of Chora (OF1-OF13) and conventionally cultivated groves in both target areas, where glyphosate is not used for weed control. The analysis of soil samples aimed at the examination of possible occurrence of glyphosate residues transferred from bordering sites, where glyphosate is used for weed control, or from other unpredictable routes of entry. Except for one site in which glyphosate was traced at levels of 27 µg/kg in 2013, no glyphosate was detected at any sampling event in the OF sites. The metabolite AMPA was detected in five sites, in at least one sampling event, and at concentrations ranging from 13 to 440 µg/kg (Figure 7.5-3). It is possible that glyphosate and AMPA residues were derived from neighbouring sites via drift and run-off. Glyphosate and AMPA have been previously found in soil environments in which glyphosate had never been used as a result of surface run-off from zones where it was initially applied. However, this route of entry cannot explain the elevated concentrations of AMPA in OF5 and OF7 sites. Further scrutiny revealed that the two sites were used as spots for washing of application equipment after use in nearby fields in 2012. The high AMPA levels can thus be considered as a result of point source pollution. The improper disposal of spraying remnants was not repeated at subsequent years. The quantified levels of AMPA in these two sites significantly decreased in 2013, resulting in 93-100 % dissipation of the initial amount within 1 year.

Except for one site in which AMPA amounted to 25 µg/kg, no glyphosate or AMPA residues was traced in the 16 sites in Peza in which no chemical weed control was carried out the year of sampling. Furthermore, AMPA residues ranging from 16 to 21 µg/kg were quantified in the six sites in Chora where glyphosate was not used for weed control.

Figure 7.5-2: The measure concentration (MCs) to predicted environmental concentration (PEC) ratio of AMPA residues in 2012-2014 in 11 sites from Chora and four sites in Peza (only sites for which data on all 3 years are presented)

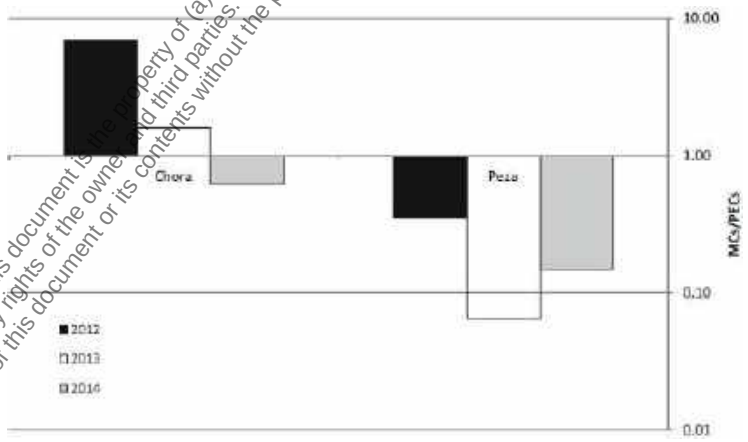


Table 7.5-13: Measured concentrations (MCs; µg/kg) and predicted environmental concentration (PECs; µg/kg) of glyphosate and AMPA in treated sites in Peza

Site code	2012				2013				2014			
	Glyphosate		AMPA		Glyphosate		AMPA		Glyphosate		AMPA	
	PECs	MCs	PECs	MCs	PECs	MCs	PECs	MCs	PECs	MCs	PECs	MCs
CFP1	137	< LOQ	79	32	0	< LOQ	82	20	nd[d]	nd[d]	nd[d]	nd[d]
CFP2	555	< LOQ	297	71	3	< LOQ	172	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP3	149	< LOQ	127	13	3	< LOQ	147	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP4	213	< LOQ	123	80	1	19	138	26	289	< LOQ	268	47
CFP5	291	< LOQ	168	26	1	< LOQ	128	17	nd[d]	nd[d]	nd[d]	nd[d]
CFP6	351	< LOQ	203	24	1	< LOQ	137	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP7	257	< LOQ	161	< LOQ	0	< LOQ	210	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP8	678	< LOQ	336	30	nd[a]	12	nd[a]	10	nd[d]	nd[d]	nd[d]	nd[d]
CFP9	399	140	249	27	1	23	156	< LOQ	231	< LOQ	231	96
CFP10	386	31	415	65	1	14	167	14	nd[d]	nd[d]	nd[d]	nd[d]
CFP11	361	< LOQ	285	12	nd[a]	14	nd[a]	16	nd[d]	nd[d]	nd[d]	nd[d]
CFP12	441	< LOQ	240	70	60	< LOQ	306	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP13	462	< LOQ	289	32	1	< LOQ	194	22	nd[d]	nd[d]	nd[d]	nd[d]
CFP14	379	240	174	21	1	< LOQ	214	15	311	< LOQ	227	< LOQ
CFP15	114	< LOQ	66	34	0	< LOQ	81	< LOQ	348	< LOQ	340	< LOQ
CFP16	629	< LOQ	312	62	0	< LOQ	168	27	nd[d]	nd[d]	nd[d]	nd[d]
CFP17	629	< LOQ	312	22	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]
CFP18	nd[b]	< LOQ	nd[b]	< LOQ	2	< LOQ	198	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP19	nd[d]	nd[d]	nd[d]	nd[d]	nd[c]	< LOQ	nd[c]	< LOQ	263	< LOQ	234	100
CFP20	nd[d]	nd[d]	nd[d]	nd[d]	9	< LOQ	62	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP21	nd[d]	nd[d]	nd[d]	nd[d]	0	< LOQ	186	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP22	nd[d]	nd[d]	nd[d]	nd[d]	3	< LOQ	119	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP23	nd[d]	nd[d]	nd[d]	nd[d]	1	< LOQ	108	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP24	nd[d]	nd[d]	nd[d]	nd[d]	1	< LOQ	112	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP25	nd[d]	nd[d]	nd[d]	nd[d]	0	< LOQ	169	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP26	nd[d]	nd[d]	nd[d]	nd[d]	0	< LOQ	169	< LOQ	nd[d]	nd[d]	nd[d]	nd[d]
CFP27	nd[d]	nd[d]	nd[d]	nd[d]	1	< LOQ	162	15	nd[d]	nd[d]	nd[d]	nd[d]
CFP28	nd[d]	nd[d]	nd[d]	nd[d]	9	< LOQ	59	18	nd[d]	nd[d]	nd[d]	nd[d]
CFP29	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	156	< LOQ	143	< LOQ
CFP30	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	160	< LOQ	147	< LOQ
CFP31	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	178	< LOQ	164	60
CFP32	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	121	< LOQ	112	10
CFP33	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	172	< LOQ	158	< LOQ
CFP34	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	143	< LOQ	132	< LOQ
CFP35	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	nd[d]	223	< LOQ	211	< LOQ

nd not determined

[a] Application of 2013 is conducted but no information on the application rate or date is available

[b] Application with glyphosate was not carried out in this year

[c] No information on sampling date is available

[d] No sampling was carried out

Table 7.5-14: Measured concentrations (MCs; µg/kg) and predicted environmental concentration (PECs; µg/kg) of glyphosate and AMPA in treated sites in Chora Trifilias

Site code	2012				2013				2014			
	Glyphosate		AMPA		Glyphosate		AMPA		Glyphosate		AMPA	
	PECs	MCs	PECs	MCs	PECs	MCs	PECs	MCs	PECs	MCs	PECs	MCs
CFC1	41	190	72	210	0	<LOQ	16	140	22	<LOQ	127	<LOQ
CFC2	39	150	70	180	0	<LOQ	40	<LOQ	1	<LOQ	81	80
CFC3	0	<LOQ	74	<LOQ	0	<LOQ	50	<LOQ	1	<LOQ	102	29
CFC4	nd[a]	<LOQ	nd[a]	<LOQ	nd[b]	nd[b]	nd[b]	nd[b]	0	<LOQ	67	100
CFC5	0	<LOQ	176	250	0	<LOQ	62	10	0	<LOQ	44	150
CFC6	0	230	176	260	0	<LOQ	62	19	0	<LOQ	35	34
CFC7	0	350	177	650	0	<LOQ	63	14	0	<LOQ	35	16
CFC8	0	<LOQ	6	260	0	<LOQ	27	26	0	<LOQ	152	10
CFC9	0	<LOQ	35	100	0	<LOQ	58	30	0	<LOQ	109	<LOQ
CFC10	0	<LOQ	32	330	0	<LOQ	58	120	0	<LOQ	107	<LOQ
CFC11	0	<LOQ	87	310	nd[b]	nd[b]	nd[b]	nd[b]	42	<LOQ	118	<LOQ
CFC12	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	<LOQ	11	<LOQ	0	<LOQ	102	<LOQ
CFC13	0	<LOQ	93	180	0	70	118	30	0	<LOQ	162	<LOQ
CFC14	10	140	52	310	nd[b]	<LOQ	50	50	0	<LOQ	76	47
CFC15	0	nd[b]	11	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	0	<LOQ	36	<LOQ
CFC16	0	<LOQ	53	<LOQ	nd[b]	nd[b]	nd[b]	nd[b]	0	<LOQ	60	34
CFC17	nd[b]	nd[b]	nd[b]	nd[b]	0	<LOQ	26	70	nd[c]	<LOQ	nd[c]	<LOQ
CFC18	39	<LOQ	54	320	nd[b]	nd[b]	nd[b]	nd[b]	0	<LOQ	22	80
CFC19	28	<LOQ	40	140	nd[b]	nd[b]	nd[b]	nd[b]	0	<LOQ	16	140
CFC20	0	<LOQ	19	20	0	<LOQ	3	<LOQ	nd[c]	<LOQ	nd[c]	20
CFC21	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	0	<LOQ	35	18

nd not determined

[a] Application with glyphosate was not carried out in this year

[b] No sampling was carried out

[c] No information on glyphosate application available

Figure 7.5-3: Glyphosate and AMPA residues in 2012-2014 in nine sites from organic farms in Chora (only samples with data on more than 1 year are presented)

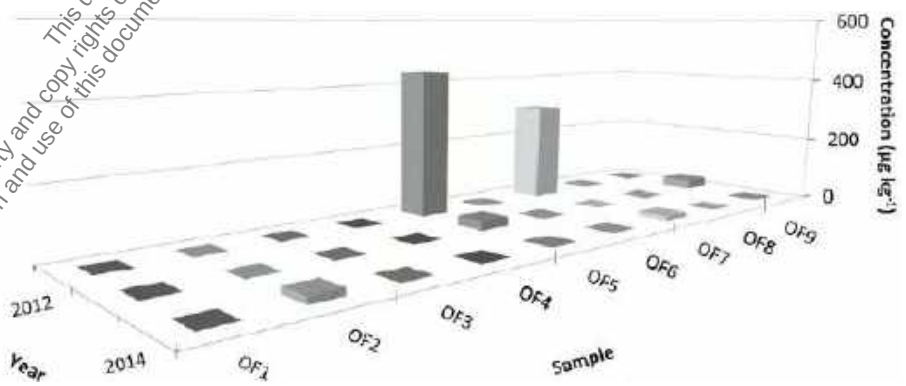


Table 7.5-15: Percentage of parcels in Chora and Peza in various score classes for each of the three triplets which are associated with the pesticide handling and the risk for contamination of the environment via point sources in the years 2011 to 2013

Triplet	Score class	Percentage of sites					
		Chora			Peza		
		2011	2012	2013	2011	2012	2013
Pesticide wastewater handling	0–0.1	8.4	7.9	6.3	0	6.6	5.5
	0.1–0.2	33.6	47.4	59.4	80.0	71.9	72.4
	0.2–0.3	53.8	41.2	32.0	16.1	18.2	21.3
	0.3–0.4	4.2	3.5	2.3	3.4	3.3	0.8
	0.4–0.5	0	0	0	0	0	0
	> 0.5	0	0	0	0	0	0
Pesticide transport	0–0.1	13.4	23.7	38.2	25.5	7.4	5.5
	0.1–0.2	58.0	46.5	49.4	52.7	70.3	76.4
	0.2–0.3	28.6	29.8	12.3	13.6	16.5	18.1
	0.3–0.4	0	0	0	7.3	5.8	0
	> 0.5	0	0	0	0.9	0	0
Storage	0–0.1	77.0	76.5	80.5	2.0	2.0	2.0
	0.1–0.2	23.0	23.5	19.5	54.5	54.5	54.0
	0.2–0.3	0	0	0	1.0	1.0	1.0
	0.3–0.4	0	0	0	0.5	0.5	1.0
	0.4–0.5	0	0	0	14.5	14.5	14.5
	> 0.5	0	0	0	27.5	27.5	27.5
Management of obsolete containers	0–0.1	48.3	50.9	57.0	79.1	79.3	78.0
	0.1–0.2	51.3	44.7	39.1	20.9	20.6	22.1
	0.2–0.3	0.3	4.4	3.9	0	0	0
	0.3–0.4	0	0	0	0	0	0
	0.4–0.5	0	0	0	0	0	0
	> 0.5	0	0	0	0	0	0

Conclusion

Glyphosate and the primary metabolite AMPA were present at maximum concentrations of 350 and 650 µg/kg, respectively, in soil sampled from olive groves in two monitoring areas in Greece. The residual amount of both contaminants differed between areas. Reduction of pesticide losses in the environment, which was one of the objectives of the SAGE10 project, was achieved by a combination of reduced glyphosate loads (especially in Chora, Trifilias, Peloponnese) and decreased glyphosate point source entries. The steep reduction of MCs/PECs values at the second and third year of monitoring was mirrored in the IAP Method triplet score, where aspects related to point source contamination were decreased, which in turn can be considered as a result of the targeted training of operators.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports monitoring data for glyphosate and AMPA in Greek agricultural soils associated with olive production. Glyphosate and AMPA were present at maximum concentrations of 350 and 650 µg/kg, respectively.
The article is therefore considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/004
Report author	Silva, V. *et al.*
Report year	2018
Report title	Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union
Document No	Anal Bioanal Chem (2012) 402:2335-2345
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Reliable with restrictions (extrapolated values for EU)

2. Full summary

Approval for glyphosate--based herbicides in the European Union (EU) is under intense debate due to concern about their effects on the environment and human health. The occurrence of glyphosate residues in European water bodies is rather well documented whereas only few, fragmented and outdated information is available for European soils. We provide the first large-scale assessment of distribution (occurrence and concentrations) of glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) in EU agricultural topsoils and estimate their potential spreading by wind and water erosion. Glyphosate and/or AMPA were present in 45 % of the topsoils collected, originating from eleven countries and six crop systems, with a maximum concentration of 2 mg/kg. Several glyphosate and AMPA hotspots were identified across the EU. Soil loss rates (obtained from recently derived European maps) were used to estimate the potential export of glyphosate and AMPA by wind and water erosion. The estimated exports, result of a conceptually simple model, clearly indicate that particulate transport can contribute to human and environmental exposure to herbicide residues. Residue threshold values in soils are urgently needed to define potential risks for soil health and off-site effects related to export by wind and water erosion.

Methods

The soil samples
Glyphosate and AMPA distributions were assessed in 317 topsoil samples: 300 samples from the LUCAS 2015 survey Land Use/Cover Area Frame Survey, a harmonized assessment of topsoil characteristics across EU Member States, and 17 samples from three independent vineyards in northcentral Portugal, where a parallel study on transport of pesticide residues by water erosion was conducted. The samples from the LUCAS 2015 survey were collected between April and October of 2015 as described in ESTAT (2015a)

and represent the uppermost 15/20 cm of soil. The samples selected for this work followed two main criteria: they were collected in i) the countries of each EU region with the highest percentage of agricultural area and pesticide use per hectare of arable and permanent croplands and ii) the crops with the highest pesticide use per hectare or highest extension of cultivated area in those countries. Pesticide use included, but was not restricted to, glyphosate-based herbicides (GlyBH) use since other pesticide residues were also analyzed in the samples. These sample selection criteria provide a worst case estimate of distribution of multiple pesticide residues in EU agricultural topsoils.

The countries selected by EU region were, from largest to smallest in order of pesticide use per hectare, in the northern region: United Kingdom (UK) and Denmark (DK); southern region: Italy (IT), Greece (EL) and Spain (ES); eastern region: Hungary (HU) and Poland (PL); western region: The Netherlands (NL), France (FR) and Germany (DE). The crops selected were cereals (wheat, barley, rye, maize, triticale, oats), root crops (potatoes, sugar beet), non-permanent industrial crops (sunflower, rapeseed), dry pulses and fodder crops (floriculture, alfalfa, temporary grassland), permanent crops (citrus, vines, olives, other fruit trees and berries), vegetables (tomatoes, other fresh vegetables). Additionally, some bare soils, which were croplands in the previous LUCAS 2009 and 2012 surveys, were included in the category others. The exhaustive list of crops within each LUCAS category is available in ESTAT (2015b). Not all the crops of each category were covered by the samples selected for this study, the covered ones are listed between brackets. Preference was then given to samples having the same land cover in previous LUCAS surveys and from different regions. All EU Member States are subdivided into regions, according to the Nomenclature of Territorial Units for Statistics (NUTS) classification, to ensure comparable regional statistics. The NUTS classification includes three hierarchical levels: NUTS 1 - major socio-economic regions, NUTS 2 - basic regions for the application of regional policies, and NUTS 3 - small regions for specific diagnoses. In this study, results are presented for basic regions (NUTS 2), defined according the NUTS 2013 classification.

The samples from the LUCAS 2015 survey were air dried and stored in the Joint Research Centre (JRC) installations in Ispra, Italy. The 300 LUCAS samples selected for this study were homogenized (by stirring the soil with a spoon until obtain a visually homogeneous sample) and sub-samples (of approximately 50 g dry weight) were collected for pesticide analysis. The sub-samples were sieved with a 2-mm sieve and frozen until chemical analysis. The Portuguese (PT) soil samples were collected in September of 2015, also following method described in ESTAT (2015a), and treated as the LUCAS (sub-) samples, i.e. air dried, 2-mm sieved and frozen until chemical analysis.

Glyphosate and AMPA analysis
The day before the analytical determinations, the soil samples were thawed and homogenized as described above for the selected LUCAS samples. Two aliquots of 2 g were collected from each sample. Glyphosate and AMPA concentrations were determined in the aliquots through HPLC-MS/MS using the same extraction and derivatisation method (see the Supporting Information for full details), chemicals, mobile phases, column characteristics and instrumentation conditions as described in Bento *et al.* (2016) and Yang *et al.* (2015).

All the validation parameters and quality control criteria were in line with those described in the guidance document for pesticides residues analysis in food and feed. Briefly, glyphosate and AMPA analytes were identified according to the retention time and peak shape of isotopically-labelled internal standards, glyphosate (1,2-^{13}C, ^{15}N) and AMPA (^{13}C, ^{15}N). Two transitions were measured by analyte [the quantification (Qn) and confirmation transitions (Ql)], and all positive results/samples presented an ion ratio of the two transitions within ± 30 % of the mean ion ratio of the solvent standards. The responses of the analytes were normalized according to the response of the isotopically-labelled internal standards. Glyphosate and AMPA concentrations were calculated based on one-point calibration, the solvent standard of 0.1 µg/mL, which analyzed every 10-15 injections/samples. A calibration curve (of the solvent standards 0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1 and 2 µg/mL) was injected at the start, middle and end of the sample sequences. All calibration curves presented satisfactory linearity of response versus concentration, with correlation coefficients ≥0.99 and individual residuals within ± 20 %. Blank soil standards fortified with a mixture of glyphosate and AMPA standards (0.25 µg/g) presented a recovery of both analytes between 70 and 120 %. Similar recovery values (75-120 %) were observed in soil samples fortified with the same

mixture of glyphosate and AMPA standards (a third aliquot was prepared from approximately 10 % of the soil samples). The concentration of glyphosate and AMPA measured in each of the two aliquots (replicates) collected per sample was typically within ± 30 %, and always within ± 35 %, the mean concentration of both aliquots. The mean concentrations of glyphosate and AMPA of aliquots were adopted as the concentrations of the sample. The limit of detection (LoD) of glyphosate and AMPA were 0.02 and 0.03 mg/kg, respectively, while the limit of quantification (LoQ) of both compounds was 0.05 mg/kg.

Data analysis
Only measurements/samples with glyphosate or AMPA (≥the LoQ 0.05 mg/kg) were considered in data analysis. Distribution of the concentrations of glyphosate and AMPA in the soils was presented in box-and-whisker plots per country and crop systems. Normality and homogeneity of variances of glyphosate and AMPA concentrations were tested with, respectively, Shapiro-Wilk W and Levine's tests. As the parametric assumptions were not met, even after log, ln, square root or arcsine transformation, differences among EU regions, countries and crop systems were tested with Kruskal-Wallis H tests. At the presence of significant differences (p <0.05), Pairwise Mann-Witney U test with Bonferroni corrections were performed to test differences between each two EU regions, countries or crop systems. The box-and-whisker plots and the statistical analyses were performed using SPSS 22.0.

Wind erosion rates in European agricultural soils were estimated by Borrelli *et al.* (2017) using a GIS version of the Revised Wind Erosion Equation model (GIS-RWEQ), while Panagos *et al.* (2015) used a modified version of the Revised Universal Soil Loss Equation (RUSLE) model to estimate water erosion rates in Europe. The complete wind and water erosion datasets are available via the European Soil Data Centre. Glyphosate and AMPA concentration data is represented at the basic region NUTS 2 level and not on exact locations due to privacy issues, and plotted together with erosion rates (although the different time scales; the erosion maps are annual maps and the soil samples were from a single time point) to indicate immediately if high concentrations in soil appear in areas vulnerable to wind and water erosion, to present a first idea of the dimension of the potential problem which was relevant to be further studied. Since the application pattern of GlyBH in croplands is similar each year, it is expected that concentration data is representative of a normal, recurrent soil situation. The maps of frequency of detection and maximum concentration of glyphosate and AMPA by NUTS 2 region were produced in ArcGIS 10.4.1. To estimate the potential export of glyphosate and AMPA to other locations, glyphosate and AMPA concentrations in top soils were multiplied by the potential annual soil loss rates from wind and water erosion at the sample collection points (extracted with ArcGIS from soil loss by wind and water erosion datasets). Export values were obtain for individual soil sampling points, if glyphosate or AMPA concentration in soil ≥0.05 mg/kg and there was a risk of wind or water erosion >0 Mg/ha year. Export rates of individual soil sampling points were then aggregated by (i) content of residues in soil, i.e. low to medium (defined in this study as 0.05-0.5 mg/kg) or high glyphosate or AMPA contents (>0.5 mg/kg), (ii) EU region, (iii) country, (iv) NUTS 2 region and (v) crop system. The threshold of 0.5 mg/kg used in this work corresponds to the 80th and 85th percentile of glyphosate and AMPA overall concentrations, respectively.

The proportion of AMPA to glyphosate in soil was determined for each sample containing glyphosate and/or AMPA (≥0.05 mg/kg), as the ratio of AMPA concentration in soil to the combined glyphosate and AMPA concentration in the soil, [AMPA / (Glyphosate + AMPA)] ∗ 100.

Results

Overall distribution of glyphosate and AMPA in topsoils
Glyphosate and/or AMPA were present (≥0.05 mg/kg) in nearly half (45 %) of the soil samples, with 18 % of the tested soils containing both compounds. AMPA was the predominant form, being present in 42 % of the soils while glyphosate was present in 21 %. Both compounds were present at higher frequencies in northern soils, while eastern and southern regions generally had the most glyphosate- and AMPA- free soils (<0.05 mg/kg), respectively. At national levels, the frequency of soils with glyphosate ranged from 7 % in Poland to 53 % in Portugal, while the frequency of soils with AMPA ranged from 17 % in Italy and Greece to 80 % in Denmark (Figure 7.5-4A and Table 7.5-16). Samples from permanent crops and root crops had the highest frequency of soils with glyphosate and AMPA (30 and 52 %, respectively), and dry pulses and fodder crops the lowest for both compounds (5 and 29 %, respectively, see Figure 7.5-4B and Table 7.5-16).

The highest concentrations of glyphosate and AMPA in soil were observed in southern parts of the EU (Figure 7.5-4C and Table 7.5-16), suggesting higher application rates of GlyBH in this region. Nevertheless, only concentrations of glyphosate were significantly higher in this region [glyphosate: Kruskal-Wallis (H) = 3.03, degrees of freedom (df) = 3, p <0.001, n = 67; AMPA: H = 20.50, df = 3, p = 0.387, n = 133].

Table 7.5-16: Distribution of glyphosate and AMPA in agricultural topsoils (015/20 cm) by EU region, country and crop system

	N	Glyphosate				AMPA				AMPA prop.
		pos. Samp.	Range (mg/kg)	Median		positive Samples	Range (mg/kg)	Median		Mean (%)
Overall	317	67 (21 %)	0.05 - 2.05	0.14		133 (42 %)	0.05 - 1.92	0 15		77
EU Reg.										
North	60	16 (27 %)	0.05 - 0.34	0.12	b	42 (70 %)	0.05 - 0.61	0 14		87
South	107	24 (22 %)	0.07 - 2.05	0.48	a	30 (28 %)	0.06 - 1.92	0 19		54
East	60	6 (10 %)	0.05 - 0.57	0.11	b	20 (33 %)	0.06 - 0.73	0 15		91
West	90	21 (23 %)	0.05 - 0.59	0.1	b	41 (46 %)	0.05 - 1.03	0 14		79
Country										
United Kingdom	30	8 (27 %)	0.05 - 0.21	0.15	ab	18 (60 %)	0.07 - 0.59	0 15	b	89
Denmark	30	9 (27 %)	0.06 - 0.34	0.11	ab	24 (80 %)	0.05 - 0.61	0 14	b	85
Portugal	17	9 (53 %)	0.43 - 2.05	1.14	a	9 (53 %)	0.42 - 1.92	0.73	a	42
Italy	30	5 (17 %)	0.09 - 0.18	0.13	ab	5 (17 %)	0.06 - 1.38	0 1	ab	54
Greece	30	3 (10 %)	0.39 - 0.63	0.54	ab	5 (17 %)	0 16 - 0.38	0 21	ab	61
Spain	30	7 (23 %)	0.07 - 0.95	0.22	ab	11 (37 %)	0.06 - 0.27	0.09	b	60
Hungary	30	4 (13 %)	0.05 - 0.57	0.1	ab	6 (20 %)	0.06 - 0.73	0 23	ab	79
Poland	30	2 (7 %)	0.08 - 0.23	0.16	ab	14 (47 %)	0.06 - 0.42	0 14	b	96
The Netherlands	30	7 (23 %)	0.05 - 0.59	0.13	ab	12 (40 %)	0.05 - 1.03	0 13	ab	75
France	30	9 (30 %)	0.05 - 0.27	0.08	b	15 (50 %)	0.06 - 0.78	0 13	ab	77
Germany	30	5 (17 %)	0.07 - 0.24	0.13	ab	14 (47 %)	0.07 - 0.54	0 15	b	83
Crop system										
Cereals	112	18 (16 %)	0.05 - 0.60	0.11		46 (41 %)	0.05 - 0.62	0 13		84
Root crops	27	6 (22 %)	0.05 - 0.59	0.33		14 (52 %)	0.05 - 1.03	0 12		80
Non-permanent industrial crops	23	5 (22 %)	0.05 - 0.21	0.07		11 (48 %)	0.06 - 0.59	0 16		86
Dry pulses and Fodder crops	21	1 (5 %)	0.06			6 (29 %)	0.07 - 0.17	0 11		86
Permanent crops	101	30 (30 %)	0.07 - 2.05		0.17	41 (41 %)	0.06 - 1.92	0 21		64
Vegetables	9	2 (22 %)	0.13 - 0.14		0.14	3 (33 %)	0.07 - 0.32	0 17		75
Others	24	5 (21 %)	0.05 - 0.95		0.15	12 (50 %)	0.06 - 0.74	0.08		79

Only samples containing glyphosate or AMPA (≥0.05 mg/kg) were considered for the range, median concentrations. For the AMPA proportion, samples containing only glyphosate or AMPA (≥0.05 mg/kg), with respectively an AMPA proportion of 0 or 100 %, were considered in mean values. Different letters represent significant differences [(p <0.05): a >b] between regions, countries or crop systems. N - number of topsoil samples tested, Range - minimum - maximum concentrations, AMPA Prop. - AMPA proportion = [AMPA / (Glyphosate + AMPA)]×100.

Soils from southern parts of the EU also presented the lowest proportion of AMPA (Table 7.5-16), suggesting more recent GlyBH applications and/or slower degradation of glyphosate into AMPA under drier conditions. Portuguese topsoils (all from vineyards) presented significantly higher amounts of

glyphosate (H = 31.97, df = 10, p <0.001, n = 67) and AMPA (H = 27.73, df = 10, p = 0.02, n = 133) than the other countries, with both compounds reaching concentrations as high as 2 mg/kg (Figure 7.5-4 and Table 7.5-16). NUTS 2 regions such as FR71, EL51, NL23, ES24 or ITC4 seem to contain low herbicide residues or be residue free (<0.05 mg/kg). Other NUTS 2 regions, including DK04, HU10, ES62, PT16 and ITH1, appear to have hotspots of glyphosate and/or AMPA contamination (N 0.5 mg/kg; Table 7.5-17).

Table 7.5-17: Distribution of glyphosate and AMPA in agricultural topsoils (0-15/20 cm) by NUTS 2 region. Only NUTS 2 with at least one sample containing glyphosate and/or AMPA (≥0.05 mg/kg)

NUTS 2	N	Glyphosate			AMPA			AMPA prop.
		positive Samples	Range (mg/kg)	Median	positive Samples	Range (mg/kg)	Median	Mean (%)
UKE3	1	0	-		1 (100 %)	0.07		100
UKF1	1	1 (100 %)	0.15		1 (100 %)	0.29		65
UKF3	2	1 (50 %)	0.21		1 (50 %)	0.57		73
UKG1	2	1 (50 %)	0.14		1 (50 %)	0.31		69
UKG2	3	0	-		3 (100 %)	0.07 - 0.08	0.07	100
UKJ1	1	0	-		1 (100 %)	0.13		100
UKK1	3	0	-		1 (33 %)	0.07		100
UKK2	2	0	-		1 (50 %)	0.09		100
UKM2	6	3 (50 %)	0.05 - 0.18	0.05	4 (67 %)	0 16 - 0.59	0.33	86
UKM3	1	0	-		1 (100 %)	0.07		100
UKM5	1	1 (100 %)	0.19		1 (100 %)	0.44		69
UKN0	2	1 (50 %)	0.07		2 (100 %)	0.09 - 0.43	0.26	93
DK02	6	0	-		5 (83 %)	0.07 - 0.17	0.11	100
DK03	7	1 (14 %)	0.10		5 (71 %)	0.06 - 0.54	0.17	96
DK04	15	6 (40 %)	0.06 - 0.33	0.12	13 (87 %)	0.05 - 0.61	0.13	77
DK05	2	1 (50 %)	0.06		1 (50 %)	0.26		82
PT16	17	9 (53 %)	0.43 - 2.05	1.14	9 (53 %)	0.42 - 1.92	0.73	42
ITC1	5	1 (20 %)	0.09		2 (40 %)	0.07 - 0.15	0.11	71
ITF3	1	1 (100 %)	0.12		0	-		0
ITG1	5	1 (20 %)	0.13		1 (20 %)	0.06		50
ITH1	1	1 (100 %)	0.13		1 (100 %)	1.38		91
ITH5	5	0	-		1 (20 %)	0.10		100
ITI1	3	1 (33 %)	0.18		0	-		0
EL52	10	1 (10 %)	0.38		3 (30 %)	0 16 - 0.38	0.18	83
EL61	2	1 (50 %)	0.53		1 (50 %)	0.20		28
EL65	7	1 (14 %)	0.63		1 (14 %)	0.26		29
ES11	3	1 (33 %)	0.22		1 (33 %)	0.07		50
ES23	3	2 (67 %)	0.07 - 0.43	0.25	3 (100 %)	0 12 - 0.27	0.15	69
ES41	4	0	-		1 (25 %)	0.08		100
ES42	5	1 (20 %)	0.11		2 (40 %)	0.06 - 0.09	0.08	69
ES61	2	1 (50 %)	0.16		1 (50 %)	0.14		47
ES62	8	2 (25 %)	0.6 - 0 95	0.78	3 (38 %)	0.06 - 0.21	0.08	45
HU10	2	1 (50 %)	0.57		1 (50 %)	0.73		56
HU21	2	0	-		1 (50 %)	0.23		100
HU22	8	1 (13 %)	0.07		1 (13 %)	0.23		77
HU32	9	2 (22 %)	0.05 - 0.13	0.09	2 (22 %)	0 12 - 0.36	0.24	71
HU33	8	0	-		1 (13 %)	0.06		100
PL22	2	0	-		1 (50 %)	0.08		100
PL23	2	0	-		1 (50 %)	0.06		100
PL31	9	2 (22 %)	0.08 - 0.23	0.16	7 (78 %)	0.06 - 0.42	0.15	92
PL33	2	0	-		1 (50 %)	0.08		100
PL41	5	0	-		1 (20 %)	0.10		100
PL51	4	0	-		1 (25 %)	0.20		100
PL52	1	0	-		1 (100 %)	0.21		100
PL61	1	0	-		1 (100 %)	0.07		100

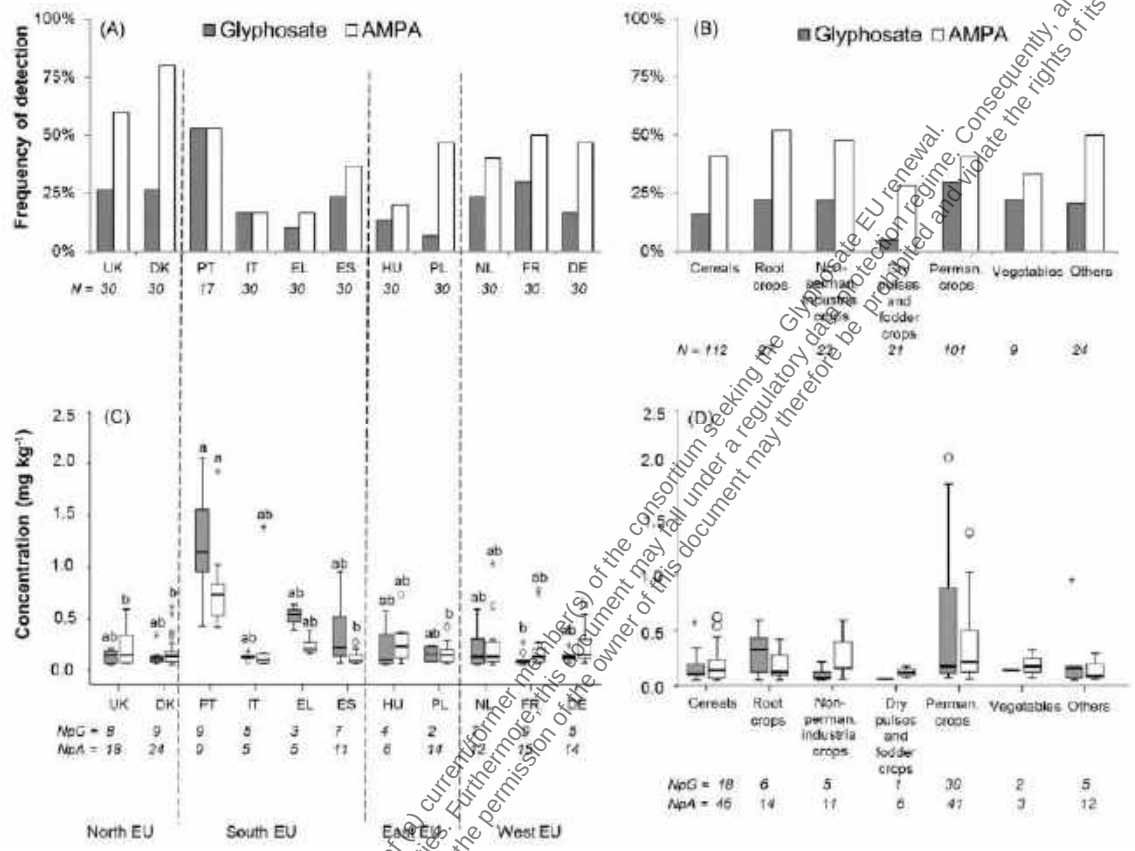
Table 7.5-17: Distribution of glyphosate and AMPA in agricultural topsoils (0-15/20 cm) by NUTS 2 region. Only NUTS 2 with at least one sample containing glyphosate and/or AMPA (≥0.05 mg/kg)

NUTS 2	N	Glyphosate			AMPA			AMPA prop.
		positive Samples	Range (mg/kg)	Median	positive Samples	Range (mg/kg)	Median	Mean (%)
NL11	5	2 (40 %)	0.07 - 0.59	0.33	4 (80 %)	0.06 - 1.02	0.18	85
NL13	4	3 (75 %)	0.05 - 0.42	0.19	4 (100 %)	0.09 - 0.62	0.22	70
NL21	4	0	-		2 (50 %)	0.08 - 0.08	0.08	100
NL23	9	1 (11 %)	0.05		1 (11 %)	0.05		50
NL34	4	1 (25 %)	0.13		1 (25 %)	0.17		57
FR22	1	1 (100 %)	0.17		1 (100 %)	0.74		82
FR25	1	1 (100 %)	0.06		0	-		0
FR51	1	0	-		1 (100 %)	0.23		100
FR52	6	2 (33 %)	0.09 - 0.10	0.10	4 (67 %)	0.09 - 0.16	0.12	79
FR53	3	2 (67 %)	0.05 - 0.07	0.06	2 (67 %)	0.06 - 0.27	0.16	66
FR61	2	0	-		1 (50 %)	0.13		100
FR81	7	3 (43 %)	0.07 - 0.27	0.08	5 (71 %)	0.06 - 0.78	0.09	80
FR82	4	0	-		1 (25 %)	0.07		100
DE11	3	0	-		1 (33 %)	0.11		100
DE91	1	1 (100 %)	0.24		1 (100 %)	0.38		62
DE92	1	1 (100 %)	0.11		1 (100 %)	0.31		73
DE93	1	0	-		1 (100 %)	0.13		100
DE94	3	0	-		2 (67 %)	0 10 - 0.16	0.13	100
DEA3	4	0	-		2 (50 %)	0 13 - 0.19	0.16	100
DEA4	1	0	-		1 (100 %)	0.07		100
DEA5	1	0	-		1 (100 %)	0.54		100
DEB1	1	1 (100 %)	0.13		1 (100 %)	0.30		70
DEB2	2	0	-		1 (50 %)			100
DEB3	6	2 (33 %)	0.07 - 0.14	0.10	2 (33 %)	0 12 - 0.21	0.16	49

Only samples containing glyphosate or AMPA were considered for the range and median concentrations. For the AMPA proportion, samples containing only glyphosate or AMPA (≥0.05 mg/kg), with respectively an AMPA proportion of 0 or 100 %, were considered in mean values. N - number of topsoil samples tested, Range - minimum and maximum concentrations, AMPA Prop. - AMPA proportion = [AMPA/(Glyphosate + AMPA)]×100.

Glyphosate and AMPA contents in soil were highest under permanent crops and lowest with dry pulses and fodder crops (Figure 7.5-4D and Table 7.5-16), yet no significant effect of the crop system was observed (glyphosate: $H = 10.29$, $df = 6$, $p = 0.113$, $n = 67$; AMPA: $H = 11.57$, $df = 6$, $p = 0.72$, $n = 133$). Vineyards presented the highest concentrations of glyphosate, yet at lower levels than those expected in soils of this crop, with maximum predicted environmental concentration (PEC) of 3.0646 mg/kg. On the other hand, the measured glyphosate concentrations in cereals occasionally exceed the respective maximum PEC value of 0.30 mg/kg. Maximum PEC values for AMPA, of 3.0862 mg/kg, available only for the worst-case scenario of a single application of 4.32 kg glyphosate/ha, were never been exceeded. Discrepancies between field measured concentrations and maximum PEC values probably result of an application regime by the farmers different from the recommended (in terms of number of treatments and on the amounts applied), of the growth stage (and interception) of the crop or of different edaphic, management or environmental conditions. In the calculation of PEC values, a worst case interception of 90 (cereals) and 0 % (orchards and vineyards), a fixed bulk density of 1.5 g/cm^3, a tillage depth of 5 cm (permanent crops) or of 20 cm (annual crops) and a halflife time (DT_{50}) of 143.3 days for glyphosate and of 514.9 days AMPA are assumed.

Figure 7.5-4: **Overall distribution of glyphosate and AMPA in EU topsoils (0–15/20 cm). Frequency of detection of glyphosate and AMPA (≥0.05 mg/kg) in soils from different (A) EU countries and (B) crop systems. Box-and-whisker plot representation of the distribution of glyphosate and AMPA contents in soils by the same factors: (C) country and (D) crop system.**

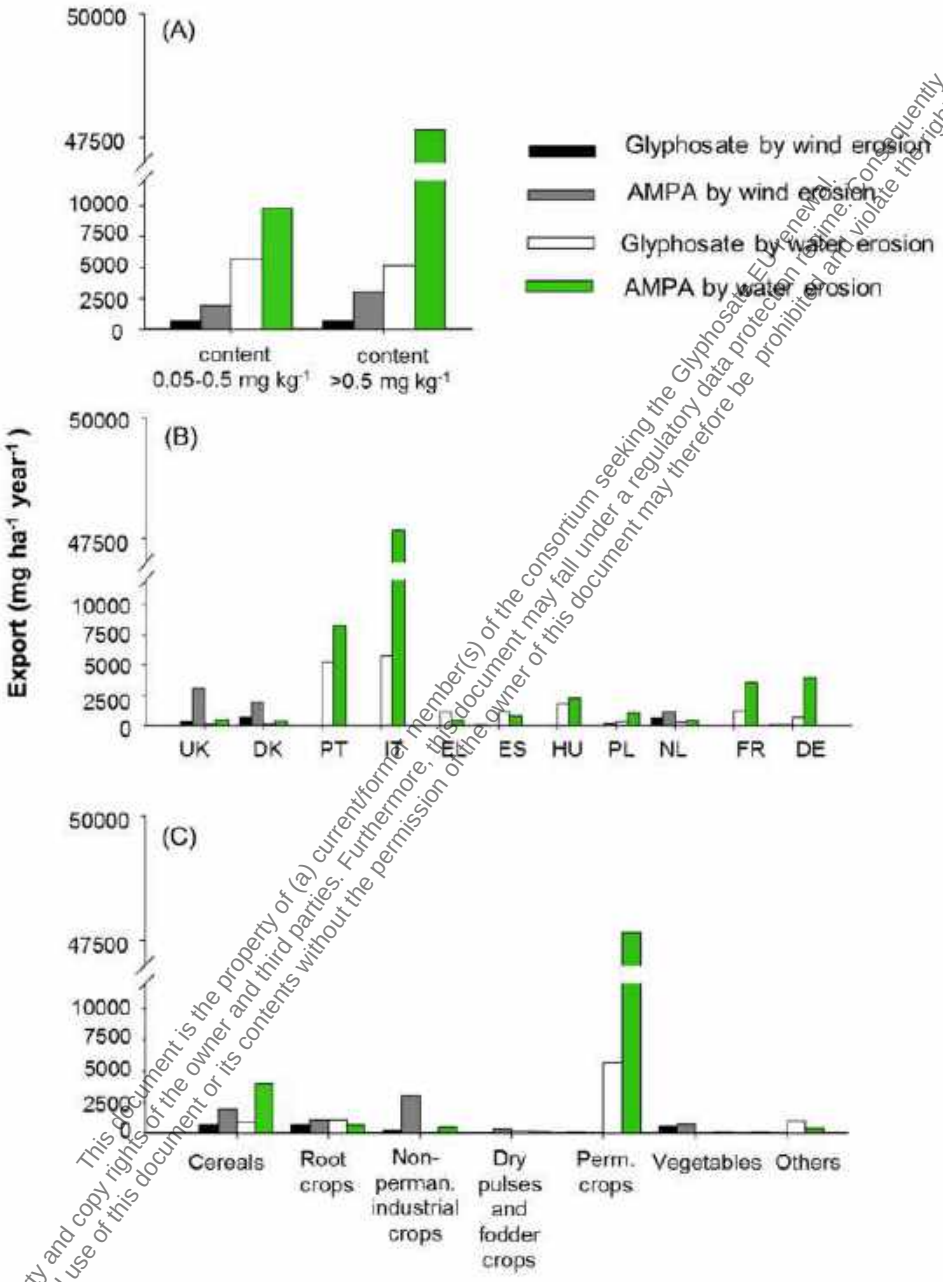


Only measurements ≥0.05 mg/kg were considered in the box-and-whisker plots. Each box represents the 25th percentile, median and 75th percentile. Whiskers represent 1.5 times the interquartile range or minimum and maximum concentrations of glyphosate or AMPA. Outliers (1.5–3 times the interquartile range) are marked with points and extreme outliers (N3 times the interquartile range) with asterisks. Different letters represent significant differences [($p < 0.05$): a > b] in glyphosate or AMPA concentrations between countries or crop systems. N – number of samples tested, Np = number of positive samples ≥0.05 mg/kg, G – glyphosate, A – AMPA.

Off-site transport by wind and water erosion

In areas with low to medium glyphosate or AMPA contents in soil (0.05-0.5 mg/kg), estimated glyphosate and AMPA removal by wind erosion reaches 1941 mg/ha year, while in areas with contents > 0.50 mg/kg it could exceed 3000 mg/ha year. Water erosion could lead to higher potential losses/exports of glyphosate and AMPA, with estimated maximum exports of 9753 mg/ha year in soils with low to medium herbicide contents, and of 47,667 mg/ha year in soils with higher contents (Figure 7.5-5A). The highest export potentials are observed in Southern parts of the EU (Figure 7.5-5B), in areas highly vulnerable to water erosion. Different crop systems, with different soil covers, lead to different transport potentials of glyphosate and AMPA: non-permanent industrial crops and root crops show the highest potential exports through wind erosion, while permanent crops and cereals present the highest exports through water erosion (Figure 7.5-5C).

Figure 7.5-5: **Potential export of glyphosate and AMPA by wind and water erosion. Maximum export estimations according to (A) glyphosate or AMPA content in topsoil, (B) country and (C) crop system. Perm. – Permanent**



A ratio between these potential exports and the typical GlyBH application rates (the exact application rates in the soil sampling points are not known) could provide an indication of the % of the initially applied products lost by erosion processes, potentially reaching water systems and atmosphere. The highest estimated potential export of glyphosate by water erosion (5715 mg/ha year), for example, would correspond to loss 0.13 % of the recommended maximum application rate of 4.32 kg glyphosate/ha year. As only glyphosate is applied to fields, no ratio can be calculated for AMPA, the most common compound in soils. Furthermore, such ratio can led to misleading results because glyphosate and AMPA are persistent compounds in soil, and their concentrations in soil (the ones used to estimate the potential exports by wind

and water erosion) often result of more than one year of treatments. Therefore, the ratio should consider not only the amount applied but also the amount accumulated from previous treatments.

Recent experimental and monitoring studies confirm wind-driven transport of glyphosate and AMPA. Bento *et al*. (2017) demonstrated in a wind tunnel experiment that contents of AMPA and especially of glyphosate were particularly high (respectively > 0.6 and > 15 µg/g) in the finest soil particle fractions (<10 µm), which can be inhaled by humans directly. In addition, both glyphosate and AMPA were often (>50 %) detected in air samples collected from agricultural areas in the U.S.A, reaching concentrations of respectively 9.1 and 0.97 ng/m^3. The presence of glyphosate in atmosphere can result of spray drift during the application and/or wind erosion of contaminated soil particles. However, for AMPA, which is formed in soil, wind erosion is the only source. The contribution of wind erosion to the atmospheric concentration of glyphosate is still unknown. In a comprehensive environmental survey conducted in the U.S.A., Battaglin *et al.* (2014) observed the presence of glyphosate and AMPA in over 70 % of the precipitation samples analyzed, at maximum concentrations of respectively 2.5 and 0.5 µg/L. In Europe, lower frequencies of detection are reported, with glyphosate and AMPA present in respectively 10 and 13 % of the rainwater samples, but with higher maximum concentrations, 6.2 and 1.2 µg/L, respectively. Glyphosate is supposed to degrade rapidly in the atmosphere by photochemical oxidative degradation, but the results from air and rain analyses indicate that glyphosate and AMPA can persist in the atmosphere and can be washed out and redistributed by rain (wet deposition).

Particulate transport via water erosion is an important pathway for glyphosate and AMPA towards surface water bodies. In fact, after a 60 min rain simulation at a rain intensity of 1 mm/min, Yang *et al.* (2015) observed that 4-5 % of the initially applied glyphosate was lost/transported by runoff in the dissolved phase while 8-11 % of the applied glyphosate was transported by the suspended load. Glyphosate and AMPA are frequently detected in U.S. large rivers (53-89 %, respectively), streams (53-72 %, respectively), lakes, ponds and wetlands (34-30 %, respectively) at maximum levels of respectively 300 and 48 µg/L. In Europe, glyphosate and AMPA have been analyzed in respectively 75,350 and 57,112 surface water samples, and detected in 33 % and 54 % of the samples at levels up to 370 µg/L and > 200 µg/L. Correlations between these concentrations in waters and the concentrations measured in this study in soils would be too speculative given the different time collection and location between the information that is available for glyphosate in streams and the soil samples analyzed for this study. However, the spatial relationship between erosion rates and pesticide distribution in soils and water bodies should be further explored. Particulate transport processes are particularly important for the off-site transport of pesticides strongly adsorbed to soil particles, just like glyphosate and AMPA. Quantification of the extent of transport off the field to surface waters (or to the atmosphere) should be explored, too. It should be noted that current EU legislation presents environmental quality standards in the field of water policy for only some pesticides, not including glyphosate or AMPA.

Conclusion

Within the context of this study, some considerations can be made. First, soil samples used in this study were collected during the spring and summer of 2015. No information is available regarding prior GlyBH application dates and rates per sample location, indicating that the 317 samples represent a mixture of real-field conditions, ranging from samples with no trace of glyphosate and/or AMPA to samples with very high levels. Despite the European Commission (EC) recommendations on the frequency of treatments and application rates, information on the actual use/sales of GlyBH in the EU, or of the active substance glyphosate, is not available and the amounts applied per crop system is confidential in almost all countries. The half-life times of glyphosate and AMPA, also of importance in the respect of the amounts found in soils, are highly variable, ranging from a few days up to one or two years, depending on edaphic and environmental conditions, namely temperature and soil moisture. AMPA is more persistent than glyphosate, and the degradation of both compounds is slower at colder and dryer conditions. The drier soils in southern EU might then explain the lower AMPA proportion found there. Second, it is well-known that glyphosate and AMPA strongly adsorb and accumulate in the top centimeter(s) of soils. As glyphosate and AMPA contents determined in this study are average values for entire topsoil layers up to 15/20 cm depth (a consequence of using topsoil samples from an already established survey), actual contents in the surface layer could be higher than the determined average, implying that the presented potential erosion-driven transport rates of glyphosate and AMPA could be underestimated. The distribution of glyphosate and

AMPA at the surface layer (the region most prone to soil erosion) and within topsoil should be considered in future work and should cover different soil management practices, as tillage results in the incorporation/redistribution of contaminants accumulated in surface into deeper layers. Third, pesticide residue transported by wind and water erosion do not necessarily end up in the atmosphere and surface water systems alone; other land and even ocean regions can be reached by such phenomena, with deposition of transported compounds as a result. This stresses the need for better monitoring of the occurrence and spatial distribution of glyphosate and AMPA across the interlinked environmental domains of soil, water and air. Fourth, from a regulatory and legislation perspective, greater effort is needed to more thoroughly assess glyphosate and AMPA contents in soils, to define critical limits to protect soil quality and soil biodiversity, and to minimize the risk of further distribution of these compounds by wind and water erosion. Some EU countries have legislation and screening values for pesticide residues in soil but they are mainly limited to persistent organochloride pesticides. Air quality monitoring programs should also target pesticide residues in transported soil dust, in particular glyphosate and AMPA, and the potential risk of inhalation by humans. Finally, despite its limitations, the results of this study are concerning; high levels of glyphosate and of its main metabolite AMPA have been often detected in agricultural soils across the EU. The presence of glyphosate and AMPA in agricultural soils may not only form a risk for soil health but also a potential risk of further spreading of these compounds across land, water, and air domains. Indeed, besides potential effects on local edaphic communities and on humans, that can be exposed to these substances by inhalation of contaminated dust particles, dermal contact or ingestion of contaminated surface water, wind and water erosion have the potential to transport contaminants to all the environmental compartments: atmosphere, other soils and surface waters. This information should be fully accounted for in reconsidering approval and use of GlyBH. Additional efforts should be made to fully quantify the extent of soil contamination by glyphosate residues in agricultural soils worldwide, and to assess the related risk for humans and the environment.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the result from a field study to measure the distribution of glyphosate and AMPA in European topsoils. The study should give a basis for the understanding of glyphosate loss from soils via wind and water erosion, i.e. experimental information from the sample sites were extrapolated to the EU area. A detailed and tabulated overview on the results is given in the supporting information. The maximum measured concentrations of 2.05 mg/kg for glyphosate and 1.92 mg/kg for AMPA were from vineyards in central Portugal.
The article is therefore considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/005
Report author	Napoli, M. *et al.*
Report year	2016
Report title	Transport of Glyphosate and Aminomethylphosphonic Acid under Two Soil Management Practices in an Italian Vineyard
Document No	Journal of Environmental Quality 45:1713-1721 (2016)
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Worldwide, glyphosate is the most widely used herbicide in controlling the growth of annual and perennial weeds. An increasing number of studies have highlighted the environmental risk resulting from the use of this molecule in aquatic and terrestrial ecosystems. The objective of the study was to determine the transport of glyphosate and its degradation product, aminomethylphosphonic acid (AMPA), through runoff and transported sediment (TS) from a vineyard under two different soil management systems: harrowed inter-row (HR) and permanent grass covered inter-row (GR). The study was performed over a period of 4 yr. Glyphosate and AMPA concentrations were found to be higher in runoff and in transported sediment from HR compared with GR, regardless of the amount of runoff and transported sediment. The mean annual percentages of glyphosate loss, via runoff and transported sediment, were about 1.37 and 0.73 % for HR and GR, respectively. Aminomethylphosphonic acid represented approximately 30.9 and 40.0 % of the total glyphosate losses in GR and HR, respectively. Moreover, results suggested that rains occurring within 4 wk after treatment could cause the transport of glyphosate and AMPA in high concentrations. Soil analyses indicated that glyphosate content was below detection within 1 yr, whereas AMPA remained in the soil profiles along the vine row and in the inter-row. Results indicated that GR can reduce soil and herbicide loss by runoff in vineyard cropping system.

Materials and Methods

Vineyard Plots Set-up and Herbicide Application

The activity was performed in Montepaldi-San Casciano Val di Pesa, Italy. The experiment was conducted over a period of 4 yr from March 2007 to February 2011. According to a nearby weather station, during the decade 1995 to 2014, the pluviometric pattern was sub-Mediterranean, with an average annual rainfall of 854 mm and an average annual temperature of 14.9°C (Napoli *et al.*, 2013).

Runoff and soil loss measurements were performed in 25yr old trained vines (*Vitis vinifera* L.) of the Sangiovese red variety cultivated on a southsouthwest facing convex slope (average slope, 16 %) and located approximately 550 m from the weather station. The soil was classified as a siltyclay, with 16 % sand, 43 % silt, 41 % clay, organic matter content of 0.8 %, total carbonate content of 15 %, and pH of 8.0. The vine rows were 89.5 m long oriented up and down the slope. Vines were planted in a 0.8 m by 2.7 m pattern and lowcordon trained. The interrows were colonized by spontaneous herbaceous species (C*ynodon dactylon* L., >90 %). Every year, a commercial formulation of glyphosate was distributed in the middle of March (360 g/L a.i.) in a 1m wide strip along each vine row at a dose of 2 L/ha (34.8 g of a.i. for plot). Within the vineyard, four plots of about 283 m^2, each including two inter-rows and three rows (about 5.4 m in length), were delimited with a 0.2m high earth bank, forming the plot boundaries. Two management systems were applied: harrowed interrow (HR) and interrow permanent grass covered (GR). The soil in HR was superficially harrowed (810 cm) once a year in late April. In GR, the interrow soil remained undisturbed. Grass height on both HR and GR interrows was kept below 0.15 m with periodical shredding.

The average monthly ground cover was 17.6 % (range, 433 %) and 22.2 % (range, 639 %) for HR and GR, respectively.

Water and Soil Core Sampling and Herbicide Residues Analysis
On 26 Feb. 2007 and then at the end of each consecutive year (i.e., the last week of February), a total of 54 undisturbed soil cores (0.05 × 0.05 m) were collected on each plot. The sampling was performed on three transects within each plot (at 15, 45, and 75 m from the top). Within each transect, samples were taken from three sampling areas: on the vine row, at 0.675 m from the vine row (on the tractor wheels traces on soil), and at the center of the inter-row (at 1.35 m apart from the vine row). The samples were collected in duplicate at depths of 0 to 0.05, 0.20 to 0.25, and 0.35 to 0.4 m, respectively. The soil samples were air-dried, weighed, sieved, and then used for analyses according to Napoli *et al.* (2015). Runoff and associated sediment from each plot were intercepted by a Gerlach trough placed along the lower side of the plot. A downstream automated runoff gauge was used for measuring the runoff volume (RV) for separate rainfall events. The runoff gauges collected runoff aliquots of about 0.2 L every 300 L of RV. These aliquots were then poured into a single external poly(p-phenylene oxide) tank to produce a single sample of the entire runoff event. The runoff samples were collected after each rainfall from 1 Mar. 2007 to 28 Feb. 2011. To limit degradation, runoff samples were immediately analyzed for determining TS weight and then preserved in the dark at -20ºC for a maximum of 25 days until analysis. An aliquot of each sample, corresponding to approximately 10 % of the sample volume (0.2 L minimum), was decanted and dried at 105°C and then weighed to determine the TS concentration in each runoff sample.

Water samples were filtered through 1-mm glass fiber filters. The liquid was immediately derivatized with fluorenemethoxycarbonyle. The herbicide residues in TS were determined only when the amount of material collected in the sieve of the suspended solid samples was sufficient (>5 g of sediment) to perform reliable measurements. Then the herbicide residues in TS, along with the residues in the soil samples, were extracted first by ultrasonic extraction in methanol and then using the derivatization procedure. Water and soil samples were dispensed in parallel into plastic vials to reduce the adsorption of glyphosate and AMPA from the methanol-extracted solutions onto glassware surfaces. After derivatization, glyphosate and AMPA were quantified using liquid chromatography electrospray ionization tandem mass spectrometry with a TSQ Vantage triple quadrupole mass spectrometer. The lower limits of glyphosate and AMPA quantification in water and soil samples (method detection limit) were 0.1 mg/L and 10 mg/kg, respectively. Therefore, the glyphosate and AMPA concentrations were set to zero for calculating the occurrence and loading when lower than the quantification limit. The glyphosate lost by runoff as a percentage of the annual amount applied was calculated as reported in Imfeld *et al.* (2013).

Statistical Analysis
Time series were used to evaluate the measured data. Samples with herbicide concentrations less than the detection limits were assigned a value of zero when calculating flow weighted average concentrations and transport amounts. Statistical comparisons of tillage treatments on glyphosate and AMPA concentration in RV and TS were made with Student's *t* test. Statistical comparisons of soil core samples were made with ANOVA. Thereafter, pairwise comparisons were performed using the post hoc Tukey test.

Results and Discussion

Rainfall, Runoff, Soil Loss, and Dissolved Herbicide Concentrations in Runoff and Transported Sediment
Daily rainfall, runoff volumes, soil losses, and glyphosate and AMPA concentrations in RV and TS from HR and GR for the first, second, third, and the fourth year of the experiment are presented in Figure 7.5-6, Figure 7.5-7, Figure 7.5-8 and Figure 7.5-9, respectively. The cumulative rainfall amounts over the period extending from 1 March to 28 February of the subsequent year were 524, 751, 678, and 1043 mm for the first, second, third, and the fourth year of the experiment, respectively. In the observation period, a total of 145 and 146 separate runoff events were recorded and sampled for each replicate on HR and GR fields, respectively. The RV for separate events differed within tillage treatments ($p = 0.02$). In particular, the RV for separate events ranged from 0.001 to 16.26 mm (average, 2 ± 2.7 mm) and from 0.004 to 14.07 mm (average, 1.6 ± 2.3 mm), respectively, for HR and GR plots, thereby generating a total volume of 286.6 ± 1.7 and 238.4 ± 0.9 mm for HR and GR plots, respectively. The annual RV during the first, second,

third, and fourth year of the experiment were 14 ± 0.3, 62.1 ± 0.8, 68.8 ± 1.4, and 142.6 ± 0.8 mm for HR fields, respectively, and 18.8 ± 0.1, 56.8 ± 1, 72.3 ± 0.9, and 91.9 ± 1.2 mm for GR fields, respectively.

In the same period, TS were sampled in 130 ± 2.8 and 123 ± 1.4 separate runoff events from the HR and GR fields, respectively. The sediment concentration in RV differed within tillage treatments ($p = 0.003$). The soil losses for separate events ranged from 0.001 to 8.364 *t*/ha (average, 0.27 ± 0.91 *t*/ha) and from 0.001 to 1.029 *t*/ha (average, 0.07 ± 0.12 *t*/ha), respectively, for HR and GR plots. The annual soil loss during the first, second, third, and fourth year of the experiment were 0.34 ± 0.01, 3.21 ± 0.06, 5.53 ± 0.03, and 26.49 ± 0.41 *t*/ha for HR fields, respectively, and 0.15, 0.63 ± 0.02, 2.25 ± 0.01, and 5.04 ± 0.01 *t*/ha for GR fields, respectively. Regardless of the inter-annual variability observed during the study period, results showed that permanent grass cover reduced the average annual RV and the average annual soil loss with respect to HR treatment.

Figure 7.5-6: Rainfall, runoff, soil losses, and glyphosate and aminomethylphosphonic acid (AMPA) concentration in runoff and in transported sediment from harrowed (HR) and permanent grass-covered (GR) plots during the first year

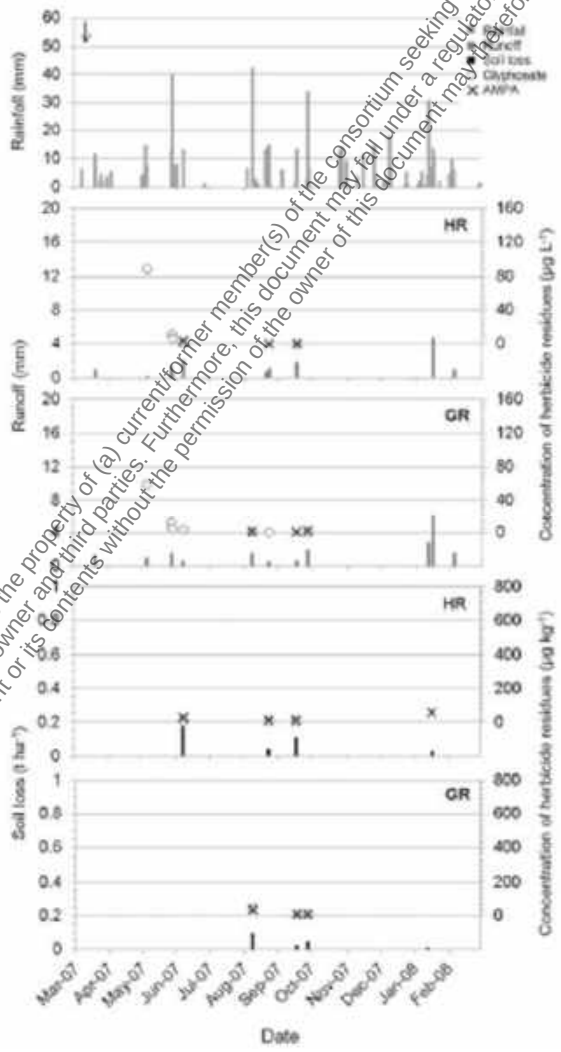
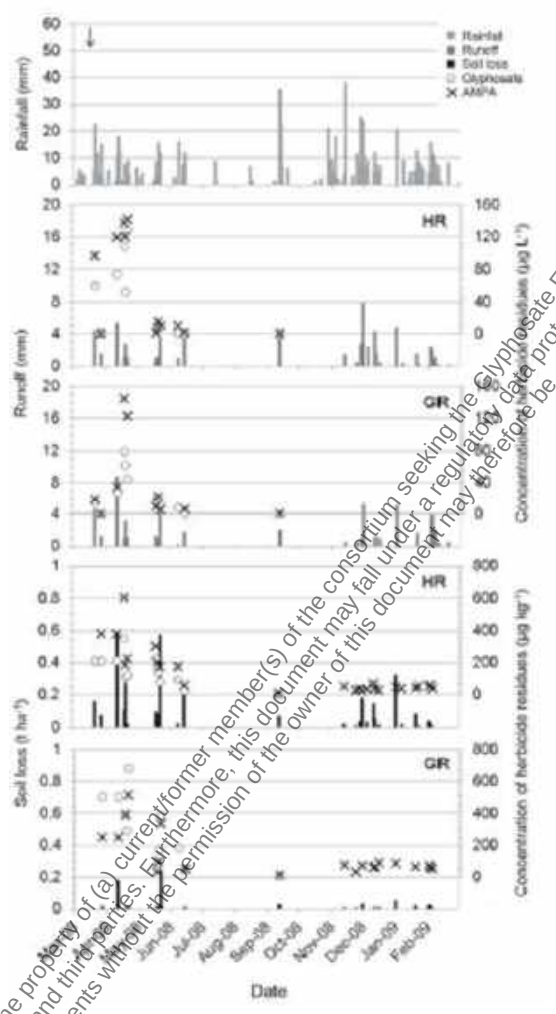


Figure 7.5-7: Rainfall, runoff, soil losses, and glyphosate and aminomethylphosphonic acid (AMPA) concentration in runoff and in transported sediment from harrowed (HR) and permanent grass-covered (GR) plots during the second year

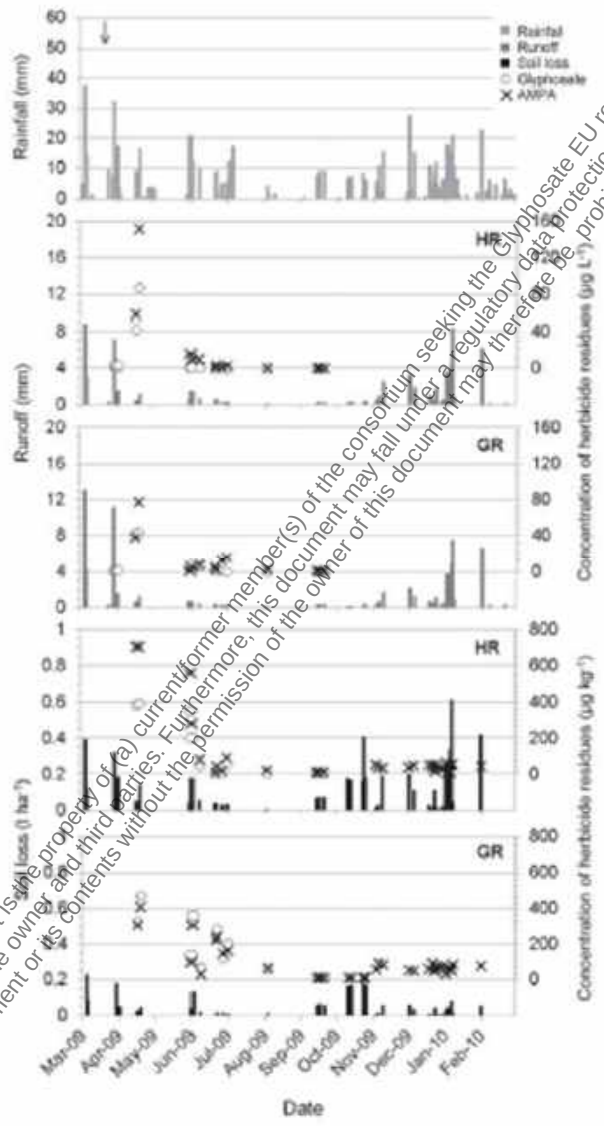


Glyphosate and Aminomethylphosphonic Acid Dissolved in Runoff

Glyphosate was detected in 33.6 ± 1 and 40.4 ± 0.6 % of the RV from HR and GR, respectively. Glyphosate concentrations ranging from 1 to 10 µg/L were detected in 8.2 ± 0.3 % of the RV from HR. Glyphosate concentrations ranging from 10 to 128.9 µg/L were detected in 10.9 ± 0.3 % of the RV from HR. Moreover, concentrations ranging from 1 to 10 µg/L were detected in 8.3 ± 0.2 % of the RV from GR, whereas concentrations ranging from 10 to 78.4 µg/L were detected in 13.7 ± 0.7 % of the RV from GR. After approximately 25.1 ± 13.8 d from the most recent application (days after application [DAA]), glyphosate appeared at high concentrations of about 68.1 ± 20.7 and 37.8 ± 19.3 µg/L in the RV from HR and GR, respectively. In 2008, the highest glyphosate concentrations in the runoff were measured after 37 DAA in HR and 34 DAA in GR. In the same year at 27 DAA, the highest glyphosate losses in runoff were about 3932.7 and 2388.9 mg/ha for HR and GR, respectively. Glyphosate was detected in the RV at concentrations exceeding 1 µg/L over a period of 68.8 ± 4.3 DAA (average, 34.0 ± 13.0 µg/L) and 76.5 ± 11.4 DAA (average, 21.0 ± 6.5 µg/L), respectively, after treatments on HR and GR. During the latter, an average RV of 11.2 ± 7.4 mm (cumulative rainfall amount, 164.4 ± 33.5 mm) and 13.8 ± 10.6 mm (cumulative rainfall amount, 180.8 ± 46.6 mm) was measured for HR and GR, respectively. Thereafter, the final glyphosate peaks appeared in the RV between early August and late September after approximately 173.5 ± 13.9 DAA and 186.8 ± 17.6 DAA in HR and GR, respectively.

Aminomethylphosphonic acid was detected in 33.6 ± 2.1 and 32.9 ± 1.6 % of the RV measured in HR and GR, respectively. In particular, AMPA at concentrations of 1 to 10 and 10 to 151.9 µg/L were detected in 7.5 ± 0.2 and 11.6 ± 0.2 % of the RV from HR, respectively.

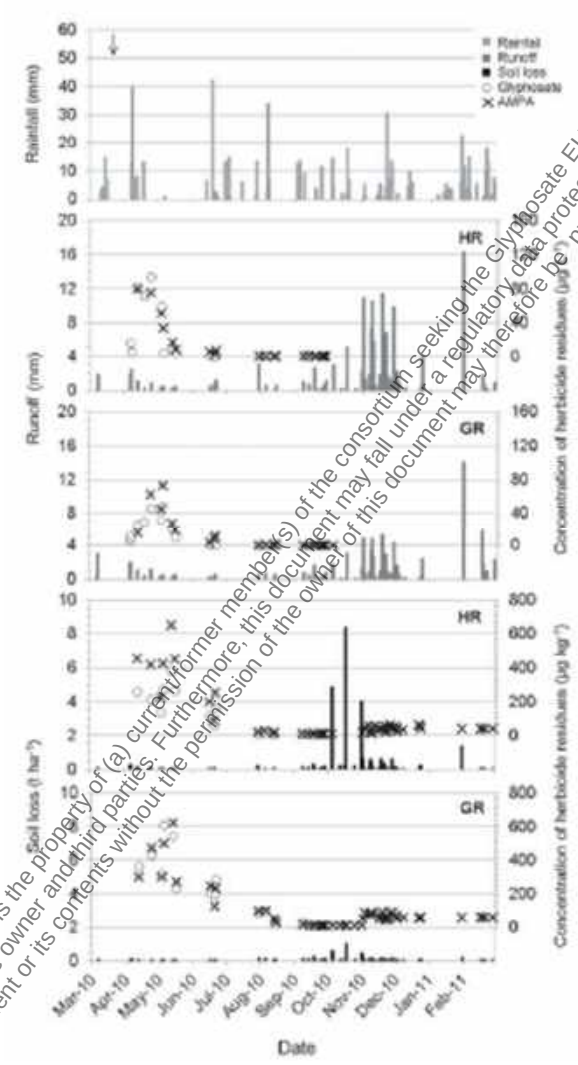
Figure 7.5-8: Rainfall, runoff, soil losses, and glyphosate and aminomethylphosphonic acid (AMPA) concentration in runoff and in transported sediment from harrowed (HR) and permanent grass-covered (GR) plots during the third year



Aminomethylphosphonic acid concentrations of 1 to 10 µg/L were detected in 10.3 ± 0.3 % of the RV from GR, whereas concentrations of 10 to 144.8 µg/L were detected in 11 ± 0.2 % of the RV from GR. After about 33.5 ± 27.7 DAA, AMPA appeared at high concentrations (approximately 59.7 ± 40.6 µg/L) in the RV from HR. In the RV from GR, AMPA appeared at a concentration of about 18.3 ± 15.5 µg/L after about 49 ± 57.6 DAA after the annual treatment. In the RV from GR, AMPA was detected at concentrations exceeding 1 mg/L for about 127.5 ± 39.4 DAA, with an average concentration of about 22.8 ± 17.0 µg/L. Instead, AMPA was detected for 90.8 ± 14.0 DAA, with an average concentration of 31.4 ± 24.1 µg/L in the RV from HR. During the latter, an average RV of 13.7 ± 9.1 mm (cumulative rainfall amount, 200.8 ± 52.9 mm) and 14.9 ± 11.0 mm (cumulative rainfall amount, 218.1 ± 51.6 mm) was measured for HR and GR, respectively. The final AMPA peaks in the RV appeared after approximately 184.3 ± 10.1 and

188.5 ± 11.1 DAA in HR and GR, respectively. The average glyphosate and AMPA concentrations in RV were significantly (p <0.001) greater in the HR than in the GR. No significant correlations were found between the glyphosate and AMPA concentration in RV and either seasonal or annual rainfall.

Figure 7.5-9: Rainfall, runoff, soil losses, and glyphosate and aminomethylphosphonic acid (AMPA) concentration in runoff and in transported sediment from harrowed (HR) and permanent grass-covered (GR) plots during the fourth year



Results indicate that glyphosate concentrations in the runoff peaked shortly after each application, similar to results observed by Shipitalo and Owens (2011) and Shipitalo *et al.* (2008). Moreover, the appearance of AMPA within the first week of monitoring in 2008 was consistent with the degradation rate of glyphosate reported by Landry *et al.* (2005) and Screpanti *et al.* (2005). Runoff events that occurred in autumn and winter did not produce any detectable concentrations of glyphosate and AMPA in RV as previously observed (Screpanti *et al.,* 2005; Shipitalo and Owens, 2011). When considering the glyphosate and AMPA concentrations in RV, both substances may have significantly contaminated surface waters only under conditions where runoff occurs shortly after herbicide application (Screpanti *et al.,* 2005).

Glyphosate and Aminomethylphosphonic Acid Bound to Transported Sediments

During the study period, glyphosate load associated with TS was detected in 38.1 ± 1.4 and 41.2 ± 0.7 % of the TS samples in HR and GR, respectively. Glyphosate was detected in 19.2 ± 0.6 % of the TS in HR

at concentrations lower than 50 mg/kg and in 18.9 ± 0.3 % of the TS at concentrations ranging from 50 to 390 µg/kg. Instead, glyphosate concentrations lower than 50 µg/kg and from 50 to 680 µg/kg were detected in 21.1 ± 0.4 and 4.2 ± 0.1 % of the TS in HR, respectively. The glyphosate associated with TS appeared after about 33.3 ± 27.8 DAA in HR at a concentration of about 220 ± 150 µg/kg. In GR, glyphosate associated with TS appeared after about 50.3 ± 56.3 DAA at a concentration of approximately 310 ± 190 µg/kg. The highest glyphosate concentrations in TS were determined after 30 DAA in the HR in 2009 and after 37 DAA in the GR in 2008. The highest glyphosate losses in TS were about 126.2 and 91.1 µg/ha for HR and GR, respectively, after 27 DAA in 2008. With the exception of the first year, glyphosate was detected in the TS at concentrations exceeding 10 times the method detection limit (100 µg/kg) for a period of 61.3 ± 14.6 DAA (average concentration, 260 ± 70 µg/kg) and 77.3 ± 29.3 DAA (average concentration, 330 ± 80 µg/kg), respectively, after treatments on HR and GR. During the latter, about 1009.5 ± 341.0 and 392.7 ± 159.8 kg/ha of TS were measured on HR and GR, respectively. Thereafter, the final glyphosate peaks appeared in TS in late September, after approximately 184.0 ± 8.3 and 188.3 ± 9.2 DAA in HR and GR, respectively.

Aminomethylphosphonic acid load associated with TS was detected in 85.4 ± 6.6 and 90.2 ± 3.4 % of the TS measured in HR and GR, respectively. Aminomethylphosphonic acid concentrations of 10 to 50, 50 to 500, and >500 µg/kg were detected in 51.5 ± 1.2, 30.0 ± 1.4, and 3.8 ± 0.1 % of the TS from HR, whereas these levels were detected in 28.5 ± 1.9, 60.2 ± 2.2, and 1.6 ± 0.1 % of the TS from GR. Aminomethylphosphonic acid load associated with TS appeared at concentrations of about 390 ± 290 mg/kg after approximately 34.8 ± 25.9 DAA after the treatments in HR and at concentrations of about 220 ± 130 µg/kg after approximately 50.3 ± 56.3 DAA after the treatment in GR. The highest AMPA concentration in TS from HR (710 µg/kg) was measured after 30 DAA in 2009, whereas the highest AMPA concentration in TS from GR (630 µg/kg) was measured after 37 DAA in 2008. The highest AMPA losses in TS from HR (223 µg/ha) and from GR (80.3 µg/ha) were measured in 2008 after 27 and 68 DAA, respectively. Except for the first year, AMPA was detected in TS at concentrations exceeding 100 µg/kg for a period of 85.7 ± 13.0 DAA (average concentration, 400 ± 140 µg/kg) in HR and 104.7 ± 38.1 DAA (average concentration, 260 ± 40 µg/kg) in GR. During the latter, about 1320.6 ± 574.8 and 514.7 ± 59.2 kg/ha of sediment losses were measured on HR and GR, respectively. Thereafter, the final AMPA peaks appeared in TS in late February, after approximately 321.8 ± 22.2 and 294.5 ± 74.9 DAA on HR (average concentration, 50 ± 10 µg/kg) and GR (average concentration, 50 ± 30 µg/kg). The average glyphosate and AMPA bound to TS was significantly ($p < 0.001$) greater from HR than from GR. This was attributable to higher soil losses from HR in comparison to GR. During the study period, no significant correlations were found between seasonal and annual rainfall and the glyphosate (and AMPA) load associated with TS. Unlike AMPA, no detectable concentrations of glyphosate in TS were found in runoff events that occurred in autumn and winter.

Percentage of Applied Glyphosate Lost by Runoff

The amounts of glyphosate and AMPA, in terms of applied glyphosate, measured in RV and TS were summed on a yearly basis (Table 7.5-18). Results indicated that tillage increased herbicide loss. On average, approximately 1.37 ± 0.03 and 0.73 ± 0.07 % of the total glyphosate applied was lost annually from HR and GR, respectively. Results indicated that AMPA represents about the 30.9 and 40.0 % of the total glyphosate losses on GR and HR, respectively. Glyphosate and AMPA bound to TS in runoff is able to reach the bed sediments of streams, lakes, and reservoirs.

Table 7.5-18: **The annual glyphosate and aminomethylphosphonic acid amount recovered in runoff volume and transported sediment from harrowed and permanent grass-covered plots for the four experimental years**

Plot† and year	Herbicide recovered‡				Total residues
	RV		TS		
	Glyphosate	AMPA§	Glyphosate	AMPA	
	------------------------------ % ------------------------------				
HR					
First year	0.05¶	0.01	0.00	0.00	0.06
Second year	1.53 ± 0.01	2.43 ± 0.03	0.05	0.08 ± 0.01	4.08 ± 0.05
Third year	0.21 ± 0.01	0.32 ± 0.01	0.02	0.04	0.59 ± 0.02
Fourth year	0.36	0.29	0.02	0.07% ± 0.02	0.74 ± 0.02
GR					
First year	0.09 ± 0.01	0.00	0.00	0.00	0.09 ± 0.01
Second year	0.96 ± 0.02	0.96 ± 0.02	0.03	0.02	1.97 ± 0.05
Third year	0.14	0.17	0.01	0.02	0.34
Fourth year	0.24 ± 0.01	0.24 ± 0.01	0.02	0.03	0.53 ± 0.02

† GR, permanent grass covered; HR, harrowed.

‡ RV, runoff volume; TS, transported sediment.

§ Aminomethylphosphonic acid.

¶ Results are average values and SDs of replicates based on the amount of glyphosate applied.

Glyphosate and Aminomethylphosphonic Acid Distribution in the Soil Profile

No extractable glyphosate was detected in the soil profiles. This result is in agreement with the degradation rate of glyphosate (DT_{50} = 10 ÷ 27 d) and its persistence of <1 yr measured in outdoor conditions (Feng and Thompson, 1990; Newton *et al.*, 1994). On the contrary, AMPA was found as deep as 45 cm in the soil profile of both HR and GR plots (Table 7.5-19). Some authors reported a reduced mobility of AMPA caused by absorption onto organic matter and clay in the soil (Grunewald *et al.*, 2001; Newton *et al.*, 1994). During the 4-yr study period, no statistically significant variation in the concentration of AMPA in any layers of the profile was noted. Thus, the measured inter-annual variation cannot be attributed to an accumulation effect but rather is due to different weather conditions. In the same way, the variations observed along the slope were not statistically significant and did not indicate any trend. Along the vine rows, the first and the third layers in GR contained significantly ($p < 0.01$) more AMPA in comparison to the corresponding layers in HR, whereas no significant differences were observed for the second layers. Results indicated that the amounts of AMPA recovered decreased significantly ($p < 0.01$) with depth both for HR and GR, as observed by Landry *et al.* (2005).

Table 7.5-19: Concentration of aminomethylphosphonic acid in the soil 1 yr after the application on the harrowed and permanent grass-covered plots

Plot†	Distance from the row	Layer deep	Concentration of AMPA‡ recovered in soil
	m	cm	µg kg^{-1}
GR	0	0–5	65.5 ± 6a§
HR	0	0–5	56.3 ± 9b
GR	0	20–25	36.4 ± 8.3cd
HR	0	20–25	34.6 ± 10.1d
GR	0	40–45	22.5 ± 8e
HR	0.675	0–5	19.2 ± 4.7f
HR	0.675	20–25	18.8 ± 4.8fg
GR	0.675	0–5	18 ± 5fg
HR	0.675	40–45	15.9 ± 4g
HR	0	40–45	14.6 ± 4.8g
HR	1.375	0–5	14 ± 7.0g
HR	1.375	20–25	12.6 ± 2.4gh
GR	0.675	20–25	12 ± 4.8hi
HR	1.375	40–45	11.6 ± 3i
GR	0.675	40–45	9.2 ± 1.6ij
GR	1.375	0–5	0 ± 0k
GR	1.375	20–25	0 ± 0k
GR	1.375	40–45	0 ± 0k

† GR, permanent grass covered; HR, harrowed.

‡ Aminomethylphosphonic acid.

§ Values are average ± SD of 24 soil core samples (4 yr × 3 transects × 2 duplicates). Lowercase letters indicate different means ($p < 0.01$) according to the Tukey post hoc test.

At a distance of 0.675 m from the vine rows, no significant differences were observed between the first layer in GR and HR. In contrast, significantly ($p < 0.01$) more AMPA was recovered from the second and the third layers in HR with respect to the corresponding layers in GR. The AMPA recovered decreased significantly ($p < 0.01$) with depth for GR, whereas no statistical differences were observed between the first and the second layer for HR. According to these results, soil tillage could have contributed to the distribution of AMPA within the soil profile. To the contrary, the grass cover in GR seemed to favor the adsorption of AMPA in the soil surface, as was suggested by Landry *et al.* (2005). In the middle of the inter-rows, AMPA concentrations were below the detection limit for all layers in GR, whereas AMPA was recovered from all layers in HR. Although not statistically significant, results indicated that AMPA decreased with depth for HR. Moreover, significantly ($p < 0.01$) less AMPA was recovered from the all the layers in HR compared with the corresponding layer in GR measured at a distance of 0.675 m from the row. Results indicated that AMPA amounts decreased significantly ($p < 0.01$) with depth for HR and GR. Similar results were observed by Veiga *et al.* (2001), who found that the concentration of AMPA reduced with increasing depth on a 0.35-m profile. According to these results, AMPA was distributed throughout the soil profiles, as observed previously (Landry *et al.*, 2005; Napoli *et al.*, 2015). Leaching of AMPA by preferential flow in macropores may have contributed to the deep penetration of this substance in the soil layers (de Jonge *et al.*, 2001, Fomsgaard *et al.*, 2003; Gjettermann *et al.,* 2009).

Conclusions

Results from this study indicate that transport of glyphosate and AMPA on a hillslope varies over time and according to the soil management practices. The concentrations of glyphosate and AMPA tended to be higher in RV and TS from HR plots than from GR plots. This was evident regardless of the amount of RV and TS. The mean annual glyphosate loss percentages via RV and TS were about 1.37 ± 0.03 and 0.73 ± 0.07 % in HR and GR, respectively. Aminomethylphosphonic acid represented approximately 30.9 and 40.0 % of the total glyphosate losses in GR and HR, respectively. Moreover, results suggested that rainfall, occurring within 4 wk after the treatment, can cause the transport of high concentrations of glyphosate and AMPA. Maximum glyphosate concentrations of 128.9 and 78.4 µg/L were transported from

HR and GR, respectively. Maximum AMPA concentrations of 151.9 and 144.8 µg/L were similarly transported from HR and GR, respectively. Soil analyses indicated that glyphosate was below detection within 1 yr, whereas AMPA was recovered in the soil profiles along the vine rows and in the inter-rows. Overall, results indicated that GR can be used in a vineyard cropping system to reduce soil and herbicide loss by runoff.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes a runoff experiment with glyphosate in a vineyard in Italy. The runoff was measured for glyphosate and AMPA residues. Maximum concentrations of glyphosate and AMPA dissolved in runoff were 128.9 µg/L and 151.9 µg/L, respectively. Maximum concentrations of glyphosate and AMPA associated with runoff sediment were 680 µg/kg and 710 µg/kg respectively.

Soil residues after 12 months were also assessed. No extractable glyphosate was detected in the soil profiles. The maximum AMPA concentration was 65.5 ± 6 µg/kg measured in the top 5 cm of a permanently grassed vineyard soil 0 m from the row.

The article is therefore considered reliable

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5.006
Report author	Székács, A. *et al.*
Report year	2014
Report title	Monitoring and biological evaluation of surface water and soil micropollutants in Hungary
Document No	Carpathian Journal of Earth and Environmental Sciences, August 2014, Vol. 9, No. 3, p. 47 - 60
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

In the development of a complex soil contamination monitoring system including the detection of agriculture-related micropollutants, heavy metal contamination and ecotoxicity, a survey has been carried out in Békés county and at certain water catchment areas in Hungary, using different techniques for the characterisation of soil and surface water status. Besides the representativity optimisation of the sampling technique, instrumental analysis, biological tests (soil biology and aquatic toxicity) were also applied, and results obtained were presented in a spatial informatics system. The target analyte group, indicators and methodology is in compliance with recommendations of the European Environment Agency monitoring working group. Contaminant concentrations of soil profiles have been characterised down to the ground water table. Pesticide residues were monitored by using gas chromatography coupled with mass spectrometry and enzyme-linked immunosorbent assay. Target analytes included triazine, phenoxyacetic

acid, acetanilide, dinitroaniline and phosphonomethylglycine type herbicides, chlorinated hydrocarbons (CHCs), organophosphate and carbamate insecticides, an insect hormonal agonist and a triazole fungicide. Besides banned persistent CHC insecticides (DDT, HCH, etc.), atrazine and acetochlor herbicides are common contaminants in Hungary, reaching 200 ng/g and 300 ng/mL concentration in the soil and surface water samples studied, and trifluralin, glyphosate and metolachlor were also detected in some cases. Heavy metal and other microelement contamination was detected by inductively coupled plasma atomic emission spectroscopy, and within-plot heterogeneities were studied throughout soil profiles. Nickel has been found as a relatively common contaminant in arable lands in the area, however, relation of the contamination pattern to fertiliser usage in the region could not be confirmed. Total microbiological activity was analysed by using fluoresce in diacetate (FDA) hydrolysis. The results of this measurement did not show correlation with heavy metal content or with land use types. Toxic effects of water and soil samples were determined on *Daphnia magna* Straus (Cladocera, Crustacea) according to the ISO 6341:1996 standard. The vast majority of the samples exerted no observable toxicity on this bioindicator organism. Overall toxicity often occurred not as the sum of the reported toxicity of the individual contaminants found: cases of antagonistic and synergistic effects in toxicity were both observed.

Materials and methods

Chemicals; Chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Sigma Chemical Co. (St. Louis, MO), unless otherwise stated. Analytical standards of the target analyte pesticides were provided by the Hungarian Central Agricultural Office, Plant Protection, Soil Conservation and Agri-environment Directorate, from official standard reference materials received from the manufacturers/distributors of acetochlor, atrazine (Nitrokémia Rt., Fűzfőgyártelep, Hungary), carbofuran (Agro-Chemie Kft., Budapest, Hungary), diazinon, fenoxycarb, prometryn (Syngenta Kft., Budapest, Hungary), metribuzin (Bayer HungáriaKft., Budapest, Hungary), phorate (BASF HungáriaKft., Budapest, Hungary), terbutryn (Agrosol Bt., Gödöllő, Hungary) and trifluralin (BudapestiVegyiművek Rt., Budapest, Hungary). Solvents purchased from Merck kgaA (Darmstadt, Germany) were of analytical grade. CarboPrep-90 (500 mg, 6 mL) and Carbograph (200 mg, 6 mL) columns were purchased from Restek (Bellefonte, PA, USA) and Alltech Associates, Inc. (Deerfield, IL, USA), respectively. HPLC grade distilled water was prepared on a MilliQ RG ion-exchanger from Millipore (Bedford, MA, USA). MN (MachereyNagel) 640W filter paper was obtained from Reanal Rt. (Budapest, Hungary).

<u>*Sampling and sample extraction*</u>

Sample collection
In the scope of a national monitoring program, 423 soil samples and 202 surface and ground water samples were collected between 2008 and 2013, in uneven annual distribution, from agricultural fields and industrial sites. Contamination in arable lands and industrial areas has been investigated on 13 plots in 5 replicates. Among agricultural areas, three types of land usage have been involved: arable lands under intensive cultivation, organic farming and pasture. The study area in the case of contamination of agricultural origin covered 4 settlements in Békéscounty (Köröstarcsa, Medgyesegyháza, Csorvás, Battonya). Both intensive and organic parcels were chosen in all 4 settlements (4 organic and 4 intensive), the pasture was designated in Csorvás. Contamination of industrial origin was examined in 3 settlements (Orosháza, Gyomaendrőd, Békéscsaba), at 5 sites (Orosháza - Linamar, Public Road Manager Corp., Glass Factory; Gyomaendrőd - Nagylapos; Békéscsaba - Sludge Desposition Site). Spatial setting of sampling accuracy was supported by a global positioning system. Soil sampling was carried out according national standard MSZ 21470-1:1998 (Hungarian Standards Institution 1998) during the April-May period by using a motorised Eijkelkamp soil drilling equipment. Contaminant concentrations of soil profiles from topsoil to subsoil were characterised down to ground water table, creating one sample in every 30 or 50 cm. Parcels of diffuse agricultural load were further narrowed to define a 5 ha Representative Parcel Part (RPP), preferably as a quadrat. RPP was designed on the representative, homogeneous part of the parcel. This was carried out to characterise the nutrient content of the surface soil layer. In our study, a sampling allocation in regular design was used for the mechanical drillings, thus a parcel of 50 m x 50 m territory was designated in one corner of the RPP. The soil samples were taken from drillings in the corners and in the centre point of this part of the RPP, in five replications each. Water sampling was carried out according to national standard MSZ ISO 5667 (Hungarian Standards Institution 1995), twice a year, before and after

agricultural pesticide applications, during the months of April-May and June-September. Surface water samples (from depths not exceeding 50 cm) were collected by immersion of a sampling vessel, while groundwater samples were taken from the soil drillings or from already existing groundwater monitoring wells. Both kinds of water samples were transferred into clean, 2.5 litre volume dark glass bottles sealed with a watertight screw-cap insulated with teflon lining, and were transported in cool boxes to the laboratory.

Sample preparation
To provide appropriate sample preparation for gas chromatography - mass spectrometry (GC-MS) determinations, solvent extraction and solid phase extraction (SPE) methods were applied. Soil samples were air-dried, ground on a Retsch GM 200 cutting mill (Retsch GmbH, Haan, Germany), and subjected to solvent extraction. Thus, 10 g dried soil was extracted with 15 mL of hexane/acetone (1:1) and centrifuged at 4000 rpm. Finally, 10 mL of the supernatant was evaporated and resuspended in 1 mL of ethyl acetate. Water samples were filtered in a suction filtration apparatus using MN 640W filter paper to remove floating particles, stirred for 1 min, left to settle for 10 min, and then subjected to SPE using graphitized carbon based SPE cartridges. SPE columns (CarboPrep-90, 500 mg, 6 mL) were conditioned, applying low eluent flow velocity, with 5 mL of dichloromethane/methanol (8:2), 2 mL of methanol, and 10 mL of distilled water containing 10 mg/mL ascorbic acid. After the conditioning step, 1000 mL of the water sample was passed through the column at a flow rate of 10-15 mL/min. The column was rinsed with 7 mL of distilled water, air-dried for 10 min with suction by vacuum, washed with 1 mL of methanol/distilled water (1:1), and air-dried again. Neutral and alkaline components absorbed into the column were eluted, at a low eluent flow velocity, with 1 mL of methanol and 1 mL of dichloromethane/methanol (8:2). Combined eluates were concentrated to 0.1 mL under nitrogen gas flow. Then 2 mL of isooctane was added to the extract, and the solution was evaporated to a final volume of 1 mL. Extract samples were applied for measurement with GC-MS.

In order to evaluate the SPE/GC-MS process, water samples were spiked with standards of the target compounds at concentrations between 0.001 and 25 ng/mL, and subjected to the above SPE protocol and to instrumental analysis. Analytical standards of the active ingredients were added to HPLC grade distilled water (MilliQ) in methanol stock solutions, except for phorate, where stock solution was prepared in acetone. Spike levels included 2- and 5-fold values of the limit of detection (LOD), except for fenoxycarb. Five parallel detections were carried out at these levels for each active ingredient.

Instrumental analysis

GC-MS
GC-MS analyses were carried out on a Saturn 2000 workstation (Varian Inc., Walnut Creek, CA, USA), consisting of a Chrompack CP 3800 gas chromatograph and a Saturn 2000R ion-trap detector. The gas chromatograph was equipped with a Varian 1079 split/splitless injector and a CP 8200 autosampler. GC-MS determinations were carried out using electron impact (EI) or chemical ionization (CI) ion sources, detecting total ion count (TIC) in full scan mode or selected ion(s) in selective ion monitoring (SIM) mode. A capillary column CP-Sil 8 CB filled with 5 % diphenyl polysiloxane and 95 % dimethyl polysiloxane (30 m, 0.25 mm, film thickness 0.25 µm) (Chrompack, Middelburg, the Netherlands) was used. The carrier gas was helium 5.0 at a flow rate of 1.0 mL/min. The mode of injection was splitless (0-1.5 min), then the split ratio set to 50. Both isothermal injection (ITI) and temperature programmed injection (TPI) were applied. During ITI, the injection temperature was set to 230°C. The injection volume was 1 µL. The corresponding column temperature, following an initial period of 120°C for 1 min, was increased to 270°C at 10°C/min, and kept at 270°C for 14 min. During TPI, the injection temperature was 60°C for 0.50 min, raised to 260°C at 200°C/min rate, held for 5 min, raised further to 60°C at 200°C/min rate, held for 20.00 min. The injection volume was 5 µL. Solvent venting was not applied. The corresponding column temperature, following an initial period of 70°C for 0.5 min, was increased to 100°C at 60°C/min, further increased to 240°C at 10°C/min and kept finally at 240°C for 20 min. The transfer line temperature was 270°C. The mass spectrometer was operated in electron impact (EI) or chemical ionization (CI) mode using methanol as reagent gas with CI storage level of 19.0 m/z. The temperature of the ion trap was 150°C. The maximum ionization time was 2000 µs, the maximum reaction time 40 ms, the ionization level 25 u, the reaction level 40 amu, reagent reaction time 9000 µs, scan time 0.60 s/scan, between 45 and 400 amu in

full-scan mode. Chlorophenoxyacetic acid type herbicides (2.4-D, dichloprop, MCPA, etc.) were determined upon derivatization with trimethylsilyl N,N-dimethyl carbamate and t-butyldimethylsilyl N,N-dimethyl carbamate.

Enzyme-linked immunosorbent assay (ELISA)
The determination of herbicide active ingredient glyphosate was carried out by the validated commercial immunoassay (PN 500086 by Abraxis LLC, Warminster, PA, USA) using antibodies specific for glyphosate. Measurements were carried out in 96-well microtiter plates according to manufacturer instructions. Acyl-derivatized samples or analytical standards were incubated with glyphosate-specific antibodies immobilized on the walls of the microtiter wells, and an enzyme conjugate of glyphosate was added. Upon washing, the bound enzyme quantity was determined by a colorimetric reaction providing optical signals at 620 nm and 450 nm wavelengths. Glyphosate concentrations were determined using standard calibration curves of linear or sigmoid fit.

Toxicity tests

Soil microbiology
Microbial enzymatic activity in the soil was measured by fluorescein diacetate (FDA) hydrolysis, optimised for soil samples. Samples were stored at the temperature of 4°C until analysis. FDA reagent (stock solutions at 1 and 10 g/L FDA in acetone) was added to 1 g of soil per replication in 15 mL phosphate buffer (pH 7.6). Upon shaking for 2 hours at 30°C. Then, the reaction was terminated by acetone (1:1 suspension in the solvent), applying a 1.5 hour long glass bead pre-shaking step to reach a proper level of suspension. Upon centrifugation of the suspensions at 3000 rpm), the amount of fluorescein developed was measured from the supernatant of each sample on a spectrophotometer at 490 nm. Statistical analyses of FDA data have been performed using one-way analyses of variances (ANOVA), effects of pesticides residues, soil humus content and soil texture have been analysed by multiple regression.

Immobilization test on Daphnia magna
Aquatic biotests using the giant water flea (*Daphnia magna* Straus) were carried out on soil and water samples with highest contamination rates detected. Immobilization tests were performed according to the ISO 6341:1996 standard (International Organisation for Standardisation 1996). Test animals were kept in 16/8 hr light/dark photoperiod with the testing atmosphere kept at 20-22°C and free from poisonous vapours or dusts. The breeds and the controls were kept in aqueous solution containing $CaCl_2$, $MgSO_4$, $NaHCO_3$ and KCl at concentrations of 220.5, 61.6, 64.8 and 5.75 mg/L, respectively. The bioanalytical accuracy of the test was assessed in potassium dichromate test: the mortality caused by $K_2Cr_2O_7$ was measured at 0.25, 0.5, 0.75, 1 and 1.25 mg/L concentrations, and the sensitivity of the test animals was considered proper according to the standard protocol if the EC_{50} value obtained for potassium dichromate fell between 0.6 and 1.7 mg/L. Water samples and aqueous soil extracts were applied directly to the test in volumes of 10 mL per replication. Floating matter when occurred was removed from the water samples by centrifugation for 5 min at 3000 rpm. For extraction of soil samples 300 g of soil was extracted with 500 mL of distilled water, the mixture was ultrasonicated for 10 min, and filtered in a suction filtration apparatus through MN 640W filter paper. Tests were carried out at the first larval stage (6-24 hours) for 48 hours, when the immobilization of the subject animals was recorded (10 animals per test) in quadruplicates. Mortality (immobilisation) rates were calculated by the Henderson-Tilton formula, correcting the measured mobility inhibition with that detected for untreated control and eliminating the effect of varying number of test individuals applied at the repetitions. Therefore, percentage mortality/immobilisation refers to values corrected with the Henderson-Tilton formula. EC_{50} values were calculated using probit transformation and log-linear regression, the data were statistically evaluated by one way ANOVA.

Computing accurate sample sizes

Reliability of estimates depends on both accuracy and precision. Accuracy is about how close the estimate is to its true value on average. Precision is about how similar repeated estimates are to each other. Percentage relative precision (PRP) of the estimation at heavy metal and pesticide residues contamination, i.e. was used to estimate precision of the measurements. PRP is the difference between the estimated mean of the measurements and its 95 % confidence limits, expressed as a percentage of the estimate. However,

because confidence limits are sometimes asymmetrically distributed around the estimate, the mean difference between them and the estimate was used. Estimation of sample sizes needed to attain a fixed percentage relative precision has been calculated on the basis of the following equation:

$$m_0 = \left(\frac{200}{Q}\right)^2 \left(\frac{s}{\bar{N}}\right)^2$$ where

Q: the required percentage relative precision
$\bar{N}$: mean value in the sample unit
s: standard deviation in the sample unit
m_0: sample size required for there to be a 95% chance of obtaining a PRP of Q or less.
PRP: percentage relative precision = 50 x (CL_2-CL_1)/$\bar{N}$, where CL_2 and CL_1 are the 95% upper and lower confidence limits, respectively. If m_o< 25, than the calculated sample size must be increased by two samples.

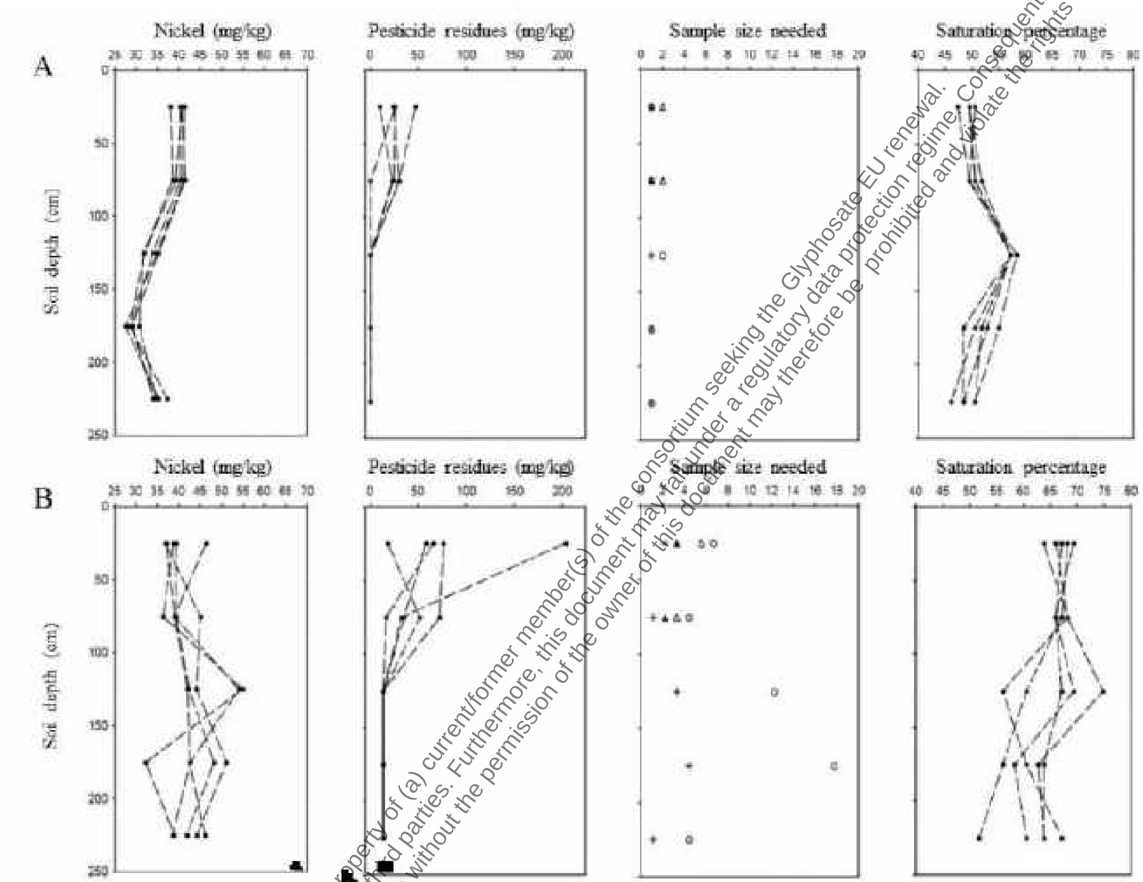
Results and discussion

Examination of sampling effort

For reliability assessment, percentage relative precision of the pollution level estimation was calculated at each site. As expected, precision was highly influenced by the heterogeneity of the sites and thus directly depended on the variance of the data. To show the consequences of small-scale heterogeneity of sites, contamination characteristics of a homogeneous and a heterogeneous site are presented on Figure 7.5-10, showing the curves of nickel concentration, pesticide residue levels and soil texture with soil depth. Similar slopes in saturation percentage indicated identical soil textures among the five samples at the homogeneous site (Figure 7.5-10A), whereas soil textures differed substantially in the heterogeneous set (Figure 7.5-10B), probably due to complex sedimentation. Sample sizes needed for 10 % and 20 % precision in nickel concentration varied between 3-4 drillings and 3-16 drillings at the homogeneous and heterogeneous sites, respectively. For pesticide residues, appropriate sample sizes have been determined between 3-8 drillings, considering the higher percentage values of the precision (50 % and 100 %). 100 % percentage relative precision actually indicates only the occurrence of the contamination. This result pointed out that the level of site heterogeneity highly influences the required sample sizes for a given precision, and also indicated the extreme importance of the composite sampling design and homogenisation in the course of sample preparation in environmental monitoring.

Figure 7.5-10: Contamination profiles at a homogenous (site A, Battonya 1) and a heterogeneous (site B, Köröstarcsa 2) sampling site

Nickel concentration was proven to exceed the "B" limit value (40 mg/kg) in both cases. The amount of total pesticide residues at site *B* is regarded to be significant; 10 % PRP (percentage relative precision) at nickel (open circle); 20 % PRP at nickel (cross); 50 % PRP at pesticide residues (open triangle), 100 % PRP at pesticide residues (closed triangle) are shown in the last column.



Chemical analysis

Pesticides

Thirty-three active ingredients and metabolites (acetochlor, alachlor, aldrin, atrazine, butylate, carbofuran, carbofuran phenol, DDD, DDE, DDMU, DDT, diazinon, dibutilphtalate, dieldrin, endrin, endrin ketone, EPTC, α-, β- and γ-HCH, heptachlor, heptachlor epoxide, isodrin, metolachlor, phenkapton, phorate, prometryn, propachlor, sulfotep, TBP (tributil phosphate), terbutryn, trifluralin) and 14 related compounds (AMPA, 2,4-D, 2,4-DB, dichloprop, dimetachlor, fenoxycarb, glyphosate, MCPA, MCPB, mecoprop, metribuzine, propisochor, simazine) or compound groups (camphechlor) were monitored by GC-MS. Of the 423 soil samples analysed, 77 samples contained detectable contamination by one or more target compounds (contamination was marginal in four cases). Therefore, contamination rate found was 17-19 %. Of the 202 water samples analysed, 76 samples contained detectable contamination by one or more target compounds (contamination was marginal in 11 cases). Therefore, contamination rate found was 18-67 %. The most common soil contaminants appeared to be atrazine (10-580 ng/g), trifluralin (3-200 ng/g), acetochlor/metolachlor (5-80 ng/g), as well as DDT/DDE (38-460 ng/g) and lindane/HCH (7-103 ng/g); the most common water contaminants were acetochlor (0.02-3900 µg/L), atrazine (0.5-100 µg/L), metolachlor (0.001-56 µg/L), trifluralin (0.8-9 µg/L) and diazinon (0.001-0.85 µg/L). The found contamination levels are in certain cases alarming as the corresponding harmonised EC Directive effective

in Hungary as well sets the maximum residue limit of 100 ng/L for a given pesticide compound and 500 ng/L for all pesticide residues in subsurface water. The herbicide active ingredient glyphosate was detected as water contaminant at concentrations of 0.54-0.98 ng/mL by a commercial ELISA method, at very high or high levels in 5 and 16 cases, respectively (relative to the substantial background signal level of the immunoanalytical method). As the reported cross-reactivity of the commercial ELISA kit used with the main glyphosate metabolite aminomethyl phosphonic acid (AMPA) is reported to be below 0.0002 %, the method only detected the parent compound and not its degradation product. Frequent occurrence of glyphosate is of major concern due to the high water contaminating potential of glyphosate, and due to its known ecotoxicological (cytotoxic, endocrine disruptive and mutagenic/teratogenic) effects, particularly when exerted in co-exposure or synergy with polyethoxylated tallowamine often used as adjuvant for this herbicide active ingredient.

<u>Toxicity testing</u>

Soil microbial activity
Soil microbial activity on arable lands (nine sites) and industrial locations (four sites) were measured by using FDA analysis with 5 replicates per site (65 drillings). Soil microbial activity differed significantly between arable lands and industrial sites ($F_{(1, 63)} = 74.5$, $p < 0.001$), arable soils showed 14 times higher microbiological activities than industrial ones ($F_{(1, 38)} = 0.39$, $p > 0.05$). Such pattern can be explained by the more favourable ecological conditions for the soil microflora occurring in arable lands than those of industrial sites. FDA activity correlated with humus content in the upper soil layer ($R^2 = 0.6$), constituting another sign for the effect of biotic conditions on soil microbiological activity. However, agricultural land use practice (intensive vs. organic farming) did not affect FDA activity. The reason for such phenomenon may be the fact that the overall duration of organic farming practices at these locations after decades of intensive agriculture was too short for the spontaneous development of a mature microbiological community with higher biomass. Soil microbiological activity is influenced by numerous biotic and abiotic environmental factors, of which contamination is only one driving force. We examined how abiotic soil factors and contamination affected soil microbiological activity. By using multiple linear regression modelling humus content, soil texture and soil pesticide residues were set as independent variables against FDA, as a dependent variable. The partial regression coefficients were obtained respectively -0.08 at herbicide and insecticide residues, 0.4 at soil texture and 0.84 at humus content, ($R^2 = 0.7$). This result showed a statistically not significant, weak negative effect of herbicide and insecticide pollution on FDA, whereas humus content and soil texture did influence microbial activities in soils. Therefore, such a general microbiological activity pattern generated from FDA analysis alone cannot be regarded as a predictor for examined soil contamination.

Aquatic toxicity detected on Daphnia magna indicator organism
There apply strict regulations in pesticide registration regarding aquatic toxicity of the candidate compounds. If the pesticide preparation is dangerous for aquatic organisms, specific protective distances (200, 50 or 20 metres) apply from water courses. As a result, toxicity exerted on *D. magna* is required to be determined for each registered pesticide active ingredient and is listed among the chemical and biological features of pesticides. Zooplankton, *Daphnia* is widely used as a test organism in order to evaluate the toxicity of several contaminants as well as their mixture. A recent report of the effects of herbicides on zooplanktons gives a comprehensive overview of the highly varying EC_{50} values on *D. magna* and other daphnids, revealing possible deviation patterns. Organisms in the environment are permanently exposed to complex mixtures of low concentrations of contaminants from mainly anthropogenic sources. Particularly aquatic organisms are endangered by toxicants since they spend their life entirely or majorly in their aquatic media, and therefore, may suffer exposure to single or multiple water contaminants all over their lifetime. The evaluation of the additive effects of multiple contaminants (e.g. pesticides) in water at low concentrations has received great attention lately. Addition and synergism were observed among sublethal concentrations of diazinon, malathion and chlorpyrifos on coho salmon (*Oncorhynchus kisutch*). A recent review reports a number of combined toxicological interactions of pesticide mixtures such as pyrethroids, carbaryl and triazine herbicides at molecular level. Exposure to pesticides at sublethal doses not only exert combined toxitity to affected organisms, but may also induce their increased sensitivity to other stress factors such as predator stress, parasite infection or UV-radiation. As reported in the scientific literature, toxic effects of low concentration pesticide mixtures on zooplanktons (including *D. magna*) and on algae

are typically close to the sum of the effects for each pesticide compound applied independently, therefore, overall toxicity levels are estimated on the basis of toxicity exerted by single compounds. Such phenomenon has been reported not only for insecticides, but for herbicide (acetochlor, metolachlor, glyphosate, 2,4-D, atrazine) mixtures as well. As expected, no toxic effect was observed in the *D. magna* immobilization test in the case of the vast majority of the water samples. In contrast, significant or salient aquatic toxicity was detected in all soil and water samples heavily contaminated with pesticide residues and/or toxic heavy metals (Table 7.5-20), indicating that these contaminants do cause toxicity on *D. magna*. Nonetheless, a clear superposition of the individual toxicity of the contaminants tested was not seen in the statistical analysis of the aquatic toxicity results. Among pesticide type organic micropollutants, mostly insecticides are expected to be considered toxic to *D. magna*: compounds designed to have toxic effects on insects are more likely to cause similar effects on other arthropods, than substances optimized for their effects on plants or on fungi. This is well reflected among the target analyte pesticides in the present study by the outstandingly low EC_{50} value of diazinon on *D. magna* (0.96 µg/L). The toxicity of microelements on *D. magna* is highly dependent on element speciation, therefore, toxicity values reported in the literature commonly refer to the most abundant forms of the given elements. Besides, the toxicity of metals in aquatic environment varies widely, depending both on environmental conditions and on the sensitivity of the exposed organisms. Most prevalently found contaminating microelements in this study (As, B, Ni and Se) exert minor toxicity of *D. magna*. As a result, significant toxicity was expected only from the most contaminated surface water samples or aqueous soil sample extracts, particularly from those contaminated with insecticides. Dibutyl phthalate, commonly reported as ubiquitous water contaminant, has been found in certain water samples, in some cases at concentrations as high as 100 ng/mL (e.g. W4A1, W5D1), yet no toxicity on *D. magna* was observed, in accordance with the reported marginal toxicity of the compound on *D. magna* (EC_{50} = 3.0-5.2 mg/L). Samples W1E1 and W1G1 heavily contaminated with acetochlor and atrazine and containing elevated levels of boron caused full mortality in the *D. magna* biotest, when applied undiluted. These two water samples were measured in 5-, 10- and 25-fold dilutions as well, and it was found that 50 % mortality (EC_{50}) was reached when the samples were applied at dilutions of 6.4- and 13.3-fold, respectively. The strong mortality caused by these samples was a clear result of the synergistic effect of the individual contaminants, as the actual (although apparently high) levels were far below of the individual EC_{50} values. Sample W1D1 represented a similar case with slight diazinon and acetochlor, and considerable metolachlor contamination (the latter still not reaching even 1 % of the EC_{50} value of metolachlor) and causing 65 % mortality on *D. magna*. As toxicity of that high magnitude would not be expected on the test animal neither from the pesticide residue, nor from the microelement contamination detected in the sample, the observed biological effect is either due to an unidentified component or caused by synergistic interactions among the detected contaminants. In contrast, a case of low or no toxicity, when significant effect on *D. magna* was expected, was also recorded: sample W2F1 caused no immobilisation *of D. magna* larvae. This was rather intriguing, because the measured diazinon content of the sample was close to the EC_{50} value of the compound on the test animal. In such case at least limited mortality would have been expected to be observed. To test whether the *D. magna* population used in these experiments shows sufficient sensitivity to diazinon the EC_{50} value of the compound was experimentally determined and was found to be 0.34 µg/L (0.27 to 0.39 µg/L).

Table 7.5-20: Toxicity of water and soil samples contaminated with pesticides and heavy metals on *Daphnia magna* as indicator organism

Samples w-water s-surface	Pesticide contamination (µg/L or mg/kg)							Element content (µg/L or mg/kg)				Mortality (*D. magna*)
	acetochlor	atrazine	diazinon	metolachlor	terbutryn	trifluralin	glyphosate	As	B	Ni	Se	
W1D1	0.18±0.04	–	0.008±0.001	55.9±4.9	–	–	–	8.0	145	5.0	–	+
W1E1	> 1000	~100	< 0.001	1.66±0.22	0.18±0.03	0.8±0.1	–	–	609	6.0	12.6	+++
W1F1	35.9±3.68	1.0±0.005	0.011±0.003	0.039±0.007	–	–	–	40.5	69.5	–	–	–
W1G1	> 1000	> 100	< 0.001	0.56±0.13	0.35±0.07	9.0±1.2	–	9.6	360	15.9	23.3	+++
W2F1	–	–	0.84±0.008	–	–	–	0,75±0.08	1.8	367	2.5		–
W2F1[a]	–	–	1.18±0.05[b]	–	–	–	0,75±0.08	1.8	367	2.5	–	–
W3A0	–	–	< 0.001	–	–	–	–	–	1544	0.8		+
W3A0[a]	–	–	0.34±0.01[b]	–	–	–	–	–	1544	0.9	–	+++
W4A1	–	–	–	–	–	–	–	–	846	–	–	–
W4A0	–	–	–	0.004±0.001	–	–	–	2.0	121	3.0	–	–
W5A1	–	–	–	–	–	–	–	–	183		5.3	–
W5D1	–	–	–	–	–	–	–	–	152	–	3.7	–
W5A0	–	–	–	< 0.001	–	–	–	2.6	118	5.0	–	–
W6B1	1.54±0.28	0.50±0.11	–	0.044±0.005	–	–	0,60±0.05	–	780	2.0	–	–
W6A0	–	–	–	–	–	–	–	12.9	130	2.6	–	–
W7F1	0.26±0.005	–	0.012±0.004	–	–	–	–	–	216	2.7	–	–
S1E3	0.005±0.001	0.20±0.04	–	0.011±0.002	–	0.011±0.01	–	7.81	22.7	40.1	–	++
S1A2	0.014±0.002	–	–	–	–	–	–	10.5	22.6	35.4	–	–
S1D3	0.011±0.002	–	–	0.019±0.004	–	–	0,56±0.26	16.6	49.2	53.1	–	–
S2A1	–	–	–	0.081±0.008	–	–	–	16.3	40.5	51.2	–	–
S3A0	–	–	–	–	–	0.20±0.03	–	15.4	30.8	37.1	0.742	+++
D. magna EC_{50}	9000[c]	87000[f]	0.96[c]	25000[c]	2660[c]	250[c]	780000[c]	7500-15040[d]	56000-141000[e]	7300[g]	430-4070[h]	

[a] Fortified with diazinon at EC_{50} value obtained in laboratory *D. magna* colony / [b] Diazinon level specified as the sum of the measured and spiked concentration. / [c] Reported for the parent compound (Tomlin 2000) / [d] Measured as As(III), As_2O_3 (Lilius et al. 1995, Guilhermino et al., 2000) / [e] 14000 µg/l for B(III), tetraborate (Maier & Knight 1991), 56000-68000 µg/L for elemental B nanoparticles (Strigul et al., 2009) / [g] Measured as Ni(II), $NiCl_2$ (Pedersen & Petersen 1996, Kencu et al., 2010) / [h] 430-3000 µg/L for Se (IV), selenite and 550-5300 µg/L for Se (VI), selenate (Martins et al., 2007)

Diazinon was spiked into water sample W2F1 at this concentration, verified to cause substantial mortality, yet mortality still not appeared in the *D. magna* immobilisation test. This observation indicates a clear antagonist effect among contaminants such as sub-lethal concentrations of diazinon and copper. Similar antagonistic patterns observed when crustacean *Ceriodaphniadubia* or mayfly *Ephoronvirgo* were exposed to a mixture of copper and diazinon. Another critical water sample (W3A0) of high boron content and of limited (40 %) toxicity on *D. magna* was also spiked with diazinon at 0.34 µg/L concentration, and resulted in full (100 %) mortality in the *D. magna* immobilization test. This verified assay sensitivity to diazinon, and indicated a slight synergism between diazinon and the boron content of the sample. Sample S1E3 contained various pesticide and microelement contaminants, primarily nickel at a substantially high level of 40.1 mg/kg. The aqueous extract of this soil sample caused 95 % immobilisation on *D. magna*. Soil sample S3A0,containing (along with other microelements) high level (15.4 mg/kg) of arsenic, the aqueous soil extract caused 100 % immobilisation, and required a 2.54-fold dilution to reach EC_{50}. Detectable toxicity to *Daphnia magna* has not been observed on water samples with detected content of glyphosate residues. This is in accordance with the known toxicity of glyphosate and AMPA to *D. magna* (780 and 690 mg/L, respectively), escalated by polyethoxylated tallowamine detergents used as formulating agents. Nonetheless, recent literature data indicate sublethal effects of glyphosate and its formulations on aquatic organisms. They may cause reduction of juvenile size and affect the growth, fecundity and abortion rate of daphnids and inhibit cholinesterase activity of mussel and fish as well.

Conclusion

The present study combines chemical analysis of pesticide residues and microelements from topsoil and subsoil, as well as surface and ground water samples with biotests on total soil microbiological activity using fluorescein diacetate (FDA) hydrolysis and on aquatic toxicity using the ISO 6341:1996 standard immobilisation protocol on *Daphnia magna* Straus. Contamination by organic micropollutants, mainly pesticide residues occurred more frequently in surface water (18-67 %), than in soil (17-19 %); the most contaminated samples arrived from an identified illegal contamination site scheduled for remediation. Residues of herbicide active ingredients atrazine, acetochlor/metolachlor and trifluralin were found both as water and as soil contaminants at various concentrations up to 3900 ng/mL and 580 ng/g, respectively. In

addition, residues of the insecticide active ingredients diazinon also occurred as water contaminant below 1 ng/mL. Of the 14 microelements monitored, 18 % and 53 % contamination frequencies above the legal threshold value was detected for soil and water samples, respectively, with Ni, As and Ba as most common soil contaminant microelements, and B and Se as major water contaminant microelements. While a clear correlation between detected soil contamination and microbiological activity determined by FDA analysis could not be established, toxicity tests with *D. magna* showed substantial toxicity in 6 cases. The survey indicated that biotests are worthwhile to be carried out even if analytical measurements reveal sublethal level contamination to the given test organism, because contaminant interactions may result in lethal effects. Interactions may appear synergistic, antagonistic and additive.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports measurements of glyphosate among other pesticides, organic pollutants, heavy metals and other microelements in soils, surface waters and groundwater bodies in Hungary from agricultural and industrial settings. The effect of the found concentrations of the different substance on *D. magna* was investigated. The reported glyphosate findings cannot be assigned to the respective sampling site. Furthermore, no comprehensive list of glyphosate findings is presented. A maximum concentration of glyphosate at 0.98 µg/L was reported as an unspecified (SW/GW) water contaminant. The maximum glyphosate soil concentration reported was 0.56 ± 0.26 mg/kg.
The article is therefore considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5.007
Report author	Daouk, S. *et al.*
Report year	2013b
Report title	The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part II: The role of infiltration and surface runoff
Document No	Journal of Environmental Science and Health, Part B (2013) 48, 725–736
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

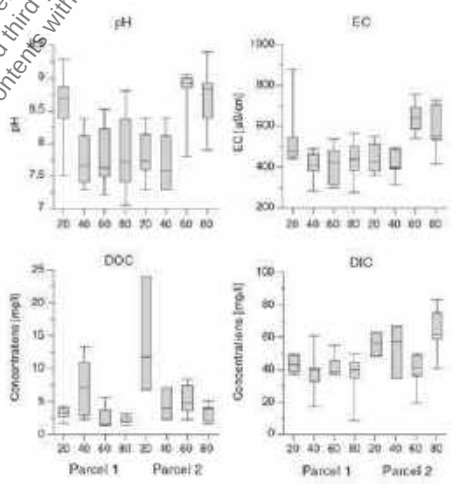
Two parcels of the Lavaux vineyard area, western Switzerland, were studied to assess to which extent the widely used herbicide, glyphosate, and its metabolite aminomethylphosphonic acid (AMPA) were retained in the soil or exported to surface waters. They were equipped at their bottom with porous ceramic cups and runoff collectors, which allowed retrieving water samples for the growing seasons 2010 and 2011. The role of slope, soil properties and rainfall regime in their export was examined and the surface runoff/throughflows ratio was determined with a mass balance. Our results revealed elevated glyphosate and AMPA concentrations at 60 and 80 cm depth at parcel bottoms, suggesting their infiltration in the upper parts of the parcels and the presence of preferential flows in the studied parcels. Indeed, the succession of rainy days induced the gradual saturation of the soil porosity, leading to rapid infiltration through macropores, as well as surface runoff formation. Furthermore, the presence of more impervious weathered marls at 100 cm depth induced throughflows, the importance of which in the lateral transport of the herbicide molecules was determined by the slope steepness. Mobility of glyphosate and AMPA into the unsaturated zone was thus likely driven by precipitation regime and soil characteristics, such as slope, porosity structure and layer permeability discrepancy. Important rainfall events (>10 mm/day) were clearly exporting molecules from the soil top layer, as indicated by important concentrations in runoff samples. The mass balance showed that total loss (10–20 %) mainly occurred through surface runoff (96 %) and, to a minor extent, by throughflows in soils (4 %), with subsequent exfiltration to surface waters.

Materials and Methods

Study area and soil features

The Lavaux is a vineyard area located in western Switzerland. This landscape is composed of moraine deposits and, with its steep slopes from 13 to 43 % and the light reflection on the Lake of Geneva, it represents a very suitable environment for the growth of grapevines. The bedrocks are composed of Tertiary molasse deposits, which include conglomerates, sandstones and marls from the upper Oligocene epoch. Soils of both parcels are colluvial calcosols, according to the French classification. Both soils showed a silt loam texture and light differences were observed between plots and depths.

Figure 7.5-11: pH, electrical conductivity (EC), dissolved organic and inorganic carbon (DOC/DIC) contents in soil water samples at 20, 40, 60 and 80 cm of both parcels



Sampling and analytical methods

In both parcels, the herbicide glyphosate was applied the same day and only under the rows, leaving a grass band in between them. It is mainly applied in spring time to avoid a nutrient and water competition between grapevines and weeds during the growing season. Application data were obtained from winegrowers and are summarized in Table 7.5-21. In previous years, the same amounts had been applied, but we assumed

that all glyphosate and AMPA degrade from year to year according to their properties. Precipitation data were obtained from the closest meteorological station. In order to sample the soil solution, both parcels were equipped at their bottom with porous ceramic suction cups at four different depths: 20, 40, 60 and 80 cm. The herbicide glyphosate and its metabolite AMPA were quantified by LC-MS/MS with a previously developed method, based on their pre-column derivatization with FMOC-Cl and their enrichment by solid phase extraction. The limit of quantification was 10 ng/L and it was tested successfully for the matrix effect that could occur by analyzing soil solution and runoff samples. Dissolved organic and inorganic carbon (DOC/DIC) concentrations were measured with a C-analyzer. A principal component analysis (PCA) was performed on the soil water samples using the R software to help interpreting all the analyses and discriminating the observations made in the two different parcels. In order to determine the surface runoff/throughflows ratio, a mass balance was done for both surface runoff and soil solution samples of parcel 2 (surface = 845 m^2). As glyphosate was applied only under the grapevine rows, the initial quantities correspond to half of the surface. The mass (M) of glyphosate and AMPA were obtained by multiplying the concentrations (C) with cumulative precipitations that fell on the parcel surface between two sampling events (mmINT): M [g] = C [g/L] × mmINT [L].

Table 7.5-21: Quantities of applied product and application dates

Parcel	Product	Quantity (L/ha)	Active Ingredient (A.I)	A.I. applied [g/ha]	Application date
1, 2	Glyfos® [1]	3	Glyphosate, isopropyl amine salt	1080	20 April 2010 15 April 2011

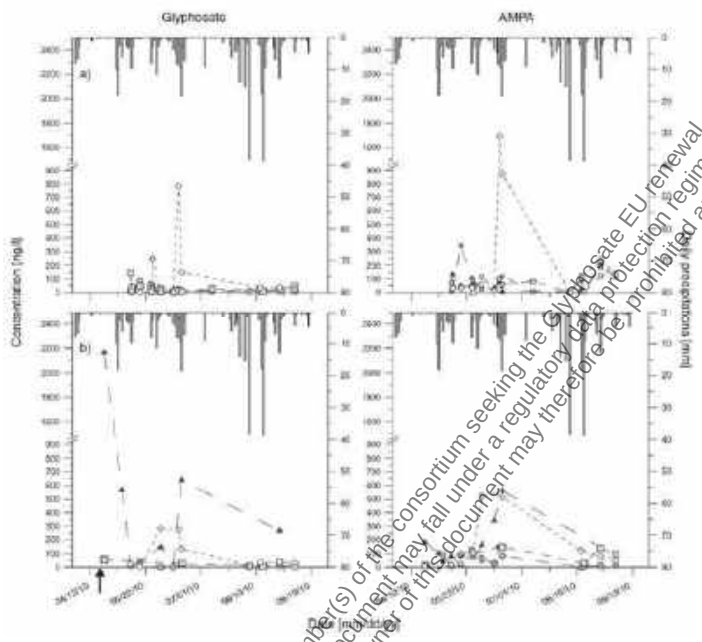
[1]® Cheminova Inc., Lenvig, Denmark

Results and discussion

Soil water samples

Soil water samples had in general pH values between 7.3 and 8.4 and electrical conductivities (EC) between 300 and 550 µS/cm (Figure 7.5-11). Higher range of values was observed at 20 cm in parcel 1 and at 60 and 80 cm in parcel 2, with pH and EC values of 8.4–9 and 450–700 µS/cm, respectively. This certainly reflects the presence of throughflows deep in the profile as previously observed. Dissolved organic carbon (DOC) contents varied in general between 2 and 10 mg/L, except in parcel 2 at 20 cm, where they were between 7 and 24 mg/L. Inorganic carbon (DIC) concentrations were found between 35 and 60 mg/L, with slightly higher values at 80 cm in parcel 2 (between 60 and 75 mg/L). Ion analysis revealed a calcium-dominated composition, with variable magnesium, sodium, nitrate and sulphate contents (data not shown). The variability for the latter was certainly due to the application of sulphur in the two parcels to prevent fungal diseases. A surprising difference in HCO_3^- discriminated samples from the two parcels, with high content for half of the samples from parcel 1 and very low ones for the others. For both parcels, in 2010, glyphosate and AMPA in soil solution were generally found at concentrations higher than 300 ng/L only at 20 and 80 cm (Figure 7.5-12).

Figure 7.5-12: **Growing season 2010. Concentrations of glyphosate and AMPA in soil solution at the bottom of parcel 1 (a) and 2 (b), at 20(♦), 40(□), 60(●) and 80 cm(▲) for the period April–September 2010. The black arrow indicates the date of glyphosate application and the daily precipitations are shown as bars (scale at the right side of the graph).**



In 2011, much higher concentrations were observed, especially in the surface layers: up to 4.7 and 1.75 µg/L at 20 cm in parcel 1 and 12 and 6.5 µg/L at 40 cm in parcel 2, respectively (Figure 7.5-13). Concentration peaks were always related to cumulative rainfall during the previous days, leading to the observed punctual water logging at the surface of this parcel. The succession of important rainfall events in August (>10 mm/day) in the end, induced the presence of AMPA at all depths, revealing a higher mobility than for glyphosate, despite its lower water solubility. In 2011 (Figure 7.5-13a), glyphosate concentrations were in general higher at 20 and 60 cm than at 40 and 80 cm, but only the 20 cm-samples exhibit concentrations at the µg/L level in April-May. Glyphosate concentrations in the 60 cm samples decreased with time, but showed important variations, from 50 to 700 ng/L, linked to important rain events. They first decreased from 700 to 100 ng/L and then re-increased in mid-May to up to 500 ng/L and also in late June up to 400 ng/L. AMPA concentrations in parcel 1 at the same depth showed similar variations, but in contrast, often increased with time (Figure 7.5-13a). Important rain events of more than 20 mm in one day, such as the one of early June, induced also a rise in concentrations at 40 and 80 cm. Furthermore, a much more important increase in concentrations was noticed at 80 cm than with more than 40 mm precipitation in two days, such as on June 17 to 18. Concerning the infiltration processes in parcel 1, the important rainfall events of June 2010 seem to have significantly leached the soil surface layer, desorbing in part glyphosate and AMPA molecules. In parcel 2, in 2010 (Figure 7.5-12b), the highest concentration of glyphosate in soil solution (2170 ng/L) was found at 80 cm depth, two days after its application (22 April 2010).

The gradual rise observed in AMPA concentrations in parcel 2 in June 2010 at 80 cm depth (Figure 7.5-12b) suggests an increasing water saturation of the soil pore space, and certainly the further use of preferential pathways by the soil solution in this parcel. In contrast to parcel 1, the occurrence of these peaks at 80 cm is certainly explained by the steeper hillslope, which represents, with the presence of more impervious reddish marls remnants in the subsoil, favourable conditions for the formation of lateral water circulation within the unsaturated zone. In 2011, still in parcel 2 (Figure 7.5-13b), a surprisingly high concentration peak of glyphosate in late July and a corresponding one of AMPA in early August was noticed at 40 cm. This could reflect either a second application in the neighbourhood parcels or a change in the pore system at this depth, with different inferred water pathways. Figure 7.5-14 shows glyphosate and AMPA

concentrations as a function of cumulative rainfall between two sampling periods. In general, with the exception of highly concentrated samples (>2.5 µg/L) and the effect of degradation with time, cumulative rain fall seem to govern glyphosate and AMPA concentration dynamics in the vadose zone in the following way: 1) cumulative rainfalls up to 10 mm decrease herbicide concentrations due to a dilution effect, 2) quantities between 10 and 30 mm lead to a concentration rise, certainly due to an increase in the kinetic energy of the soil solution, with the consequent formation of preferential flow in the parcels with colloid-associated transport, and 3) from 30 mm of cumulative rainfall, the increased surface runoff and dilution are responsible for the decrease in concentration.

Figure 7.5-13: **Growing season 2011. Concentrations of glyphosate (left) and AMPA (right) in soil solution at the bottom of parcel 1 (a) and 2 (b), at 20(♦), 40(□), 60(●) and 80 cm(▲) for the period April–September 2011. The black arrow indicates the date of glyphosate application and the daily precipitations are shown as bars (scale at the right side of the graph).**

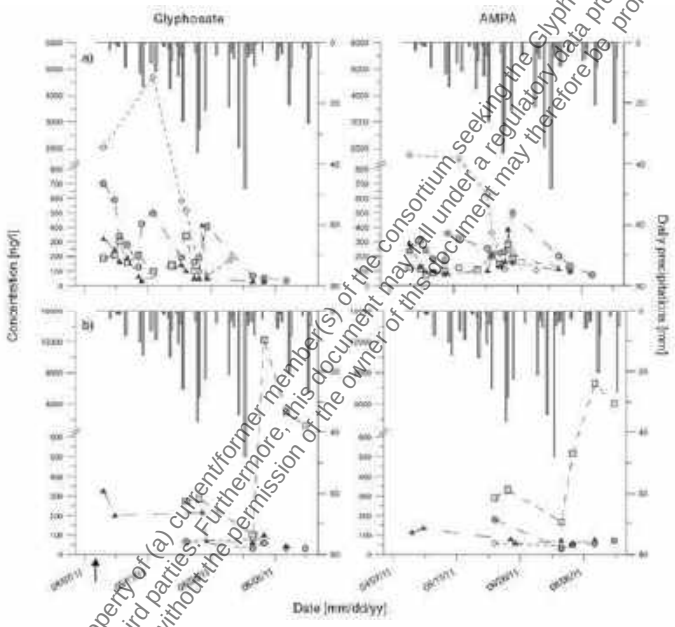


Figure 7.5-14: **Influence of cumulated rainfall between two samples on glyphosate (●) and AMPA (□) concentrations in soil waters of both vineyard parcels. Three phases can be distinguished. 1: An important decrease due to a dilution effect, 2: At medium rainfall, an important increase due to preferential flows and colloid associated transport and 3: At cumulated rain amounts greater than 30 mm, a levelling-off decrease, due to the combined effect of increased surface runoff and dilution.**

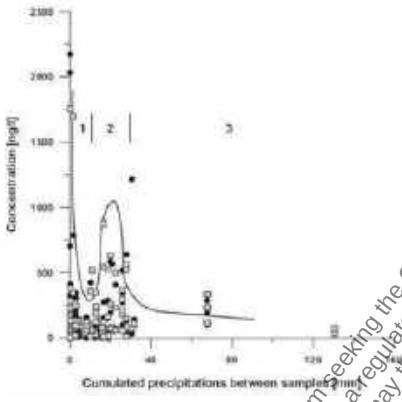
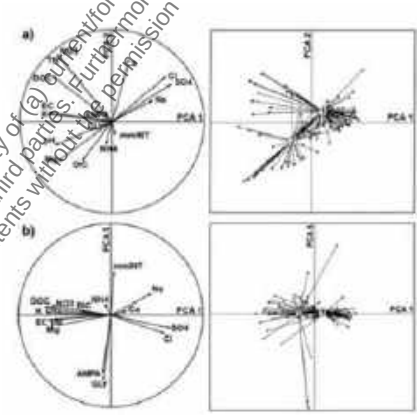


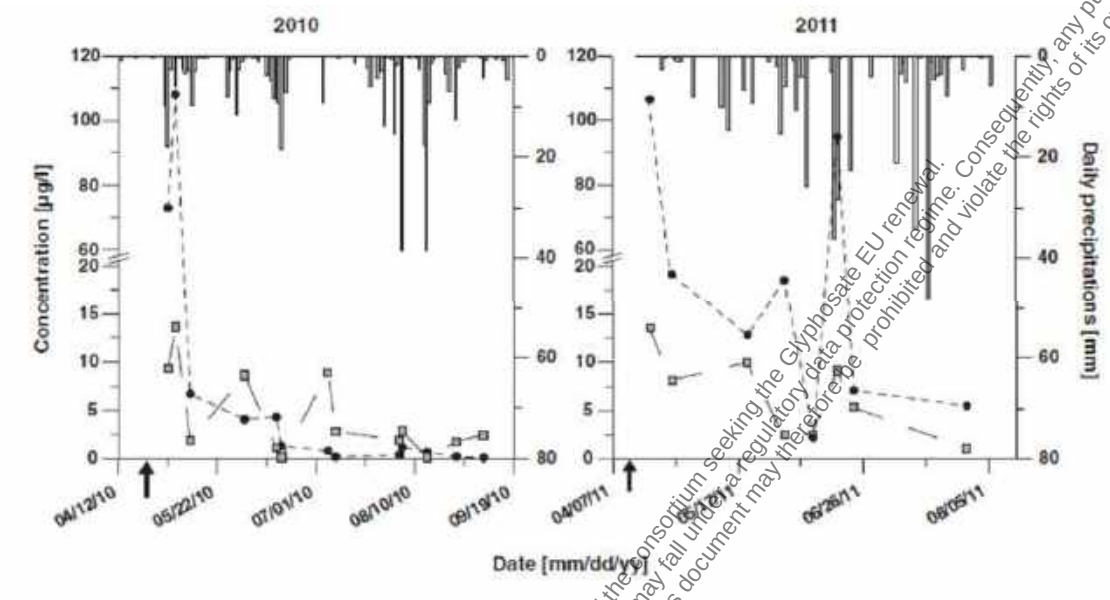
Figure 7.5-15: **Principal Component analysis (PCA) done with R-software on different normalized parameters of the soil water samples: a) correlation circle and projection of the different parameters on the 1st (X) and 2nd (Y) component axis diagram, accounting for 40.8 % of the variance, and relative positions of the observations with parcels as gravity centres; b) Idem on the 1st (X) and 5th (Y) component axis diagram, accounting for 32.4 % of the variance.**



Surface runoff water

Runoff water samples collected in parcel 2 showed pH between 8.3 and 8.7 and electrical conductivity between 50 and 105 µS/cm. In 2010, high concentrations of glyphosate and AMPA were found in the two first unfiltered (but decanted) runoff samples after the application (Figure 7.5-16): 73 and 110 µg/L, respectively 9 and 14 µg/L. This result is in agreement with the relatively high concentration found (567 ng/L) in the soil solution at 80 cm for the same period (05/05/10). Glyphosate concentrations dropped down to 7 µg/L in May and then to 4 µg/L in early June, before decreasing to 1 µg/L after the succession of rainy days in mid-June.

Figure 7.5-16: **Runoff of parcel 2. Glyphosate (•) and AMPA (□) concentrations in unfiltered, but decanted runoff water samples in 2010 (left) and 2011 (right); the black arrow again indicates the date of glyphosate application and the daily precipitations are shown as bars (scale at the right side of the graph).**



For AMPA, the decrease in concentrations was less drastic, what can be explained by the fact that it is assumed to be constantly produced by glyphosate degradation. The high concentration (~9 µg/L) observed in early July occurred after only one rainy day after a dry period that probably allowed Soil microorganisms to decay glyphosate into AMPA more actively. In 2011, concentrations were in the same range of values and their decrease was also observed, but to a lower extent. In contrast to 2010, AMPA concentrations were never higher than those of glyphosate. At the end of June, high concentrations were observed again with 95 µg/L of glyphosate and 9 µg/L of AMPA. These values are in same range than right after the application in late April, revealing an application on neighbourhood parcels. Indeed, the important rainfall of more than 40 mm in two days induced certainly a huge runoff, possibly passing across the road situated above the parcel, and penetrating it. In order to determine whether glyphosate and AMPA were transported in the dissolved state or bound to soil particles, a syringe filtration (Nylon filters) of runoff samples was made: the fraction <0.45 µm still carried between 70 and 90 % of the total concentration, with medians of 78 % and 73 % for glyphosate and AMPA respectively (n = 10, data not shown). Thus, transport of glyphosate and AMPA associated to coarse particle (>0.45 µm) accounted for 20–30 %, which is more than in previous studies despite a smaller cut-off (0.24 µm).

Mass balance

The total amount of glyphosate and AMPA retrieved in both type of samples from parcel 2 (surface = 845 m²), and likely to be exported from it, was 4.3 g in 2010 and 9.1 g in 2011 (Table 7.5-22). This represents respectively 10 and 20 % of the initial amount, which, despite the uncertainty of such kind of calculations, is in agreement with previous studies. The 80–90 % remaining were either retained, and possibly as bound residues after some time, or degraded in the soil, as volatilization is not likely to happen due to their properties. The relative contribution of throughflows in the unsaturated zone versus surface runoff in our case was 3–5 % versus 95–97 %.

Table 7.5-22: Mass balance for glyphosate in parcel 2 for both growing seasons, with amounts retrieved in both types of samples, soil solution and surface runoff, according to cumulated precipitations, as well as percentages of the applied amount and of the relative contribution of throughflows and surface runoff. One g of AMPA was considered arising from 1g of glyphosate.

Year	Applied amount [g]	Soil water [g]	[%]	Runoff [g]	[%]	Throughflows [%]	Runoff [%]
2010	45.6	0.145	0.32	4.179	9.16	3.36	96.64
2011	45.6	0.466	1.02	8.659	18.98	5.10	94.90

Conclusion

This study presents clear evidence for the mobility of the herbicide glyphosate and its metabolite AMPA in the vadose zone despite their high sorption abilities. Though the chemistry of soil solution does not play an important role in their transport, which was mainly governed by the rainfall regime and soil permeability, the presence of copper and the alkaline pH conditions in the studied vineyard soils certainly participate in their mobility by influencing their sorption. Thus, in fine-textured layered soils with significant slope, the increase in moisture content leads to the formation of throughflows just above the more impervious layer, which actively participate in the downhill transport of glyphosate and AMPA. Nevertheless, their transfer from fields to adjacent surface water happens mainly by surface runoff, in a dissolved state or bound to small colloids, representing potential threats for aquatic organisms.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article reports the mobility of glyphosate and AMPA in soil after application of the parent to a vineyard soil in Switzerland. The maximum reported soil pore water concentrations were <14 µg/L and <8 µg/L (inferred from figure) for glyphosate and AMPA, respectively. The loss to surface waters via surface runoff and throughflows in soils with subsequent exfiltration to surface waters was considered. The reported parameters are insufficient to allow a complete assessment of the validity of the study. The article is therefore considered reliable with restrictions.

Assessment and conclusion by RMS:

B. Water

B.1 Groundwater

Concentrations of glyphosate (GLY), AMPA and HMPA in groundwater arising from public monitoring datasets have been collected from regional/national environment agencies as well as published peer reviewed publications from literature searches and rated as potentially relevant/reliable are reported in this section.

There are five new applicant studies presented on groundwater. [redacted] (2020, CA 7.5/001) describes the collection of public monitoring data (1995 – 2019) for European countries for the compartment soil, water, sediment and air for glyphosate, AMPA and HMPA. [redacted] (2020, CA 7.5/002) assesses the data collected by [redacted] (2020, CA 7.5/001). These two recent studies were designed to be the more comprehensive than previous studies by considering additional metabolites, compartments and time periods. [redacted] (2020, CA 7.5/002) covers a range of environmental compartments, however, the study summary below only includes the results relevant to the environmental compartment groundwater. [redacted] (2016, CA 7.5/010) updates a previous investigation period described by an existing study of [redacted] (2012, CA 7.5/013). Studies specific to France include [redacted] (2019a, CA 7.5/008) which focuses on a more recent period (2008-2014) of the same dataset investigated by [redacted] (2016, CA 7.5/009) which considered the years 1997-2013. There is a large degree of overlap between the datasets used in these various studies, for example the French dataset is common to all studies and given its size often comprises the majority of compiled European datasets.

The four existing applicant studies by Anonymous (2012, CA 7.5/011), [redacted] (2012, CA 7.5/013), [redacted] (2006, CA 7.5/014) as well as [redacted] (2005, CA 7.5/015) are presented for completeness.

Several publications are presented outlining concentrations found in groundwater:

- Rosenbom *et al.* (2019, CA 7.5/016), Rosenbom *et al.* (2015, CA 7.5/019) and Norgaard *et al.* (2014, CA 7.5/021) present data interpretations from the Danish Pesticide Leaching Assessment Programme which comprises 53 highly instrumented field sites.
- Poiger *et al*. (2017, CA 7.5/017), Di Guardo and Finizio (2016, CA 7.5/018), Mörtl *et al.* (2013, CA 7.5/024), [redacted] (2011, CA 7.5/028) and [redacted] (2010, CA 7.5/029) present site investigations focused on locations with groundwater quality issues. Bruchet *et al*. (2011, CA 7.5/027) present data in shallow boreholes exploited for drinking water following bank filtration.
- McManus *et al.* (2014, CA 7.5/020) present data for Ireland while Sanchis *et al.* (2012, CA 7.5/025 and CA 7.5/026) present regional data for Catalonia, Spain. Both datasets likely overlap fully with those in existing applicant studies presented e.g. [redacted] (2020, CA 7.5/002).

A summary of maximum concentrations of glyphosate (GLY) and AMPA in groundwater reported by the applicant studies and publications is presented in Table 7.5-23 while the maximum reported rates of exceedance of various thresholds by these datasets are summarised in Table 7.5-24.

Maximum measured concentrations of GLY up to 1005 µg/L are reported, however, the most extreme values are likely anomalous. [redacted] (2020, CA 7.5/002) identified 10 outliers in their dataset, including this maximum value of 1005 µg/L, which when removed brought the maximum value to 39.2 µg/L. This is still the highest reported value and is well below the Surface Water Regulatory Acceptable Concentration (SW RAC) for groundwater fed ecosystems of 400 µg/L and the lifetime health-based (Acceptable Daily Intake, ADI) concentration of 1500 µg/L used for consumer risk assessment. Several of the applicant studies provide statistical summaries of the concentration datasets investigated and these shed additional light on the few extreme values in these datasets, for example [redacted] (2020, CA 7.5/002) calculate that the arbitrary regulatory threshold of 0.1 µg/L represents the 98.98th percentile GLY concentration in their dataset, including these anomalous values.

The maximum measured AMPA concentration in groundwater was 19.0 µg/L which is well below the SW RAC of 1200 µg/L (for groundwater fed ecosystems) and the lifetime health-based (ADI) concentration of 3960 µg/L. Several of the applicant studies provide statistical summaries of the concentration datasets investigated and these shed additional light on the few extreme values in these datasets, for example [redacted] (2020, CA 7.5/002) calculate that the arbitrarily defined regulatory threshold of 10 µg/L for non-relevant metabolites represents the 99.998th percentile concentration in their dataset. It should also be borne in mind that AMPA may arise from other sources than glyphosate use, e.g. detergents, particularly in groundwater affected by surface water or flooding. [redacted] (2006, CA 7.5/014) identified not only the influence of surface waters on groundwater detections, but also the influence of wastewater from sewage treatment works transporting AMPA from household and industrial detergent uses.

Assessment of rates of exceedance of thresholds requires the dataset to be large enough to capture a range of agronomic, geographical, pedoclimatic and hydrogeological situations. The datasets analysed by [redacted] (2020, CA 7.5/002) and [redacted] (2016, CA 7.5/010) best meet this criterium. Those of [redacted] (2019a, CA 7.5/008) and [redacted] (2016, CA 7.5/009) meet this criterium to a lesser extent as they only focus on France as well as only presenting annual summaries which are influenced by small sample sizes in the 1990s. The datasets of [redacted] (2020, CA 7.5/002), [redacted] (2016, CA 7.5/010) and [redacted] and [redacted] (2019a, CA 7.5/008) demonstrate that compliance for GLY with the arbitrary regulatory threshold of 0.1 µg/L is very high being >99.3 % of samples and >99.9 % when considering consecutive samples that exceed the threshold. [redacted] (2020, CA 7.5/002) demonstrate that compliance for AMPA with the arbitrarily defined regulatory threshold of 10 µg/L for non-relevant metabolites is >99.998 % of samples. This conclusion is further supported by [redacted] (2019a, CA 7.5/008) that calculate compliance as >99.9 % against a threshold of 2 µg/L, a guideline threshold for raw waters destined for drinking water supply in France.

The site investigations presented in the individual publications highlight the difficulty of interpreting groundwater monitoring data without site specific context. [redacted] (2006, CA 7.5/014), through investigation of locations in Germany with GLY and AMPA detections, concluded that all detections could be explained as false positives or were the result of surface or wastewater influences. [redacted] (2011, CA 7.5/028) investigating GLY detections in Lombardy Italy conclude that the cause in most cases was point source contamination of unsealed well heads in courtyards of sprayers, direct application to well heads or the influence of surface runoff as the borehole head was not sealed. These observations mirror those of [redacted] (2010, CA 7.5/029) following investigation of wells in the Netherlands where they conclude that poor well head protection and ingress of surface runoff was the primary cause for GLY detections. [redacted] (2005, CA 7.5/015) investigating detections in a shallow ground water catchment in Sweden concluded that the installation of agricultural drains to 4 m depth to address undulating topography had inadvertently allowed the interaction of surface water and groundwater and that GLY detections were the result of this interaction. Anonymous (2012, CA 7.5/011) through investigation of vulnerable sites in France with GLY and AMPA detections, concluded that while these occasionally occur, often at very vulnerable sites, they are not systematic and generally not at wells that are used for drinking water. They also add that for those wells that might be used for drinking water there was no protection zone in place to protect the well and that this protection would address any perceived risk. Case studies presented in [redacted] (2020, CA 7.5/002) investigating situations where public monitoring suggested elevated rates of detection demonstrate that local factors like open hand dug wells may influence detections of GLY and AMPA and that localised investigations to understand the situation better with a view to adapting local practice through targeted stewardship programs is the most appropriate means of addressing these situations where they arise. Despite the public monitoring data assessed including such erroneous and anomalous data the rates of compliance reported are very high.

The data presented in this section demonstrate that the environmental concentrations typically encountered in this environmental compartment do not pose a risk for ecosystems or human health *via* drinking water. Safe use with respect to groundwater is demonstrated for the vast majority of use environments in Europe.

Table 7.5-23: Summary of reported maximum concentrations of glyphosate (GLY) and AMPA in groundwater

Reference	Context	Maximum Concentration (µg/L)	
		GLY	AMPA
[redacted] 2020, CA 7.5/002	EU Summary	1005.0 39.2[1]	16.0 16.0[1]
[redacted] 2019a, CA 7.5/008	FR Summary	1005.0 10.7[5]	9.3 2.5[5]
[redacted] 2016, CA 7.5/010	EU Summary	28.0	19.0
[redacted] 2016, CA 7.5/009	FR Summary	1005.0 8.9[5]	19.0 2.2[5]
Anonymous, 2012, CA 7.5/011	Site investigation of highly vulnerable sites	12.9	3.4
[redacted] 2012, CA 7.5/013	EU Summary	24.0	19.0
[redacted] 2006, CA 7.5/014	DE site investigation	0.32[2]	0.5
[redacted] 2005, CA 7.5/015	SE site investigation	0.18	NA
Rosenbom, A. et al., 2019, CA 7.5/016	DK PLAP Sites – Soil pore water in variably saturated zone	0.05	0.14
	DK PLAP Sites – Groundwater in saturated zone	0.13	0.02
Poiger, T. et al., 2017, CA 7.5/017	CH investigation of 14 vulnerable sites, including karst	0.025	0.65
Di Guardo A., Finizio A., 2016, CA 7.5/018	IT investigations in Lombardy	NR	NR
Rosenbom, A. et al., 2015, CA 7.5/019	DK PLAP Sites – Soil pore water ~1 m	31.0	1.6
	DK PLAP Sites – Groundwater	0.67	0.08
McManus, S., et al., 2014, CA 7.5/020	Ireland investigation (158 sites)	<0.1[3]	<0.1[3]
Norgaard, T., et al., 2014, CA 7.5/021	Drainflow at 1.1 m depth	31.0	~1.6[3]
Mörtl, M., et al., 2013, CA 7.5/024	36 ground water samples; 14 sampling sites in Békés county, Hungary	0.98	NA
Sanchís, J., et al., 2012, CA 7.5/025	Catalonia (NE Spain) 139 samples from 69	2.56	NA
Bruchet, A., et al., 2011, CA 7.5/027	Groundwater following bank filtration	<0.1[7]	<0.1[7]
[redacted] 2011, CA 7.5/028	Lombardy IT site investigation of 5 sites	1.375[6]	NA
[redacted] 2010, CA 7.5/029	NL site investigations	4.74	0.23[4]

NR/NA – Not reported/Assessed
[1] Excluding outliers
[2] Wastewater from sewage plant
[3] Inferred from graph
[4] Was not target of the investigation
[5] 99th percentile
[6] Confirmed point source contamination
[7] Bank infiltration

Table 7.5-24: Summary of reported rates of concentrations of various thresholds for measured concentrations of glyphosate (GLY) and AMPA in groundwater

Reference	Context	Exceedance threshold and rate		
		Threshold (µg/L)	GLY (%)	AMPA (%)
[redacted] 2020, CA 7.5/002	EU Summary	0.1	0.62	0.68
		10	NA	0.002
[redacted] 2019a, CA 7.5/008	FR Summary	0.1	0.7[4]	1.1[4]
		2	<0.1[4]	<0.1[4]
[redacted] 2016, CA 7.5/010	EU Summary	0.1	0.6	0.75
[redacted] 2016, CA 7.5/009	FR Summary	0.1	3.0[2]	11.4[2]
		2	<0.1[2]	<0.1[2]
Anonymous, 2012, CA 7.5/011	Site investigation of highly vulnerable site	NR	NR	NR
[redacted] 2012, CA 7.5/013	EU Summary	0.1	0.64	0.77
[redacted] 2006, CA 7.5/014	DE site investigation	NR	NR	NR
[redacted] 2005, CA 7.5/015	SE site investigation	NR	NR	NR
Rosenbom, A. et al., 2019, CA 7.5/016	DK PLAP Sites – Soil pore water in variable saturated zone	0.1	0.0	3.1[6]
	DK PLAP Sites – Groundwater in saturated zone	0.1	0.9[5]	0.0
Poiger, T. et al., 2017, CA 7.5/017	CH investigation of 14 vulnerable sites, including karst	NR	NR	NR
Di Guardo A., Finizio, A., 2016, CA 7.5/018	IT investigations in Lombardy	0.1	1.75[1]	NR
Rosenbom, A. et al., 2015, CA 7.5/019	DK PLAP Sites	0.1	NR	NR
McManus, S. et al., 2014, CA 7.5/020	Ireland investigation (158 sites)	0.1	0.0	NR
Norgaard, T. et al., 2014, CA 7.5/021	Drainflow at 1.1m depth	NA	NA	NA
Mörtl, M. et al., 2013, CA 7.5/024	36 ground water samples; 6 sampling sites in Békés county, Hungary	0.1	100[3]	NA
Sanchís, J. et al., 2012, CA 7.5/025	Catalonia (NE Spain) 139 samples from 69	NR	NR	NA
Bruchet, A. et al., 2011, CA 7.5/027	Bank filtration	0.1	0.0	0.0
[redacted] 2011, CA 7.5/028	Lombardy IT site investigation of 5 sites	NR	NR	NR
[redacted] 2010, CA 7.5/029	NL site investigations	NR	NR	NR

NA/NR – Not Assessed/Reported
[1] 5 out of 285 samples
[2] Maximum annual value of 14/15 years (AMPA/GLY) and influenced by small sample sizes
[3] Atypical results from a very small study focussing on contaminated industrial sites
[4] Maximum annual value of 7 years
[5] 1 out of 116 samples
[6] 2 out of 65 samples

Applicant studies

New studies/assessments

1. Information on the study

Data point:	CA 7.5/001
Report author	[redacted]
Report year	2020
Report title	Collection of public monitoring data for European countries for the compartments soil, water, sediment and air for Glyphosate, AMPA and HMPA
Document No	110057-1
Guidelines followed in study	Methodology is based on the Groundwater Monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations') Minimum quality criteria of monitoring data described by the FOCUS Ground Water Work Group chapter 9.5 (European Commission, 2014)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The report provides information about the outcome of a search for readily accessible and available monitoring data in European countries at a regional/national level for the time period 1995-2019. The main focus was on the time period 2012-2019 while earlier years are already covered by existing data. The search included raw data, requested from regional/national authorities or downloadable from their websites, as well as aggregated data extracted from reports compiled by authorities.

Data from 14 European countries were considered: Austria, Belgium, Denmark, France, Germany, Hungary, Ireland, Italy, The Netherlands, Poland, Romania, Spain, Sweden and the United Kingdom. The countries represent the major markets of products containing glyphosate sold in the EU. The data compilation included the active substance glyphosate and its metabolites AMPA and HMPA, in the soil, groundwater, surface water, tidal water, drinking water, sediment and air environmental compartments.

As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities and other bodies of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were not actually included in any of the monitoring programs.

Groundwater Compartment Conclusion

A large groundwater public monitoring dataset was compiled, comprising raw datasets from 11 countries (AT, BE, DE, DK, ES, FR, IE, IT, NL, SE and UK) and aggregated datasets from published reports for 9

countries (AT, DE, DK, ES, HU, IE, IT, NL, SE). Collectively these cover a wide range of pedoclimatic and hydrogeological settings typically spanning more than a decade.

I. MATERIAL AND METHODS

The general methodology of data collection of public monitoring data and minimum quality criteria is based on existing guideline documents for groundwater monitoring programs. The underlying principles have been applied to all environmental compartments, especially where no specific guidance is at hand. Data search, acquisition and processing approaches are described below. The same approach was applied for each country, compartment and substance. Country specific adaptations to the general procedure were made in order to generate a harmonized database. The data collected for this report refers to third party organization data regarding all environmental compartments (SOIL, GW, SW, TD, DW, SD, AIR) and was further differentiated into the two different data types, i.e. raw data and aggregated data. Aggregated data refers to information provided in publicly available reports, e.g. from environmental agencies or research institutes. Such reports might hold only summary information on substance findings over space and time and may intersect with the raw data. Raw data refers to mid to long term time series of data that are provided on request by e-mail or by database from governmental authorities and are therefore recognized as official monitoring data. These datasets hold the information of sampling values, quality information (sampling, treatment, limit of detection - LOD, limit of quantification - LOQ) as well as information of location and time of sampling.

The following data source types were investigated in order to collect monitoring data:

- E-mail requests: a general e-mail was sent to the national responsible authorities with regard to the required information.
- Governmental webpages: the official webpages of the national responsible authorities were searched for information regarding available reports and datasets.
- Public online databases: available data from online databases were downloaded as provided by the webpages of governmental authorities and other institutions.

The data search resulted in a very heterogeneous collection of tabular data and reports in different formats and structure. Data were processed into a harmonized tabular format by selecting relevant information and adapting data organisation. In general, the complete datasets were included in the final harmonized database as provided by the authorities, but obvious duplicates were deleted. In general, all entries for the digital database were checked for consistency and plausibility. For the raw data it was assumed that information was already subjected to critical scrutiny by the respective organization. For the aggregated data the same assumption was made with quality assurance of the data (mostly summaries) being the responsibility of the authors of the respective reports.

II. RESULTS AND DISCUSSION

The final data collection of raw data and aggregated data is summarised for each compartment and each country in Table 7.5-25.

Groundwater

- Austria (AT)
  - Raw monitoring data from national authorities for groundwater were downloaded from the H2O-Fachdatenbank.
  - Aggregated monitoring data from reports published by national authorities for groundwater were downloaded from several sources.

- Belgium (BE)
  - Raw monitoring data for groundwater for both Flanders and Wallonia compiled by the Belgian association for the plant protection products industry were received by e-mail.
  - No aggregated monitoring data from reports published by national authorities were considered in case of the compartment groundwater, because of the good data availability *via* raw data.

- Germany (DE)
  - Raw monitoring data from national authorities for groundwater were provided by the German EPA, the regional authorities of Brandenburg, Bavaria, Bremen, Mecklenburg-Vorpommern, North Rhine – Westphalia, Schleswig-Holstein, Saxony, Saarland and Hesse.
  - Aggregated monitoring data from reports published by national authorities were obtained from LAWA, the German parliament, from the German EPA, and the environmental authorities of Mecklenburg-Vorpommern, North Rhine – Westphalia, Rhineland-Palatinate and Schleswig-Holstein.

- Denmark (DK)
  - Raw monitoring data from national authorities for groundwater were provided by GEUS from the GRUMO monitoring programme.
  - Aggregated monitoring data from reports published by national authorities for groundwater were downloaded from GEUS and the National Center for Environment and Energy.

- Spain (ES)
  - Raw monitoring data from national authorities for groundwater were provided from the Ministry of Agriculture, Fisheries and Food after contacting the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) per e-mail.
  - Aggregated monitoring data from reports in one scientific paper published by the Spanish Geological and Mining Institute.

- France (FR)
  - In France monitoring data for groundwater are published by the Public Water Information Service (eaufrance). Raw monitoring data from national authorities for groundwater were downloaded from ADES.
  - No aggregated monitoring data from reports published by national authorities were considered, because of the very good data obtained *via* raw monitoring data.

- Hungary (HU)
  - Raw monitoring data from national authorities for groundwater were not available.
  - Aggregated monitoring data from reports published by national authorities for groundwater were obtained in the form of a peer-reviewed paper from the National Agricultural Research and Innovation Centre published in Journal of Chemistry.

- Ireland (IE)
  - Raw monitoring data from national authorities for groundwater were provided by the Irish EPA by e-mail.
  - Aggregated monitoring data from reports published by national authorities for groundwater were downloaded from the Irish EPA and from the governmental page on the Water Framework Directive.

- Italy (IT)
  - Raw monitoring data from national authorities for groundwater were downloaded from the provincial environment agency of Lombardia.
  - Aggregated monitoring data from reports published by national authorities for groundwater were downloaded from ISPRA.

- The Netherlands (NL)
  - Raw monitoring data from national authorities for groundwater were obtained in the form of the stand-alone software tool "Groundwater Atlas for pesticides in The Netherlands".
  - Aggregated monitoring data from reports published by national authorities for groundwater were downloaded from RIVM and the Dutch Water Quality portal. Further reports were downloaded from Wageningen University & Research.

- Poland (PL)
  - The responsible authorities for monitoring data in Poland are the Polish Geological Institute and the Chief Inspectorate Of Environmental Protection. The latter authority confirmed by e-mail that in Poland there is currently no public monitoring of glyphosate or its metabolites in groundwater.

- Romania (RO)
  - The responsible authority for monitoring data is the Ministry of Water and Forests. The Water Resources Management Directorate confirmed on behalf of the Ministry of Water and Forests that no public monitoring of glyphosate or its metabolites is carried out in any water compartment in Romania.

- Sweden (SE)
  - Raw monitoring data from national authorities in Sweden for groundwater were provided by SLU *via* e-mail. Additional raw monitoring data for groundwater were directly downloaded from the SLU homepage. Moreover, SLU provided another database containing raw data for groundwater issued from other sources than national monitoring, e.g. regional monitoring and private wells.
  - Aggregated monitoring refer to a report downloaded with aggregated groundwater monitoring data from the environment department of the municipality of Stockholm and aggregated national monitoring reports in tabular form for groundwater downloaded from the SLU homepage.

- United Kingdom (UK)
  - Raw monitoring data from national authorities for groundwater were downloaded from the Environment Agency for England and Northern Ireland *via* e-mail.
  - No aggregated monitoring data from reports were identified.

Table 7.5-25: Overview of public monitoring data availability of raw data (R) and aggregated data (A)

Country	Soil	Water				Sediment	Air
		Ground	Surface	Tidal	Drinking		
Austria	-	R, A	R, A	-	A	-	-
Belgium	-	R	R	-	A (Flanders)	-	-
Denmark	-	R, A	A	-	A	-	-
France	-	R	R	-	A	R	-
Germany	R (Brandenburg)	R, A	R, A	R	R (Schleswig-Holstein), A	-	-
Hungary	-	A (one research article)	A (one research article)	-	-	-	-
Ireland	-	R, A	R, A	-	R, A	-	-
Italy	-	R (Lombardia), A	R, A	-		-	-
The Netherlands	-	R, A	R, A	-	R	-	-
Poland	Confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Romania	Confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Spain	-	R, A	R, A	-	A	-	-
Sweden	-	R, A	R	-	R, A	R	-
UK England	-	R	R	R	A	-	-
UK Northern Ireland	-	R	-	-	-	-	-
UK Scotland	-	-	R	-	-	-	-
UK Wales	-	-	R	-	A	-	-

R: Raw data available; A: Aggregated data from reports available; -: No raw or aggregated data available

III. CONCLUSIONS

The collection of public monitoring data for glyphosate, AMPA and HMPA in soil, groundwater, surface water, drinking water, tide water, sediment and air resulted in a comprehensive database of 'raw monitoring data from national authorities' and 'aggregated monitoring data from reports published by national authorities'. As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were not actually included in any of the monitoring programs.

A large groundwater public monitoring dataset was compiled, comprising raw datasets from 11 countries (AT, BE, DE, DK, ES, FR, IE, IT, NL, SE and UK) and aggregated datasets from published reports for 9 countries (AT, DE, DK, ES, HU, IE, IT, NL, SE). Collectively these cover a wide range of pedoclimatic and hydrogeological settings typically spanning more than a decade.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes the collection process of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA.
The study is therefore considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Document No	-
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
The report provides information about the outcome of an analysis of public monitoring data comprising environmental concentrations of glyphosate (GLY) and its primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA) collated from readily available public monitoring databases held by national/regional environment agencies. This data collection and analysis was designed to expand previous reviews to include other compartments and supplement them for surface water, groundwater and drinking water. Public monitoring data from the following Member States (MS) were assessed for the water, sediment and soil compartments: Austria (AT), Belgium (BE), Denmark (DK), France (FR), Germany (DE), Ireland (IE), Italy (IT), Netherlands (NL), Spain (ES), Sweden (SE) and the United Kingdom (UK). Three MS, namely Poland (PL), Hungary (HU), and Romania (RO) confirmed that they do not conduct analyses for GLY, AMPA and HMPA in any environmental compartment. No data for HMPA was identified for any MS or compartment. Note that at the time the study was started the UK was a Member State and is referred to as a Member State throughout the report.

Analyses of the large spatial and temporal dataset of measured concentrations occurring in several environmental compartments, namely surface water, groundwater, drinking water, tidal water, sediment and soil, were conducted to assess their state. This analysis not only sought to assess the state of the environmental compartment but also to consider the potential impacts this might have on biota, ecosystems and human health by using regulatory endpoints and thresholds from a range of European (EU) Directives. These included the Water Framework Directive (Directive 2000/60/EC) and associated Groundwater (2006/118/EC), Drinking Water (1998/83/EC) and Priority Substances (2008/105/EC28) Directives in addition to the Plant Protection Products Directive (1107/2009/EC).

Groundwater

Groundwater (GW) data from AT, BE, DE, DK, ES, FR, IE, IT, NL, SE and UK were analysed for compliance with a range of regulatory endpoints and thresholds. The data were assessed against the following regulatory endpoints, 0.1 µg/L for GLY and the arbitrarily defined 10 µg/L for AMPA (for which there is no legal limit as AMPA is a non-relevant metabolite). In addition, case study investigations were conducted in ES and UK to investigate atypical elevated frequencies of detection.

Glyphosate

The large GLY public monitoring dataset (>251 000 samples collected from >37 800 sampling sites) was dominated by French data (~79.1 %) with smaller contributions from Denmark (~5.8 %), Germany (~5.7 %) and Austria (~3.8 %). Detection of GLY in GW samples was ~2 % which compared well with the 1.3 % of samples in the previous data collection ([redacted] 2012, CA 7.5/013 and 2016, CA 7.5/010). Compliance with the 0.1 µg/L threshold was very high (99.4 % samples from 97 % of sites) with very few exceedances (~0.6 % of samples from ~3.0 % of sites) and compared well with aggregated report values (ranging from 0.0 % in DE to ~7.0 % in ES) and the 0.6 % of samples from the previous data collection. Only 0.089 % of samples are consecutively above the threshold indicating the rare exceedances are non-systematic. The assessment of outliers identified 10 outliers in the dataset and if these are excluded the maximum concentration is reduced to 39.2 µg/L which is well below the SW RAC (for groundwater fed ecosystems) and the life time health-based ADI concentration of 1500 µg/L. Case studies exploring elevated rates of groundwater detection in ES and the UK, suggest these findings are most likely a function of direct contamination, like spray drift into open wells.

AMPA

The large AMPA public monitoring dataset (>230 000 samples collected from >34 400 sampling sites) was dominated by the French data (~82.4 %) with smaller contributions from Denmark (~6.4 %) and Germany (~5.2 %). Detection of AMPA in GW samples was ~2.9 % which compared well with the ~2.1 % of samples in the previous data collection. Compliance with the arbitrarily defined 10 µg/L regulatory threshold for a non-relevant metabolite was very high (99.998 % of samples from 99.994 % of sites) given exceedances were rare (~0.002 % of samples from ~0.006 % sites). The maximum concentration of 16 µg/L is well below the SW RAC (for groundwater fed ecosystems) and the lifetime health-based ADI concentration of 3960 µg/L. It should be borne in mind that AMPA may originate from sources other than GLY, for example detergents. In order to compare these AMPA results with previously published and aggregated results, assessment against the threshold of 0.1 µg/L was also undertaken. Compliance with the arbitrarily defined regulatory threshold of 0.1 µg/L was very high (99.3 % of samples) with few exceedances (~0.7 % of samples) indicated, which compared well with aggregated report exceedance (ranging from 0.0 % in SE to ~3 % in the IT) of ~0.75 % of samples from the previous data collection.

Groundwater Compartment Conclusion

The analysis of the large groundwater dataset for GLY and AMPA indicates they are both occasionally detected above the LOQ in this compartment, however, compliance against regulatory endpoints and thresholds is very high with the frequency of exceedance being very low as would be expected given that the compounds are only slightly mobile in soil. The environmental concentrations typically encountered do not pose a risk for ecosystems or human health from drinking water.

I. MATERIAL AND METHODS

The dataset analysed comprised individual groundwater analysis records as well as existing aggregated analyses extracted from reports sourced from regional/national environment agencies (see [redacted], 2020, CA 7.5/001). The approach taken for the data processing was precautionary in that it preserved samples in the analysis where there was any doubt regarding their reliability. As such the number of records excluded from the analysis was small, especially relative to the total number of samples prior to removal. Similarly, no attempt to remove outliers was undertaken despite the presence of extreme values in the datasets. In order to explore the extreme nature of some of the values included in the groundwater dataset and assess the implications for this analysis, an outlier analysis was performed on the combined EU dataset using the same approach as the European Commission's Joint Research Centre (JRC) in the evaluation of candidate compounds for the priority substance watch list (Carvalho *et al.*, 2016). Analysis and assessment of the data against thresholds was undertaken using the statistical software R (R Core Team, 2019) and graphs produced with the R package ggplot2 (Wickham, 2009). The groundwater public monitoring data was evaluated against the following thresholds:

- Drinking water endpoint: A threshold of 0.1 µg/L for parent compounds and relevant metabolites was used for GLY
- Regulatory threshold: The arbitrarily defined threshold of 10 µg/L for non-relevant metabolites was used for AMPA;
- Drinking water threshold: An additional threshold of 0.75 µg/L for AMPA is also presented. This threshold is based on the tiered testing requirements given in the guidance document on non-relevant metabolites (SANCO 221/2000 rev.10) above which data to set a lifetime safe drinking water limit is required to be obtained. The lifetime safe drinking water limits for glyphosate and AMPA are 1500 µg/L and 3960 µg/L, respectively.

II. RESULTS AND DISCUSSION

The input data collated for analysis of GLY residues in GW were dominated by data sourced from France (~79.1 %) with smaller contributions from Denmark (~5.8 %), Germany (~5.7 %) and Austria (~3.8 %). This pattern was also apparent for AMPA residues with French data dominating the combined dataset (~82.4 %) with lower, but important contributions from Denmark (~6.4 %) and Germany (~5.2 %). As such the French dataset is likely to influence statistics and conclusions derived from analysis and consideration of the combined European dataset. The exact nature of a groundwater body and how these are sampled is not generally known from the publicly available data, e.g. how deep the groundwater is or the manner in which it is sampled e.g. piezometer, borehole, well or spring. It is not typically known what the groundwater from these locations is used for or why the water at this location was selected for monitoring. Temporally the GLY (see Figure 7.5-17) and AMPA (see Figure 7.5-18) data indicates some bias at a MS level with fewer samples typically collected in the winter and spring months resulting in a unimodal distribution, e.g. IE, or a bimodal distribution with data collection in spring and autumn during key usage periods being greater than at other times of the year, e.g. FR, DE, NL, SE. The spatial distribution of GLY and AMPA public monitoring locations for MS where data is collected is biased (see Figure 7.5-19 and Figure 7.5-20). For some MS, e.g. DE, IT and ES, this is a function of data only arising from some provincial/regional environment agencies while for others, e.g. the UK, this is likely a function of spatial targeting.

Figure 7.5-17: Bar chart of monthly groundwater glyphosate (GLY) sampling effort

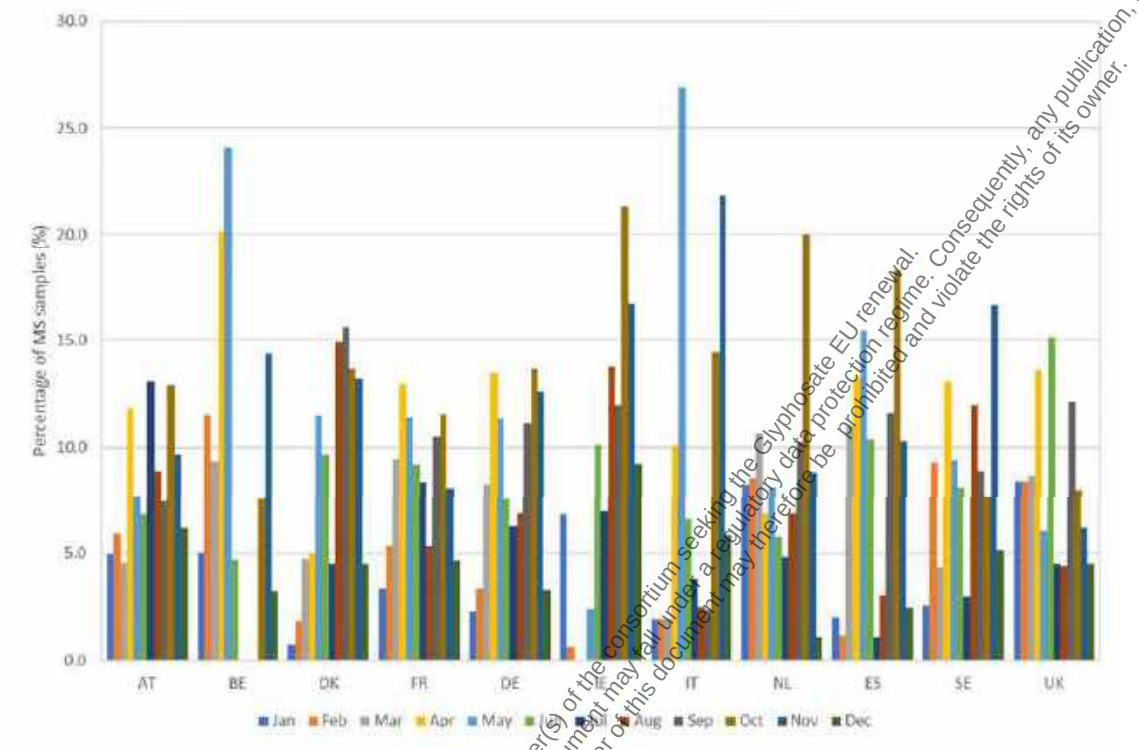


Figure 7.5-18: Bar chart of monthly groundwater AMPA sampling effort. No data is available for AT, IE and UK.

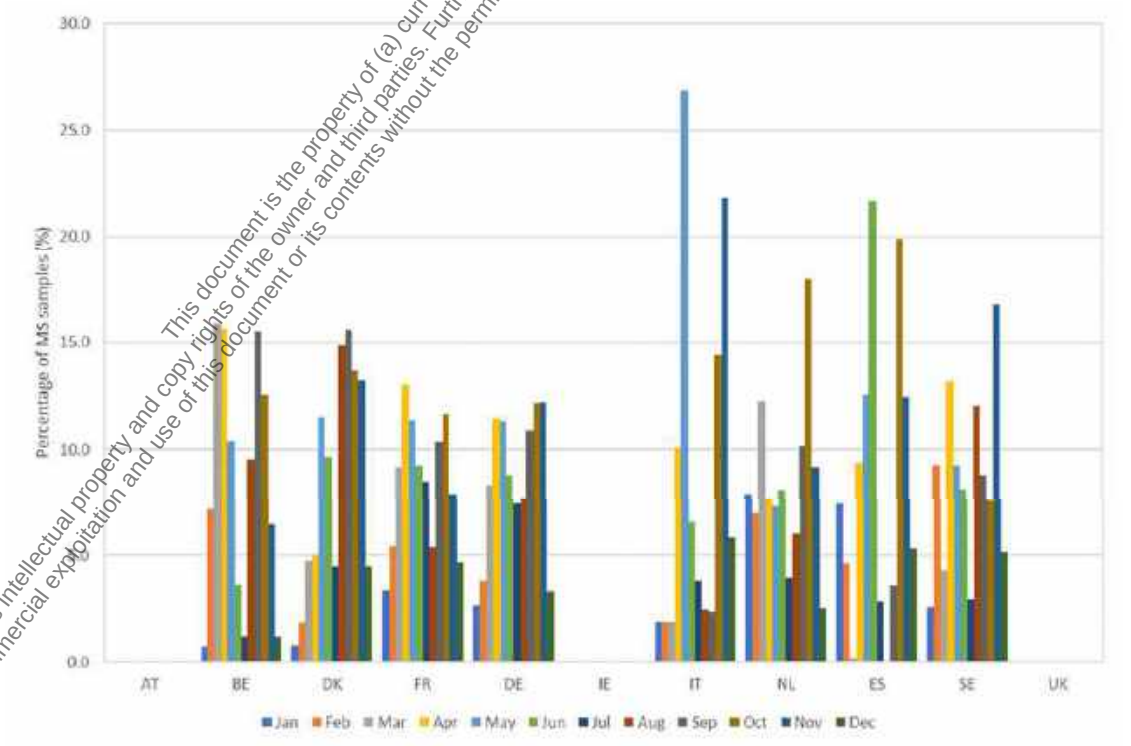


Figure 7.5-19: Map illustrating the distribution of glyphosate (GLY) groundwater sampling locations. Also illustrated are the number of exceedances of the GW regulatory concentration at each location.

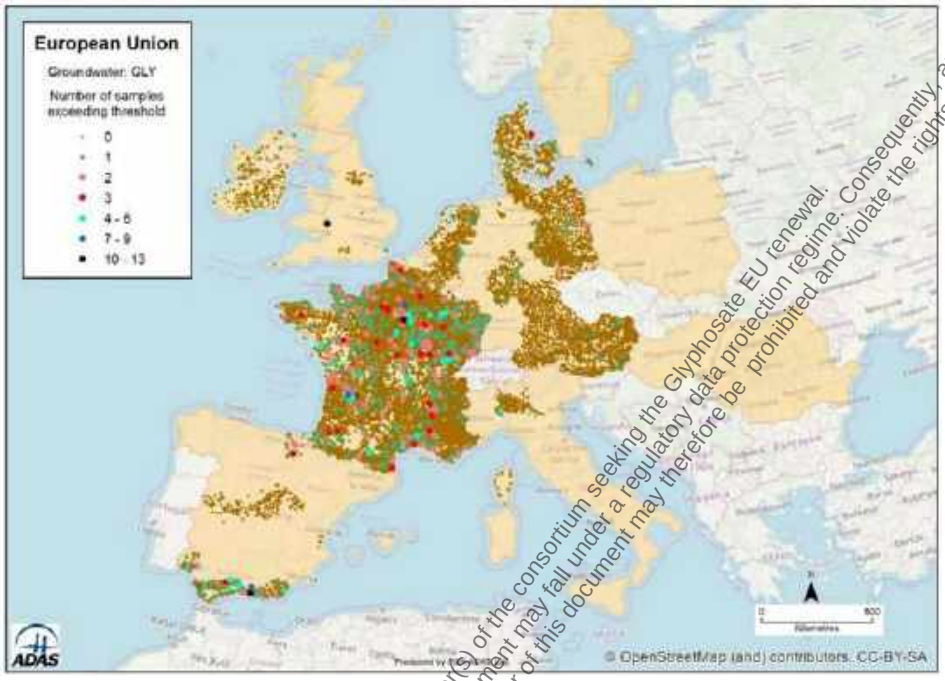
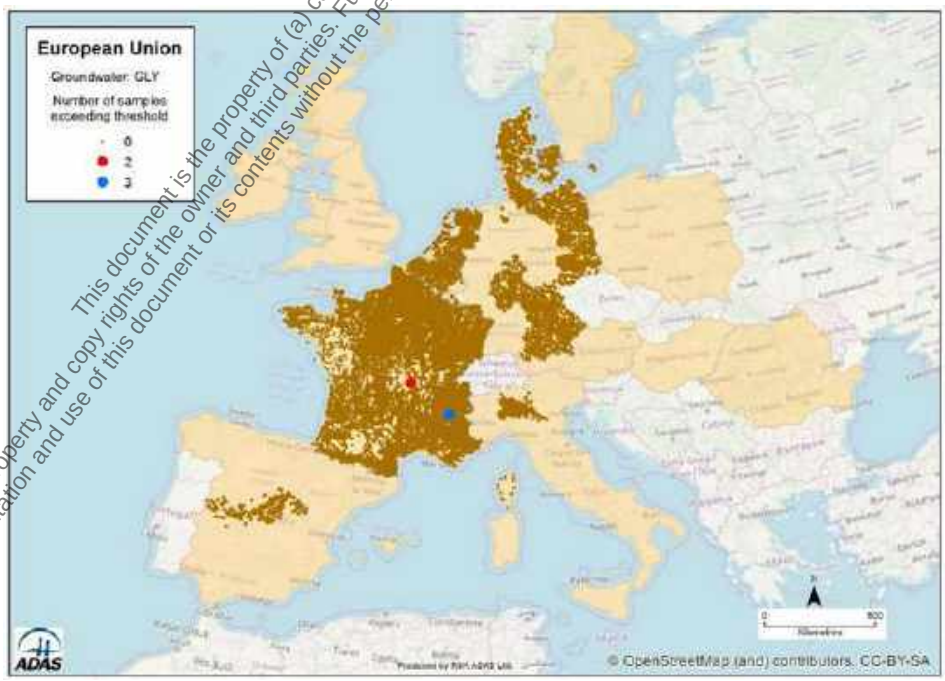


Figure 7.5-20: Map illustrating the distribution of AMPA groundwater sampling locations. Also illustrated are the number of exceedances of the GW regulatory concentration at each location.



Glyphosate
Across all MS the GLY public monitoring dataset compiled comprised >251 000 samples collected from >37 800 sampling sites (see Table 7.5-26). Detection of GLY in GW was ~2 %, ranging from as low as 0.2 % in AT to as high as 10.3 % in SE, relative to a varying LOQ with an average of 0.06 µg/L (min: 0.01 – max: 100 µg/L). These compare well with the previous data collection (Horth, 2012, CA 7.5/013 and 2016, CA 7.5/010) where GLY was detected in 1.3 % of samples (see Table 7.5-29).

Compliance with the 0.1 µg/L threshold was very high (99.4 % samples from 97 % of sites) with very few exceedances (~0.6 % of samples from ~3.0 % of sites) and compared well with aggregated report values (ranging from 0.0 % in DE to ~7 % in ES; see Table 7.5-27) and the 0.6 % of samples from the previous data collection. Consideration of whether these exceedances were consecutive, an indicator of more systematic groundwater quality issues rather than one off events, indicates that only 0.089 % of samples (n = 216) are consecutively above the threshold (see Table 7.5-30). The spatial distribution of the GLY exceedance locations (see Figure 7.5-19) does not indicate any specific patterns or bias.

Maximum measured concentrations up to 1005 µg/L are reported, however, these extreme values are likely anomalous. The 99th percentile concentration, the concentration that 99 % of samples is below, is 0.19 µg/L (see Table 7.5-28) while the 0.1 µg/L threshold represents the 98.976th percentile concentration. In line with the precautionary data processing approach adopted in this study possible outliers were not removed from the dataset prior to analysis. However, an additional analysis step was conducted to identify likely outliers in the dataset and the implications of these for the analysis assessed. This identified 10 outliers which if excluded, suggest the maximum concentration would be 39.2 µg/L (see Table 7.5-28) which is well below the SW RAC (for groundwater fed ecosystems) of 100 µg/L and the lifetime health-based ADI concentration of 1500 µg/L.

AMPA
Across all MS the AMPA public monitoring dataset compiled comprised >230 000 samples collected from >34 400 sampling sites (see Table 7.5-26). Detection of AMPA in GW was ~2.9 %, ranging from as low as 0.4 % in ES to as high as 19.5 % in BE, relative to a varying LOQ with an average of 0.05 µg/L (min: 0.01 – max: 5 µg/L). These compare well with the previous data collection where AMPA was detected in ~2.1 % of samples (see Table 7.5-29).

Compliance with the arbitrarily defined regulatory threshold of 10 µg/L for a non-relevant metabolite was very high (99.998 % of samples from 99.994 % of sites) given exceedances were rare (~0.002 % of samples from ~0.006 % sites, ranging from 0 % in BE to ~0.003 % in FR; see Table 7.5-27) and occurred on a single occasion (see Table 7.5-30). Compliance with the testing requirement 0.75 µg/L threshold for a non-relevant metabolite was very high (99.93 %) given the small number of exceedances (~0.07 %, ranging from 0.0 % in ES to ~0.8 % in FR).

The maximum concentration is 16 µg/L which is well below the SW RAC (for groundwater fed ecosystems) and the lifetime health-based ADI concentration of 3960 µg/L. The 99th percentile concentration, the concentration that 99 % of samples is below, is 0.14 µg/L (see Table 7.5-28) while the arbitrarily defined regulatory threshold of 10 µg/L represents the 99.998th percentile concentration. No outliers were identified in the dataset (see Table 7.5-28). It should be borne in mind that AMPA may originate from sources other than GLY, for example detergents, particularly in GW affected by SW or flooding. In order to compare these AMPA results with previously published and aggregated results, assessment against the arbitrarily defined regulatory threshold of 0.1 µg/L was also undertaken. Detection above the threshold of 0.1 µg/L was ~0.7 %, ranging from 0.1 % in ES to 2.3 % in NL. These compare well with the aggregated values extracted from reports (see Table 7.5-27) ranging from 0.0 % in SE to ~3 % in IT. Similarly, these are comparable with the previous data collection where ~0.75 % of samples were found to exceed 0.1 µg/L.

Annual and monthly investigations of sampling effort and compliance were also documented within the report. These have not been summarised as they do not alter the conclusions of the primary study, but instead provide additional detail.

Table 7.5-26: Member State and combined European dataset public monitoring summaries for glyphosate (GLY) and AMPA in groundwater

MS	Substance	Number of Sites	Number of Samples	Years	LOQ (µg/L)	Samples with LOQ<DrW		Detected >LOQ		Detected >0.1 µg/L		Detected >0.75 µg/L		Detected >10.0 µg/L		Measured Concentration (µg/L)
					Mean (min – max)	Sites	Samples	Samples	%	Samples	%	Samples	%	Number	%	Median (min – max)[1]
AT	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
AT	GLY	2172	9475	2004 - 2014	0 05 (0 04 - 0 06)	2172	9475	22	0 23	14	0 15	NA	NA	NA	NA	0 03 (0 015 - 2 6)
BE	AMPA	599	5540	2008 - 2018	0 01 (0 01 - 0 50)	599	5539	1078	19 46	98	1 77	10	0 18	0	0 000	0 01 (0 000 - 2 6)
BE	GLY	242	278	2008 - 2017	0 01 (0 01 - 0 01)	242	278	1	0 36	0	0 00	NA	NA	NA	NA	0 00 (0 000 - 0 1)
DE	AMPA	3604	11957	1996 - 2019	0 06 (0 01 - 5 00)	3598	11855	1102	9 30	113	0 95	13	0 11	0	0 000	0 03 (0 000 - 6 0)
DE	GLY	4198	14210	1995 - 2019	0 05 (0 01 - 5 00)	4190	14008	948	6 77	40	0 29	NA	NA	NA	NA	0 04 (0 000 - 7 0)
DK	AMPA	1806	14671	1997 - 2018	0 01 (0 01 - 0 50)	1806	14670	122	0 83	28	0 19	7	0 05	0	0 000	0 01 (0 010 - 9 1)
DK	GLY	1806	14681	1997 - 2018	0 01 (0 01 - 1 00)	1806	14671	145	0 99	23	0 17	NA	NA	NA	NA	0 01 (0 010 - 4 7)
ES	AMPA	241	995	2014 - 2017	0 05 (0 05 - 0 05)	241	995	4	0 40	1	0 10	0	0 00	0	0 000	0 05 (0 050 - 0 4)
ES	GLY	650	3869	2008 - 2017	0 05 (0 01 - 0 30)	650	3855	396	10 27	248	6 43	NA	NA	NA	NA	0 05 (0 010 - 9 6)
EU Trans	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
EU Trans	GLY	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
FR	AMPA	26048	190218	2000 - 2019	0 06 (0 06 - 2 00)	25982	183247	3943	2 15	1218	0 66	118	0 06	5	0 003	0 05 (0 000 - 16 0)
FR	GLY	26219	198622	1999 - 2019	0 06 (0 06 - 100)	26140	191114	3204	1 68	1067	0 56	NA	NA	NA	NA	0 05 (0 000 - 1005)
IE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IE	GLY	227	1584	2007 - 2014	0 01 (0 01 - 0 02)	227	1584	32	2 02	5	0 32	NA	NA	NA	NA	0 01 (0 005 - 0 5)
IT	AMPA	213	893	2015 - 2017	0 10 (0 05 - 0 10)	213	893	16	1 79	16	1 79	7	0 78	0	0 000	0 10 (0 050 - 5 3)
IT	GLY	213	893	2015 - 2017	0 10 (0 05 - 0 10)	213	893	7	0 78	7	0 78	NA	NA	NA	NA	0 10 (0 050 - 3 4)
NL	AMPA	660	1831	1996 - 2016	0 12 (0 01 - 1 00)	586	1309	95	7 26	30	2 29	1	0 05	0	0 000	0 05 (0 010 - 1 3)
NL	GLY	657	1882	1996 - 2016	0 12 (0 02 - 1 20)	592	1370	74	5 40	32	2 34	NA	NA	NA	NA	0 05 (0 015 - 14 3)
SE	AMPA	1328	4876	1996 - 2017	0 28 (0 05 - 1 00)	1324	4856	25	0 54	7	0 15	2	0 04	0	0 000	0 00 (0 000 - 7 9)
SE	GLY	1334	4898	1996 - 2017	0 27 (0 03 - 1 00)	1329	4827	43	0 89	7	0 15	NA	NA	NA	NA	0 00 (0 000 - 0 2)
UK	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
UK	GLY	124	866	2000 - 2018	0 21 (0 10 - 1 00)	124	727	51	7 02	51	7 02	NA	NA	NA	NA	0 10 (0 100 - 39 2)
EU All	AMPA	34499	230981	1996 - 2019	0 05 (0 01 - 5 00)	34339	223164	6385	2 86	1511	0 68	158	0 07	5	0 002	0 05 (0 000 - 16 0) 0 05 (0 000 – 16 0)[2]
EU All	GLY	37842	251258	1995 - 2019	0 06 (0 01 - 100)	37638	242792	4923	2 03	1496	0 62	NA	NA	NA	NA	0 05 (0 000 - 1005) 0 05 (0 000 – 39 2)[2]

[1] Values <LOQ and <LOD are treated as equal to LOQ and LOD as a precautionary estimate of the median
[2] Statistics with outliers excluded
ND = Non identified within the timeframe
NA – Not applicable

Table 7.5-27: Summary of monitoring data aggregated in reports for glyphosate (GLY) and AMPA in groundwater

MS	Substance	Number of reports identified	Reports with data relating to threshold						Maximum value (µg/L)
			Number of reports	Date range	Number of samples	Threshold (µg/L)	Samples above threshold	% samples above threshold	
AT	AMPA	4	4	2003 - 2015	18928	3	10	0.06	0.75
	GLY	4	4	2003 - 2016	30495	0.1	5[illegible]	0.17	NS
BE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
DE	AMPA	5	1	2007-2013	764	10	0	0	NS
	GLY	8	1	2007-2013	643	0.1	0	0	0.43
DK	AMPA	2	2	1990-2017	>34854	0.1	>53	0.15/0.34[1]	9.10
	GLY	2	2	1990-2017	>34901	0.1	>70	0.20/0.46[1]	5.00
ES	AMPA	1	1	2000-2000	55	0.1	1	1.8	0.8
	GLY	1	1	2000-2000	55	0.1	4	7.3	0.6
EU Trans	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
FR	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
IE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	1	1	2005-2006	> 5[illegible]	0.1	0	0	NS
IT	AMPA	8	8	2007 - 2016	>2473	0.1	>74	2.99/2.82[1]	NS
	GLY	8	8	2007 - 2016	>2412	0.1	95	3.94/5.20[1]	NS
NL	AMPA	3	2	2003-2016	1756	0.1	40	2.3	5.12
	GLY	3	2	2003-2016	1756	0.1	19	1.1	5.5
SE	AMPA	1	1	2011-2012	10	0.1	0	0	0.085
	GLY	1	1	2011-2012	10	0.1	0	0	NS
UK	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND

[1] Report data includes sample counts and % values – The first value is the average using count data only while the second is the average of report averages
ND – No data identified; NS – Not specified; > As missing values to calculate total

Table 7.5-28: Summary statistics for glyphosate (GLY) and AMPA groundwater concentration data considering the influence of outliers

Compound	Outlier Status	Concentration (µg/L)										Percentile of 0.1 µg/L	Percentile of 10.0 µg/L	Number of outliers
		Minimum	Mode	25th Percentile	Median	Mean	75th Percentile	90th Percentile	95th Percentile	99th Percentile	Maximum			
GLY	Included	0	0.05	0.03	0.05	0.070	0.1	0.1	0.1	0.19	1005	98.756	NA	NA
	Excluded	0	0.05	0.03	0.05	0.061	0.1	0.1	0.1	0.19	39.2	98.760	NA	10
AMPA	Included	0	0.05	0.02	0.05	0.058	0.1	0.1	0.1	0.14	16	NA	99.9978	NA
	Excluded	0	0.05	0.02	0.05	0.058	0.1	0.1	0.1	0.14	16	NA	99.9978	0

NA – Not applicable as not considered

Table 7.5-29: Summary of glyphosate (GLY) and AMPA data in groundwater in Europe (*after* [redacted] 2016, CA 7.5/010)

Country	Compound	Date	Sites	Samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max Conc	LoQ (LoD)
		Range	No.	No.	No.	%	No.	%	µg/L	µg/L
Austria	GLY	2004	~950	3633	7	0.19	2	0.06	>0.1	0.1
	AMPA	2004	~950	3636	44	1.2	11	0.3	0.75	0.1
Belgium (Flanders and Wallonia)	GLY F	2006-08	≥448	1488	4	0.03	1	0.01	≤0.5	0.01
	AMPA F	2007-14	≥504	4515	789	17.5	≥8	≥0.18	1.8	0.01
	GLY W	2000-06	450	≥450	0	-	0	-	<0.025	<0.025
	AMPA W	2000-06	450	≥450	13	3	0	-	<0.05	<0.025
Denmark	GLY	1990-13	4941	15552	142	0.9	28	0.2	4.7	(0.01-<0.1)
	AMPA	1990-13	4946	15541	106	0.7	23	0.15	9.1	(0.01-<0.1)
Finland	GLY	2002-08	81	81	0	-	0	-	-	0.1
	AMPA	2002-08	81	81	0	-	0	-	-	0.05
France	GLY	99-2012	≥7028	78431	859	1.1	563	0.7	28	0.01-0.2
	AMPA	2000-12	≥6904	70492	1122	1.6	643	0.9	19	0.01-0.2
Germany	GLY	96-2008	≥430	≥2599	35	1.3	9	0.34	<1.0	<0.1
	AMPA	96-2008	≥387	≥1986	64	3	34	1.7	≥1.0	<0.1
Ireland	GLY	2007-09	92	679	6	0.8	1	0.1	0.19	<0.1
Italy (Lombardia Region)	GLY	2005-12	≥359	1497	8	0.6	5	0.2	1.2	0.1
	AMPA	2007-12	≥359	1156	14	1.2	11	0.9	1.3	0.1
Malta	GLY	2009	18	≥18	0	-	0	-	-	0.01
Norway	GLY	99-00	7	8	0	-	0	-	-	0.01
	AMPA	99-00	7	8	1	12.5	0	-	0.02	0.01
Spain	GLY	2009-12	≥461	963	325	34	86	8.9	25	0.03-0.3
Sweden	GLY	2000-14	24	5989	26	0.43	10	0.17	0.23	<0.03
	AMPA	2000-14	24	5930	31	0.52	≤26	0.43	7.9	<0.05
Switzerland	GLY	2005-06	117	≥234	≥4	1.7	≥3	1.3	0.21	0.05
	AMPA	2005-06	117	≥232	17	7.3	11	4.7	0.46	0.05
Netherlands	GLY	2003-06	<691	691	4	0.58	4	0.58	4.7	(<0.1)
	AMPA	2003-06	<691	691	21	3	21	3	5.1	(<0.1)
UK	GLY	95-2015	≥264	1680	16	0.95	≤6	≤0.35	1.38	(0.01-0.1)
Total	**GLY**	**90-2015**	**≥16160**	**≥113993**	**1437**	**1.3**	**724**	**0.6**	**<0.05-28**	**0.01-0.2**
	AMPA	**90-2013**	**15417**	**≥104718**	**2222**	**2.1**	**788**	**0.75**	**0.02-19**	**0.01-0.2**

Table 7.5-30: Summary of sites and samples exceeding investigated thresholds for glyphosate (GLY) and AMPA in groundwater

Compound	GLY		AMPA			
Threshold	DrW: 0.1 µg/L	LTHAC: 1500 µg/L	Threshold: 0.1 µg/L	Threshold: 0.75 µg/L	DrW: 10.0 µg/L	LTHAC: 3980 µg/L
Number of sites	37638	37842	34349	34451	34457	34499
Number of samples	242792	251258	223164	224293	224545	230981
Number of samples > threshold	1496	0	1511	158	5	0
% of samples > threshold	0.6	0.0	0.7	0.1	0.002	0.0
Number of sites > threshold	1128	0	994	112	2	0
% of sites > threshold	3.0	0.0	2.9	0.3	0.006	0.0
Number of consecutive samples > threshold	216	0	359	39	3	0
% of samples that are consecutive samples > threshold	0.089	0.0	0.16	0.017	0.002	0.0
Maximum number of samples > threshold at a single site	13	0	37	13	3	0
Maximum number of consecutive samples > threshold at a single site	8	0	15	13	3	0

LTHAC - Lifetime health-based ADI concentration

Case Studies

Case studies were initiated for Spain and the UK to assess why the compliance rates with the 0.1 µg/L threshold are lower in these MS (Spain 93.6 % and the UK 93.0 %). Several monitoring sites were included in the case studies and were elucidated by a desk-based approach. The aim was to determine whether the findings of glyphosate residues in groundwater relates to urban or agricultural uses and whether the reasons for exceedance are from compliant glyphosate use or could be related to point sources or other contaminations. (The detailed results of the case studies are provided in Appendix 2 of the report).

For Spain it was found that the geographical distribution of monitoring sites exceeding 0.1 µg/L is mainly concentrated in the south of Spain; 128 out of 137 sites showing glyphosate exceedances are situated in the region of Andalucia. Eleven sites which showed the most consecutive exceedances were investigated in more detail. The results showed that all sites are located in rural areas dominated by agricultural use. Most of the monitoring points comprise large diameter wells which are not constructed to a standard of a water quality monitoring well. Almost all wells were used for water abstraction (domestic or irrigation). It was concluded that all 11 monitoring sites that were examined in this case study show deficiencies, mostly due to inappropriate well construction or a location of the well which makes them susceptible to overspray. The sites are therefore not suited for water quality monitoring and for a subsequent assessment of the leaching potential of glyphosate.

In addition, it was noted that in the vicinity of many sites there are orchards (e.g. citrus or olives) where glyphosate may have been used compliantly and repeatedly in high application doses. Glyphosate is reported to strongly sorb which makes the substance only slightly mobile reducing the leaching risk. However, the soils in southern Spain typically have a low organic content (OC) and it may be possible that the combination of depleted soil OC together with repeated high dosage applications in orchards may cause leaching of glyphosate to shallow groundwater. Further work is ongoing in these localised areas to understand the situation better with a view to adapting local practice through targeted stewardship programs.

For the UK the vast majority of glyphosate exceedances >0.1 µg/L, and all multiple exceedances, relate to a small area near Hereford in west-central England. All detections in this area relate to the time period 2008-2009, as monitoring for glyphosate discontinued in 2010. Most detections, and the highest glyphosate concentrations, relate to the vicinity of a large plant nursery, with some further detections, lower in concentration and less frequent, in the urban area of Hereford. The local focus on glyphosate analyses together with a dense monitoring network and frequency may indicate that a problem with glyphosate occurred in the area and that the contamination spread and temporal course was examined. A possible scenario may have been an accident with glyphosate containing products.

Despite the fact that the glyphosate exceedances in the Hereford area could not be elucidated conclusively, it is evident that the cluster of detections is very local. These contradict the monitoring data from elsewhere in the UK, or indeed in EU member states that were considered as part of the public monitoring data assessment. As such, the glyphosate detections in the Hereford area should be considered as atypical and non-representative.

The UK glyphosate detection rate of 7.0 % is strongly driven by the large number of detections in the Hereford area. Excluding the atypical data from this area, the exceedance rate for the UK is 1.1 % and therefore much more comparable with that observed for the EU member states that were considered as part of the public monitoring data assessment. Even the 1.1 % exceedance may be biased as it relies on a small sample number and is based on only 4 single detections across all of the UK. Further work is ongoing in these localised areas to understand the situation better with a view to adapting local practice through targeted stewardship programs.

III. CONCLUSIONS

The analysis of the large groundwater dataset for GLY and AMPA indicates they are both occasionally detected above the LOQ in this compartment. However, compliance is very high with the frequency of quantification above the regulatory acceptable concentrations very low and non-systematic as would be expected given that the compounds are only slightly mobile in soil. It should be borne in mind that AMPA may originate from sources other than GLY, for example detergents, particularly in GW affected by SW or flooding. The environmental concentrations typically encountered in this environmental compartment do not pose a risk for ecosystem or human health *via* drinking water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the analysis of public monitoring data for key European countries for the compartments soil, water and sediment for Glyphosate and AMPA. The maximum GLY concentration in GW of 1005 µg/L is likely anomalous and once outliers are identified and excluded would be 39.2 µg/L which is well below the SW RAC (for groundwater fed ecosystems) of 400 µg/L and the lifetime health-based ADI concentration of 1500 µg/L. The maximum AMPA concentration in GW is 16 µg/L which is well below the SW RAC of 1200 µg/L (for groundwater fed ecosystems) and the lifetime health-based ADI concentration of 3960 µg/L. The available data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in the groundwater compartment.
The study is therefore considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/008
Report author	[redacted]
Report year	2019a
Report title	Phase 1: Traitements et analyses statistiques sur les données SOES UIPP 2008 - 2014 Analyses des données de suivi de glyphosate et de l'AMPA dans les eaux de France Période 2008-2014 (Original in French: Phase 1: Processing and statistical analysis of the 2008-2014 SOES UIPP data. Analysis of the 2008-2014 water monitoring data for glyphosate and AMPA in France)
Document No	REA-DOC-026
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No (but conducted by testing facilities accredited by the Member State)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

This report is an update of a previous report "Analysis of monitoring data for glyphosate and AMPA in French waters in the period 1997-2013" ([redacted] 2016, CA 7.5/009). It includes the 2014 monitoring data for glyphosate and AMPA in ground and surface waters (extracted from the SOES UIPP database in July 2017). The dataset extracted from the SOES UIPP database is analysed in several ways.

Number of measurements and monitoring stations

At the combined national and French overseas level, the entire dataset for surface waters consists of 148561 analyses, of which 74271 are for AMPA and 74290 are for glyphosate. The number of unique stations is 3006 for the whole dataset. The present study only considers data from mainland France. Therefore, the surface water database selected for the study comprises 148295 analyses (74138 for AMPA and 74157 for glyphosate) from 2980 stations (Table 7.5-31).

For groundwater, the database consists of 129364 analyses, of which 64249 are for AMPA and 65115 are for glyphosate. The number of distinct water quality monitoring stations is 14 831 for the whole database (France mainland only).

Both glyphosate and AMPA were monitored every year between 2008 and 2014 in surface waters and groundwater. The majority of stations extracted from the $SOES_{uipp}$ database have both AMPA and glyphosate monitoring data.

I. MATERIAL AND METHODS

Groundwater

In the $SOES_{uipp}$ database, the number of analyses for groundwater increased between 2008 and 2011 to a maximum of 13396 analyses. From 2012, this number decreased to roughly the same value as that of 2008 (Figure 7.5-21).

The number of groundwater monitoring stations (Figure 7.5-22) increased between 2008 and 2009, then remained constant until 2011 (approximately 7000 stations monitoring AMPA and glyphosate). After this, the numbers reduced to about 2000 stations monitoring AMPA and glyphosate.

Table 7.5-31: Number of analyses for glyphosate and AMPA performed during 2008-2014 period

Year	Total $SOES_{uipp}$ data points	AMPA	Glyphosate	Year	Total $SOES_{uipp}$ data points	AMPA	Glyphosate
Groundwater				**Surface water**			
2008	1 421 369	7048	7246	2008	2 074 007	4862	4862
2009	2 446 506	11662	11783	2009	4 000 041	7559	7559
2010	2 833 373	12514	12663	2010	4 428 556	10001	10001
2011	3 136 242	13258	13396	2011	5 100 625	12456	12457
2012	1 887 369	6106	6373	2012	5 123 717	11395	11417
2013	2 122 877	6811	6808	2013	7 039 438	13067	13066
2014	2 431 470	6850	6846	2014	6 944 879	14798	14795
TOTAL	**16 279 206**	**64249**	**65115**	**TOTAL**	**34 710 663**	**74138**	**74157**

Figure 7.5-21: Evolution of the annual number of groundwater analyses carried out for AMPA and glyphosate

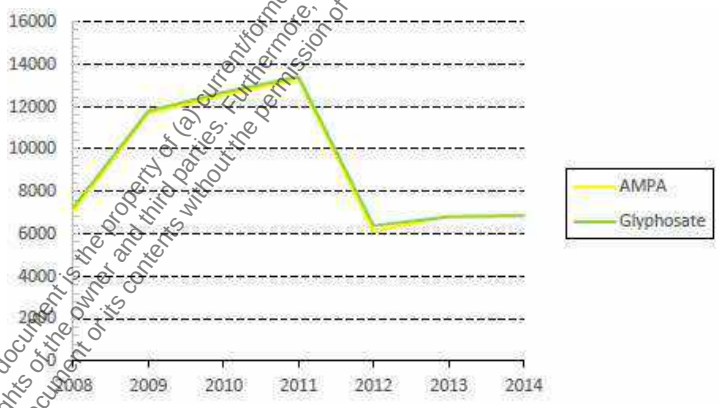
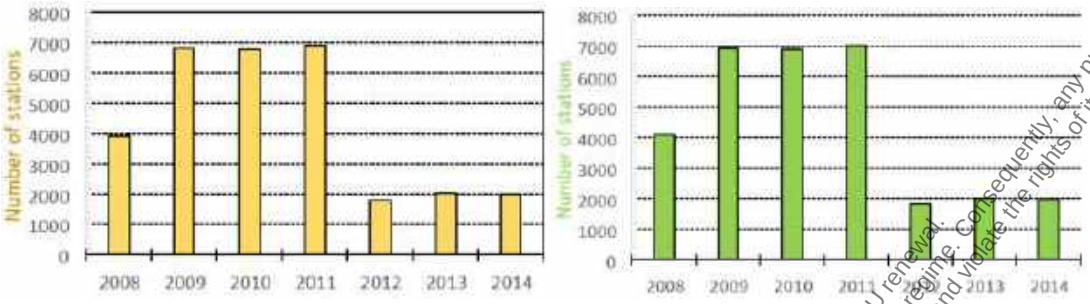


Figure 7.5-22: Number of stations involved in the groundwater monitoring of (a) AMPA (yellow) and (b) glyphosate (green)



(a) (b)

Surface water

For surface water, the number of analyses has constantly increased between 2008 and 2014 (except in 2012). The number of analyses increased threefold across seven years with 14700 analyses for each substance in 2014 (Figure 7.5-23).

For surface water monitoring (Figure 7.5-24), there was a gradual increase in stations monitoring for glyphosate and AMPA between 2008 and 2014. There were 909 stations in 2008 and 2154 in 2014 monitoring both AMPA and glyphosate.

Figure 7.5-23: Evolution of the annual number of surface water analyses carried out for AMPA and glyphosate.

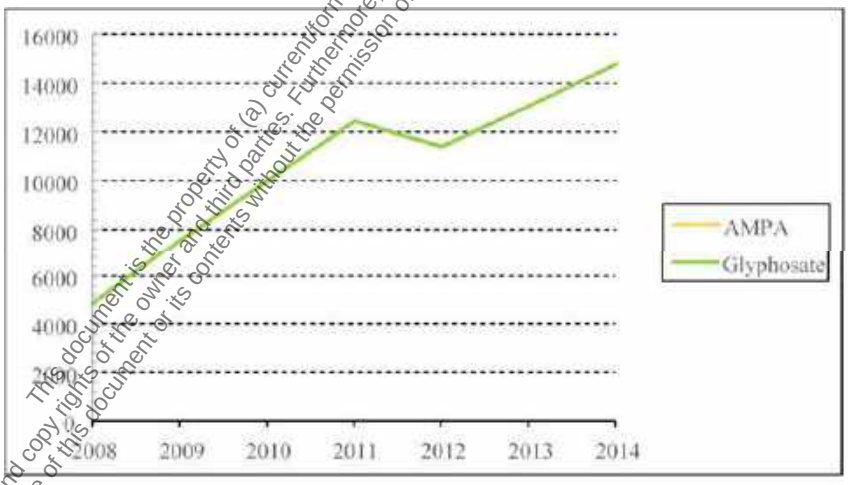
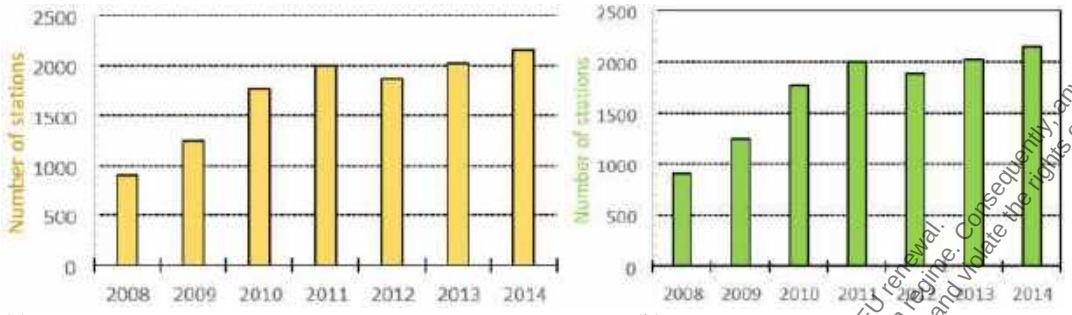


Figure 7.5-24: Number of stations involved in the surface water monitoring of (a) AMPA and (b) glyphosate



(a) (b)

II. RESULTS AND DISCUSSION

Multi-year continuity analysis

Based on the number of years of monitoring, this section looks at the continuous measurements within the time period and therefore on the ability to draw conclusions in terms of how the multi-annual trends evolved. Taking into account the inter- and intra-annual climatic variability as well as crop rotations, it is necessary to have several years of monitoring data to assess such trends and this does not necessarily require data to be based on consecutive years.

Groundwater

For groundwater (Table 7.5-32), only 8 % of stations in the database have monitoring data for AMPA and glyphosate over the seven years studied. More than half of the stations only measured for one year (52 % and 54 % for AMPA and glyphosate, respectively). This proportion dropped to 23 % and 24 % of stations over two years, then dropped further to 5 % and 6 % over 3 years.

Table 7.5-32: Number of follow-up years of groundwater monitoring from stations between 2008-2014

	AMPA		Glyphosate	
No of follow-up years	**No of stations**	**% of stations**	**No of stations**	**% of stations**
One year	7783	52 %	7948	54 %
2 years	3411	23 %	3520	24 %
3 years	812	5 %	823	6 %
4 years	567	4 %	582	4 %
5 years	200	1 %	199	1 %
6 years	351	2 %	331	2 %
7 years	1127	8 %	1154	8 %
Total no of stations	14251	-	14557	-

Surface water

For surface waters (Table 7.5-33), the number of stations for which monitoring is carried out over the seven years is greater than for groundwater with 22 % of stations carrying out measurements. The percentage of stations monitoring for one year is 25 %. The results are similar for AMPA and glyphosate. However, the surface water stations performed more systematic measurements in comparison to groundwater monitoring stations.

Table 7.5-33: Number of years of surface water monitoring for stations between 2008-2014

	AMPA		Glyphosate	
No of years	**No of stations**	**% of stations**	**No of stations**	**% of stations**
One year	731	25 %	751	25 %
2 years	340	11 %	339	11 %
3 years	209	7 %	210	7 %
4 years	170	6 %	170	6 %
5 years	467	16 %	467	16 %
6 years	389	13 %	389	13 %
7 years	655	22 %	655	22 %
Total no of stations	2961	-	2981	-

Analysis of the annual number of measurements
The examination of the continuity of research across multiple years includes an assessment of the annual number of monitoring data. The data are presented as seven ranges to reflect the number of measurements made per station per year: 1 p.a; 2-3 p.a; 4-5 p.a; 6-9 p.a; 10-14 p.a; 15-49 p.a; >50 p.a.

Groundwater
For groundwater monitoring, the greatest majority of stations only had one measurement per year between 2008 and 2011. The number of measurements per station per year increased thereafter. In 2012, 42 % of stations had 4-5 measurements per year, and in 2013 and 2014, 41-45 % of stations had 2-3 measurements per year. Only 1 % of stations recorded more than 10 measurements per year, and this could correspond to a monthly monitoring schedule. No station performed as many as 50 measurements a year. The number of stations sampled per year decreased by about half between 2008 and 2014. Although the number of stations carrying out measurements seems to decrease, the frequencies of measurements increased.

Surface water
For surface water monitoring stations, the number of measurements per station and per year is generally greater than seen for groundwater. Except in 2008 where the number of analyses per station was mostly 4-5 per year, the number of monitoring events per station was mostly 6-9 per year (for 50 %-61 % of stations across all years). Between 5 % and 15 % of stations carried out more than 10 monitoring events per year, and this could correspond to a monthly measurement schedule. No station performed as many as 50 measurements a year. In contrast to groundwater, the number of stations with sampling doubled between 2008 and 2014.

Assessment of the multi-year trend in measurements greater than LOQ (code 1)
In this section, the results of the quantifiable analytical results of glyphosate and AMPA are studied i.e. those results where the concentration of the target molecule is reported as being greater than the limit of quantification (LOQ). The measured concentration values are compared against the regulatory values provided for the provision of drinking water: greater or equal to 0.1 µg/L for drinking water and greater or equal to 2 µg/L for water destined for drinking water.

AMPA
The percent of annual measurements for AMPA > LOQ in groundwater is low (< 5 % in all years studied, Table 7.5-34). The lowest such values for AMPA were in 2008-2010 with 0.7-0.9 %. From 2013 to 2014, this value for AMPA is *ca.* 2.5 % of analyses. In mainland France, fewer than 100 analyses for AMPA exceeded 0.1 µg/L in any one year, except in 2011 where there were 133 exceedances. The concentrations greater than 0.1 µg/L were always <1.1 % in groundwater over the seven years of the study. Fewer than five analyses per year exceeded the 2 µg/L limit.

The annual percentage of AMPA measurements >LOQ in surface water were much higher than in groundwater. These vary between 46 % and 62.5 % with a median quantification rate of 54 % (AMPA is detected in more than half of the measurements). Concentrations of AMPA are often > 0.1 µg/L with 33.2 % - 53.6 % of measurements exceeding this threshold. Concentrations for AMPA >2 µg/L comprise <3 % (between 106 and 268 values p.a.) of samples.

Table 7.5-34: Annual measurements above LOQ for AMPA

	2008	2009	2010	2011	2012	2013	2014
Groundwater							
Number of analyses	7048	11662	12514	13258	6106	6811	6850
Number of analyses with conc. > LOQ	63	101	101	201	254	164	169
% >LOQ (code remarque = 1)	**0.9 %**	**0.9 %**	**0.8 %**	**1.5 %**	**4.2 %**	**2.4 %**	**2.5 %**
Number of analyses > LOQ and >= 0.1 µg/L	58	78	85	133	66	47	49
% of analyses > LOQ and >=0.1 µg/L	**0.8 %**	**0.7 %**	**0.7 %**	**1.0 %**	**1.1 %**	**0.7 %**	**0.7 %**
Number of analyses > LOQ and >= 2 µg/L	3	2	2	2	2	2	4
% of analyses > LOQ and >=2 µg/L	**<0.1 %**	**<0.1 %**	**<0.1 %**	**<0.1 %**	**<0.1 %**	**<0.1 %**	**0.1 %**
Surface water							
Number of analyses	4862	7559	10001	12456	11395	13067	14798
Number of analyses with conc.> LOQ	2557	3881	4597	7789	6148	7307	7983
% >LOQ (code remarque = 1)	**52.6 %**	**51.4 %**	**46.0 %**	**62.5 %**	**54.0 %**	**55.9 %**	**53.9 %**
Number of analyses > LOQ and >= 0.1 µg/L	2130	3389	4068	6681	4054	4348	4913
% of analyses > LOQ and >=0.1 µg/l	**43.8 %**	**44.8 %**	**40.7 %**	**53.6 %**	**35.6 %**	**33.3 %**	**33.2 %**
Number of analyses > LOQ and >= 2 µg/L	106	190	172	268	136	162	191
% of analyses > LOQ and >=2 µg/L	**2.18 %**	**2.51 %**	**1.72 %**	**2.15 %**	**1.54 %**	**1.24 %**	**1.29 %**

Glyphosate

The annual percentage of measurements > LOQ for glyphosate in groundwater is low (<2 % in all years studied, Table 7.5-35). The lowest percentages >LOQ in groundwater were 2008-2011 (0.4-1.0 % of measurements). From 2012 to 2014, this value is 1.5-1.7 %. Fewer than 100 measurements exceeded 0.1 µg/L. Fewer than eight analyses per year exceeded the 2 µg/L limit.

The annual percentage of measurements > LOQ in surface waters are much higher than in groundwater. These vary between 25.3 % and 39.1 % with a median quantification rate of 32.3 % (indicating that glyphosate is detected in one third of analyses). As well as being identified as higher than LOQ, the analyses were often > 0.1 µg/L (14-21.7 % of measurements compared to the whole dataset). In addition, <1 % (between 25 and 52 values p.a.) of analyses were >2 µg/L.

Table 7.5-35: Annual measurements above LOQ for glyphosate

	2008	2009	2010	2011	2012	2013	2014
Groundwater							
Number of analyses	7246	11783	12663	13396	6373	6808	6846
Number of analyses with conc. > LOQ	NA	53	105	132	109	105	118
% >LOQ (code remarque = 1)		**0.4 %**	**0.8 %**	**1.0 %**	**1.7 %**	**1.5 %**	**1.7 %**
Number of analyses > LOQ and >= 0,1 µg/L	NA	48	92	94	27	31	37
% > LOQ and >=0,1 µg/l		**0.4 %**	**0.7 %**	**0.7 %**	**0.4 %**	**0.5 %**	**0.5 %**
Number of analyses > LOQ and >= 2 µg/L	NA	2	7	3	3	3	1
% of analyses > LOQ and >=2 µg/L		**<0.1 %**	**<0.1 %**	**<0.1 %**	**<0.1 %**	**<0.1 %**	**<0.1 %**
Surface water							
Number of analyses	6862	7559	10001	12457	11417	13066	14795
Number of analyses with conc. > LOQ	1550	1936	2535	4026	4189	5048	5791
% >LOQ (code remarque = 1)	**31.9 %**	**25.6 %**	**25.3 %**	**32.3 %**	**36.7 %**	**38.6 %**	**39.1 %**
Number of analyses > LOQ and >= 0,1 µg/L	1051	1404	1751	2697	1937	2004	2072
% of analyses > LOQ and >=0,1 µg/L	**21.6 %**	**18.6 %**	**17.6 %**	**21.7 %**	**17.0 %**	**15.3 %**	**14.0 %**
Number of analyses > LOQ and >= 2 µg/L	25	43	36	32	40	41	47
% of analyses > LOQ and >=2 µg/L	**0.5 %**	**0.6 %**	**0.4 %**	**0.4 %**	**0.4 %**	**0.3 %**	**0.3 %**

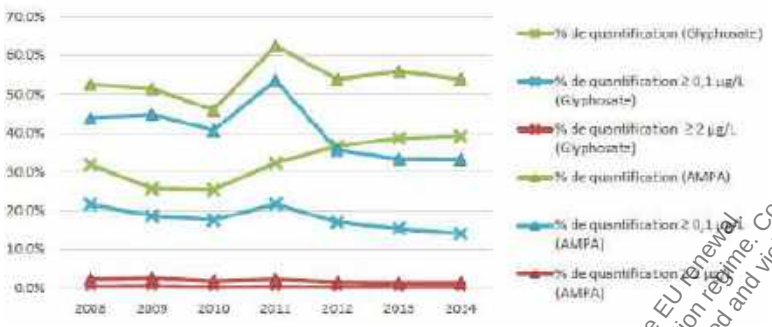
Comparison of concentration levels (greater than LOQ) of AMPA and glyphosate

Surface water

For surface waters, the annual percentage of measurements with concentrations > 0.1 µg/L for AMPA varied between 33 % and 54 %, whilst for glyphosate the variability was less, between 15 % and 22 % of analyses (Figure 7.5-25).

There appears to be a correlation between the annual percentages for AMPA and glyphosate as they both follow similar trends between years. They generally decrease over the period 2008-2014. Values for annual % of measurements for concentrations >2 µg/L were always low and below 0.6 % for glyphosate and 2.5 % for AMPA.

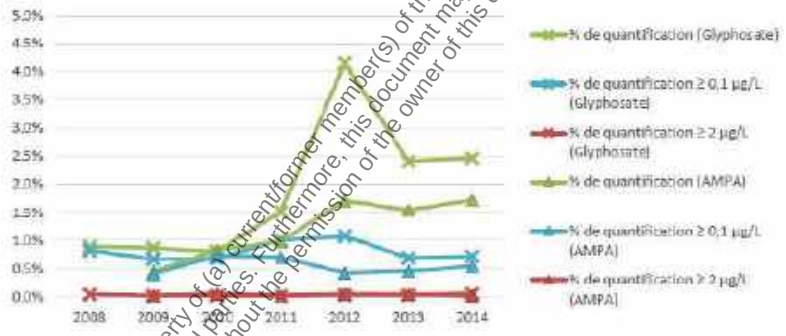
Figure 7.5-25: Examining the trend in yearly % of measurements in surface water with respect to regulated concentrations



Groundwater

For groundwater, the annual percentage of measurements with concentrations > 0.1 µg/L were relatively similar for AMPA and glyphosate and were between 0.4 % and 1.1 % (Figure 7.5-26). Analyses > 2 µg/L were always ≤0.1 % for AMPA and glyphosate.

Figure 7.5-26: Examining the trend in yearly % of measurements in surface water with respect to regulated concentrations.



Maximum concentrations, 90th, 95th and 99th percentiles

The measured concentrations of AMPA and glyphosate in groundwater and surface waters each year for the period 2008-2014 are described according to their maximum value and their 90th, 95th and 99th percentiles (Table 7.5-36 to Table 7.5-39).

The glyphosate and AMPA maximum concentrations vary between the years, covering a range of ~1 to 3369 µg/L. There is no logical explanation for these maximum concentration values. Hypotheses put forward are:

- The maximum value can be due to pollution events upstream from the monitoring station with minimal dilution.
- This maximum value could simply be erroneous (transcription error, unit error, etc.)

The 99th percentile concentrations range between 1.6 and 26.3 µg/L.

The 95th percentile concentrations range between 0.3 and 2.9 µg/L. While the 90th percentile concentrations range between 0.2 and 1.2 µg/L.

The data shows opposite trends for surface water and groundwater as follows:

- For surface water, glyphosate maximum concentrations during 2008-2014 are less than those measured for AMPA in all percentile assessments.
- On the contrary, groundwater maximum glyphosate concentrations during 2008-2014 are higher than those for AMPA in all percentile assessments.

The surface water concentrations for both AMPA and glyphosate tend to decrease in all percentiles since 2009.

Table 7.5-36: Annual summary of maximum concentrations (µg/L)

	Period	2008	2009	2010	2011	2012	2013	2014
Ground water								
AMPA	9.3	2.36	5.7	9.3	7.78	6.3	5.05	4.07
Glyphosate	1005	0.96	3.91	22	11	1005	140	23.3
Surface water								
AMPA	3369	20.3	33.5	106	3369	80	59.1	61.4
Glyphosate	2237	17.3	19.7	21	2237	66	37.9	558

Table 7.5-37: Annual summary of 90th percentile concentrations (µg/L)

	Period	2008	2009	2010	2011	2012	2013	2014
Ground water								
AMPA	0.32	0.70	0.48	0.78	0.26	0.21	0.18	0.21
Glyphosate	0.47	0.50	0.62	0.86	0.44	0.21	0.25	0.42
Surface water								
AMPA	0.91	1.11	1.20	1.02	1.04	0.88	0.68	0.73
Glyphosate	0.45	0.54	0.70	0.55	0.53	0.42	0.32	0.34

Table 7.5-38: Annual summary of 95th percentile concentrations (µg/L)

	Period	2008	2009	2010	2011	2012	2013	2014
Ground water								
AMPA	0.68	1.09	0.79	1.10	0.43	0.31	0.31	0.52
Glyphosate	0.91	0.76	1.40	2.86	0.91	0.82	0.46	0.75
Surface water								
AMPA	1.50	1.74	1.97	1.68	1.65	1.40	1.20	1.21
Glyphosate	0.74	0.84	1.19	0.89	0.81	0.71	0.55	0.56

Table 7.5-39: Annual summary of 99th percentile concentrations (µg/L)

	Period	2008	2009	2010	2011	2012	2013	2014
Ground water								
AMPA	2.52	2.26	3.80	2.00	1.77	1.55	2.24	3.35
Glyphosate	10.67	0.92	3.02	9.74	7.92	26.25	11.41	1.77
Surface water								
AMPA	4.17	4.70	4.80	4.57	5.16	4.04	3.85	2.90
Glyphosate	2.11	2.85	3.49	2.34	2.22	1.90	1.70	1.80

Assessment of quantification (concentrations greater than LOQ) with respect to monitoring stations
These results mirror those from the preceding section in that both target molecules are not frequently measured and quantified during groundwater monitoring (Figure 7.5-27). For surface water, AMPA is measured and quantified in ~80 % of monitoring stations quoted compared to ~70 % for glyphosate (Figure 7.5-28).

Figure 7.5-27: Groundwater Red- % of stations with glyphosate concentrations measured/quantified; Blue- % of stations with AMPA concentrations measured/quantified; Yellow- % of stations with glyphosate concentrations measured/quantified less than/equal to 0.1 µg/L; Green- % of stations with AMPA concentrations measured/quantified less than/equal to 0.1 µg/L

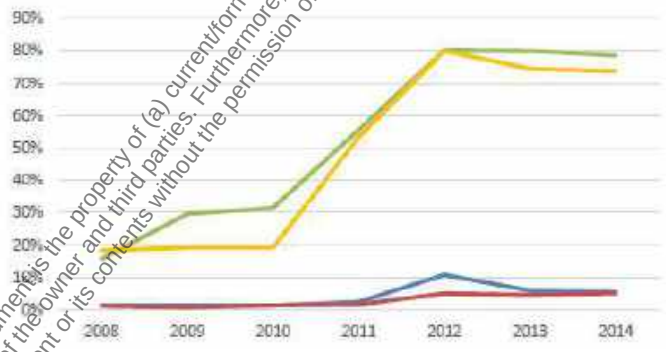
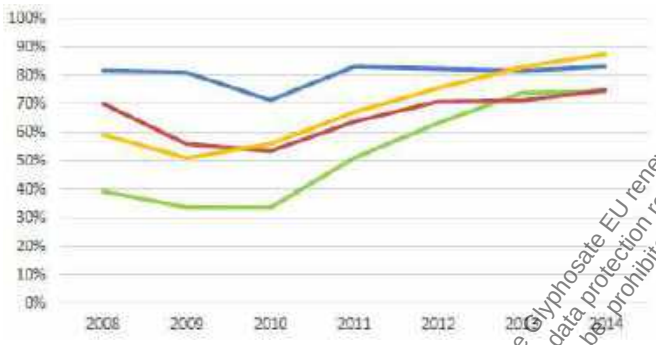


Figure 7.5-28: **Surface water Red- % of stations with glyphosate concentrations measured/quantified; Blue- % of stations with AMPA concentrations measured/quantified; Yellow: % of stations with glyphosate concentrations measured/quantified less than/equal to 0.1 µg/L; Green- % of stations with AMPA concentrations measured/quantified less than/equal to 0.1 µg/L**



Seasonal assessment of quantifications (concentrations greater than LOQ)
Glyphosate is mainly applied between March and June. Analytical measurements of glyphosate occurred mainly in the Spring. For AMPA, higher concentrations were mostly seen in the summer and "rest of the year". The lowest concentrations of both glyphosate and AMPA were in winter.

For groundwater, on average half of concentrations above LOQ for AMPA and glyphosate are between July and October (Figure 7.5-29 and Figure 7.5-30).

Figure 7.5-29: **Groundwater distribution of glyphosate from the dataset**

	2008	2009	2010	2011	2012	2013	2014	2008-
Jan to Mar	33%	0%	22%	0%	0%	0%	10%	9%
Apr to Jun	11%	13%	44%	14%	20%	0%	20%	16%
Jul to Sep	22%	60%	22%	57%	80%	71%	40%	48%
Oct to Dec	33%	27%	11%	29%	20%	29%	30%	27%
No of quantifications	9	15	9	7	5	7	10	64

Figure 7.5-30: **Groundwater distribution of AMPA from the dataset**

	2008	2009	2010	2011	2012	2013	2014	2008-14
Jan to Mar	100%	0%	0%	0%		0%	33%	16%
Apr to Jun	0%	0%	0%	0%		40%	0%	11%
Jul to Sep	0%	100%	67%	100%		40%	0%	53%
Oct to Dec	0%	0%	33%	0%		20%	67%	21%
No of quantifications	2	4	3	2	0	5	3	19

For surface water, the measurements are taken during spring for glyphosate and summer for AMPA (which aligns well with the main usage of the active substance). In fact, on average a third of measurements occur between April and June as well as July and September (Figure 7.5-31 and Figure 7.5-32).

Figure 7.5-31: Surface water distribution of glyphosate from the dataset

	2008	2009	2010	2011	2012	2013	2014	2008-14
Jan to Mar	24%	13%	17%	17%	26%	10%	18%	18%
Apr to Jun	24%	28%	37%	31%	38%	29%	39%	32%
Jul to Sep	42%	33%	28%	32%	29%	42%	25%	33%
Oct to Dec	11%	25%	19%	20%	7%	19%	18%	17%
No of quantifications	395	402	440	608	422	426	320	3 013

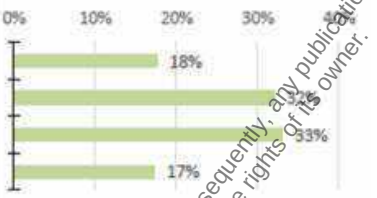
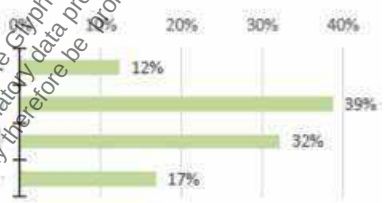


Figure 7.5-32: Surface water distribution of AMPA from the dataset

	2008	2009	2010	2011	2012	2013	2014	2008-2014
Jan to Mar	16%	7%	7%	18%	18%	11%	13%	12%
Apr to Jun	45%	35%	42%	13%	38%	40%	41%	39%
Jul to Sep	31%	32%	31%	30%	32%	33%	28%	32%
Oct to Dec	8%	25%	20%	37%	12%	16%	19%	17%
No of quantifications	203	170	178	199	221	241	148	1360



III. CONCLUSIONS

The present study only considers data from mainland France. The surface water database selected for the study comprises 148295 analyses (74138 for AMPA and 74157 for glyphosate) from 2980 stations.

For groundwater, the database consists of 129364 analyses, of which 64249 are for AMPA and 65115 are for glyphosate. The number of distinct water quality monitoring stations is 14831 for the whole database (France mainland only).

Both glyphosate and AMPA were monitored every year between 2008 and 2014 in surface waters and groundwater. The majority of stations extracted from the $SOES_{uipp}$ database have both AMPA and glyphosate monitoring data. The analysis focusses on those concentrations measured/detected which are above the LOQ, then assesses from those measurements which are ≤ 0.1 µg/L and greater than 2.0 µg/L. The surface water concentrations for both AMPA and glyphosate tend to decrease in all percentiles since 2009.

The data shows opposite trends for surface water and groundwater as follows:

- For surface water, glyphosate maximum concentrations during 2008-2014 are less than those measured for AMPA in all percentile assessments.
- On the contrary, groundwater maximum glyphosate concentrations during 2008-2014 are higher than those for AMPA in all percentile assessments.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the analyses for both surface water and groundwater for glyphosate and AMPA across mainland France during the monitoring period of 2008-2014. The analysis focusses on those concentrations measured/detected which are above the LOQ, then assesses from those measurements which are ≤ 0.1 µg/L and greater than 2.0 µg/L.
The study is therefore considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/009
Report author	[redacted]
Report date	2016
Report title	Analyse des données de suivi du glyphosate et de l'AMPA dans les eaux de France - Période 1997-2013 (Original in French: Analysis of monitoring data for glyphosate and AMPA in French waters – Time period 1997-2013)
Document No	Rapport_AMPA_Glyphosate_1997-2013(V3)
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No (but conducted by testing facilities accredited by the Member State)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
This report is an update of a previous report « analyse des données de suivi du glyphosate et de l'AMPA dans les eaux de France, période 1999-2012 » of July 2015 (Analysis of monitoring data for glyphosate and AMPA in French waters, time period 1999-2012). It includes the 2013 monitoring data for glyphosate and AMPA in ground and surface waters (extracted from the $IFEN_{uipp}$ database in 2015).

Glyphosate was monitored in surface waters since 1997 and in groundwaters since 1999. AMPA was monitored in surface waters since 1998 and in groundwaters since 2000. Both substances are followed simultaneously in groundwaters and surface waters between 2000 and 2013.

The dataset extracted from the $IFEN_{uipp}$ database was analysed, for each substance, in terms of 1) the volume of individual measurements and 2) the number of stations contributing to the measurements, on an annual basis. The dataset for surface waters consists of 93302 and 103583 analyses, for AMPA and glyphosate, respectively. There were 4392 and 4632 stations associated with the monitoring, for AMPA and glyphosate, respectively. The dataset for groundwater consists of 76951 and 85067 analyses, for AMPA and glyphosate, respectively. There were 17130 and 18216 stations associated with the monitoring, for AMPA and glyphosate, respectively.

I. MATERIAL AND METHODS

Surface water

There is a trend of stations to increasingly monitor for AMPA and glyphosate in surface water over time (Figure 7.5-33, Figure 7.5-34 and Figure 7.5-35).

Figure 7.5-33: Annual progression in the number of analyses for glyphosate and AMPA in surface water

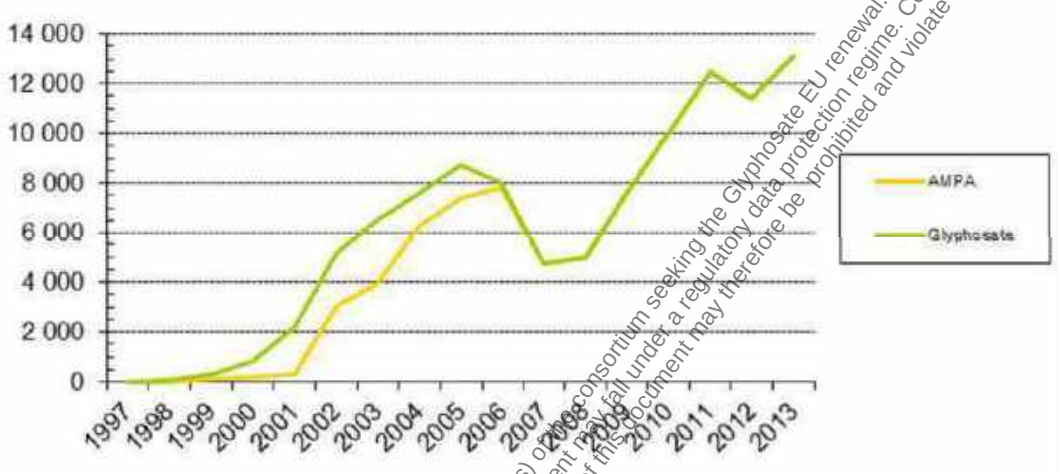


Figure 7.5-34: Evolution of the number of stations monitoring for AMPA in surface waters (left axis: Number of stations as bar chart; right axis: Share of stations of the IFFEN database as a line chart)

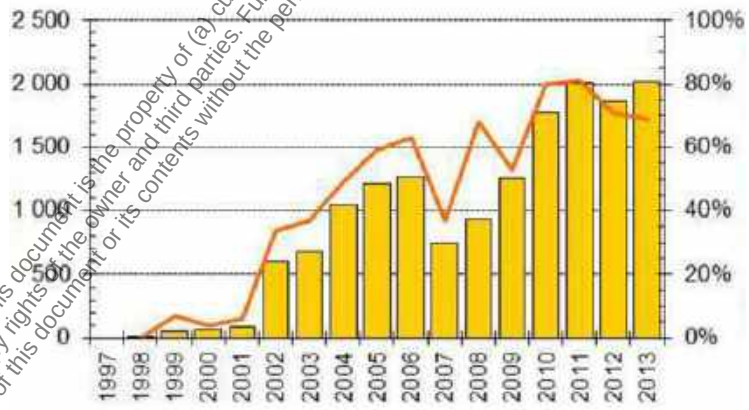
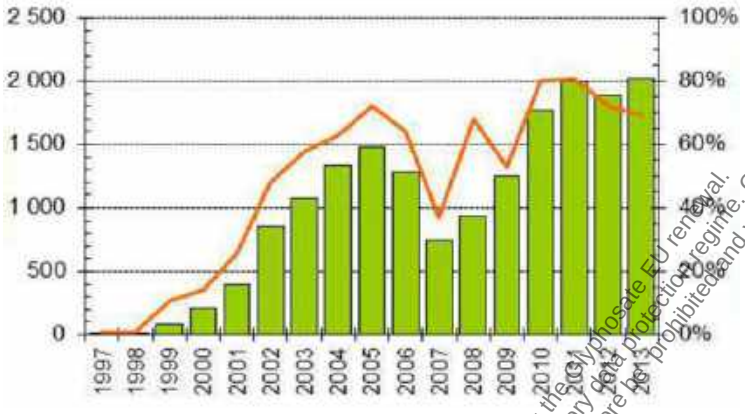


Figure 7.5-35: Evolution of the number of stations monitoring for glyphosate in surface waters (left axis: Number of stations as bar chart; right axis: Percent of stations of the IFFEN database as a line chart)



Groundwater

There is a general move over the years for stations to more routinely monitor AMPA and glyphosate in groundwater (Figure 7.5-36, Figure 7.5-37, Figure 7.5-38).

Figure 7.5-36: Annual progression in the number of analyses for glyphosate and AMPA in groundwater

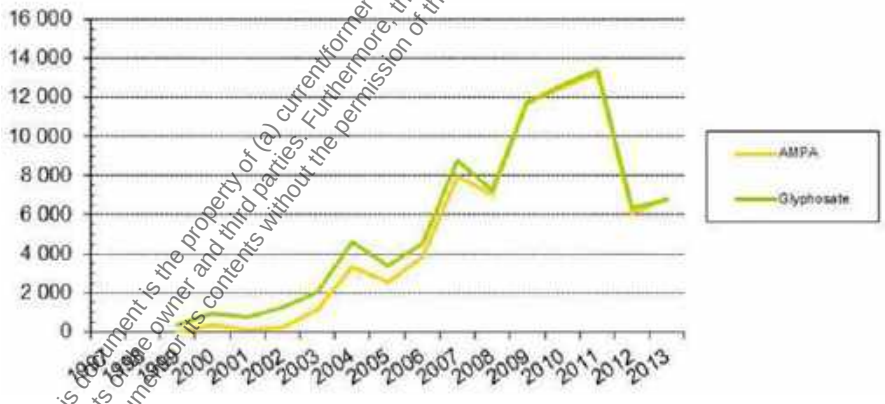


Figure 7.5-37: Evolution of the number of stations monitoring for AMPA in groundwater (left axis: Number of stations as bar chart; right axis: Share of stations of the IFFEN database as a line chart)

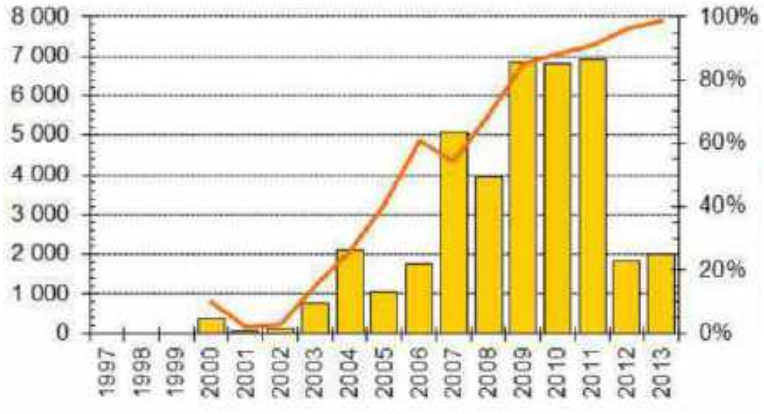
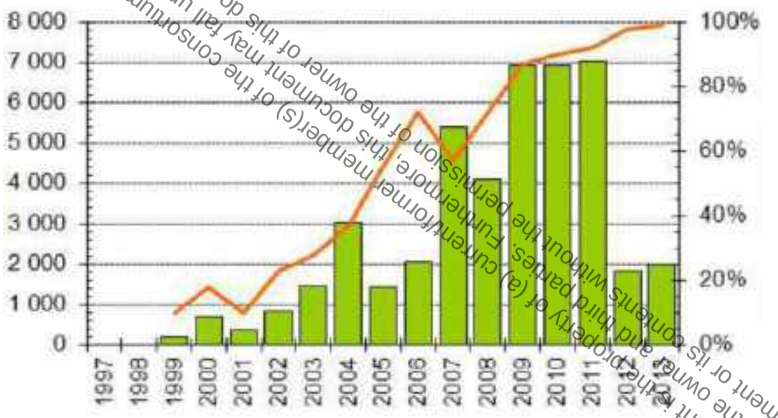


Figure 7.5-38: Evolution of the number of stations monitoring for glyphosate in groundwater (left axis: Number of stations as bar; right axis: Percent of stations of the IFFEN database as a line chart)



Multi-year continuity analysis of measurements

Based on the number of years of monitoring, an assessment was conducted to look at the continuous measurements within the time period and therefore on the ability to draw conclusions in terms of how the multi-annual trends evolve. It is worth noting that the stations are ordered by years of monitoring without the monitoring being necessarily in consecutive years (e.g. a station may be included in 5 years, corresponding to 1999, 2005, 2010, 2011, 2012).

Though the dataset corresponds to 15 years of AMPA and glyphosate monitoring (1999-2013), no station is monitored on an annual basis within the 15-year period. At best, some stations are monitored for 14 years (for glyphosate).

In the case of surface waters, while the monitoring duration may theoretically be up to 17 years (1997-2013), the actual monitoring duration is up to 15 years at a maximum. Some stations are monitored only during a single year (36 % and 32 % for AMPA and glyphosate, respectively) while 30 % and 34 % of stations have 5 or more years, for AMPA and glyphosate, respectively (Table 7.5-40).

Table 7.5-40: Number of years of monitoring of 'surface water' stations on the 1999-2013 period

	AMPA		Glyphosate	
Number of years of monitoring	Number of stations	% of stations	Number of stations	% of stations
A single year	1 592	36%	1 480	32%
2 years	632	14%	739	16%
3 years	317	7%	358	8%
4 years	501	11%	492	11%
5 years	414	9%	529	11%
6 years	318	7%	342	7%
7 years	126	3%	139	3%
8 years	165	4%	142	3%
9 years	56	1%	74	2%
10 years	46	1%	71	2%
11 years	91	2%	93	2%
12 years	113	3%	123	3%
13 years	9	<1%	37	1%
14 years	9	<1%	10	<1%
15 years	3	<1%	3	<1%
16 years	0	0%	0	0%
17 years	0	0%	0	0%
Total number of stations	**4 392**	-	**4 632**	-

For groundwater, most stations cannot contribute to a multi-year analysis (Table 7.5-41; 54 % and 53 % of stations are monitored for only a single year for AMPA and glyphosate, respectively).

Table 7.5-41: Number of years of monitoring of 'groundwater' stations on the 1999-2013 period

	AMPA		Glyphosate	
Number of years of monitoring	Number of stations	% of stations	Number of stations	% of stations
A single year	9 199	54%	9 591	53%
2 years	3 182	19%	3 317	18%
3 years	1 871	11%	2 007	11%
4 years	659	4%	775	4%
5 years	502	3%	553	3%
6 years	432	3%	446	2%
7 years	597	3%	503	3%
8 years	167	1%	189	1%
9 years	209	1%	280	2%
10 years	149	1%	295	2%
11 years	151	1%	172	1%
12 years	9	<1%	27	<1%
13 years	3	<1%	37	<1%
14 years	0	0%	24	<1%
15 years	0	0%	0	0%
Total number of stations	**17 130**	-	**18 216**	-

Analysis of the frequency of measurements within a monitoring year
The multi-year continuity analysis comprises an analysis of the frequency of measurements within a year of monitoring. For groundwater, the frequency varies between once and twice a year, with a majority of measurements being carried out once a year. The exception to this is 2012, where 4 or 5 measurements per year were conducted for more than 40 % of stations.

For surface waters, annual measurement frequencies are higher. There is a general increase from 2-3 times a year in 2000 to 6-9 times in the later years (2011 - 2013).

II. RESULTS AND DISCUSSION

Assessment of the multi-year trend in measurements higher than LOQ
Quantification rates of AMPA in groundwater (Table 7.5-40) are typically below 2.5 % (except 2002, 2003 and 2012). The period between 2008 and 2010 has the lowest levels of quantification (0.8 to 0.9 % of measurements). Quantification rates ≥2 µg/L are typically <0.1 %.

Quantification rates of AMPA in surface waters (Table 7.5-42) are higher and vary around a median of 54 %. Quantification rates ≥2 µg/L are typically <3 %.

Quantification rates of glyphosate in groundwater (Table 7.5-43) are low and vary, depending on the years, around 1.5 %. Quantification rates ≥2 µg/L are typically <0.1 %.

In surface waters, the annual glyphosate quantification rate (Table 7.5-43) varies around 30 % during the 2000-2012 period; without a clear tendency toward increase or decrease. Quantification rates ≥2 µg/L are typically <1 %.

Comparison of concentration level (above LOQ) of AMPA and glyphosate
The annual quantification rates above 0.1 µg/L were compared on the 2000-2013 time period. Quantification rates above 0.1 µg/L vary between 40 and 60 % for AMPA and 20 and 40 % for glyphosate. There is no apparent correlation in terms of quantification rate value and consecutive annual variations.

Maximum concentrations, 90th, 95th and 99th percentiles
The maximum concentration values (Table 7.5-44) show an erratic profile indicative of temporal pollution events. With regards to the higher measured concentration values, and in particular values greater than 1000 µg/L, it is likely that they do not correspond to raw water sampling (e.g. aquatic organisms, sediments, etc.). They may also correspond to a unit mistake (g/kg, for example). The analysis of 90th, 95th and 99th percentile concentrations (Table 7.5-45, Table 7.5-46 and Table 7.5-47) does not show any recognisable pattern for glyphosate or AMPA. This is more so as the percentile increases.

Analysis of measurements depending on their detection/quantification status ("Code Remarque")
This assessment considered measurement results associated with each analysis status ("code remarque") that may have one of 4 values:

- 1: concentration is above the limit of quantification
- 2: concentration is below limit of detection
- 7: concentration is above the limit of detection and below the limit of quantification
- 10: concentration is below the limit of quantification (no indication given as to whether the substance was detected)

Table 7.5-42: Quantification rates of AMPA in groundwater and surface water

Year/Statistics	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Groundwater																	
Number of analyses				380	107	272	1124	3347	2527	3840	7985	7048	11672	12514	13258	6106	6811
Number >LOQ				0	1	59	41	69	48	54	115	63	101	101	201	254	164
% >LOQ				0 %	0.9 %	21.7 %	3.6 %	2.1 %	1.9 %	1.4 %	1.4 %	0.9 %	0.9 %	0.8 %	1.5 %	4.2 %	2.4 %
Number ≥0.1 µg/L				0	1	31	39	64	44	49	98	58	78	85	133	66	43
% ≥0.1 µg/L				0 %	0.9 %	11.4 %	3.5 %	1.9 %	1.7 %	1.3 %	1.2 %	0.8 %	0.7 %	0.7 %	1.0 %	1.1 %	0.6 %
Number ≥2 µg/L				0	0	0	0	1	1	0	1	3	2	2	2	2	2
% ≥2 µg/L				0 %	0 %	0 %	0 %	<0.1 %	<0.1 %	0 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %
Surface water																	
Number of analyses		2	108	218	291	3050	3974	6280	7382	7810	4714	4954	7600	10001	12456	11395	13067
Number >LOQ		0	63	106	179	1602	2351	3449	4355	3995	2030	2558	3893	4597	7789	6148	7307
% >LOQ		0 %	58.3 %	48.6 %	61.5 %	52.5 %	59.2 %	54.9 %	59.0 %	51.2 %	43.1 %	51.6 %	51.2 %	46.0 %	62.5 %	54.0 %	55.9 %
Number ≥0.1 µg/L		0	63	94	157	1453	2282	3284	4161	3865	1908	2130	3393	4068	6681	4054	4134
% ≥0.1 µg/L		0 %	58.3 %	43.1 %	54.0 %	47.6 %	57.4 %	52.3 %	56.4 %	49.5 %	40.5 %	43.0 %	44.6 %	40.7 %	53.6 %	35.6 %	31.6 %
Number ≥2 µg/L		0	1	2	5	50	200	141	222	219	68	106	190	172	268	176	162
% ≥2 µg/L		0 %	0.9 %	0.9 %	1.7 %	1.6 %	5.0 %	2.2 %	3.0 %	2.8 %	1.4 %	2.1 %	2.5 %	1.7 %	2.2 %	1.5 %	1.2 %

Table 7.5-43: Quantification rates of glyphosate in groundwater and surface water

Year/Statistics	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Groundwater																	
Number of analyses			370	948	735	1296	2064	4639	3404	4553	8795	7246	11793	12663	13396	6373	6808
Number >LOQ			5	6	29	37	23	94	57	96	59	50	53	105	132	109	105
% >LOQ			1.4 %	0.6 %	3.9 %	2.9 %	1.1 %	2.0 %	1.7 %	2.1 %	0.7 %	0.7 %	0.4 %	0.8 %	1.0 %	1.7 %	1.5 %
Number ≥0.1 µg/L			5	6	22	25	23	84	57	90	53	46	48	92	94	27	30
% ≥0.1 µg/L			1.4 %	0.6 %	3.0 %	1.9 %	1.1 %	1.8 %	1.7 %	2.0 %	0.6 %	0.6 %	0.4 %	0.7 %	0.7 %	0.4 %	0.4 %
Number ≥2 µg/L			0	0	0	2	2	2	1	3	4	0	2	7	3	3	3
% ≥2 µg/L			0 %	0 %	0 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %	0 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %	<0.1 %
Surface water																	
Number of analyses	4	51	289	840	2218	5172	6452	7589	8740	7989	4714	4954	7600	10001	12457	11417	13066
Number >LOQ	3	39	105	282	594	1903	2112	2713	2979	2114	1048	1551	1938	2535	4026	4189	5048
% >LOQ	75.0 %	76.5 %	36.3 %	33.6 %	26.8 %	36.8 %	32.6 %	35.7 %	34.1 %	26.5 %	22.2 %	31.3 %	25.5 %	25.3 %	32.3 %	36.7 %	38.6 %
Number ≥0.1 µg/L	3	39	103	249	563	1715	2022	2553	2837	1963	947	1051	1406	1757	2697	1937	1873
% ≥0.1 µg/L	75.0 %	76.5 %	35.6 %	29.6 %	25.4 %	33.2 %	31.3 %	33.6 %	32.5 %	24.6 %	20.1 %	21.2 %	18.5 %	17.6 %	21.7 %	17.0 %	14.3 %
Number ≥2 µg/L	0	2	5	7	17	52	62	38	73	37	26	25	43	36	52	40	40
% ≥2 µg/L	0 %	3.9 %	1.7 %	0.8 %	0.8 %	1.0 %	1.0 %	0.5 %	0.8 %	0.5 %	0.6 %	0.5 %	0.6 %	0.4 %	0.4 %	0.4 %	0.3 %

Table 7.5-44: Maximum concentrations recorded per year (in µg/L)

	Period	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Groundwater																		
AMPA	19				1	0.9	0.59	1.65	8	19	0.97	3.4	2.36	5.7	9.3	7.78	6.3	5.05
Glyphosate	1005			0.4	0.4	0.54	2.17	6.78	4.95	24	4.87	12.9	0.96	3.91	22	11	1005	140
Surface water																		
AMPA	3369			5.05	2.99	4.2	48.9	48.1	17	30	27.5	16.5	20.3	33.5	106	3369	80	59.1
Glyphosate	3257	1.5	3.4	35	6.36	41	40.6	3257	50	17	34	28	17.3	19.7	21	2237	66	37.9

Table 7.5-45: 90th percentile concentrations recorded per year (in µg/L)

	Period	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Groundwater																		
AMPA	0.4					0.9	0.21	0.72	0.43	0.55	0.41	0.47	0.7	0.48	0.78	0.26	0.21	0.18
Glyphosate	0.54			0.36	0.26	0.4	0.79	1.4	0.47	0.44	0.72	0.7	0.5	0.62	0.85	0.44	0.21	0.25
Surface water																		
AMPA	1.1			1.05	0.91	0.93	0.89	1.66	1.2	1.3	1.3	1	1.11	1.2	1.1	1.04	0.88	0.68
Glyphosate	0.6	1.23	0.86	0.92	0.72	0.7	0.73	0.8	0.62	0.8	0.66	0.65	0.54	0.7	0.59	0.53	0.42	0.32

Table 7.5-46: 95th percentile concentrations recorded per year (in µg/L)

	Period	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Groundwater																		
AMPA	0.7					0.9	0.24	0.8	0.53	0.84	0.55	1	1.09	0.79	1.1	0.43	0.31	0.31
Glyphosate	1.1			0.38	0.33	0.4	1.36	2.3	1.1	0.55	1.32	2.12	0.76	1.4	2.85	0.91	0.82	0.46
Surface water																		
AMPA	1.7			1.24	1.13	1.56	1.44	2.71	1.8	2	2.1	1.5	1.74	1.97	1.79	1.65	1.4	1.2
Glyphosate	0.95	1.37	1.22	1.63	1.3	1.2	1.25	1.3	0.93	1.13	1.08	1.27	0.84	1.19	0.95	0.81	0.71	0.55

Table 7.5-47: 99th percentile concentrations recorded per year (in µg/L)

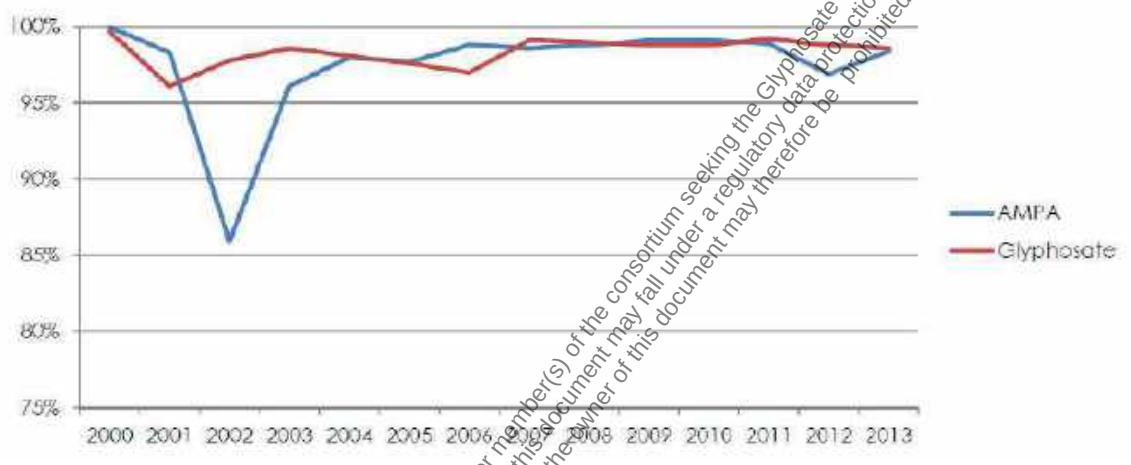
	Period	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Groundwater																		
AMPA	2.21					0.9	0.51	1.64	3.58	10.8	0.91	1.4	2.26	3.8	2	1.77	1.55	2.24
Glyphosate	8.94			0.4	0.39	0.5	2.14	5.81	2.95	11	4.21	0.91	0.92	3.02	9.73	7.92	26.25	11.41
Surface water																		
AMPA	4.6			3.06	2	2.59	4.22	6.25	3.45	4.47	5.33	3.77	4.7	4.8	4.67	5.16	4.04	3.85
Glyphosate	2.83	1.47	3.1	11.7	3.22	5.54	4.4	4.6	2.3	3.64	3.29	4.41	2.85	3.49	2.36	2.22	1.9	1.7

Assessment of quantification (concentrations above LOQ) with respect to stations

To avoid an assessment limited by its focus on the number of measurements, the number of stations without quantification >0.1 µg/L is considered. For the vast majority of stations looking at groundwater, measurements do not show the presence of AMPA and glyphosate (Figure 7.5-39):

- In 1999-2013, 96.6 % and 96.1 % of stations do not show a quantification greater than 0.1 µg/L for AMPA and glyphosate.
- From 2006 for AMPA and 2007 for glyphosate, every year more than 98 % of stations did not quantify the substances above 0.1 µg/L, with the exception of 2012 (although 2012 is characterized by a drop in the number of stations recorded)

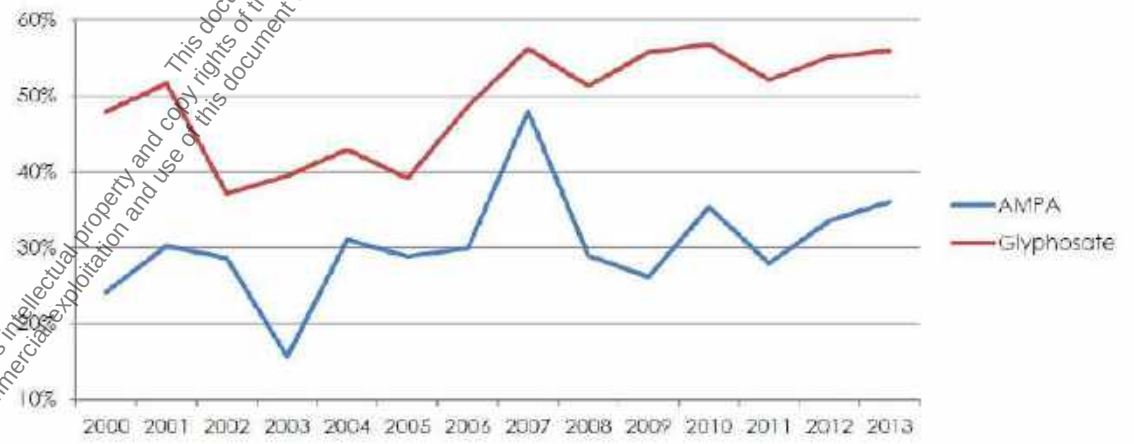
Figure 7.5-39: Annual evolution of the percent of stations without measurements quantified at >0.1 µg/L in groundwater



This is in contrast with the conclusion for surface waters (Figure 7.5-40):

- Less than a third of stations monitored between 1997 and 2013 show measurements with quantifications of AMPA that are not >0.1 µg/L.
- During the period 1997-2013, 38 % of stations do not show quantification of glyphosate above 0.1 µg/L.

Figure 7.5-40: Annual evolution of the share of stations without measurements quantified >0.1 µg/L in surface water



Analysis of measurement results by Department
An analysis of the geographical breakdown of the preceding results is also presented. This does not alter the primary observations.

Analysis of a smaller dataset composed of higher-frequency measurements
Complementary investigations were carried out by limiting the type of data used to only the higher-frequency monitoring programmes. Observations that complement preceding sections are presented.

Analysis of the seasonality of the quantifications, based on a subset composed of higher-frequency measurements
For groundwater (Figure 7.5-41 and Figure 7.5-42), there is no apparent relationship between quantifications and time of year. This may demonstrate the randomness of groundwater quantifications, linked to temporary pollution. The lack of correlation may also be due to the travel time to groundwater.

For surface water (Figure 7.5-43 and Figure 7.5-44), there is a clear relationship between quantifications and spring and summer periods. This is consistent with the pattern of glyphosate usage, showing diffuse pollution directly linked to periods of use.

Figure 7.5-41: Seasonal distribution of AMPA quantification in groundwater - smaller dataset

	2006	2007	2008	2009	2010	2011	2012	2013	2006-2013
January to march									
April to june	34%	86%		20%	100%				42%
July to september	66%	14%		80%			50%	100%	35%
October to december		20%	100%	20%		100%	50%		23%
Quantification number	3	15	1	5	1	1	2	3	

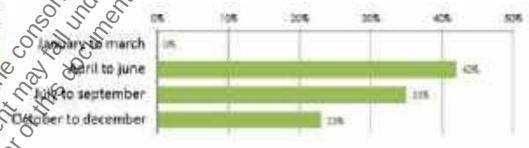


Figure 7.5-42: Seasonal distribution of Glyphosate quantification in groundwater - smaller dataset

	2006	2007	2008	2009	2010	2011	2012	2013	2006-2013
January to march	75%	100%						12%	36%
April to june	12%							25%	14%
July to september				100%	100%			63%	32%
October to december	12%						100%		18%
Quantification number	8	1		1	1		3	8	22

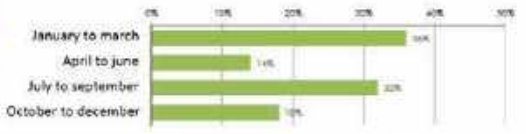


Figure 7.5-43: Seasonal distribution of AMPA quantification in surface water - smaller dataset

	2006	2007	2008	2009	2010	2011	2012	2013	2006-2013
January to march	15%	18%	17%	14%	24%	22%	30%	16%	20%
April to june	28%	31%	20%	26%	31%	27%	36%	32%	29%
July to september	34%	38%	42%	39%	25%	29%	25%	32%	30%
October to december	23%	13%	11%	20%	20%	23%	7%	20%	21%
Quantification number	444	445	123	159	242	336	256	274	2 330

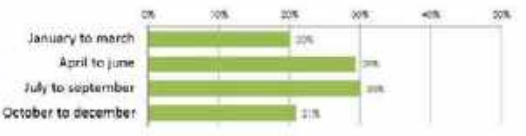
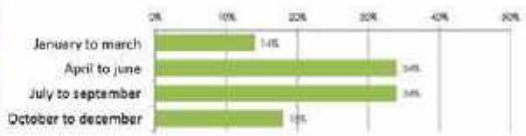


Figure 7.5-44: Seasonal distribution of Glyphosate quantification in surface water - smaller dataset

	2006	2007	2008	2009	2010	2011	2012	2013	2006-2013
January to march	16%	13%	17%	5%	7%	18%	20%	15%	14%
April to june	31%	32%	36%	26%	33%	34%	36%	36%	34%
July to september	37%	33%	32%	37%	36%	28%	34%	33%	34%
October to december	16%	22%	13%	26%	25%	20%	10%	13%	18%
Quantification number	186	196	87	63	117	130	154	159	1 092



Surface Water Load calculations

When available, streamflow data for the stations were used in combination with the concentration measurements to calculate loads. This analysis is useful to put the analysis based on concentration measurements into perspective. This was conducted for stations with at least monthly monitoring data in 2012 or 2013. Of the 64 stations with suitable concentration data 9 had associated streamflow data. Concentration data were treated as monthly averages, where more than one value was present monthly averages were calculated. Daily flow data were summed to produce a corresponding monthly flow total. Monthly load calculations were summed and normalised by the catchment area to produce loads in g/year/km^2.

Glyphosate loads vary (Table 7.5-48), depending on the watershed, between 0.67 and 31 g/year/km². The AMPA loads vary between 13 and 94 g/year/km².There is a lack of consistency between the calculated loads of AMPA and glyphosate: in 7 cases out of 9, AMPA loads are much higher than glyphosate, with a ratio of 1.3 to 20.7.

Table 7.5-48: Glyphosate and AMPA loads from 9 stations

Monitoring station	Associated Hydro Station	AMPA Load (g/km^2)	Glyphosate Load (g/km^2)	AMPA/GLY Ratio
03091000	H5091010	13	19	0.7
03109000	H5321010	38	29	1.3
04131500	M3823010	31	10	3.2
04134700	M5300010	16	6	2.5
04155500	N3511610	28	31	0.9
04207400	J7214010	60	5	13
04179500	J3821820	13	4	3
04211000	J7483010	94	9	10.7
04216000	J9300611	14	0.67	20.7

Analysis of 6 AOC vineries

An analysis of AMPA and Glyphosate measurements, over the years, for stations associated with 6 vineyards is also presented.

III. CONCLUSIONS

Glyphosate was monitored in surface waters since 1997 and in groundwaters since 1999. AMPA was monitored in surface waters since 1998 and in groundwaters since 2000. Both substances are followed simultaneously in groundwaters and surface waters between 2000 and 2013. The dataset extracted from the $IFEN_{uipp}$ database was analysed, for each substance, in terms of 1) the volume of individual measurements and 2) the number of stations contributing to the measurements, on an annual basis.

The dataset for surface waters consists of 93302 and 103583 analyses, for AMPA and glyphosate, respectively. There were 4392 and 4632 stations associated with the monitoring, for AMPA and glyphosate, respectively.

The dataset for groundwater consists of 76951 and 85067 analyses, for AMPA and glyphosate, respectively. There were 17130 and 18216 stations associated with the monitoring, for AMPA and glyphosate, respectively.

For the vast majority of stations looking at groundwater, measurements do not show the presence of AMPA and glyphosate:

- In 1999-2013, 96.6 % and 96.1 % of ESO stations do not show a quantification greater than 0.1 µg/L for AMPA and glyphosate, respectively.
- From 2006 for AMPA and 2007 for glyphosate, every year more than 98 % of stations did not quantify the substances above 0.1 µg/L, with the exception of 2012 (although 2012 is characterized by a drop in the number of stations recorded).

This is in contrast with the conclusion for surface waters:

- Less than a third of stations monitored between 1997 and 2013 show measurements with quantifications of AMPA that are not >0.1 µg/L.
- During the period 1997-2013, 38 % of stations do not show quantification of glyphosate above 0.1 µg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the analyses of both surface water and groundwater for glyphosate and AMPA across France during the monitoring period of 1997-2013. The data analysis focusses on those concentrations measured/detected which are quantified above 0.1 µg/L.
The study is therefore considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/010
Report author	[redacted]
Report year	2016
Report title	Survey of glyphosate and AMPA in groundwaters and surface waters in Europe - 2015/16 update review – final report
Report No	MSL0027535
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The report represents a review of glyphosate and AMPA monitoring results for surface (fresh) waters and groundwater across Europe, i.e. all 28 Member States of the European Union, as well as Norway and Switzerland, where information was available. The review is based on an earlier review carried out in 2012, which has been updated to include the latest available information.

Information has been obtained from professional contacts across Europe (government departments and research organisations). In addition, some data provided by Monsanto Europe, as well as from web and literature searches, and on-line databases are included. Some data from the previous review has been omitted where more up-to-date information has become available.

Additional data were collected for 13 countries, i.e. Belgium, the Czech Republic, Denmark, Finland, France, Germany, Italy, Norway, the Slovak Republic, Spain, Sweden, the Netherlands and the UK, as well as the Danube River Basin. In total, there is data for 17 countries, 16 countries plus the Danube River Basin for surface water, and 15 countries for groundwater, with most countries including both.

Surface water

Glyphosate and AMPA have been frequently detected in surface waters, AMPA usually at higher concentrations and in a larger proportion of samples. Glyphosate has been analysed in over 143,000 samples from over 4,400 sites (from 1993-2015) and detected in 31 % of samples, with 21 % above 0.1 µg/L. AMPA has been analysed in over 115,000 samples from over 3,500 sites (1997-2015) and detected in 50 % of samples, with 39 % above 0.1 µg/L. Concentrations vary widely, with maximum concentrations for glyphosate in the range 0.07-3400 µg/L and AMPA from 0.07-393 µg/L. The more persistent presence of AMPA in surface waters throughout the year may be mainly derived from aminophosphonate containing complexing agents in detergents and cooling waters, entering surface waters via wastewater treatment effluents, rather than from the degradation of glyphosate.

Generally, results are rather variable and not suggesting an increase in detection frequency or concentration observed over the years. However, a trend analysis from the Netherlands over the years 1997-2014 indicates a slight upward trend for glyphosate and a slight downward trend for AMPA.

Groundwater

Glyphosate and AMPA have been increasingly analysed and occasionally detected in groundwater. Glyphosate has been analysed in about 114,000 samples from over 16,000 sites (1990-2015) and detected in 1.3 % of samples, with 0.6 % above 0.1 µg/L. AMPA has been analysed in almost 105,000 samples from over 15,000 sites (1990-2013) and detected in 2.2 % of samples, with 0.8 % above 0.1 µg/L. The highest numbers of detections have been reported from Denmark, France and Spain. These seem to occur in shallow water or spring water, which is often included in groundwater surveys, sometimes associated with contamination incidents, and even unsuitable sampling sites and analytical techniques.

From a current perspective, there seems to be no evidence of any persistent and confirmed groundwater contamination with glyphosate or AMPA. In many cases, detections occur in isolated samples rather than consistently at the same sampling site. Where the necessary information is available, it is frequently shown that glyphosate detections are only observed in shallow groundwater (e.g. Denmark and the Netherlands) or wells with direct surface water influence.

The majority of detections occurred only once, which is a clear indication that there is no real groundwater contamination. The small number of multiple detections occurred in shallow groundwater (spring water) or wells unsuitable for groundwater monitoring, suggesting superficial short-term contamination.

I. MATERIAL AND METHODS

The report represents a review of glyphosate and AMPA monitoring results for surface (fresh) waters and groundwater across Europe, i.e. all 28 Member states of the European Union, as well as Norway and Switzerland, where information was available. The review is based on an earlier review carried out in 2012, which has been updated to include the latest available information.

Information has been obtained from professional contacts across Europe (government departments and research organisations). In addition, some data provided by Monsanto Europe, as well as from web and literature searches, and on-line databases are included. Some data from the previous review has been omitted where more up-to-date information has become available.

Additional data were collected for 13 countries, i.e. Belgium, the Czech Republic, Denmark, Finland, France, Germany, Italy, Norway, the Slovak Republic, Spain, Sweden, the Netherlands and the UK, as well as the Danube River Basin.

In total, there is data for 17 countries, 16 countries plus the Danube River Basin for surface water, and 15 countries for groundwater, with most countries including both. However, the Czech and Slovak Republics monitor only surface water, whereas for Malta only groundwater was monitored in a special investigation. Data was mainly collated at national level, but in some cases at regional level, e.g. for Belgium (two regions) and Germany (surface water data Rhine and some individual Länder). 11 countries have confirmed that there is no monitoring of glyphosate and AMPA (Bulgaria, Croatia, Cyprus, Greece, Hungary, Latvia, Lithuania, Luxembourg, Poland, Portugal and Romania).Although it has been confirmed that glyphosate and AMPA are monitored in Slovenia, it has not been possible to obtain any data to date, nor has any information been received from Estonia. Although overall most data are considered reasonably reliable, it was not possible to fully assess their reliability, notably the French database which provides a comprehensive source of data for surface water and groundwater, includes several extremely high values, which were considered 'outliers' and excluded from this analysis.

II. RESULTS AND DISCUSSION

Table 7.5-49 and Table 7.5-50 provide an overview of the main data for surface water and groundwater, respectively. The summarised data is not precise but presents a best estimate, mainly because of the various forms in which the data was obtained, e.g. some results in terms of samples, others in terms of sites, and other information gaps.

Surface water

Table 7.5-49 Summary of glyphosate and AMPA data in surface water in Europe

Country / Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
Austria									
AMPA	2001-2002	?	345	≥90	≥26	90	26	3.4	?
Belgium (Flanders-F and Wallonia-W)									
Glyphosate F	2007-2015	≥131	6802	5510	81.0	1628	23.9	139	0.02-0.4
AMPA F	2007-2015	≥132	6801	6256	92.0	3844	56.5	47	0.02-0.4
Glyphosate W	2001-2014	≥171	6118	≥961	≥15.7	961	15.7	15.5	(0.05)
AMPA W	2007-2014	≥171	5891	≥148(s)	≥86.6(s)	≥148	≥86.6(s)	35.8	(0.025-0.1)
Czech Republic									
Glyphosate	2010-2014	≥290	6358	2547	40.0	≤2476	≤38.9	52	0.025-1.0
AMPA	2010-2014	≥236	4845	3185	65.7	≤3020	≤62.3	83	0.05-10
Denmark									
Glyphosate	2004-2013	≥20	370	281	76	<281	<76	2.7[1]	0.01-0.1
AMPA	2010-2014	≥20	363	296	81	<269	<81	0.28[1]	0.01-0.2

Table 7.5-49 Summary of glyphosate and AMPA data in surface water in Europe

Country / Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
Finland									
Glyphosate	2007-2011	4	82	5	6.1	5	6.1	0.9	0.1
AMPA	2007-2011	4	84	14	16.7	≤13	≤15.5	0.22	0.05
France									
Glyphosate	1997-2012	≥2003	91044	27999	30.7	19505	21.4	88	0.01-2.5
AMPA	1998-2012	≥2001	80817	42855	53	36053	44.6	106	0.01-0.25
Germany									
Glyphosate	1997-2013	>204	≥2018	831	41	≤712	≤35	4.7	0.02-1.5
AMPA	1997-2013	≥71	≥1362	≤837	61.4	≤719	52.8	1.4	0.05-0.5
Ireland									
Glyphosate	2005-2012	≥256	≥2544	142	5.6	≤142	≤5.6	186	0.08-0.1/20
AMPA	2010-2012	≥70	870	2	0.2	≥2	≥0.2	>200	20
Italy (Lombardia Region)									
Glyphosate	2005-2012	≥274	2851	754	26.4	673	23.6	37.6	0.1
AMPA	2008-2012	≥274	2229	1386	62.2	1386	62.2	393	0.1
Norway									
Glyphosate	1997-2015	12	98	88	89.8	≤71	≤72	0.93	0.01-0.05
AMPA	1997-2015	12	98	90	91.8	≤59	≤60	0.54	0.01-0.05
Slovak Republic									
Glyphosate	2006-2014	≥142	5018	835	16.6	775	15.4	4.2	0.05-0.5
Spain [2]									
Glyphosate	2009-2014	≥343	5418	1847	34	1218	22	3400	0.03-30
AMPA	2012-2014	≥ 84	830	543	65	534	64	9.2	0.05-0.2
Sweden									
Glyphosate	2000-2014	≥ 21	1439	442	30.7	≤433	≤30	370	<0.06-<1
AMPA	2000-2014	≥ 21	1418	320	22.6	≤312	≤22	36.0	<0.07-<1
Switzerland									
Glyphosate	2006	5	≥10	≥8	80	1	≤10	0.1	0.0007
AMPA	2006	5	≥11	≥11	100	≥3	27	0.29	0.0008
The Netherlands									
Glyphosate	2006-2014	≥373	9316	≥1223	≥13	≤1223	≤13	0.142)	?
AMPA	2006-2014	≥373	9270	≥1358	≥15	≤1358	≤15	0.07 2)	?
UK									
Glyphosate	1993-2015	≥102	3916	754	19.2	754	19.2	8.2	0.1-1
Danube									
Glyphosate	2013	68	68	5	7.3	0	-	0.07	0.03
AMPA	2013	68	68	66	97	≤66	≤97	0.96	0.03
Total									
Glyphosate	1993-2015	≥4419	≥143470	444232	31	30858	21	0.07-3400	Mainly 0.01-2.5
AMPA	1997-2015	≥3543	≥115302	≥57457	50	≥47876	41	0.07-393	Mainly 0.01-0.5

LoQ = Limit of quantification (LoD = Limit of detection)
(s) Sites (number of samples not known, but assumed ≥1 per site)
[1] Maximum 90 percentile value
[2] Maximum annual average concentration

Glyphosate has been analysed in over 143,000 surface water samples from over 4,400 sites (from 1993-2015) and detected in 31 % of samples, with 21 % above 0.1 µg/L. AMPA has been analysed in over 115,000 samples from over 3,500 sites (1997-2015) and detected in 50 % of samples, with 39 % above 0.1 µg/L. Concentrations vary widely, with maximum concentrations for glyphosate in the range 0.07-3400 µg/L and AMPA from 0.07-393 µg/L.

Glyphosate has a high usage rate and has been rated among the most frequently detected herbicides in some countries, notably in the Netherlands. It has been suggested that urban run-off can be a significant source of glyphosate in surface waters (France and the Netherlands). Where data allowed interpretation, glyphosate was linked to application periods (from spring through to autumn) and run-off events and does not seem to

persist. The more persistent presence of AMPA in surface waters throughout the year may be mainly derived from aminophosphonate containing complexing agents in detergents and cooling waters, entering surface waters *via* wastewater treatment effluents, rather than from the degradation of glyphosate.

Generally, results are rather variable and not suggesting an increase in detection frequency or concentrations observed over the years. However, a trend analysis from the Netherlands over the years 1997-2014 indicates a slight upward trend for glyphosate from an annual average concentration of 0.102 µg/L in 1997 to 0.138 µg/L in 2014, and a slight downward trend for AMPA from 0.209 µg/L to 0.188 µg/L over the same period.

Some countries have proposed (or implemented) various environmental quality standards (EQS) or objectives for glyphosate in surface water, ranging from an EQS of 60 µg/L in Ireland, to a Proposed No Effect Concentration (PNEC) of 10 µg/L and a Maximum Admissible Concentration (MAC) of 100 µg/L in Belgium (Flanders), and in the Netherlands a Maximum Tolerable Risk (MTR) standard at 77 µg/L, and a pesticide authorisation standard of 64 µg/L. Some professionals (Belgium-Wallonia and Rheinland-Pfalz in Germany) suggested that an EQS should be set. However, none of these (proposed) standards have been exceeded on regular basis. Perhaps more importantly, the Netherlands apply the drinking water standard of 0.1 µg/L for pesticides to surface water intakes at waterworks, and LAWA in Germany has set a target value of 0.1 µg/L for the same purpose.

Groundwater

Table 7.5-50: Summary of glyphosate and AMPA data in groundwater in Europe

Country / Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg l^{-1}		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
Austria									
Glyphosate	2004	~950	3633	7	0.19	2	0.06	>0.1	<0.1
AMPA	2004	~950	3636	44	1.2	11	0.3	0.75	<0.1
Belgium (Flanders & Wallonia)									
Glyphosate (Flanders)	2006-2008	≥448	1488	4	0.03	1	0.01	≤0.5	0.01
AMPA (Flanders)	2007-2014	≥504	4513	789	17.5	≥8	≥0.18	1.85	0.01
Glyphosate (Wallonia)	2000-2006	450	≥450	0	-	0	-	<0.025	<0.025
AMPA (Wallonia)	2000-2006	450	≥450	13 (s)	3 (s)	0	-	< 0.05	<0.025
Denmark									
Glyphosate	1990-2013	4941	15552	142	0.9	28	0.2	4.7	(0.01-<0.1)
AMPA	1990-2013	4946	15541	106	0.7	23	0.15	9.1	(0.01-<0.1)
Finland									
Glyphosate	2002-2008	81	81	0	-	0	-	-	0.1
AMPA	2002-2008	81	81	0	-	0	-	-	0.05
France									
Glyphosate	1999-2012	≥7028	78431	859	1.1	565	0.7	28	0.01-0.2
AMPA	2000-2012	≥6904	70492	1122	1.6	643	0.9	19	0.01-0.2
Germany									
Glyphosate	1996-2008	≥430	≥2599	35	1.3	9	0.34	<1.0	<0.1
AMPA	1996-2008	≥387	≥1986	64	3.2	34	1.7	≥1.0	<0.1
Ireland									
Glyphosate	2007-2009	92	679	6	0.8	1	0.1	0.19	<0.1
Italy (Lombardia Region)									
Glyphosate	2005-2012	≥359	1497	9	0.6	5	0.2	1.2	0.1
AMPA	2007-2012	≥359	1156	14	1.2	11	0.9	1.3	0.1
Malta									
Glyphosate	2009	18	≥ 18	0	-	0	-	-	(0.01)

Table 7.5-50: Summary of glyphosate and AMPA data in groundwater in Europe

Country / Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg l^{-1}		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
Norway									
Glyphosate	1999-2000	7	8	0	-	0	-	-	(0.01)
AMPA	1999-2000	7	8	1	12.5	0	-	0.02	(0.01)
Spain									
Glyphosate	2009-2012	≥461	963	325	34	86	8.9	25	0.03-0.3
Sweden									
Glyphosate	2000-2014	≥ 21	5989	26	0.43	10	0.17	0.23	<0.03
AMPA	2000-2014	≥ 21	5930	31	0.52	≤26	0.43	7.9	<0.05
Switzerland									
Glyphosate	2005-2006	117	≥ 234	≥4	1.7	≥3	1.3	0.21	(0.05)
AMPA	2005-2006	117	≥ 232	17	7.3	11	4.7	0.46	(0.05)
The Netherlands									
Glyphosate	2003-2006	<691	691	4	0.58	4	0.58	4.7	(<0.1)
AMPA	2003-2006	<691	691	21	3.0	21	3.0	5.1	(<0.1)
UK									
Glyphosate	1995-2015	≥264	1680	16	0.95	≤6	≤0.35	1.38	(0.01-0.1)
Total									
Glyphosate	1990-2015	≥16160	≥113993	1437	1.3	724	0.6	<0.05-28	0.01-0.2
AMPA	1990-2013	15417	≥104718	2222	2.1	788	0.75	0.02-19	0.01-0.2

LoQ = Limit of quantification (LoD = Limit of detection)
(s) Sites (number of samples not known)
- Not relevant

Glyphosate and AMPA have been increasingly analysed and occasionally detected in groundwater. Glyphosate has been analysed in about 114 000 samples from 16 000 sites (1990-2015) and detected in 1.3 % of samples, with 0.6 % above 0.1 µg/L; AMPA has been analysed in 105 000 samples from over 15 000 sites (1990-2013) and detected in 2.2 % of samples, with 0.8 % above 0.1 µg/L. The highest numbers of detections have been reported from Denmark, France and Spain. These seem to occur in shallow water or spring water, which is often included in groundwater surveys, sometimes associated with contamination incidents (where the information is available), and even unsuitable sampling sites and analytical techniques (investigations in Denmark, France, Germany and the Netherlands-although no details are available for the detection in Spain).

From a current perspective, there seems to be no evidence of any persistent and confirmed groundwater contamination with glyphosate or AMPA. In many cases, detections occur in isolated samples rather than consistently at the same sampling site. Where the necessary information is available, it is frequently shown that glyphosate detections are only observed in shallow groundwater (e.g. Denmark and the Netherlands) or wells with direct surface water influence, where the surface water contaminated groundwater.

Reports from some countries stated that groundwater contamination with glyphosate and AMPA was not of concern, e.g. Belgium-Wallonia, Finland, Norway, the Czech and Slovak Republics, nor does it seem to be an important issue in the Netherlands. Some countries have reduced or discontinued glyphosate monitoring in groundwater as a result of special investigations or routine monitoring, where it was rarely found, e.g. Austria, Belgium-Flanders, Baden-Württemberg (Germany), Finland, Ireland, Malta, Norway, Sweden and the UK. Portugal decided on the basis of risk assessments that it was not necessary to monitor glyphosate and AMPA.

III. CONCLUSION

Ground and surface water monitoring data were gathered from 17 European countries, 16 countries plus the Danube River Basin for surface water, and 15 countries for groundwater, with most countries including both.

Glyphosate and AMPA have been extensively monitored and frequently detected in surface water above the 0.1 µg/L drinking water standard (21 % of the samples for glyphosate and 39 % for AMPA), but typically below the proposed environmental quality standards or objectives (ecotoxicologically relevant concentration).

In groundwater, glyphosate and AMPA have been increasingly monitored and occasionally detected above the 0.1 µg/L limit (0.6 % of the analysed samples for glyphosate and 0.8 % for AMPA). From a current perspective, there seems to be no evidence of any persistent and confirmed groundwater contamination with glyphosate or AMPA.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides an overview on monitoring data (up to 2015) for groundwater and surface water from 15 and 17 European countries, respectively. No specific guideline is applicable to this data point.
The study is therefore considered valid.

Assessment and conclusion by RMS:

Existing studies/assessments

1. Information on the study

Data point:	CA 7.5/011 CA 7.5/012 (Translation)
Report author	Anonymous
Report year	2012
Report title	Analysis of groundwater contamination with glyphosate/AMPA
Report No	GDY/MGI/10332_Rapport_final_V5
Document No	
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (no experimental work performed)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The annual reports of the French Environmental Institute (Institut Français de l'Environnement, Ifen) monitoring the plant protection products in the French waters mentioned the detection of glyphosate and its degradation product, AMPA, above 0.1 µg/L in several groundwater sampling sites. A selection of 27 sites for further investigation was performed based on the information available in the ADES database

(Accès au Données sur les Eaux Souterraines – access to groundwater data) at the start of the project (April 2010). The sites were selected based on the following criteria:

- Sites with multiple glyphosate detections
- Sites with glyphosate and AMPA detection,
- Priority wells as defined by the WFD (Water Framework Directive) with at least one glyphosate detection
- Detection of glyphosate without subsequent confirmatory analysis after the detection.

Two sites were rejected early in the study, due to their low vulnerability (confined water body) suggesting that the reported detects were not reliable. An in-depth investigation was conducted on the remaining 25 wells to verify the analytical data and the site vulnerability. Based on the information gathered during the investigation, a confidence (reliability) index related to the glyphosate/AMPA detect was estimated for each site.

Eight different labs were involved in the analysis of the samples and in most cases the analytical method included a direct FMOC-Cl derivatization (no or limited sample clean-up) followed by HPLC quantification with either fluorescence or MS detection. Of the 25 sites, 19 reported a single detect of glyphosate and out of those, 16 had follow-up analyses <0.1 µg/L reported in the ADES database since the start of the project, whilst no analysis were available after the detection for three sites. Six sites reported multiple detects: two sites (used for drinking water supply) showed two detects the same year, but samplings the years after showed glyphosate results <0.1 µg/L. The four other sites with multiple detects had no well protection, and were not suited for groundwater monitoring (fire well, piezometer, spring, private well).

The geological investigation showed that the groundwater vulnerability of 21 out of the 25 sites was high to very high and the detection of numerous other plant protection products were observed in several wells. Overall, the detects from seven wells have been given an estimated confidence index ≥8 (very high confidence that the detect is real): five of these are sites with multiple detects, one is a private well with no well protection and one is a WFD priority site (spring in karstic soil, shallow water which showed many detects of plant protection products including one isolated glyphosate detect >0.1 µg/L).

None of the detects could be attributed to long-term contamination of typical groundwater. The majority of detections occurred once only, which is a clear indication that there is no real groundwater contamination, and the small number of multiple detections occurred in shallow groundwater (spring water) or wells unsuitable for groundwater monitoring, suggesting superficial short-term contamination.

I. MATERIAL AND METHODS

The investigation of the groundwater contamination with glyphosate/AMPA included three phases:

- Phase 1: Survey on the sampling and analysis conditions: first, the parties organizing the analyses were contacted with a view of identifying the laboratories that performed the analyses with glyphosate/AMPA detections. After identification, those laboratories were contacted in order to identify the various elements of the sampling and analysis process.
- Phase 2: Investigation of the wells and their environment:
  - The hydrogeological characteristics of each well is described mainly on the basis of available information from existing databases. The collected elements were later corroborated during site visits. The various researched data included the use of the well, the existence of a well protection (water supply well), water yield, depth of the works and, if possible, groundwater level, the geology at the well and in its proximity. The analysis of these elements enabled the assessment of the "hydrogeological vulnerability of the well".
  - The analysis of the soil characteristics was performed based on soil cores taken within 1 km around the well within the boundaries of the catchment area. Therefore, three soil cores were taken at each site. When the topographic features allowed it (slopes, break of slope, deep ditch), a soil profile study was performed, in order to describe the existing soils in a more detailed manner. The collected soil information included the soil texture, colour of the

different horizons, soil depth, depth of rock weathering layer occurrence and depth of occurrence for the parent rock itself, load of coarse elements, hydromorphy, organic matter content, and characteristics of the underlying geological layer.

- Within 1 km around the well and depending on the well catchment area, the land use was surveyed for the following elements:
  - agricultural area: crop type for each plot (when the plot is ploughed and where possible, the previous crop was identified on the basis of crop residues);
  - non-agricultural area: residential areas, industrial and commercial areas, road infrastructure. Any development likely to contribute to groundwater contamination through glyphosate use.

For each well, a cartographic representation on an orthophoto base was prepared, comprising the above elements.

- Phase 3: Summary of data and definition of a confidence (or reliability) index related to groundwater glyphosate/AMPA contamination. This index is a crossing between the risk of groundwater contamination by glyphosate (based on land (glyphosate potential) use and site/aquifer vulnerability) and the characteristics of the analyses performed (laboratory, method, detection frequency, presence of other plant protection products, nitrates, coliforms). It does not take into account the inherent physico-chememical properties of glyphosate and its metabolite AMPA. A reliability index from 1 to 10 (low to high confidence) has been estimated for each site.

II. RESULTS AND DISCUSSION

The table below provides information on the method used by laboratories that performed the glyphosate and AMPA analyses, as well as the number of sites covered by the lab.

Table 7.5-51: Methods of analyses

Laboratory	Clean-up step	Derivatization	Detection	Quantification	Number of sites
IPL (Maxeville)	Concentration and clean-up on FPC cartridge	FMOC-Cl	LC/MS/MS	Internal standard (cysteic acid)	13
SGS laboratory	Acidification and sample concentration	O-Phthalaldehyde	HPLC - Fluorescence	External standard	1
CAR (Illkirch)	Sample concentration and acidification	O-Phthalaldehyde	HPLC - Fluorescence	External standard	1
IPL (Lille)	No	FMOC-Cl	LC/MS/MS	Internal standard ($^{13}C^{15}N$ glyphosate)	2
LASAT (La Rochelle)	No	FMOC-Cl	HPLC - Fluorescence	External standard	1
ASPOSAN (Montbonnot)	No	FMOC-Cl	HPLC - Fluorescence	External standard	2
LD26 (Valence)	No	FMOC-Cl	HPLC - Fluorescence	External standard	4
Labo des Pyrénées (Lagor)	Concentration after derivatization	FMOC-Cl	HPLC - Fluorescence	External standard	1

The most reliable methods are the ones involving LC/MS/MS with an internal standard, which is the case for the IPL laboratories (Lille and Maxeville). However, the laboratory from Maxeville has only been acquired by IPL in 2008, and the analytical method previously used may not have been the one described in the table above.

The quantification limit of most of the analytical methods was at 0.1 µg/L; suggesting that a relatively high margin of error will be associated to detects close to this value (below 0.15 µg/L).

The table below summarizes the results of the investigation for the 27 wells.

Table 7.5-52: Investigation results

Site (use of the well)	Well water table (m)	Well depth (m)	Findings	Results investigation	Confidence index[1]
Sites rejected (not investigated)					
Lureuil	81	-	0.14 µg/L glyphosate (04/2008)	Confined aquifer- low vulnerability	low
Sarreinseming	256	-	0.23 µg/L glyphosate/2 µg/L AMPA (04/2008)	Low vulnerability	low
Sites with single glyphosate detects (no AMPA) (2007-2008)					
Nort sur Erdre (DW supply)	63	up to 8 m	0.17 µg/L glyphosate (06/2007) 15 subsequent analyses <0.1 µg/L(2007-2010)	Highly vulnerable water body – permeable soils - Numerous pesticides>0.1 µg/L + nitrates >50 mg/L Glyphosate use likely	high
Avant-les-Ramerupt (DW supply)	50	Up to 10.7 m	0.15 µg/L glyphosate (10/2007) 2 subsequent analyses <0.1 µg/L (2010)	Highly vulnerable water body – permeable soils Some pesticides >0.1 µg/L Glyphosate use likely	high
Boissy le Repos (Petroleum research)	64	-	0.3 µg/L glyphosate (04/2007) Single analysis	Water body of medium vulnerability - No well protection Glyphosate use unlikely	low
Bouy (DW supply)	28	up to 8.3 m	0.13 µg/L glyphosate (10/2007) 4 subsequent analyses <0.1 µg/L (2009-2010)	Water body of medium to high vulnerability – permeable soils Glyphosate use likely	medium
Vernoy (DW supply)	6.6	up to 0.9 m	0.27 µg/L glyphosate (10/2007) Analysis 2 days later : <0.1 µg/L 21 subsequent analyses <0.1 µg/L (2008-2010)	Highly vulnerable water body Numerous pesticides>0.1 µg/L – regular detection of coliforms Glyphosate use likely	high
Tonnay Charente (DW supply)	9	up to 6.2 m	0.19 µg/L glyphosate (11/2007) No subsequent analysis	Highly vulnerable water body and permeable soils – nitrates > 50 mg/L Glyphosate use likely	high
Castagnède (DW supply)	-	-	1.19 µg/L glyphosate (06/2007) One subsequent analysis <0.1 µg/L (2008)	Highly vulnerable water body (possible contact with surface water) – no other pesticide detects. – permeable soils Glyphosate use unlikely	low

Table 7.5-52: Investigation results

Site (use of the well)	Well water table (m)	Well depth (m)	Findings	Results investigation	Confidence index[1]
Sites with single glyphosate detects(no AMPA) (2007-2008)					
La Chapelle Agnon (DW supply)	-	-	0.21 µg/L glyphosate (10/2008) Four subsequent analyses <0.1 µg/L (2009-2010)	Water body of average to high vulnerability – permeable soils -no other pesticides detected Glyphosate use unlikely	low
La Roche Noire (DW supply)	-	-	0.12 µg/L glyphosate (10/2008) Three subsequent analyses <0.1 µg/L	Highly vulnerable water body (possible contact with surface water) – permeable soils - one other pesticide detect Glyphosate use possible	low
Saint Cyr sous Dourdan (DW supply)	88	up to 3 m	2.06 µg/L glyphosate (09/2007) One subsequent analysis (2009) <0.1 µg/L	Highly vulnerable water body – no other pesticide detected >0.1 µg/L Glyphosate use possible	medium
Houvin-Houvigneul (DW supply)	54.5	up to 35.1 m	0.3 µg/L glyphosate (11/2007) One subsequent analysis (2010) <0.1 µg/L	Highly vulnerable water body – no other pesticide detected >0.1 µg/L Glyphosate use likely	high
Aubignan (Private well - Qualitometer)	7	-	0.2 µg/L glyphosate (10/2007) No subsequent analysis	Highly vulnerable water body – permeable soils - few other pesticide detected >0.1 µg/L Glyphosate use likely No well protection	high
Grosne (DW supply)	5.5	-	0.12 µg/L glyphosate (12/2007) One subsequent analysis <0.1 µg/L (2009)	Water body of medium vulnerability – no other pesticide detected Glyphosate use possible	low
Issans (DW supply)	5.2 m (spring)		0.2 µg/l glyphosate (08/2007) 3 subsequent analyses <0.1 µg/L	Highly vulnerable water body – many other pesticides detected – permeable soils Glyphosate use likely	very high
Villers-Farlay (DW supply)	-	-	0.31 µg/L glyphosate (07/2007) Four subsequent analyses <0.1 µg/L (2010)	Highly vulnerable water body – many other pesticides detected – permeable soils Glyphosate use likely	high
Machecoul (DW supply)	-	-	0.13 µg/L glyphosate (06/2007) 20 subsequent analyses <0.1 µg/L	Highly vulnerable water body – many other pesticides detected – very permeable soils Glyphosate use likely	high

Table 7.5-52: Investigation results

Site (use of the well)	Well water table (m)	Well depth (m)	Findings	Results investigation	Confidence index[1]
Sites with simultaneous glyphosate and AMPA single detects (2007-2008)					
Saint Georges d'Esperanche (DW supply)	56	up to 34.7 m	0.22 µg/L glyphosate (01/2008) 0.22 µg/L AMPA (01/2008) 14 subsequent analyses <0.1 µg/L (2008-2010)	Water body of medium to high vulnerability – few other pesticides detected – very permeable soils Glyphosate use likely	high
Monteynard (DW supply)	-	-	0.3 µg/L glyphosate (01/2008) 0.14 µg/L AMPA (01/2008) Two subsequent analyses <0.1 µg/L (2009-2010)	Highly vulnerable water body – no other pesticide detected >0.1 µg/L Glyphosate use unlikely	low
La Flotte (private well)	20	up to 8m	0.21 µg/L glyphosate (09/2008) 0.19 µg/L AMPA (09/2008) Four subsequent analyses <0.1 µg/L (2009-2010)	Water body of medium to high vulnerability – very permeable soils Glyphosate use very likely No well protection area	very high
Sites with multiple detects					
Chepy (fire well)	4	-	0.56 µg/L glyphosate (09/2007) 3.4 µg/L AMPA (09/2007) 2.36 µg/L AMPA (10/2008) 0.63 µg/L glyphosate (04/2009) 0.21 µg/l AMPA (0.4/2009) 0.63 µg/L glyphosate (10/2010) 1.04 µg/L AMPA (10/2010)	Very highly vulnerable water body – many other pesticides detected – nitrates >50 mg/L Glyphosate use very likely No well protection area	very high
Corbeilles (piezometer)	19	up to 9 m	0.254 µg/L glyphosate (05/2007) 0.14 µg/L glyphosate (10/2010) 0.19 µg/L glyphosate (12/2010) Regular AMPA detects 2007-2010 (average 0.2 µg/L)	Very highly vulnerable water body – many other pesticides detected – nitrates >50 mg/L Glyphosate use	very high
Blanzay (DW supply)	60	up to 9.5 m	0.2 µg/L glyphosate (10/2008) 0.5 µg/L glyphosate (12/2008) Nine subsequent analysis <0.1 µg/L (2009-2010)	Highly vulnerable water body – few other pesticides detected – nitrates >50 mg/L Glyphosate use likely	very high
Fontenay le Pesnel (DW supply)	3.8	up to 2 m	0.137 µg/L glyphosate 04/2007) 12.9 µg/L glyphosate (10/2007) 0.92 µg/L AMPA (10/2007) Six subsequent analyses <0.1 µg/L (2008-2010)	Highly vulnerable water body – few other pesticides detected Glyphosate use likely	high
Evans (Qualitometer)	spring	-	0.16 µg/L glyphosate (07/2008) 0.95 µg/L AMPA (07/2008)	Highly vulnerable water body – many other pesticides detected Glyphosate use likely	very high

Table 7.5-52: Investigation results

Site (use of the well)	Well water table (m)	Well depth (m)	Findings	Results investigation	Confidence index[1]
			0.1 µg/L glyphsoate (07/2010) 1.1 µg/L AMPA (07/2010) 0.24 µg/L glyphosate (05/2010) 0.73 µg/L AMPA (05/2010)		
Avrille (private use)	6.5	up to 1.65 m	0.68 µg/L glyphosate (05/2007) 0.3 µg/L glyphosate (03/2008) AMPA regularly detected – average 0.41 µg/L (1007-2010)	Highly vulnerable water body – many other pesticides detected – nitrates >50 mg/L permeable soils Glyphosate use very likely	very high

[1] This index provides the level of confidence in the reported detects, based on the vulnerability of the aquifer, soil permeability, land (glyphosate) use in the area, analytical method and detection frequency. It does not take into account the inherent phys-chem properties (and thus the low leaching potential) of glyphosate and AMPA.

Two sites were rejected early in the process due to their low vulnerability and no further investigation was performed. Out of the 25 remaining sites, 16 showed a single detect of glyphosate (without AMPA) and three showed a single detect for both glyphosate and AMPA. With the exception of three sites at which no subsequent glyphosate analysis were performed after the detect, all other sites had samples analyzed within the months/years after the detect showing glyphosate/AMPA results <0.1 µg/L, demonstrating that the contamination was not widespread in the aquifer and not long-term.

Multiple detects were observed in six sites, two of which were used for drinking water supply. At those sites, the detects occurred the same year, and the analyses performed the following years showed no further contamination by glyphosate and AMPA. The four other sites with multiple detects had no well protection area, and were not suited for drinking water supply (private or fire well, piezometer, qualitometer, spring).

The estimated confidence index was very high for seven of the 25: five of those are sites with multiple detects, one is a private well with no well protection area, and one is a site used for DW supply (spring in a karstic soil, shallow water which showed many detects of plant protection products including one isolated detects of glyphosate).

The estimated confidence index was low for six of the 25 sites: in those areas the use of glyphosate was questionable.

III. CONCLUSION

In an attempt to investigate the presence of glyphosate/AMPA in groundwater, a selection of 27 groundwater abstraction sites was evaluated. Two sites were rejected early in the process due to their low vulnerability, suggesting that the reported detect was not accurate.

An in-depth investigation was performed on the 25 remaining sites. The results show that in 76 % of the cases, the detections were sporadic (one sample of several analysis), demonstrating that the contamination was not widespread in the aquifer. Four sites showed a more serious contamination, with multiple detects over the years, but none of these are used for drinking water supply and none had a well protection area.
In summary, none of the glyphosate detects could be attributed to long-term contamination of typical groundwater.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study elucidates findings of glyphosate and AMPA in groundwater in France. The methods and results are sufficiently described. The study is therefore considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/013
Report author	[redacted]
Report year	2012
Report title	Survey of glyphosate and AMPA in groundwaters and surface waters in Europe
Report No	-
Document No	BVL No. 2310291
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (no experimental work performed)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
This review is based on an earlier review carried out in 2009, which has been updated to include the latest available information. The review covers glyphosate and AMPA monitoring results for surface (fresh) waters and groundwater across Europe, *i.e.* all 27 Member States of the European Union, as well as Norway and Switzerland, where available.

Information has been obtained from professional contacts across Europe (government departments and research organisations), and including some data provided by Monsanto Europe, as well as from web and literature searches, and by querying on line databases. Some data from the previous review has been omitted where more up to date information has become available.

Additional data has been obtained for twelve countries, *i.e.* Belgium, the Czech Republic, Denmark, France, Ireland, Italy, the Slovak Republic, Spain, Sweden, the Netherlands and the UK. In total, there is data for 17 countries, 14 each for surface water and groundwater, with most countries including both.
<u>Surface water</u>
Glyphosate and AMPA have been frequently detected in surface waters, AMPA usually at higher concentrations and in a larger proportion of samples. Glyphosate has been analysed in almost 75 000 samples from about 4 000 sites (from 1993-2011) and detected in 33 % of samples, with 23 % above

0.1 µg/L; AMPA has been analysed in about 56 700 samples from nearly 3 000 sites (1997-2011) and detected in 54 % of samples, with 46 % above 0.1 µg/L. The more persistent presence of AMPA in surface waters throughout the year may be mainly derived from aminophosphonate containing complexing agents in industrial and household detergents and in cooling waters, entering surface waters via wastewater treatment effluents, rather than from the degradation of glyphosate.

There have been some indications of an upward trend detections and concentrations found in recent years, e.g. in Belgium – Flanders and the Netherlands (not confirmed in recent years, and the opposite in NL - Flevoland), but the data may not be adequate to conclude on trends and coincides with higher numbers of sites and samples analysed.

Where data allowed interpretation, glyphosate has been linked to application periods (from spring through to autumn) and run-off events and does not seem to persist.

Groundwater

Glyphosate and AMPA have been increasingly analysed and occasionally detected in groundwater**.** Glyphosate has been analysed in almost 67 000 samples from about 675 sites (1993-2010) and detected in 1 % of samples, with 0.6 % above 0.1 µg/L; AMPA has been analysed in 52 000 samples from 1 345 sites (1993-2011) and detected in 2.6 % of samples, with 0.8 % above 0.1 µg/L. These seem to occur in shallow water or spring water, which is often included in groundwater surveys, sometimes associated with contamination incidents (where the information is available), and even unsuitable sampling sites and analytical techniques (investigations in France and Germany).

To date, there seems to be no evidence of any persistent and confirmed groundwater contamination with glyphosate or AMPA. In many cases detections occur in isolated samples rather than consistently at the same sampling site. Where the necessary information is available, it is frequently shown that glyphosate detections are only observed in shallow groundwater (e.g. Denmark and the Netherlands) or wells with surface water influence, for example contamination.

I. MATERIAL AND METHODS

This investigation is a desk study, and the information was obtained from professional contacts across Europe (government departments and research organisations in each of the countries), and including some data provided by Monsanto Europe, as well as from web and literature searches, and by querying on line databases.

II. RESULTS AND DISCUSSION

Table 7.5-53 and Table 7.5-54 provide an overview of the main data for surface water and groundwater, respectively. The summarised data is not precise but presents a best estimate, mainly because of the various forms in which the data were obtained, *e.g.* some results in terms of samples, others in terms of sites, and other gaps in information.

In total, there is data for 17 countries, 14 each for surface water and groundwater, with most countries including both. However, the Czech and Slovak Republics monitor only surface water, and data for Spain was available for surface water only; for Malta and Switzerland only groundwater data was obtained. Data were mainly collated at national level, but in some cases regional, as for Belgium (two regions), Italy (one region), and Germany (surface water data for several Länder). Seven countries have confirmed that there is no monitoring of glyphosate and AMPA (Bulgaria, Cyprus, Hungary, Latvia, Lithuania, Luxembourg, Romania), no information was obtained from the remaining five countries (Estonia, Greece, Poland, Portugal and Slovenia).

Surface water

Table 7.5-53: Summary of glyphosate and AMPA data in surface water in Europe

Country / Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
Austria									
AMPA	2001-02	-[1)]	345	≥90	≥26	90	26	3.4	-[1]
Belgium (Flanders - F and Wallonia - W)									
Glyphosate F	2007-11	198	5350	4450	83.2	1387	25.9	139	0.05-0.4
AMPA F	2007-11	198	5351	4967	92.8	3215	60.1	47	0.05-0.4
Glyphosate W	2001-06	26	531	≥ 429	≥ 81	429	81	1.3	≤ 0.1
Czech Republic									
Glyphosate	2010-11	41	359	168	47.8	96	28.7	5.3	0.025-0.05
AMPA	2010-11	9	165	165	100	138	83.6	1.37	0.05
Finland									
Glyphosate	2002-09	3	26	3	11.5	2	7.7	0.46	0.1
AMPA	2002-09	3	26	3	11.5	1	3.8	0.22	0.05
France									
Glyphosate	97-2009	≥2493	57171	17251	30.2	13655	23.9	50	0.03-0.2
AMPA	98-2009	≥2217	46969	24325	51.8	22062	47.0	48.9	0.02-0.5
Germany (Baden-Württemberg, Hessen, Rheinland-Pfalz, Thüringen & River Rhine combined)									
Glyphosate	97-2011	105	1298	386	29.7	96	7.4	4.7	0.02-1.5
AMPA	97-2011	66	782	571	57.5	514	65.7	3.6	0.05-0.5
Ireland									
Glyphosate	2005-11	256	2483	139	5.6	≥42	≥1.7	186	0.08-0.1/ 20
AMPA	2010-11	-[1)]	496	1	0.2	1	0.2	>200	20
Italy (Lombardia Region)									
Glyphosate	2005-08	150	919	224	24.3	224	24.3	37.6	0.1
AMPA	2008	59	239	208	87.0	208	87.0	37	0.1
Norway									
Glyphosate	97-06	11	80	74	92.5	≤ 57	≤ 71	0.93	(0.01)
AMPA	97-06	11	80	74	92.5	≤ 48	≤ 60	0.54	(0.01)
Slovak Republic									
Glyphosate	2006-10	142	2092	321	15.3	261	12.6	3.6	(0.05)
Spain [2]									
Glyphosate	2006-08	115	748	96	7.4	80	11	15.3	0.003-0.1
Sweden									
Glyphosate	2000-10	≥ 21	1306	360	27.6	≥15	≥1.1	370	<0.1
AMPA	2000-10	≥ 21	1285	244	19.0	≥14	≥1.1	4.0	<0.1
The Netherlands									
Glyphosate	2010	293	1349	254 (s)	87 (s)	198 (s)	68 (s)	>1.0	<0.1
AMPA	2010	293	1374	293 (s)	100 (s)	≥ 40 (s)	≥14 (s)	>8.0	-[1]
UK									
Glyphosate	93-2011	≥105	3730	759	20.3	759	20.3	8.2	0.1
Total									
Glyphosate	93-2011	≥3959	75350	≥24914	≥33	≥17301	≥23	1.3-370	0.003-1.5 (20)
AMPA	97-2011	≥2879	57112	≥30941	≥54	≥26331	≥46	0.22->200	0.02-0.5 (20)

LoQ = Limit of Quantification, LoD = Limit of Detection
[1] No information
[2] Data from sites with known quality problems
(s) Sites (number of samples not known)

Glyphosate has been analysed in almost 75 000 surface water samples from about 4 000 sites (from 1993-2011) and detected in 33 % of samples, with 23 % above 0.1 µg/L; AMPA has been analysed in about 56 700 samples from nearly 3 000 sites (1997-2011) and detected in 54 % of samples, with 46 % above 0.1 µg/L.

Glyphosate has a high usage rate and has been rated among the most frequently detected herbicides in some countries, notably in the Netherlands. It has been suggested that urban run-off can be a significant source of glyphosate in surface waters (France and the Netherlands). There have been some indications of an upward trend detections and concentrations found in recent years, e.g. in Belgium – Flanders and the Netherlands (not confirmed in recent years, and the opposite in NL - Flevoland), but the data may not be adequate to conclude on trends and coincides with higher numbers of sites and samples analysed.

Some countries have proposed various environmental quality standards (EQS) or objectives for glyphosate in surface water, ranging from a proposed EQS of 28 µg/L in Mecklenburg-Vorpommern (Germany) and 60 µg/L in Ireland (now accepted), to a proposed no effect concentration (PNEC) of 10 µg/L and a maximum admissible concentration (MAC) of 100 µg/L in Belgium - Flanders, and a Maximum Tolerable Risk (MTR) standard at 77 µg/L, and a pesticide authorisation standard of 64 µg/L in the Netherlands, whilst some professionals (Belgium – Wallonia and Rheinland-Pfalz (Germany) suggested that an EQS should be set. However, none of these standards have been exceeded on a regular basis. Perhaps more importantly, the Netherlands apply the drinking water standard of 0.1 µg/L for pesticides to surface water intakes at waterworks, and LAWA in Germany has set a target value of 0.1 µg/L for the same purpose.

Where data allowed interpretation, glyphosate has been linked to application periods (from spring through to autumn) and run-off events and does not seem to persist. The more persistent presence of AMPA in surface waters throughout the year may be mainly derived from aminophosphonate containing complexing agents in detergents and cooling waters, entering surface waters via wastewater treatment effluents, rather than from the degradation of glyphosate. This seems to be a fairly widely accepted view now, with more evidence having become available, and might explain why AMPA is not always found at higher concentrations, as for example in Sweden, where the population density is low.

Groundwater

Table 7.5-54: Summary of glyphosate and AMPA data in groundwater in Europe

Country / Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. Conc.	LoQ (LoD)
				No.	%	No.	%	µg/L	µg/L
Austria									
Glyphosate	2004	~950	3633	7	0.19	2	0.06	>0.1	<0.1
AMPA	2004	~950	3636	44	1.2	11	0.3	0.75	<0.1
Belgium (Flanders & Wallonia)									
Glyphosate (Flanders)	2007-08	450	1088	1	0.1	0	nr	0.011	0.01
AMPA (Flanders)	2007-11	504	3933	707	18	-[1]	nr	1.85	0.01
Glyphosate (Wallonia)	2000-06	450	≥450	0	nr	0	nr	<0.025	<0.025
AMPA (Wallonia)	2000-06	450	≥450	13 (s)	3 (s)	0	nr	< 0.05	<0.025
Denmark									
Glyphosate	1993-10	1825	9908	117	1.2	21	0.21	4.7	(0.01-<0.1)
AMPA	1993-10	1840	9906	84	0.84	18	0.18	4.2	(0.01-<0.1)
Finland									
Glyphosate	2002-08	80	80	0	nr	0	nr	nr	0.1
AMPA	2002-08	80	80	0	nr	0	nr	nr	0.05
France									
Glyphosate	99-09	≥7403	45960	515	1.1	390	0.8	24	0.01-0.1
AMPA	99-09	≥7184	30529	442	1.4	321	1.1	19	0.01-0.1
Germany									
Glyphosate	2007	196	≥ 196	7 (s)	3.6 (s)	0	nr	≤ 0.1	<0.1
AMPA	2007	326	≥ 326	10 (s)	3.1 (s)	5	1.5	≥ 1	-[1]
Ireland									
Glyphosate	2007-09	92	679	6	0.8	1	0.1	0.19	<0.1
Italy (Lombardia Region)									
Glyphosate	2005-08	359	961	0	nr	0	nr	<0.1	0.1
AMPA	2007-08	359	≥619	3	≤0.5	3	≤0.5	0.9	0.1
Malta									
Glyphosate	2009	18	≥ 18	0	nr	0	nr	nr	(0.01)
Norway									
Glyphosate	99-00	7	8	0	nr	0	nr	nr	(0.01)
AMPA	99-00	7	8	1	12.5	0	nr	0.02	(0.01)
Sweden									
Glyphosate	2009-10	≥ 21	1247	1	0.08	0	nr	0.04	(<0.03)
AMPA	2009-10	≥ 21	1242	3	0.24	1	0.08	0.72	(<0.03)
Switzerland									
Glyphosate	2005-06	117	≥ 234	4 (s)	3.4	3 (s)	2.6 (s)	0.21	(0.05)
AMPA	2005-06	117	≥ 232	≥ 10 ≤ 17 (s)	≥ 9 ≤ 14 (s)	≥ 6 ≤ 11 (s)	≥ 5 ≤ 9 (s)	0.46	(0.05)
The Netherlands									
Glyphosate	2003-06	<691	691	4	0.58	4	0.58	4.7	(<0.1)
AMPA	2003-06	<691	691	21	3.0	21	3.0	5.1	(<0.1)
UK									
Glyphosate	95-07	≥ 217	1509	13	0.9	≤ 3	≤ 0.2	0.47	(0.014-0.4)
Total									
Glyphosate	93-2010	12876	≥66662	675	1.0	424	0.64	0.01-24	0.01-0.4
AMPA	93-2011	12525	≥51652	1345	2.6	398	0.77	0.02-19	0.01-0.1

LoQ = Limit of Quantification, LoD = Limit of Detection
[1] No information
(s) Sites (number of samples not known)
nr = Not relevant

Glyphosate and AMPA have been increasingly analysed and occasionally detected in groundwater. Glyphosate has been analysed in almost 67 000 samples from about 675 sites (1993-2010) and detected in 1 % of samples, with 0.6 % above 0.1 µg/L; AMPA has been analysed in 52 000 samples from 1 345 sites (1993-2011) and detected in 2.6 % of samples, with 0.8 % above 0.1 µg/L. These seem to occur in shallow water or spring water, which is often included in groundwater surveys, sometimes associated with contamination incidents (where the information is available), and even unsuitable sampling sites and analytical techniques (investigations in France and Germany).

To date, there seems to be no evidence of any persistent and confirmed groundwater contamination with glyphosate or AMPA. In many cases detections occur in isolated samples rather than consistently at the same sampling site. Where the necessary information is available, it is frequently shown that glyphosate detections are only observed in shallow groundwater (e.g. Denmark and the Netherlands) or wells with surface water influence, for example contamination.

Reports from some countries stated that groundwater contamination with glyphosate and AMPA was not of concern, e.g. Belgium – Wallonia, Finland, Norway, the Czech and Slovak Republics. Some countries have reduced or abandoned glyphosate monitoring in groundwater as a result of special investigations or routine monitoring, where it was rarely found, e.g. Austria, Belgium – Flanders, Baden-Württemberg (Germany), Italy, Sweden and the UK.

Whereas an increase in glyphosate detection and/or concentrations before 2009 in Denmark was indicated, a decrease was reported in 2010. It would need further data and statistical analyses before any conclusions can be drawn.

III. CONCLUSION

Ground and surface water monitoring data were gathered from 17 European countries, 14 each for surface and groundwater, with most countries including both. Glyphosate and AMPA have been extensively monitored and frequently detected in surface water above the 0.1 µg/L drinking water standard (23 % of the samples for glyphosate and 46 % for AMPA), but typically below the proposed environmental quality standards or objectives (ecotoxicologically relevant concentration). In groundwater, glyphosate and AMPA have been increasingly monitored and occasionally detected above the 0.1 µg/L limit (0.6 % of the analyzed samples for glyphosate and 0.8 % for AMPA). To date, there seems to be no evidence of any persistent and confirmed groundwater contamination with glyphosate or AMPA.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study compiles drinking water quality data (up to 2012) for glyphosate and AMPA from national authorities in Europe. The methods and results are sufficiently described.
The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/014
Report author	[redacted]
Report year	2006
Report title	Clarification of well-related findings of glyphosate and AMPA in groundwater
Report No	IF-06/00603024
Document No	BVL No. 2310282
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The Federal Office of Consumer Protection and Food Safety (BVL) requested registration owners of glyphosate containing plant protection products to investigate the causes of findings ≥0.1 µg/L of glyphosate and its metabolite AMPA in the groundwater, which were reported from monitoring points in Bavaria, Baden-Wuerttemberg and Hessen since 2003. Glyphosate was found only at 5 wells, while the metabolite AMPA appeared at 21 locations. A detailed investigation was conducted, during which available information about the wells and findings were gathered and evaluated, eventually followed by a site or laboratory visit. This study has furnished a plausible explanation of the origin of glyphosate or AMPA findings for all 24 reported locations. The findings can be classified in groups of causes:

Five wells showed inflow of surface water or bank filtrate and one well was affected by a waste deposit. In one case the well was located inside a sewage plant and showed the influence of the waste water .In another site the sample was contaminated at the well which serves as a processing water well for a tank filling place. The 16 findings reported from Hessen were due to an analysis which was obviously deficient.

I. MATERIAL AND METHODS

The clarification of the reported glyphosate and AMPA findings was done in stepwise procedure. In a first step all easily accessible information was requested from the responsible authorities. Then it was checked within a pre-evaluation, if on this basis definite conclusions regarding the validity and origin of the finding are possible. Provided other causes than the normal and proper use of the active ingredient or the origin of the findings were obvious, the investigations were terminated. For findings, where this was not the case, a detailed investigation was conducted. For this purpose relevant data with regard to technical, hydrogeological information were gathered and local authorities or the owners of the wells were contacted. Then a site inspection was conducted and if possible an interview with persons who are able to contribute to the clarification, as farmers, well operators etc. was performed. In an additional step information on the analytical details were queried from the laboratories.

II. RESULTS AND DISCUSSION

The table below provides an overview of the findings and the result of the assessment

Table 7.5-55: Overview on findings of glyphosate/AMPA and results of the assessments

Well	Finding glyphosate [µg/L] (year)	Finding AMPA [µg/L] (year)	Cause of the finding
Bavaria			
Sulzbach	0.25 (2003) 0.25 (2004)	–	well contamination by surface water
Woelsbach	B18: 0.16 (2004) B26: 0.12 (2004)	–	influence from a waste deposit
Escherndorf	0.06 (2003)	0.20 (2003)	sample contamination
Bamberg (Luisenhain FB1 / FB2 and Gereuth FB9)	FB1: 0.16, FB2 0.12 FB9 0.32 (2001)	–	bank filtrate
Hessen			
Meineringhausen	–	0.11 (2004)	analysis not valid
Muehlenberg	–	0.16 (2004)	analysis not valid
Battenberg B2	–	0.11 (2004)	analysis not valid
Ronshausen	–	0.11 (2004)	analysis not valid
Schoenberg	–	0.14 (2004) 0.16 (2005)	analysis not valid
Bicken	–	0.10 (2004)	analysis not valid
Kleinlueder	–	0.18 (2005)	analysis not valid
Spring Weiher (Ober-Hoegern)	–	0.16 (2004)	analysis not valid
B5 (Ober-Hoegern)	–	0.13 (2004)	analysis not valid
BUGA (Praunheim III)	–	0.18 (2004)	analysis not valid
Geisenheim	–	0.14 (2004)	analysis not valid
Niederrad I	–	0.40 (2004)	analysis not valid
Walldorf	–	0.12 (2004)	analysis not valid
Messenhausen	–	0.11 (2004)	analysis not valid
Seeheim-Jugenheim	–	0.10 (2004	analysis not valid
Viernheim	–	0.10 (2004)	analysis not valid
Baden-Wuerttemberg			
Riesbuerg-Pflaumloch	0.17 (2002) 0.08 (2003)	0.5 (2002) 0.27 (2003)	wastewater influence from a sewage plant
Laufenburg	–	0.15 (2002) 0.12 (2003)	bank filtrate
Laudenbach	–	0.18 (2002) 0.06 (2003)	surface water inflow
Weinheim	–	0.11 (2002) 0.15 (2003)	deficient monitoring well quality / contamination by surface or sewage water

III. CONCLUSION

The detailed investigation has resulted in plausible explanations of the origin of the glyphosate and AMPA findings at the 24 locations. In all cases, if the analysis was not a false positive, the origin of the glyphosate and/or AMPA concentrations could be allocated to surface or waste water influences. There was not a single case for which the findings could be correlated with the normal and proper use of the active ingredient in the field.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study elucidates findings of glyphosate and AMPA in German groundwater wells. The methods and results are sufficiently described.
The study was seen as valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/015
Report author	[redacted]
Report year	2005
Report title	An investigation of reported borehole contamination in the Vemmenhög Catchment, Sweden
Report No	-
Document No	BVL No. 2310285
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (no experimental work performed)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
Glyphosate was detected in 2 experimental boreholes between August 2004 and February 2005 in the Vemmenhög catchment in Southern Sweden. In the first well, to the North East of the catchment, the concentration reached 0.045 µg/L whilst in the second well located in the Center/West of the catchment, glyphosate was detected at 0.18 and 0.035 µg/L. A review of the regional characteristics showed that extensive drainage systems are in place in the catchment. The tile drains were placed at 1 m depth, although due to the undulated topography they end up in much deeper depth in some places. About 23 % of the catchment was treated with glyphosate and this included application to the immediate or near vicinity of the boreholes. The historical data review and observations at the site demonstrate that there can be potential for direct hydrological connectivity between surface water and shallow groundwater at about 0.7-4 m depth via artificial drainage systems.

I. MATERIAL AND METHODS

The clarification of the reported glyphosate and AMPA findings was done in stepwise procedure. In a first step all information on the characteristics of the watershed, water management, land and glyphosate use were gathered and evaluated. The second step involved an on-site investigation of the watershed, including inspection of the boreholes and evaluation of the farm management practices.

II. RESULTS AND DISCUSSION

The table below shows the glyphosate concentrations in water samples taken from 4 boreholes of the Vemmeshög catchment.

Table 7.5-56: Overview on findings of glyphosate in the Vemmeshög catchment

Sample date/location	Glyphosate residue in µg/L (LOD)	GW depth (m)	Date of adjacent glyphosate application
19/08/2004			
North/East 1	0.045 (0.03)	-3.27	29/10/2003
North/East 2	ND (0.03)	-3.03	
Center West 1	ND (0.03)	-2.14	05/07/2003
Center West 2	ND (0.03)	-2.12	
16/11/2004			
North/East 1	ND (0.02)	-3.24	No
North/East 2	ND (0.02)	-3.13	
Center West 1	0.18 (0.02)	-1.42	20/10/2004
Center West 2	ND (0.02)	-1.13	
09/02/2005			
North/East 1	ND (0.02)	-3.24	No
North/East 2	ND (0.02)	-2.89	
Center West 1	0.035 (0.02)	-1.51	20/10/2004
Center West 2	ND (0.02)	-1.23	

The investigation showed that groundwater often remains close or above field drain depth over the winter period, responding to recharge from excess rainfall. The field drainage in the catchment has been designed to rapidly remove excess water from the surface and also from rooting layers. Although the drains are generally installed at approximately 1-1.5 m depth, they may be as deep as 4 m to accommodate the undulated topography and maintain gradient requirements. There is thus a potential for direct hydrological connectivity between surface water and shallow groundwater.

III. CONCLUSION

The detailed investigation has evidenced a potential contact between ground and surface water through the drainage system in place at this watershed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study elucidates findings of glyphosate in two experimental groundwater boreholes in Sweden. The methods and results are sufficiently described.
The study is considered valid.

Assessment and conclusion by RMS:

Relevant literature articles

1. Information on the study

Data point:	CA 7.5/016
Report author	Rosenbom, A. *et al.*
Report year	2019
Report title	The Danish Pesticide Leaching Assessment Programme
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

In 1998, the Danish Parliament initiated the Pesticide Leaching Assessment Programme (PLAP), an intensive monitoring programme aimed at evaluating the leaching risk of pesticides and/or their degradation products (metabolites) under field conditions. The specific aim is to analyse whether pesticides applied in accordance with current regulations will result in leaching of the pesticide and/or its degradation products to groundwater in unacceptable concentrations.

This update on the previous PLAP monitoring covers glyphosate and AMPA monitoring results besides leaching data on other pesticides of five selected representative fields in Denmark. In the monitoring period July 2015-June 2017, the maximum allowed dose of the specific pesticide in connection with a specific crop was applied. Data collected in this period were summarised and incorporated in the results of the complete monitoring campaign. Additionally, data on bromide leaching, soil water dynamics and water balance were analysed within the report.

During the monitoring period July 2015-June 2017, glyphosate was applied at the fields of Silstrup, Estrup and Faardrup. Glyphosate and AMPA were analysed in 65 and 116 water samples collected from the variably-saturated Zone (VZ; drains and suction cups) and saturated Zone (SZ; groundwater screens), respectively. Glyphosate was detected in 12 samples from the VZ with no detection ≥0.1 µg/L and a maximum concentration of 0.05 µg/L. In samples collected from the SZ, glyphosate was detected three times with one detection ≥0.1 µg/L and a maximum concentration of 0.13 µg/L. AMPA was detected in 51 samples from the VZ with two detections ≥0.1 µg/L and a maximum concentration of 0.14 µg/L. In samples collected from the SZ, AMPA was detected two times with no detection ≥0.1 µg/L and a maximum concentration of 0.02 µg/L.

Data on the complete PLAP-monitoring period (when glyphosate was also applied at the two sandy soil sites before 2015) revealed a negligible leaching risk on the coarse sandy soil of Jyndevad, whereas evidence of glyphosate leaching was seen on clayey till soils. Glyphosate and AMPA leached at 1 m depth in average concentrations exceeding 0.1 µg/L within the first season after application at Silstrup and Estrup. They were detected in more than three consecutive samples or in a single sample in concentrations exceeding 0.1 µg/L and an average concentration (1 m depth) below 0.1 µg/L within the first season after application at Faardrup.

The numbers of detections exceeding 0.1 µg/L in groundwater monitoring wells is very limited. In groundwater, glyphosate and AMPA leached in a concentration exceeding 0.1 µg/L within the first season

after application at Estrup. At Jyndevad, Silstrup and Faardrup, glyphosate and AMPA were detected in more than three consecutive samples or in a single sample in concentration exceeding 0.1 µg/L and concentrations below 0.1 µg/L within the first season after application.

Materials and methods

The PLAP encompasses five fields that are representative for the dominant soil types and the climatic conditions in Denmark with shallow groundwater tables, which enable pesticide leaching to groundwater to be rapidly detected. Cultivation of the PLAP fields is done in accordance with conventional agricultural practice in the area and the maximum permitted dose of the pesticides are applied in the manner specified in the regulations. Characteristics of the five fields included in the monitoring for the period 1999-2017 are shown in the table below.

Table 7.5-57: Characteristics of the five PLAP fields included in the PLAP–monitoring for the period 1999–2017

Parameter	Tylstrup	Jyndevad	Silstrup	Estrup	Faardrup
Location	Brønderslev	Tinglev	Thisted	Askov	Slagelse
Precipitation[1] (mm y^{-1})	668	858	866	862	558
Pot. evapotransp.[1] (mm y^{-1})	552	555	564	543	585
Classification of top soil texture	Loamy sand	Sand	Sandy clay loam / sandy loam	Sandy loam	Sandy loam
Clay content (%)	6	5	18–26	10–20	14–15
Silt content (%)	13	4	27	20–27	25
Sand content (%)	78	88	8	50–65	57
pH	4–4.5	5.6–6.2	6.7–7	6.5–7.8	6.4–6.6
TOC (%)	2.0	1.8	2.2	1.7 – 7.3	1.4

[1] Yearly normal based on a time series for the period 1961–90. The data refer to precipitation measured 1.5 m above ground surface.

The report presents the results of the monitoring period July 2015 to June 2017 comprising analyses conducted on water samples collected at the five PLAP-fields. During this period, PLAP has evaluated the leaching risk of 6 pesticides and 18 degradation products after applying the maximum allowed dose of 13 specific pesticides in connection with a specific crop. Glyphosate and its degradation product AMPA were evaluated within this study. Besides pesticide leaching, the leaching of bromide as well as soil water dynamics and water balance were analysed. This data is not covered within this summary.

From each of the PLAP fields, samples were collected of groundwater, drainage water and soil water in the variably-saturated zone. Throughout the years, the sample collection interval of the monitoring study changed. Until March 2002, pesticide analysis was performed monthly on water samples from the suction cups, two screens of the horizontal monitoring wells and two of the downstream vertical monitoring wells. Every four month, all sample points were monitored. Pesticide analysis was also performed on drainage water samples.

Until 2012, the number of pesticide analyses was reduced. Monthly monitoring was restricted to one monitoring well. All samples points were monitored every six month (except for Tylstrup).

The drainage system was sampled time proportionally weekly until July 2004. Additional samples were analysed during storm events. From July 2004 and onwards pesticide analysis were done weekly on water sampled flow-proportionally from the drainage water system.

In 2011, new horizontal wells with three new horizontal screens were established. A horizontal well with three PE-screens (3 m long, separated by 1 m packer-section attached 0.8 m bentonite, slits of 0.1 mm) was installed September 2011 at all five PLAP-fields to optimize monitoring of the fields both in time and space. From these wells, water samples were collected monthly at the sandy fields (Tylstrup and Jyndevad). 3 L

were sampled from each filter via applying suction onto the two tubes. A half-litre of the 3 L was passed through cells in a flow box measuring pH, temperature and conductivity. The remaining 2.5 L was pooled with equal volumes from the two other filters. Subsamples for analysis were then taken from the 7.5 L pooled sample. At clayey till fields (Silstrup, Estrup and Faardrup), water samples were collected monthly if the groundwater table nearest vertical monitoring well was situated more than 20 cm above the screen. Having saturated conditions, one litre of water sample was collected from each screen via the two tubes during approximately 10 minutes. The litre sample was passes through cells in a flow box measuring pH, temperature and conductivity. The samples from each screens are then pooled and send for analysis.

LOD and LOQ of the detection of glyphosate and AMPA were not reported. Detailed analysis methods are described in Kjær *et al*. (2002).

Results and discussion

Glyphosate was not applied at Tylstrup or Jyndevad for the period of 2011-2017. The application of Glyphosate for Silstrup, Estrup and Faardrup as well as the weighted average concentration 1 m below ground surface (C_{mean}) is shown in Table 7.5-58 and Table 7.5-59 provides an overview of the detection of glyphosate and AMPA in the variably-saturated zone and the saturated zone.

Table 7.5-58: Glyphosate and AMPA application and analysis at the PLAP–fields. Application date (Appl. date), end of monitoring period (End. mon.) are listed. C_{mean} refers to average leachate concentration [µg/L] at 1 m below ground surface the first year after application.

Crop	Applied product	Analysed Pesticide	Appl. date	End mon.	C_{mean}
Silstrup					
Red fescue 2012	Glyfonova 450 Plus	Glyphosate	Sep 12	Jun 15[1]	0.15
		AMPA	Sep 12	Jun 15*	0.067
Spring barley 2013	Glyfonova 450 Plus	Glyphosate	Aug 13	Apr 16	0.01
		AMPA	Aug 13	Apr 16	0.01
Winter wheat 2013	Glyfonova 450 Plus	Glyphosate	Jul 14	Apr 16	<0.01
		AMPA	Jul 14	Apr 16	<0.01
Estrup					
Winter wheat 2011	Roundup Max	Glyphosate	Oct 11	Jun 15	0.88
		AMPA	Oct 11	Jun 15	0.26
Pea 2013	Glyfonova 450 Plus	Glyphosate	Aug 13	Apr 16	0.10
		AMPA	Aug 13	Apr 16	0.07
Winter wheat 2013	Glyfonova 450 Plus	Glyphosate	Jul 14	May 16	0.06
		AMPA	Jul 14	May 16	0.1
Faardrup					
Spring barley and White clover 2012	Glyphogan	Glyphosate	Oct 11	Aug 12	<0.01
		AMPA	Oct 11	Aug 12	<0.01

[1] Monitoring continues the following year

Table 7.5-59: The number of water samples analysed collected from the variably–saturated Zone (VZ; drains and suction cups), saturated Zone (SZ; groundwater screens) are presented together with the results of analysis on samples from VZ and SZ given as number of detections (Det.), detections >0.1 µg/L and maximum concentration (Max conc.)

Pesticide	Analyte	Number of samples		Results of analysis					
		VZ	SZ	VZ			SZ		
				Det.	>0.1 µg/L	Max conc. [µg/L]	Det.	>0.1 µg/L	Max conc. [µg/L]
Glyphosate	Glyphosate	65	116	12	0	0.05	3	1	0.13

Table 7.5-59: The number of water samples analysed collected from the variably–saturated Zone (VZ; drains and suction cups), saturated Zone (SZ; groundwater screens) are presented together with the results of analysis on samples from VZ and SZ given as number of detections (Det.), detections >0.1 µg/L and maximum concentration (Max conc.)

Pesticide	Analyte	Number of samples		Results of analysis					
		VZ	SZ	VZ			SZ		
				Det.	>0.1 µg/L	Max conc. [µg/L]	Det.	>0.1 µg/L	Max conc. [µg/L]
	AMPA	65	116	51	2	0.14	2	0	0.02

During the monitoring period of July 2015-June 2017, glyphosate and AMPA were analysed in 65 and 116 water samples collected from the Variably-saturated Zone (VZ; drains and suction cups) and Saturated Zone (SZ; groundwater screens), respectively. Glyphosate was detected in 12 samples from the VZ with no detection ≥0.1 µg/L and a maximum concentration of 0.05 µg/L. In samples collected from the SZ, glyphosate was detected three times with one detection ≥0.1 µg/L and a maximum concentration of 0.13 µg/L. AMPA was detected in 51 samples from the VZ with two detections ≥0.1 µg/L and a maximum concentration of 0.14 µg/L. In samples collected from the SZ, AMPA was detected two times with no detection ≥0.1 µg/L and a maximum concentration of 0.02 µg/L.

The following results encompass the complete monitoring period of the PLAP study (data from 2015-2017 and data from previous years). Glyphosate (and AMPA; not distinguished in this result) revealed a leaching risk through fractured clayey tills. The frequency of glyphosate detection is shown in Table 7.5-60. The monitoring output of glyphosate and AMPA from all sample points is given in Table 7.5-61.

Table 7.5-60: Frequency of glyphosate detections in water collected from drainage and suction cups at 1 m depth and from groundwater monitoring screens

Frequency	Pesticide	Sand		Clayey till		
		Tylstrup	Jyndevad	Silstrup	Estrup	Faardrup
Drainage and suction cups a 1 m depths						
High	Glyphosate		o	X	X	•
Groundwater monitoring screens						
High	Glyphosate		•	•	X	•

X: The pesticide (or its degradation products) leached at 1 m depth in average concentrations exceeding 0.1 µg/L within the first season after application.

•: The pesticide (or its degradation products) was detected in more than three consecutive samples or in a single sample in concentrations exceeding 0.1 µg/L; average concentration (1 m depth) below 0.1 µg/L within the first season after application.

o: The pesticide either not detected or only detected in very few samples in concentrations below 0.1 µg/L.

Table 7.5-61: Monitoring output of glyphosate and AMPA from drainage at 1 m depth, suction cups at 1 m depths and from the groundwater monitoring screens given for each of the five fields. Output given as the total number (T) of samples analysed, number of detections (D), number of detections exceeding 0.1 µg/L (X) and the max conc. M (µg/L).

Substance		Jyndevad				Silstrup			
		T	D	X	M	T	D	X	M
Glyphosate	Drainage/ Suction cups	72	0	0	–	257	108	22	4.7
	Groundwater	223	0	0	–	646	40	0	0.05
AMPA	Drainage/ Suction cups	72	1	0	0.01	258	203	18	0.35
	Groundwater	223	2	0	0.02	646	40	0	0.08
Substance		**Estrup**				**Faardrup**			
		T	D	X	M	T	D	X	M
Glyphosate	Drainage/ Suction cups	601	343	109	31	236	5	0	0.09
	Groundwater	1017	53	6	0.67	451	5	0	0.03
AMPA	Drainage/ Suction cups	601	499	120	1.6	236	15	1	0.11
	Groundwater	1018	8	0	0.07	451	2	0	0.03

Glyphosate and AMPA were found to leach through the root zone in high average concentrations through clayey till soils. All applications at the clayey till fields (Silstrup and Estrup) within the total monitoring period have resulted in detectable leaching into the drainage, often at concentrations exceeding 0.1 µg/L several months after application. Higher leaching levels of glyphosate and AMPA have mainly been confined to the depth of the drainage system and were rarely detected in monitoring screens located below the depth of the drainage system. However, the detections of glyphosate in groundwater monitoring wells at Estrup seem to increase over the years.

QA of the analytical methods indicates that the true concentration of glyphosate may have been underestimated from June 2007 to July 2010.

On two occasions, heavy rain events and snowmelt triggered leaching to the groundwater monitoring wells in concentrations exceeding 0.1 µg/L, more than two years after application.

However, the numbers of detections exceeding 0.1 µg/L in groundwater monitoring wells is very limited. Glyphosate and AMPA were detected in drainage water at the clayey till field of Faardrup, but in low concentrations. Leaching risk was negligible on the coarse sandy soil of Jyndevad, whereas evidence of glyphosate leaching was seen on clayey till soils.

At the Silstrup field, glyphosate and AMPA have been detected in concentrations up to 0.66 µg/L in drainage after application in September 2012. After application in August 2013, glyphosate was detected in drainage in low concentrations up to 0.036 µg/L and AMPA in concentrations up to 0.054 µg/L. In nine groundwater samples, glyphosate and AMPA were detected in low concentrations up to 0.052 µg/L.

In drainage from Estrup, glyphosate and AMPA were detected frequently in high concentrations ≥0.1 µg/L after application in October 2011 and in August 2013. Glyphosate was detected in one groundwater sample in concentration ≥0.1 µg/L (0.13 µg/L) after the 2012 application. After the application of August 2013, glyphosate and AMPA were not detected in groundwater from Estrup. The leaching of glyphosate and AMPA were highly climate driven, controlled by the timing and intensity of the first rainfall event after glyphosate application.

The Silstrup and Estrup fields were sprayed in July 2014, 23 and 10 days, respectively, before the harvest of winter wheat. In the first sampling of drainage at Silstrup on 27 August 2014, the concentration of glyphosate was 0.27 µg/L and the concentration of AMPA was 0.089 µg/L. An additional 21 samples contained glyphosate (0.01 to 0.14 µg/L). AMPA was detected in 53 of a total 65 samples (0.012 to

0.14 µg/L). Glyphosate and AMPA were only detected in 15 and 16 groundwater samples, respectively, all having concentrations below 0.1 µg/L. For glyphosate, all samples were collected before April 2015.

Following the latter application at Estrup in July 2014, glyphosate was detected in 26 drainage samples out of 68 with two samples having concentrations of 0.13 and 0.32 µg/L. Only six detections of glyphosate were obtained on groundwater samples with the two highest concentrations being 0.09 µg/L in September 2015 and 0.13 µg/L in March 2016. These detections seem to be weather driven, in this case by heavy rain and snowmelt events, respectively. Following the July 2014 application, AMPA was not detected in the groundwater samples but in 60 samples out of 68 samples from drainage with nine exceeding 0.1 µg/L (max. conc. 0.21 µg/L).

Conclusion

The leaching of glyphosate and AMPA was reported within a monitoring program that covers the leaching risk of all together 50 pesticides and 65 degradation products in Denmark between 1998 and 2021 (so called PLAP-pesticide leaching assessment program). During the monitoring period 2015-2017, glyphosate and AMPA (among further 5 pesticides and 17 degradation products) were analysed in 65 and 116 water samples collected from the variably-saturated zone and saturated zone, respectively. Glyphosate was detected in 12 samples from the VZ with a maximum concentration of 0.05 µg/L and in three samples collected from the SZ with a maximum concentration of 0.13 µg/L. AMPA was detected in 51 samples from the VZ with a maximum concentration of 0.14 µg/L. In samples collected from the SZ, AMPA was detected two times with a maximum concentration of 0.02 µg/L. Data on the complete PLAP-monitoring period revealed a leaching risk for glyphosate and AMPA through fractured clayey tills.

Data on the complete PLAP-monitoring period revealed a negligible leaching risk on the coarse sandy soil, whereas evidence of glyphosate leaching was seen on clayey till soils. Glyphosate and AMPA leached at 1 m depth in average concentrations exceeding 0.1 µg/L within the first season after application at Silstrup and Estrup. The numbers of detections exceeding 0.1 µg/L in groundwater monitoring wells is very limited.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article is issued by the Danish Ministry of Energy, Utilities and Climate. The research program PLAP has high quality assurance measures. The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/017
Report author	Poiger, T. *et al.*
Report year	2017
Report title	Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS
Document No	Environmental Science and Pollution Research (2017) 24:1588-1596
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Agroscope)
Acceptability/Reliability:	Reliable

2. Full summary

Glyphosate is currently one of the most important herbicides worldwide. Its unique properties provide for a wide range of uses in agriculture, but also in non-agricultural areas. At the same time, its zwitterionic nature prevents the inclusion in multi-residue analytical methods for environmental monitoring. Consequently, despite its extensive use, data on occurrence of glyphosate in the aquatic environment is still scarce. Based on existing methods, we developed a simplified procedure for the determination of glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) in water samples using derivatization with fluorenylmethyl chloroformate FMOC-Cl, combined with on-line solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) detection. This method was extensively tested on over 1000 samples of surface water, groundwater, and treated wastewater and proved to be simple, sensitive, and reliable. Limits of quantification of 0.005 µg/L were routinely achieved. Glyphosate and AMPA were detected in the vast majority of stream water samples in the area of Zurich, Switzerland, with median concentrations of 0.11 and 0.20 µg/L and 95th percentile concentrations of 2.1 and 2.6 µg/L, respectively. Stream water data and data from treated wastewater indicated that non-agricultural uses may significantly contribute to the overall loads of glyphosate and AMPA in surface waters. In the investigated groundwater samples, selected specifically because they had shown presence of other herbicides in previous monitoring programs, glyphosate and AMPA were generally not detected, except for two monitoring sites in Karst aquifers, indicating that these compounds show much less tendency for leaching.

Materials and Methods

Chemicals
Glyphosate (purity 98 %), glyphosate-FMOC (99.5 %), AMPA-FMOC (97 %), $^{13}C_2$ ^{15}N-glyphosate (internal standard, 98 %), AMPA (99 %) $^{13}C_2$ $^{15}ND_2$-AMPA (100 mg/L in water) were obtained commercially. Stock solutions of the unlabeled compounds were prepared at concentrations of 500 mg/L in acetonitrile/water (7:3). To aid dissolution of glyphosate and AMPA, 100 µL 1 M aqueous NaOH solution was added to 20 mL of stock solution. All solvents were of HPLC grade.

Water samples
Grab samples from various streams in the area of Zurich, Switzerland, were collected during routine samplings by the Office for Waste, Water, Energy, and Air of the Canton of Zurich (AWEL) from 2006 to 2013. Further grab samples from a small stream in the Canton of Vaud were provided by the water protection laboratory of the canton from 2011 to 2014. Groundwater samples were collected by the Federal Office for the Environment (FOEN) at selected monitoring sites of the NAQUA National Groundwater Monitoring Program during a pilot study in 2010 and 2011, and by the official food control authority of the Canton of Zurich in 2006, 2007, and 2012. Grab samples and 24-h flow proportional composite samples of

treated wastewater from various WWTPs in Switzerland were obtained from the personnel of these plants. All samples were collected in 125-mL high-density polyethylene (HDPE) flasks, shipped in ice-cooled containers (not frozen), and stored at 4°C after addition of internal standard (see below). Storage time was usually less than 2 weeks.

Derivatization with FMOC-Cl
Upon arrival at the laboratory, exactly 100 mL of each water sample was retained in the HDPE container while the rest was discarded. Samples were fortified with 100 µL of a solution of $^{13}C_2$ ^{15}N-glyphosate and $^{13}C_2$ $^{15}ND_2$-AMPA (0.1 ng/µL each) in acetonitrile/water (7:3) to yield concentrations of 100 ng/L in the samples. Spiked samples were kept for at least 24 h at 4°C to allow for equilibration between dissolved and particulate phase. To an aliquot of water sample, 0.1 M borate buffer solution and 2 mM FMOC-Cl solution were added, shaken, and left at room temperature overnight. To remove excess reagent and side products, as well as a substantial fraction of the acetonitrile, dichloromethane was then added to the derivatized samples. The samples were shaken and left undisturbed until the phases were completely separated. Specific details of the derivatization are provided in the article. 'Matrix matched' standards were prepared in 'fossil' groundwater, which was also used for blank determination. Concentrations ranged from 10 to 2000 ng/L glyphosate and AMPA (depending on the concentrations present in the samples). The internal standards were added at the same concentration as in the water samples, and derivatization was done together with the real samples.

On-line SPE and liquid chromatography-tandem mass spectrometry
The instrumental setup was similar to the one reported earlier (Gulkowska *et al*. 2014) and consisted of an auto-sampler equipped with two six-port valves for column switching, a sample loop, and an on-line extraction cartridge. Pre-concentration of the derivatized analytes was achieved using a column switching technique. A PEEK loop was loaded with the derivatized sample solely from the upper, aqueous layer via the auto-sampler syringe. The sample was then transferred from the loop to the SPE cartridge with purified water. After valve switching, the enriched analytes were eluted backward directly on to a C-18 column equipped with a guard column followed by separation using the mobile phase program. The HPLC column was connected to an API 4000 triple quadrupole mass spectrometer equipped with a turbo ion spray (TIS) source operated in negative mode and multiple reaction monitoring (MRM). The characteristic fragmentation reaction for the primary transition was the cleavage of the FMOC moiety from the derivatized molecule. Specific HPLC and mass spectrometer conditions, and ion transitions monitored are reported in the paper. Quantification was based on peak area ratios of analyte versus internal standard in reference to standards in spiked fossil groundwater. Concentrations were determined separately using the primary (Q) and secondary ion transitions (q), and measurements were flagged when the concentration ratio Q/q was not within 0.8-1.2.

Relative response, method precision, and recovery in different matrices
The influence of the sample matrix on the intensity of MRM transitions for glyphosate and AMPA was studied in groundwater, surface water (Sagentobelbach, sampled on August 18, 2015), WWTP effluent (Dübendorf, August 18, 2015), and purified water containing calcium chloride. Standards were prepared in these matrices by appropriate dilution of a stock solution of the isotopically labeled surrogate compounds (concentrations, 62.5, 125, 250, 500, 1000, and 2000 ng/L), followed by derivatization. By using the isotopically labeled surrogate compounds, a possible influence of background levels could be excluded. The slopes of the respective calibration curves were used to calculate responses in matrix relative to purified water (Table 7.5-62). Method precision was determined by replicate analysis (N = 6) of WWTP effluent (Villars-sous-Yens, August 11, 2015), surface water (Boiron, July 17, 2015), and groundwater (Aqui, spiked with glyphosate and AMPA at concentrations of 25 and 250 ng/L, respectively). Recoveries were determined in surface water (Sagentobelbach, August 18, 2015) and WWTP effluent (Villars-sous-Yens, August 11, 2015) relative to calibration standards in groundwater (Aqui) at two spike levels each.

Table 7.5-62: Influence of sample matrix on responses of glyphosate and AMPA in groundwater, river water, and WWTP effluent; method precision; and recovery

	Relative response [%][a]		Precision				Recovery		
			Concentration (ng/L)		RSD[b] (%) (N = 6)		Spike level	Recovery[c] (%)	
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA	(ng/L)	Glyphosate	AMPA
Groundwater	72 %	111 %	25	25	3.2	1.7			
			250	250	1.5	1.1			
Surface water	70 %	100 %	66	102	2.3	3.6	100[d]	97 %	103 %
							500[d]	98 %	99 %
WWTP effluent	85 %	89 %	105	1560	1.2	1.4	500[e]	97 %	93 %
							2000[e]	107 %	97 %

[a] Slope of calibration curve in matrix relative to purified water (containing 1 mM $CaCl_2$)
[b] Relative standard deviation
[c] Relative recoveries calculated using calibration standards prepared in groundwater
[d] Background concentrations of glyphosate and AMPA were 12 and 39 ng/L, respectively; recoveries were calculated after correction for background
[e] Background concentrations of glyphosate and AMPA were 145 and 1720 ng/L, respectively; recoveries were calculated after correction for background

Quality assurance in routine monitoring

Several measures were used to assure accurate determination of glyphosate and AMPA during monitoring campaigns. Blank samples (fossil groundwater) and a control standard of spiked groundwater were analyzed with each batch of samples. Selected samples were analyzed in triplicate to determine precision (RSD values were in the range of those reported in Table 7.5-62). In longer campaigns, selected samples from previous sampling events were reanalyzed to determine intermediate precision (measured concentrations, usually within ±10 %) and storage stability (peak area of internal standards over time, usually within ±10 %). Storage stability varied between 1 and 2 months (groundwater) and 2 weeks (WWTP effluent). During storage, concentrations of the analytes and internal standards did not decrease continuously but rather dropped rapidly after a certain 'lag phase.' However, peak area ratios (analyte vs. internal standard) remained constant.

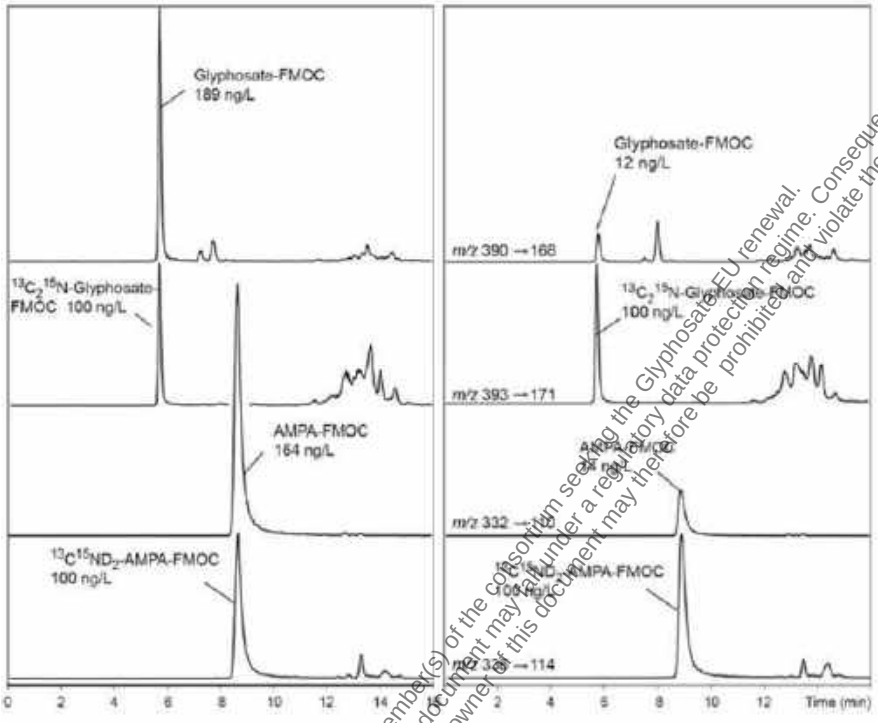
Results and Discussion

Optimization of the analytical procedure

Derivatization of glyphosate and related compounds with FMOC-Cl offers several advantages. First, the reaction proceeds directly in aqueous samples (with a certain amount of acetonitrile as co-solvent) so that there is no need for pre-concentration or solvent exchange prior to derivatization. Second, the derivative is significantly more lipophilic than the underivatized test substances and thus more easily concentrated from water and more suited for reversed-phase HPLC. Third, the main by-product of the derivatization, the FMOC alcohol, can easily be removed as it is far more lipophilic than the derivatives. The procedure requires only 5 mL of sample and no transfer steps prior to derivatization. On-line preconcentration requires no dedicated equipment except a dual injection valve and an auxiliary HPLC pump for transfer of sample from sample loop to cartridge precolumn used for preconcentration.

In chromatograms from natural water samples, some of the mass traces also contain substantial signals other than those of the target compounds (Figure 7.5-45). This is due to the fact that the major transitions observed in negative ion tandem mass spectrometry result from loss of the FMOC moiety. Therefore, we optimized the chromatographic separation between target compounds and possible interferences by using a rather high pH eluent (≈ 9.15) in combination with an HPLC column that is sufficiently stable at this high pH. As can be seen in the figure, glyphosate and AMPA elute earlier than any of the interferences.

Figure 7.5-45: Typical chromatograms of glyphosate and AMPA in samples from the river Aabach at Mönchaltorf (weekly composite sample, September 30 to October 6, 2013, left) and from Lake Greifensee (1 m depth, October 7, 2013, right)



Signal responses in different matrices, detection limits, precision, and recoveries

Relative responses (expressed in % of the response in purified water) showed fairly narrow variation between different matrices (Table 7.5-62). Limits of quantification (LOQs) for glyphosate and AMPA of 5 ng/L (signal/noise ratio of >10 for the ion trace used for quantification and S/*N* >3 for the ion trace used for confirmation) were achieved under most circumstances, except for surface water samples with high particle loads and groundwater samples with low pHs (see below). Method precision was excellent with relative standard deviations for replicate analyses ranging from 1.1 to 3.6 % with no clear trend with regard to matrix or substance. Recoveries in spiked surface water and WWTP effluent ranged from 91 to 103 %.

Field testing of the analytical procedure

The optimized procedure was extensively tested on a total number of more than 1000 samples of groundwater, surface water from different streams and lakes, and effluents from WWTPs and proved to be robust and sensitive. Some of the experiences during application of the method are discussed hereafter. In some groundwater samples, fairly low signals were obtained for internal standards as well as the test substances. This phenomenon was reported by other researchers and attributed to possible complexation of the test substances with metals (Ibanez *et al*. 2006). In our experience, low signals were limited to groundwater samples with low pHs (<6.5) and low calcium content. In these cases, adjusting the pH to ≥7 and addition of 1 mM $CaCl_2$ prior to derivatization resulted in a substantial improvement in signal intensity.

Samples were normally analyzed without prior filtration. After addition of the internal standard, the particles were allowed to settle and a subsample from the supernatant was subjected to derivatization and analysis. Assuming that the time between addition of internal standard and derivatization (24 h) was long enough to allow for an equilibration between aqueous and particulate phases, the measured concentration thus reflects the total amount in the sample, including the fraction which is adsorbed to particles. Surface water samples from storm events with very high content of organic matter (particulate and/or dissolved)

sometimes also yielded low signal intensities. Regardless of whether this was due to signal suppression, low derivatization yield, or both, dilution with blank (fossil) groundwater improved the situation.

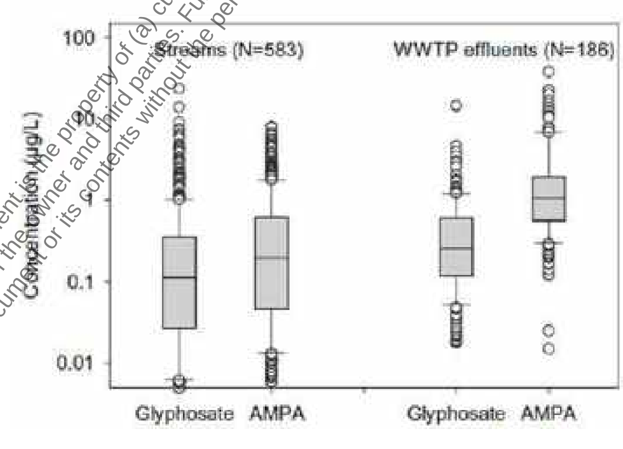
Glyphosate in groundwater samples

A total of 141 groundwater samples from 14 monitoring sites in Switzerland in 2010 and 2011 were analyzed as part of an intensive campaign conducted by the FOEN to determine the concentration dynamics of pesticides at these stations. Concentration dynamics were expected to be high at these locations due to high vulnerability. Glyphosate was detected twice above the LOQ of 0.005 µg/L at one location (0.009 and 0.025 µg/L, respectively). AMPA was regularly detected at two locations above the LOQ of 0.005 µg/L in concentrations of 0.08-0.65 and 0.017-0.070 µg/L, respectively. Both monitoring sites are located in vulnerable Karst aquifers with a shallow soil cover. During 2006, 2007, and 2012, further single groundwater samples from eight locations were analyzed with no detections above the LOQ of 0.005 µg/L. Some of these locations are known to receive substantial amounts of river bank infiltration (Buerge *et al*. 2009). Overall, these results confirm the low potential of glyphosate and AMPA for leaching to groundwater which is due to strong sorption to soil particles combined with fairly rapid dissipation (European Food Safety Authority (EFSA) 2015).

Occurrence of glyphosate and AMPA in rivers and streams

From 2006 to 2013, glyphosate and AMPA were analyzed in numerous water samples from various locations in Switzerland, particularly in the canton of Zurich. In the following, we present results from monitoring campaigns where monthly grab samples were taken between March and November (no sampling during winter and early spring) as part of the pesticide monitoring program of the canton of Zurich (AWEL 2016). Both compounds were regularly detected in the investigated streams with median concentrations of 0.11 and 0.20 µg/L and 95th percentile concentrations of 2.1 and 2.6 µg/L, respectively (Figure 7.5-46).

Figure 7.5-46: Distribution of glyphosate and AMPA concentrations in rivers and streams (*N* = 583) and WWTP effluents (*N* = 186), analyzed from 2006 to 2013. The boxes indicate median and 25th and 75th percentiles, the whiskers the 5th and 95th percentiles. Values outside this range are plotted individually.



Only 40 out of 583 samples showed glyphosate concentrations below the LOQ of 0.005 µg/L (27 for AMPA). On average, concentrations of AMPA were higher than those of glyphosate. On a sample-by-sample basis, in only 28 % of samples, concentrations of AMPA were lower than those of glyphosate. Nevertheless, the highest overall concentrations were found for glyphosate. Widespread occurrence in streams as well as the detected concentrations compare well to findings in other studies (Battaglin *et al*. 2014; Daouk *et al*. 2013; European Glyphosate Environmental Information Sources (EGEIS) 2009; Hanke *et al*. 2008; Hanke *et al*. 2010; Kolpin *et al*. 2006). The seasonal variation of glyphosate and AMPA concentrations in weekly, flow-proportional composite samples was monitored in various streams in the canton of Zurich from spring to fall. For example, in the Furtbach, a small stream in

the north of Zurich (long-term mean discharge, 655 L/s; Q347 = 208 L/s), receiving inputs from agricultural land as well as from three municipal WWTPs serving a total population of approximately 32,000, glyphosate was already present in the water samples in April and increased to a maximum of 1.9 µg/L at the end of May (Figure 7.5-47 (top)). Thereafter, the concentrations remained relatively high until mid-September, consistent with its main application window in August, and then dropped to below 0.1 µg/L at the beginning of November. The minima in June, August, and end of September correspond to weeks with no precipitation. Glyphosate was detected at elevated concentrations for a much longer part of the year than other herbicides that are applied in large quantities such as isoproturon or metolachlor and which are typically found primarily during a narrow time window during and immediately following the application period. Concentrations of AMPA in the same samples varied much less. While, overall, higher concentrations were observed in summer, the differences between summer and spring/fall were smaller than for glyphosate. Particularly, the minima in June and August were not observed for AMPA. Concentration ratios AMPA/glyphosate were calculated for all weekly composite river water samples analyzed from 2006 to 2013. To eliminate some of the variability due to different meteorological conditions, the calculated values were grouped monthly. Concentration ratios varied greatly as indicated by the wide range that is spanned by the 5th and 95th percentile whiskers in Figure 7.5-47 (bottom). Nevertheless, there is a trend toward lower ratios in summer, when glyphosate concentrations are at their maximum.

Wastewater treatment plants as a source of glyphosate and AMPA in surface waters

Glyphosate and AMPA were regularly present in treated wastewater. Concentrations tended to be higher than in rivers and streams with median concentrations of 0.38 and 1.3 µg/L, respectively (Figure 7.5-48). Comparison of glyphosate and AMPA concentrations in WWTP effluents (data not shown) do not indicate that occurrence of these two compounds is linked. Concentration ratios (AMPA/glyphosate) ranged from 1.2 to 38 and seemed to be related to the particular WWTP rather than to any other parameter (such as time of year, high or low concentrations, etc.). All these observations indicate that AMPA, although a major metabolite of glyphosate, must have other sources as well. Indeed, AMPA is also a major degradation product of a number of phosphonates used, e.g., in detergents as chelating agents (Nowack 2003). Comparison of in-stream concentrations of glyphosate and AMPA upstream and downstream of WWTPs indicated that treated wastewater indeed is a source of these compounds in surface waters (Figure 7.5-48). For glyphosate, contribution of WWTP effluent to downstream concentrations was predominant (>90 %) in 6 out of 47 cases, significant (20 to 90 %) in another 21, and negligible (<20 %) in 20 cases.

Figure 7.5-47: **Example of the seasonal variation of glyphosate and AMPA concentrations in flow-proportional weekly composite samples from a small river (Furtbach 2008, top) and of the AMPA/glyphosate concentration ratios in various small rivers in the Canton of Zurich, Switzerland (2008-2013, bottom).**

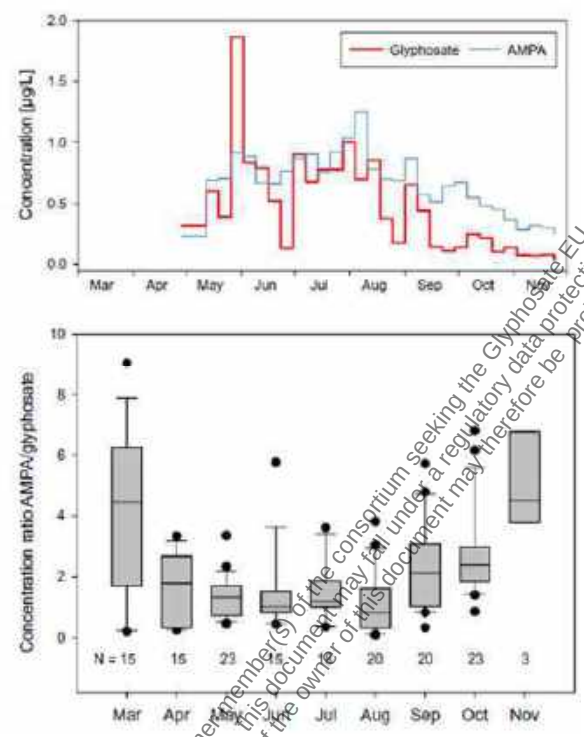
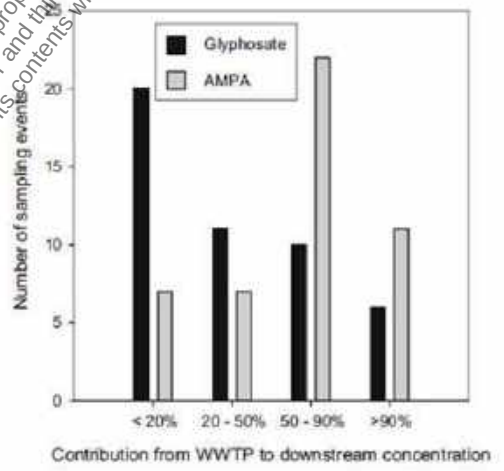


Figure 7.5-48: **Evidence of contribution of treated wastewater to total loads of glyphosate and AMPA in surface waters from a comparison of in-stream concentrations, upstream and downstream of municipal WWTPs (*N* = 47)**



Urban contribution to total load of glyphosate in surface waters

Ubiquitous occurrence of glyphosate in wastewater indicates that non-agricultural uses of glyphosate may substantially contribute to the total burden to surface waters. Potential candidates are uses for weed control along highways and railroads as well as private and semi-private application such as in gardening and weed

control in residential areas, parks, golf courses, etc. Our data from streams and WWTP effluents clearly support this finding. Due to its unique combination of properties, glyphosate has found many applications in areas where other herbicides would be expected to pose significant risk for contamination of surface and/or groundwater. Despite its favorable properties, glyphosate losses from urban uses can be quite significant (Ramwell *et al.* 2014) and may contribute substantially to the elevated concentrations in surface waters over extensive periods of time. Even though these concentrations are still well below the currently proposed environmental quality standards for surface waters in Switzerland and some EU Member States (Johnson 2012; Maycock *et al.* 2010; Oekotoxzentrum2016), it appears to be warranted to reduce the use of glyphosate particularly in those areas (e.g., application on or along sealed surfaces) where the potential losses are high.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the derivation of a simplified procedure for the determination of glyphosate and AMPA in water samples. More than 1000 samples from ground and surface waters, and from treated wastewaters in Switzerland were tested with this method and the results are reported.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/018
Report author	Di Guardo, A., Finizio, A.
Report year	2016
Report title	A moni-modelling approach to manage groundwater risk to pesticide leaching at regional scale
Document No	Science of the Total Environment 545–546 (2016) 200–209
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Historically, the approach used to manage risk of chemical contamination of water bodies is based on the use of monitoring programs, which provide a snapshot of the presence/absence of chemicals in water bodies. Monitoring is required in the current EU regulations, such as the Water Framework Directive (WFD), as a tool to record temporal variation in the chemical status of water bodies. More recently, a number of models have been developed and used to forecast chemical contamination of water bodies. These models combine information of chemical properties, their use, and environmental scenarios. Both approaches are useful for risk assessors in decision processes. However, in our opinion, both show flaws and strengths when taken alone. This paper proposes an integrated approach (moni-modelling approach) where monitoring data and modelling simulations work together in order to provide a common decision framework for the risk assessor. This approach would be very useful, particularly for the risk management

of pesticides at a territorial level. It fulfils the requirement of the recent Sustainable Use of Pesticides Directive. In fact, the moni-modelling approach could be used to identify sensible areas where implement mitigation measures or limitation of use of pesticides, but even to effectively re-design future monitoring networks or to better calibrate the pedo-climatic input data for the environmental fate models.

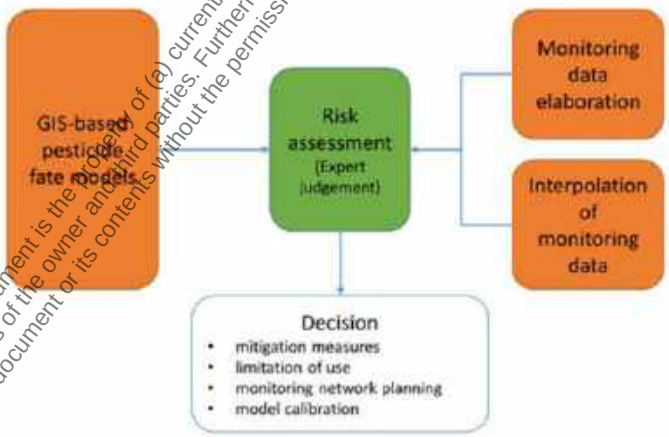
A case study is presented, where the moni-modelling approach is applied in Lombardy region (North of Italy) to identify groundwater vulnerable areas to pesticides. The approach has been applied to six active substances with different leaching behaviour, in order to highlight the advantages in using the proposed methodology.

Materials and Methods

Overview of the methodology

The moni-modelling approach, here briefly described (Figure 7.5-49), is based on coupling spatial modelling of environmental fate and long term monitoring data of Plant Protection Products (PPPs) occurrence in wells. A methodology of comparison between the results of the two types of information permits to take valuable conclusions on the effective vulnerability of the area. In the first instance, it foresees the definition of vulnerability maps at regional scale using GIS-coupled models for predicting the potential pesticide concentrations in groundwater at regional scale. On the other side, another brick of information for decision making is given by the availability of long term monitoring data of PPPs residues in groundwater. Generally, monitoring points (wells) can be easily georeferenced in a GIS map by using geographical coordinates. Considering the availability of long term data on PPPs residues in groundwater it is possible to create a map of 95th percentile of each PPP monitored observed in each monitoring site. This map can be used as input for a geostatistical analysis (i.e. using an ordinary block kriging interpolation method) to produce a new map highlighting the influence areas of different wells in the territory.

Figure 7.5-49: Flux diagram of the proposed methodology. Colours represent different spatial levels of each action (in orange at regional level, in green at local level)



By evaluating both monitoring and modelling results, decision makers will have a powerful tool to identify specific areas at risk where implement risk mitigation measures. In addition, decision maker will have useful information to plan better monitoring networks and/or better calibrations of predictive models.

Case study: Lombardy region (North Italy)

Description of the area
Lombardy region has an extension of about 23.844 km^2 which almost a half of it is plain (47 %) and the rest consists of hills (12 %) and mountains (41 %). Flat areas extend from West to East, while mountains are located at North (Alps) and in the South-West (Apennine). The last agriculture census reports that arable crops are cultivated in the 92.1 % of the available crop area of the Lombardy plain, while the remaining part is dedicated to woody crops and grasslands; maize is the main crop of the Lombardy region, where it covers almost a half of the total arable area.

Plant Protection Products under evaluation
In order to set up the methodology and to give some examples of how the outcomes could be very useful for risk managers, we considered five PPPs and a metabolite. Particularly, terbuthylazine (Tba), glyphosate (Gly), pendimethalin (Pend) and s-metolachlor (s-Met) herbicides, the insecticide chlorpyriphos (Cpyr) and the terbuthylazine metabolite desethyl-terbuthylazine (d-Tba) were considered.

Table 7.5-63, reports a summary representation of the main physical–chemical properties and persistence (degradation time in soil: $DegT_{50}$) of the selected substances; data are from the online PPDB database maintained by the Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire.

Table 7.5-63: Main physical-chemical properties of selected active ingredients from PPBD database (Pesticide Properties Database)

A.I. name	MW [g mol^{-1}]	Water solubility [mg l^{-1}]	Henry's constant [–]	Vapour pressure [mPa]	DegT$_{50}$ soil [d]	Koc [–]	Freund. exponent [–]	Kfoc [–]	GUS index	Comment on GUS index
Cpyr	350.89	1.05	4.78E−01	1.43E+00	42	8151	–	–	0.15	Not leaching
Gly	169.1	10,500	2.10E−07	1.31E−02	47	1435	9.60E−01	28,700	0.90	Not leaching
s-Met	283.79	480	2.20E−03	3.70E+00	90	226	1.06E+00	226.1	1.93	Moderately leaching
Pend	281.31	0.33	2.73E−03	1.94E+00	90	17,581	9.69E−01	15,744	−0.47	Not leaching
Tba	229.71	6.6	3.24E−03	1.20E−01	75	–	9.30E−01	151	3.41	Leaching
d-Tba	201.68	327.1	8.86E−08	3.50E−02	70.5	–	8.60E−01	78	3.90	Leaching

The modelling system
VULPES is an exposure assessment tool to identify groundwater vulnerable areas to PPPs at regional level. It focuses the attention to the interaction of active ingredients with the agricultural and environmental characteristics of the area. It uses the PELMO v.3.2 model to evaluate the pesticide fate in groundwater.

For s-Met, Pend, Cpyr, Gly simulations were made by considering the maximum allowed application rates for each active ingredient, as reported in the commercial formulation labels.

Monitoring data
The presence of the six substances in Lombardy groundwater is actually monitored by ARPA Lombardia, the environmental protection agency of the Lombardy region. We analysed five years data from 2005 to 2009 from 320 monitoring stations evenly distributed in the Po plain part of the Lombardy region.

Table 7.5-64, reports a brief summary of the main characteristics of the monitoring data provided, For each well and substance we then calculated the 95th percentile of observed values. Values below the level of detection (LOD) was taken into account into the next elaborations, assuming an observed value equal to a half of LOD (in agreement with the 2009/90/CE Directive).

Table 7.5-64: Statistical summary of monitoring data for the six substances

A.i. name	Total number of monitoring wells	Years of monitoring data	Data above LOD	Data >0.1 µg/L
Cpyr	185	2005–2006	0	0
Gly	289	2005–2009	5	1
Pend	257	2005–2007	1	0
s-Met	333	2005–2009	60	10
Tba	394	2005–2009	217	29
d-Tba	394	2005–2009	349	50

Geostatistical elaborations

Monitoring data provide information on the local contamination and they are related to a single point in space. In order to compare monitoring data with modelling output expressed as areas of vulnerability, we adopted the ordinary block kriging (an optimal interpolation technique based on regression against observed values of surrounding data points, weighted according to spatial covariance values). For each active substance, we elaborated the available monitoring data in order to obtain the 95th percentile for each well and we used the kriging tool implemented in the SAGA-GIS software to elaborate maps of interpolated observation values of substance concentrations in the water table.

Results

Maps of predicted concentration of PPPs residues in groundwater of Lombardy region (vulnerability maps)

VULPES produced six vulnerability maps, which highlight the 80th percentile of the investigated active ingredient concentration at 1 m below the soil surface taking into account all the years of meteorological data available. Results are grouped into six categories; hence, values can be directly compared with the legal limit for active ingredient concentration in the groundwater, actually set to 0.1 µg/L. No map has been reported for Cpyr, Gly and Pend because VULPES system did not identify any vulnerability related problem with these substances. Resulting maps demonstrate two different behaviours. In agreement with GUS index the simulations for Pend, Gly and Cpyr indicated a non-leaching behaviour (each polygon falls into the class below 0.02 µg/L). On the contrary, s-Met demonstrated leachability in some areas (particularly those characterised by highly permeable soils), while Tba and d-Tba are likely to leach in several parts of the region well beyond the trigger value of 0.1 µg/L.

Maps of measured concentration of PPPs residues in groundwater of Lombardy region

In Table 7.5-65, we report a general picture of the 95th percentiles values of monitoring data for each of the considered active ingredient. In order to have a direct comparison with the vulnerability maps produced by VULPES we used the same division in classes. For non-leaching substances (Cpyr, Gly and Pend) values fall into the first three classes except for a consistent presence of Gly in 5 wells. Browsing raw data, almost all are below the LOD, hence values in the three classes testify the different LOD used in several part of the region. Among leaching substances, the 96 % of s-Met data falls into the first three classes, while only the remaining 4 % lies within the higher ones. The same general trend applies for Tba and d-Tba (89 % and 87 % values falls into the first three classes respectively). However, noticeably, 6 % of values are above the trigger limit for both substances.

Table 7.5-65: Classification of 95th percentile monitoring values of each well for the six active ingredients

	Observation occurrences in classes (µg L^{-1})						
	<0.02	0.02–0.04	0.04–0.06	0.06–0.08	0.08–0.1	>0.1	Total
Cpyr	127 (69%)	58 (31%)	0	0	0	0	185
Gly	0	0	280 (98%)	0	0	5 (2%)	285
Pend	4 (2%)	194 (76%)	57 (22%)	0	0	0	255
s-Met	115 (36%)	166 (51%)	28 (9%)	5 (1.5%)	1 (0.3%)	7 (2.2%)	322
Tba	278 (59%)	123 (26%)	22 (4.6%)	16 (3.4%)	5 (1%)	29 (6%)	473
d-Tba	228 (48.2%)	137 (29%)	49 (10.4%)	15 (3.2%)	14 (2.9%)	30 (6.3%)	473

In order to get a spatial distribution of the yearly-observed monitoring data we apply the ordinary block kriging as a geostatistical interpolation method. Cpyr, Gly and Pend do not have evidences in wells; hence, no meaningful maps could be obtained by kriging interpolation.

Discussion

VULPES allows identifying potentially vulnerable areas to pesticides on a territorial scale, while monitoring data gives information on single points where contamination occurred. Analysis of map of interpolated monitoring data and vulnerability map could be done at two different spatial levels, following a top down approach. In case of leaching active ingredients (such as Tba, d-Tba and s-Met), when leachability strongly depends to environmental characteristics of the area, analysis should be focused at a local level. Particularly, both vulnerability map produced by VULPES and map of interpolated monitoring data should be analysed in deep details in order to highlight whether information are concordant or discordant. At this scale, 3 different situations could occur a) predicted and observed data are consistent (no risk or a certain level of potential pollution in the area), b) models forecast a feasible level of vulnerability, but no observations support it, c) observations denote a pollution in the area, but models indicates no vulnerability.

Case a*. Agreement between predicted and observed data*.
When there is agreement on the lack of pollution in a particular area, then the risk assessor could reasonably judge that no mitigation measures or limitation of use are necessary in that area, even if occasional controls through monitoring should be considered. On the contrary, when the agreement is on the presence of the substance in the water table, then, depending on the extension of the area or the level of pollution, the risk assessor could be confident on adopting mitigation measures or limitation of use of the active ingredient.

Case b *Vulnerability detected by model and no observed values in monitoring data*
In this case, the leaching model forecasts a high vulnerability to the active ingredient, while the surrounding wells does not provide values of it above the LOD. Analysing details of the location of wells, we could distinguish two case. If they belong to non-agricultural areas or to agricultural areas not cultivated at maize then probably their position in the area should be improved to evaluate if the vulnerability forecasted by the model could be definitively confirmed or not. The risk assessor could operate in that direction and re-evaluate the area with new data. If monitoring wells are correctly placed in areas cultivated with the studied crop and assuming the representativeness of the observed data, then there should be some weaknesses on input parameters of model simulations. They could belong to a wrong representation of the soil permeability of the area or to a lesser use of the active ingredient in the area. The risk assessor could evaluate the realisation of an in-depth analysis of the soil characteristics or take in consideration the effective average use of the active ingredient in the area and re-run the model simulation with the real amounts.

Case b *No vulnerability detected by model and positive values in monitoring data*
The opposite occurs when the map elaborated by the leaching model does not forecast vulnerability, but the monitoring wells provide values of detection near or above the legal limit for groundwater (0.1 µg/L). Several considerations could be done. In case of just one exceeding in a well while the others in proximity have values below the LOD, then the area could be interested by a point source contamination due for example to an unsustainable use of the active ingredient. However, if the vulnerability map with the map of 95th percentiles of the monitoring values is overlapped, then it can be observed that in the area there is only one point well beyond the threshold of 0.1 µg/L (exactly 0.199 µg/L), while the others are below the LOD. In case of several exceeding in nearby wells, probably the input data (such as pedology, meteorology, irrigation amounts) used as input for the model in the area do not represent its environmental characteristics. Input data should be checked with ad hoc measurement campaigns to verify their representativeness. Another important factor to be taken into account is the real pesticide usage in the area: the gap between observed and predicted could be explained if, for some reasons, commercial formulations containing the active ingredient have been used at higher rates than allowed.

Conclusion
The moni-modelling approach here presented provides risk assessors with a complete methodology to investigate the groundwater vulnerability to pesticide, raising the knowledge of the active substance presence and movement in the considered territory. It combines vulnerability maps obtained with pesticide fate models and monitoring data analysis in order to identify areas where mitigation measure or limitation of use of the investigated active ingredient should apply. Moreover, it could be useful to verify the appropriateness of the current monitoring network or to suggest its repositioning. At last, it could identify areas where simulation models could not represent the correct substance transport in the groundwater, probably due to an incorrect parameterisation of the pedo-climatic characteristic of the area.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article presents an approach for combining long-term groundwater monitoring data from the Lombardy Region, Northern Italy with regional scale vulnerability modelling. No experimental or monitoring data were generated.

The 95th percentile monitoring values indicate that the groundwater concentrations of glyphosate ranged between 0.04 – 0.06 µg/L in 280 wells (98 %) and greater than the parametric drinking water limit of 0.1 µg/L in 5 wells (2 %).

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/019
Report author	Rosenbom, A. *et al*.
Report year	2015
Report title	Pesticide leaching through sandy and loamy fields – Long-term lessons learnt from the Danish Pesticide Leaching Assessment Programme
Document No	Environmental Pollution 201 (2015) 75-90
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (div. not named commercial laboratories)
Acceptability/Reliability:	Reliable

2. Full summary

The European Union authorization procedure for pesticides includes an assessment of the leaching risk posed by pesticides and their degradation products (DP) with the aim of avoiding any unacceptable influence on groundwater. Twelve-year's results of the Danish Pesticide Leaching Assessment Programme reveal shortcomings to the procedure by having assessed leaching into groundwater of 43 pesticides applied in accordance with current regulations on agricultural fields, and 47 of their DP. Three types of leaching scenario were not fully captured by the procedure: long-term leaching of DP of pesticides applied on potato crops cultivated in sand, leaching of strongly sorbing pesticides after autumn application on loam, and

leaching of various pesticides and their DP following early summer application on loam. Rapid preferential transport that bypasses the retardation of the plow layer primarily in autumn, but also during early summer, seems to dominate leaching in a number of those scenarios.

Materials and Methods

Selection of the five fields

Five agricultural fields were selected for Pesticide Leaching Assessment Programme (PLAP) - two sandy soil fields (at Tylstrup and Jyndevad) and three loamy soil fields (at Silstrup, Estrup and Faardrup). Instrumentation was installed during 1999. Monitoring began at the Tylstrup, Jyndevad and Faardrup fields in 1999 and at the Silstrup and Estrup fields in 2000 (Table 7.5-66). The three loamy fields are characterized by preferential transport through macropores (biopores, fractures) in a low permeable soil matrix (Rosenbom et al., 2009b), while other forms of preferential transport in the soil matrix may be found in the sandy fields (Rosenbom et al., 2009a).

Monitoring design of the PLAP fields

In order to determine whether or not the yearly flux-averaged concentration 1 m b.g.s. and the groundwater concentration of a single pesticide and/or its degradation product exceeds MAC (European-Commission, 1994) the following studies were undertaken: (i) a detailed geological, pedological and hydrogeological characterization of the field; (ii) long-term detailed monitoring of the water balance of the field (Table 7.5-66), e.g. climate, soil water content, groundwater table, drainage flow); (iii) numerical modeling of the field using MACRO version 5.2 (Larsbo *et al*., 2005) to estimate the water balance, including percolation 1 m b.g.s.; and finally (iv) long-term detailed monitoring of the single pesticides and/or their degradation product/products at 1 m b.g.s. and deeper. To avoid any artificial leaching of pesticides, all installations and soil sampling deeper than 20 – 30 cm b.g.s. (plow depth) were restricted to the buffer zones surrounding the fields.

Table 7.5-66: Characteristics of the five PLAP fields

Field	Tylstrup	Jyndevad	Silstrup	Estrup	Faardrup
Precipitation[a] [mm y^{-1}]	941	1052	949	1085	682
Sim. actual evapotransp.[a] [mm y^{-1}]	515	524	474	481	474
Sim. groundwater discharge[a] [mm y^{-1}]	478	608	269	179	106
Measured drain discharge[a] [mm y^{-1}]			169	381	102
W × L [m]	70 × 166	135 × 184	91 × 185	105 × 120	150 × 160
Area [ha]	1.1	2.4	1.7	1.3	2.3
Tile drain	No	No	Yes	Yes	Yes
Depths to tile drain (m b.g.s.)			1.1	1.1	1.2
Monitoring initiated	May 1999	Sep 1999	Apr 2000	Apr 2000	Sep 1999
Groundwater table [m b.g.s.]	2.5–3.5	0.9–2.8	0.5–3.8	0.5–4.9	0.9–3.5
Geological characteristics					
Deposited by	Glaciofluvial	Meltwater	Glacier	Glacier/meltwater	Glacier
Parent material	Fine sand	Coarse sand	Clayey till	Clayey till	Clayey till
Depth to the calcareous matrix [m b.g.s.]	6	5–9	1.3	1–4[b]	1.5
Depth to the reduced matrix [m b.g.s.]	>12	10–12	5	>5[b]	4.2
Max. fracture depth[c] [m]	–	–	4	≤6.5	8
Fracture intensity 3–4 m depth [fracture m^{-1}]	–	–	<1	11	4
Ks in C horizon [m s^{-1}]	$2.0 \cdot 10^{-5}$	$1.3 \cdot 10^{-4}$	$3.4 \cdot 10^{-6}$	$8.0 \cdot 10^{-8}$	$7.2 \cdot 10^{-6}$
Topsoil characteristics					
USDA classification	Humic Psammentic Dystrudept	Humic Psammentic Dystrudept	Alfic Argiudoll/Typic Hapludoll	Abruptic Argiudoll/Aquic Argiudoll	Haplic Vermudoll/Oxyaquic Hapludoll
USDA texture class	Loamy sand	Sand	Sandy clay loam/sandy loam	Sandy loam	Sandy loam
Porosity in A horizon [cm^3 cm^{-3}]	0.45–0.50	0.46–0.48	0.42–0.46	0.41–0.46	0.35–0.46
Water saturation at 25 cm depth [%]	40–60	15–50	40–100	20–100	20–90
Clay content, <2 µm [%]	6	5	18–26	10–20	14–15
Silt content, 2–20 µm [%]	13	4	27	20–27	25
Sand content, 20–2000 µm [%]	78	88	8	50–65	57
pH	4–4.5	5.6–6.2	6.7–7	6.5–7.8	6.4–6.6
TOC in the plow layer [%]	2.0	1.8	2.2	1.7–7.3	1.4

[a] Yearly average based on a time series of hydrological years in the period 1990–2011. The precipitation data refer to measurements 1.5 m above ground.
[b] Large variation within the field.
[c] Maximum fracture depth refers to the maximum fracture depth found in excavations and wells.

The monitoring equipment used and the aspects monitored include: a) piezometers - potentiometric pressure of the groundwater; b) vertical and horizontal monitoring wells - sampling of groundwater and

measurement of groundwater level; c) suction cups - water samples from the variably saturated soil; d) automatic ISCO samplers - sampling of drainage water; e) weather stations - precipitation, air temperature, solar radiation and wind speed; f) TDR probes - soil water content; g) Pt100 sensors - soil temperature; and h) pressure sensors - barometric pressure. The location of the two nests of suction cups S1 and S2, the drainwater monitoring well and the vertical wells at all the PLAP fields was determined by the direction of the shallow groundwater flow. All suction cups and horizontal wells and all but one of the vertical wells are installed down-gradient to capture leaching from the field. The one remaining vertical well enables solute mass contributions from neighboring up-gradient fields to be accounted for. In the sandy fields, water samples from the variably saturated zone are collected using suction cups. In the loamy fields, the water samples for pesticide and/or degradation product analysis are collected from the drains (see Table 7.5-66 for drain depth) and, until March 2002, also from suction cups installed 1 m b.g.s. The latter sampling ceased due to cuts to PLAP funding when this type of sampling was found given the loamy soil texture to be less representative of the conditions in the variably saturated.

Selection of pesticide products and crops

The selection of pesticides and/or their degradation products for evaluation in PLAP for a period of at least two years focuses on compounds in the following three categories: (i) newly authorized pesticide products that are expected to be used either in large amounts and/or to be applied over a large area; (ii) pesticide products that have already been applied for several years either in large amounts and/or over a large area; (iii) authorized pesticides where there are indications of a potential risk of leaching either from the authorization procedure or from new information about them and/or their degradation products. In the latter group, not all the degradation products included in PLAP are found to be relevant metabolites according to the EU guidelines. Once the pesticide products have been selected, appropriate crops are chosen for the fields so that the pesticides can be applied to the crops for which their use is authorized and the best possible crop rotation can be maintained. Cultivation of the PLAP fields is in line with conventional agricultural practice in the locality except that the pesticides are always applied at the maximum permitted dosage. The monitoring studies thus represent the worst-case scenario since farmers often apply the pesticides in lower doses. During the 12 years of monitoring with a minimum of two years in between, a few of the pesticides included in PLAP were applied up to four times on some of the fields (e.g. glyphosate on Estrup). These pesticides and/or their degradation products are often not detectable after two years of monitoring and can therefore be applied to a different crop, if found to be appropriate. In contrast, if pesticides and/or their degradation products are found to leach in high concentrations two years after application, monitoring is often continued (up to nine years after application).

Water sampling and data processing

The concentration of the selected pesticides and/or their degradation product(s) is obtained via analysis of water samples collected from 1 m b.g.s (collected via suction cups and drains) and groundwater monitoring screens (installed 1.5 - 4.5 m b.g.s.). Soil water samples are collected monthly using 16 Teflon suction cups, each connected via a single length of PTFE tubing to a sampling bottle placed in a refrigerator in the instrument shed. The soil water is extracted by applying a continuous vacuum (approx. 80 kPa) to each of the suction cups one week before sampling. The 16 suction cups are clustered in four groups. Each group of four suction cups covers a horizontal distance of 2 m. Chemical analysis is performed on a single, pooled water sample from each of the four groups. Drainage water samples were collected using time-proportional (up to July 2004) and flow-proportional sampling (July 2004 onwards) in the loamy fields as described by Plauborg *et al*. (2003). Time-proportional sampling refers to sampling at regular intervals throughout the whole drainage season. During the period of continuous drainage, a 70-mL subsample is collected every hour regardless of the flow rate. Twenty-four samples are collected per bottle, giving 1680 mL/d. Chemical analysis is then performed on a weekly basis on a pooled sample, derived from the seven bottles. Flow-proportional sampling refers to sampling drainwater induced by sudden precipitation events. Here the flow-proportional sampler collects a 200 ml subsample for every 3000 L of drainage flow during the winter season (September-May) and for every 1500 L of drainage flow during the summer season (June-August). Every week, all the subsamples collected are pooled and a sample of these analyzed at the laboratory. Samples are refrigerated (at around 5ºC) and stored in darkness at all times. As the samples are pooled, they do not represent peak concentrations that may occur during the week. The weighted average concentration of pesticides in the tile-drainage water is subsequently calculated according to the equation described in Kjaer *et al*. (2005b). Groundwater samples are collected monthly from selected vertical and horizontal well

screens. The results of the analysis of water samples collected from the groundwater screens for each pesticide and/or degradation product are presented as the number of detections, since it is not yet possible to estimate the flux at the sampling point.

Analysis and quality assurance
All pesticide analyses are carried out at commercial laboratories selected on the basis of a competitive tender. In order to ensure the quality of the analyses, the call for tenders included a requirement that the laboratory's quality assurance (QA) system comprised both an internal and an external control procedure. In addition to specific quality control under PLAP, the laboratories are accredited by the Danish Accreditation and Metrology Fund (DANAK), based on the international standard DS/EN ISO/IEC 17025. Two types of sample are used in the quality control - samples with known pesticide composition and concentration are used for internal monitoring of the laboratory method, while externally spiked samples collected every four months are used to incorporate additional procedures, such as sample handling, transport and storage. Blank samples consisting of HPLC water are included in the external QA procedure every month to address possible blank positives and contamination risk (such as input from the atmosphere). All samples included in the control and blank sample are labeled with coded reference numbers and shipped together with conventional samples so that the analyzing laboratory is unaware of the samples used for quality control and the origin of the sample.

Results and discussion

PLAP's monitoring results for the period from May 1999 - June 2011 reveal differences in pesticide detection between the sandy and loamy fields. To describe the compounds' environmental fate properties with respect to soil degradation and sorption, the data were categorized following Hertfordshire (2013).

Pesticide detections in sandy and loamy fields
In general, the applied pesticides resulted in less frequent detections at both 1 m b.g.s. (Table 7.5-67 and Table 7.5-68) and in the groundwater (Table 7.5-69 and Table 7.5-70) in the sandy fields than in the loamy fields. This also applies in cases where the concentration exceeds 0.1 µg/L. In the sandy fields, it is primarily degradation products that are detected in high frequency, even though only a few compounds are involved. In the loamy fields, in contrast, pesticides are also detected in high frequency (Table 7.5-68 and Table 7.5-70). The degradation products diketo-metribuzin (metribuzin) and CGA108906 (metalaxyl-M) are detected in more than 60 % of the samples analyzed in both the variably saturated zone and the saturated zone in the sandy fields (Figure 7.5-50). The detection frequency is much lower in the saturated zone in the loamy fields. Here the highest detection frequency recorded is 42 % for the degradation product desethylterbuthylazine, which is frequently detected in water samples from drains approximately 1 m b.g.s. (Figure 7.5-51). Overall, the PLAP results therefore indicate that the highest risk of leaching is posed by degradation products in the sandy fields, and by a mixture of pesticides and/or their degradation products in the loamy fields.

Table 7.5-67: Leaching 1 m b.g.s. of pesticides and/or their degradation products in the five PLAP fields after application of the pesticide (analysis of water collected via suction cups and, if present, drainage)

Parent	Application time	Tylstrup	Jyndevad	Silstrup	Estrup	Faardrup
Azoxystrobin	June					
Bentazone	June					
Bifenox	April–May, *Sept–Oct*		April	*Sept*	*May*	*October*
Ethofumesate	May					
Fluazifop-P-butyl	June					
Glyphosate	Aug– *Nov*					
Metalaxyl-M	July					
Metamitron	May					
Metribuzin	May					
Pendimethalin	*Oct– Nov*					
Picolinafen	*Oct*					
Pirimicarb	July					
Propyzamide	*Nov*					
Pyridate	May					
Rimsulfuron	May–June					
Tebuconazole	*Nov*					
Terbuthylazine	May					
Amidosulfuron	April					
Bromoxynil	*Nov*					
Clomazone	Aug					
Dimethoate	June					
Epoxiconazole	May					
Flamprop-M-	May					
Fluroxypyr	May					
Ioxynil	*Nov*					
MCPA	May					
Mancozeb	June					
Mesosulfuron-	*Oct*					
Phenmedipham	May					
Propiconazole	May–June					
Prosulfocarb	*Oct*					
Triflusulfuron-	May					
Chlormequat	April					
Clopyralid	April					
Desmedipham	May					
Fenpropimorph	June					
Florasulam	June					
Iodosulfuron-	April and August					
Linuron	May					
Metsulfuron-	May and August					
Thiamethoxam	August					
Triasulfuron	May					
Tribenuron-	April					

Pesticide (or its degradation products) leached 1 m b.g.s. in average concentrations exceeding 0.1 µg L^{-1} (MAC) within the first season after application.

Pesticide (or its degradation products) detected in either several (more than three) consecutive samples or in a single sample in concentrations exceeding MAC; average concentration (1 m b.g.s.) below MAC within the first season after application.

Pesticide either not detected or only detected in a very few samples in concentrations below 0.1 µg L^{-1}.

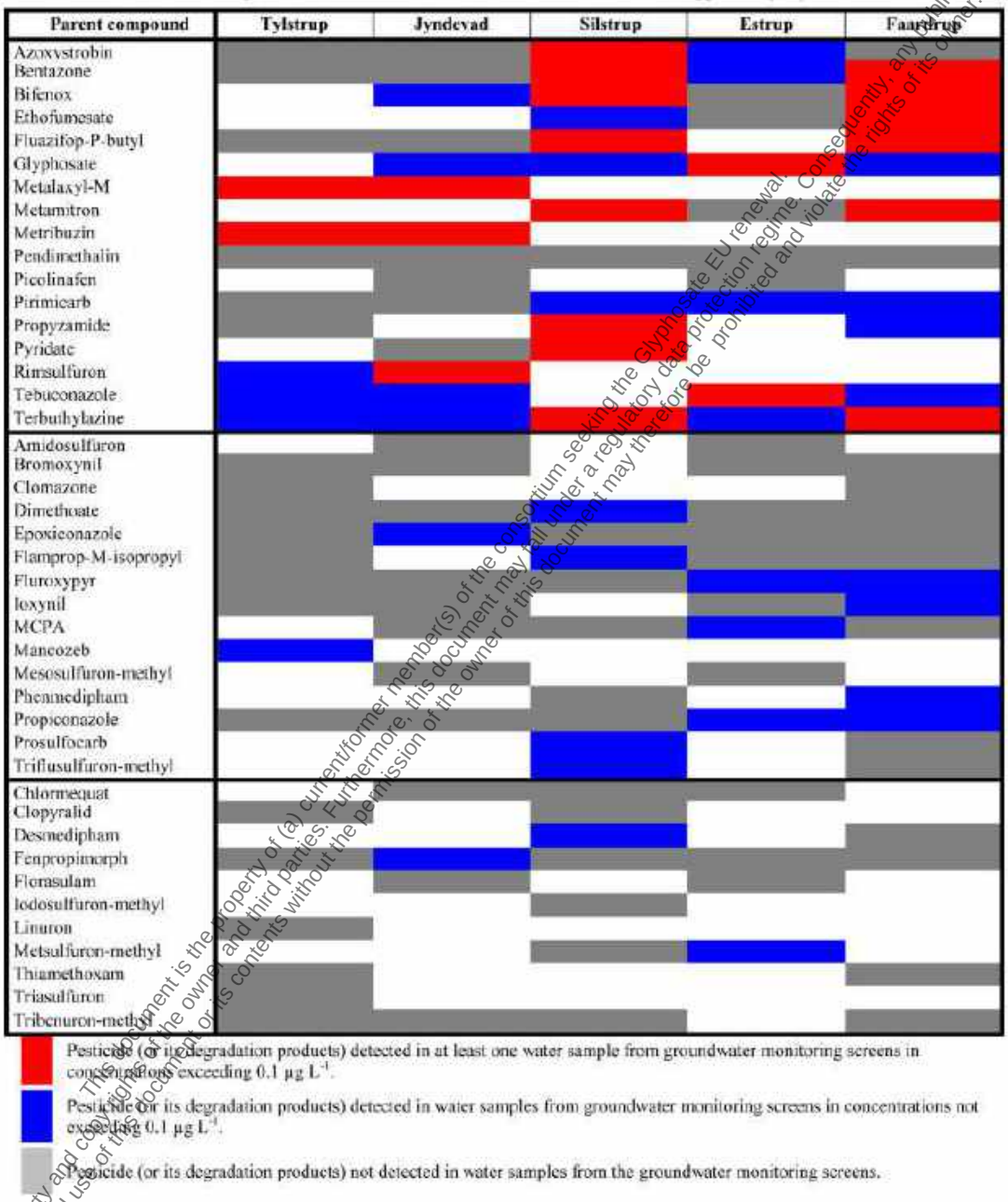
White cells indicate that the pesticide has not been included in PLAP for this field. For at least one field application, leaching to 1 m b.g.s. and/or groundwater is high for 17 pesticides (average concentration above 0.1 µg/L), medium for 15 pesticides and low for 11 pesticides. The month in which the pesticide is applied is shown. Autumn applications are indicated by italic text. Pesticides applied in spring 2011 are not included in the table.

Table 7.5-68: Number of samples from 1 m b.g.s. in which the various pesticides and/or their degradation products were detected in each field with maximum concentration (mg/L) in parentheses

Compound	Tylstrup	Jyndevad	Silstrup	Estrup	Faarup
Azoxystrobin			10(0.03)	106(1.4)	
R234886			73(0.34)	203(2.1)	4(0.05)
Bentazone	1(0.01)	39(1.6)	45(6.4)	161(20)	30(3.3)
2-amino-N-isopropylbenzamide		2(0.03)		1(0.06)	1(0.06)
Bifenox		2(0.04)	1(0.03)	3(0.15)	3(0.09)
Bifenox acid		1(0.1)	12(4.2)	12(1.9)	17(8.6)
Nitrofen			1(0.02)		6(0.16)
Ethofumesate			20(0.23)	35(3.36)	14(12)
Fluazifop-P-butyl					
Fluazifop-P					9(3.8)
TFMP			21(0.52)		
Fluroxypyr				3(1.4)	1(0.19)
Glyphosate			67(4.7)	254(31)	5(0.09)
AMPA		1(0.01)	122(0.35)	367(1.6)	15(0.11)
Metalaxyl-M	4(0.03)	7(0.04)			
CGA 108906	13(0.52)	18(2.3)			
CGA 62826	7(0.04)	17(1.2)			
Metamitron			46(1)	12(26.37)	12(1.7)
Desamino-metamitron			58(0.67)	49(5.55)	16(2.5)
Metribuzin					
Desamino-diketo-metribuzin	67(2.1)				
Diketo-metribuzin	185(0.62)	3(0.09)			
Pendimethalin			14(0.06)	41(32)	2(0.04)
Picolinafen		1(0.02)		17(0.07)	
CL153815				31(0.5)	
Pirimicarb			14(0.05)	40(0.08)	7(0.06)
Pirimicarb-desmethyl		1(0.01)	6(0.03)		6(0.05)
Pirimicarb-desmethyl-formamido				26(0.38)	3(0.04)
Propyzamide			7(1.6)		4(0.51)
RH-24580			3(0.02)		
RH-24644			15(0.05)		4(0.02)
RH-24655					1(0.02)
Pyridate					
PHCP			4(2.69)		
Rimsulfuron					
PPU	119(0.09)	16(0.13)			
PPU-desamino	27(0.03)	8(0.04)			
Tebuconazole				41(2)	4(0.05)
Terbuthylazine			60(1.55)	112(11)	41(10)
2-hydroxy-desethyl-terbuthylazine	5(0.02)		28(0.11)	87(6.3)	8(1)
Desethyl-terbuthylazine	2(0.01)	20(0.06)	108(1.08)	146(8.2)	69(8.3)
Desisopropylatrazine	17(0.04)		43(0.04)	71(0.44)	25(0.36)
Hydroxy-terbuthylazine	1(0.04)		26(0.04)	88(0.99)	21(0.58)
Amidosulfuron		3(0.11)			
Bromoxynil				3(0.6)	
Clomazone					1(0.28)
FMC 65317					1(0.3)
Dimethoate			1(1.42)		
Epoxiconazole				14(0.39)	
Flamprop-M-isopropyl			12(0.11)	20(0.07)	1(0.04)
Flamprop			7(0.1)	13(0.03)	1(0.09)
Ioxynil				20(0.25)	1(0.01)
MCPA				12(3.89)	2(0.28)
2-methyl-4-chlorophenol				1(0.05)	1(0.24)
Mancozeb					
ETU	6(0.04)				
Mesosulfuron-methyl				13(0.06)	
Phenmedipham					
MHPC					2(0.19)
Propiconazole			6(0.03)	26(0.86)	
Prosulfocarb			5(0.18)		
Triflusulfuron-methyl					
IN-E7710			5(0.01)		

The table encompasses pesticides/degradation products detected in either several (more than three) consecutive samples or in a single sample in concentrations exceeding 0.1 mg/L. Pesticides and degradation products are mentioned when analyzed. Pesticides applied in spring 2011 are not included.

Table 7.5-69: Pesticides and/or their degradation products detected in water samples from the groundwater monitoring screens in the five PLAP fields after pesticide application (see Table 7.5-69 for details)



White cells indicate that the pesticide has not been included in PLAP for this field. Pesticides applied in spring 2011 are not included in this table.

Table 7.5-70: The number of samples from the groundwater monitoring screens in which the various pesticides and/or their degradation products were detected at each field

Compound	Tylstrup	Jyndevad	Silstrup	Estrup	Faardrup
Azoxystrobin				1(0.01)	
R234886			28(0.1)	11(0.09)	
Bentazone			29(0.44)	16(0.02)	1(0.6)
2-amino-N-isopropylbenzamide				1(0.03)	
Bifenox		2(0.05)	5(0.1)		
Bifenox acid			13(3.1)		1(0.19)
Ethofumesate			5(0.04)		31(1.4)
Fluazifop-P-butyl					
Fluazifop-P			1(0.07)		6(0.17)
TFMP			48(0.29)		
Fluroxypyr				(0.05)	1(0.07)
Glyphosate		4(0.03)	42(0.67)	3(0.02)	
AMPA		2(0.02)	15(0.08)	8(0.07)	2(0.03)
Metalaxyl-M		6(0.08)	8(0.54)		
CGA 108906	63(0.26)	65(1)			
CGA 62826	2(0.02)	21(0.02)			
Metamitron		29(0.17)		24(0.63)	
Desamino-metamitron			30(0.19)		48(1.3)
Metribuzin		1(0.01)			
Desamino-diketo-metribuzin	238(0.2)	20(1.83)			
Diketo-metribuzin	456(0.55)	26(1.37)			
Pirimicarb			3(0.1)	1(0.02)	2(0.04)
Pirimicarb-desmethyl					3(0.04)
Pirimicarb-desmethyl-formamide					2(0.08)
Propyzamide			9(0.14		1(0.03)
RH-24644			2(0.03)		
Pyridate					
PHCP		0	3(0.309)		
Rimsulfuron					
PPU	19(0.05)	349(0.11)			
PPU-desamino		76(0.03)			
Tebuconazole	1(0.01)	1(0.01)		5(0.12)	1(0.01)
Terbuthylazine		36(0.12)	1(0.02)	51(1.9)	
2-hydroxy-desethyl-terbuthylazine	1(0.03)		1(0.02)		7(0.09)
Desethyl-terbuthylazine		276(0.2)	161(0.14)	7(0.05)	66(0.84)
Desisopropylatrazine	1(0.01)		4(0.05)	27(0.03)	60(0.04)
Hydroxy-terbuthylazine					34(0.07)

The maximum concentration (µg/L) is shown in parentheses. Only pesticides and or their degradation products where at least one of the compounds is detected in more than three samples from one field are included. Pesticides applied in spring 2011 are not included.

Types of leaching scenario

More detailed studies of leaching scenarios in the sandy fields reveal long-term leaching of degradation products in concentrations continuously exceeding 0.1 µg/L (Table 7.5-67 and Table 7.5-69) up to six years after application of the pesticides metribuzin in May and rimsulfuron in June to potato crops Recent PLAP results show that the slightly mobile and moderately persistent fungicide metalaxyl-M, applied in July on potatoes, exhibits the same long term leaching of its degradation products (CGA62826 and CGA108906). However, this parent compound was also detected in water samples from 1 m b.g.s in groundwater in both the sandy fields. The concentration exceeded 0.1 µg/L in 5 % of the ground water samples collected from the Jyndevad field (Figure 7.5-50).

In the loamy fields, dominant preferential flow results in leaching scenarios that differ from those seen in the sandy fields. Leaching occurred following both the early summer and autumn application of pesticides. Early summer application resulted in considerable leaching of pesticides and/or their degradation products, grouped below according to their fate properties (see paper for discussion of detection of terbuthylazine, fluazifop-P-butyl, azoxystrobin, and bentzon and/or their metabolites).

Autumn application of pesticides resulted in leaching of several strongly-sorbing pesticides. Glyphosate (sorbs to the mineral soil fraction, hydrophilic, non-persistent, application period 11 August- 9 November) and pendimethalin (sorbs to the organic soil fraction, moderately persistent, applied in May as well as October - November) were found to leach to a 1 m depth and below in the loamy fields, primarily in dissolved form. Neither of the two compounds leached in the sandy fields. In contrast, bifenox, a strongly sorbing, non-persistent herbicide (sorbs to organic sorption sites, application date: 27 April at Jyndevad, 1 May at Estrup, 9 September at Silstrup and 25 October at Faardrup), was detected 1 m b.g.s. in all three loamy fields as well as in the sandy Jyndevad field (Table 7.5-68). Furthermore, it was detected in

groundwater in both the Jyndevad and Silstrup fields (Figure 7.5-50, Figure 7.5-51, Table 7.5-70). Degradation products of bifenox were detected at some sites as well.

Figure 7.5-50: Frequency of detection in water samples from the suction cups (left) and groundwater monitoring screens located deeper than the suction cups (right) in the sandy soil fields: Tylstrup (A, B) and Jyndevad (C, D).

Frequency is estimated for the entire monitoring period during which the different pesticides and/or degradation products have been included in PLAP. When pesticides are applied several times or result in long-term leaching, the entire monitoring period can consist of multiple monitoring periods of at least two years or long-term monitoring of up to nine years. The number of analyzed samples therefore varies considerably among the different pesticides and/or degradation products. Compounds monitored for less than one year are not included. The crop on which the pesticide is applied is indicated.

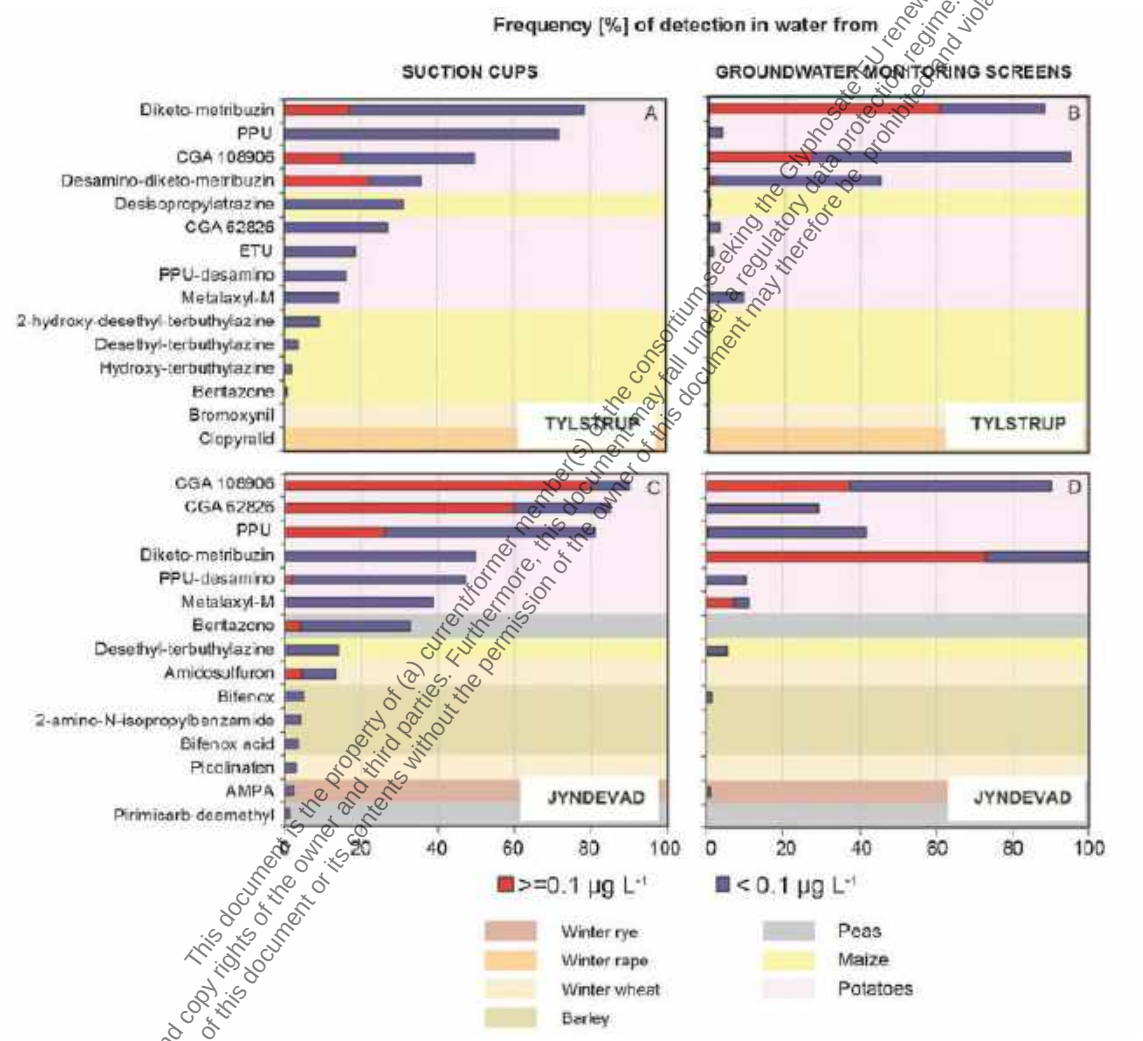
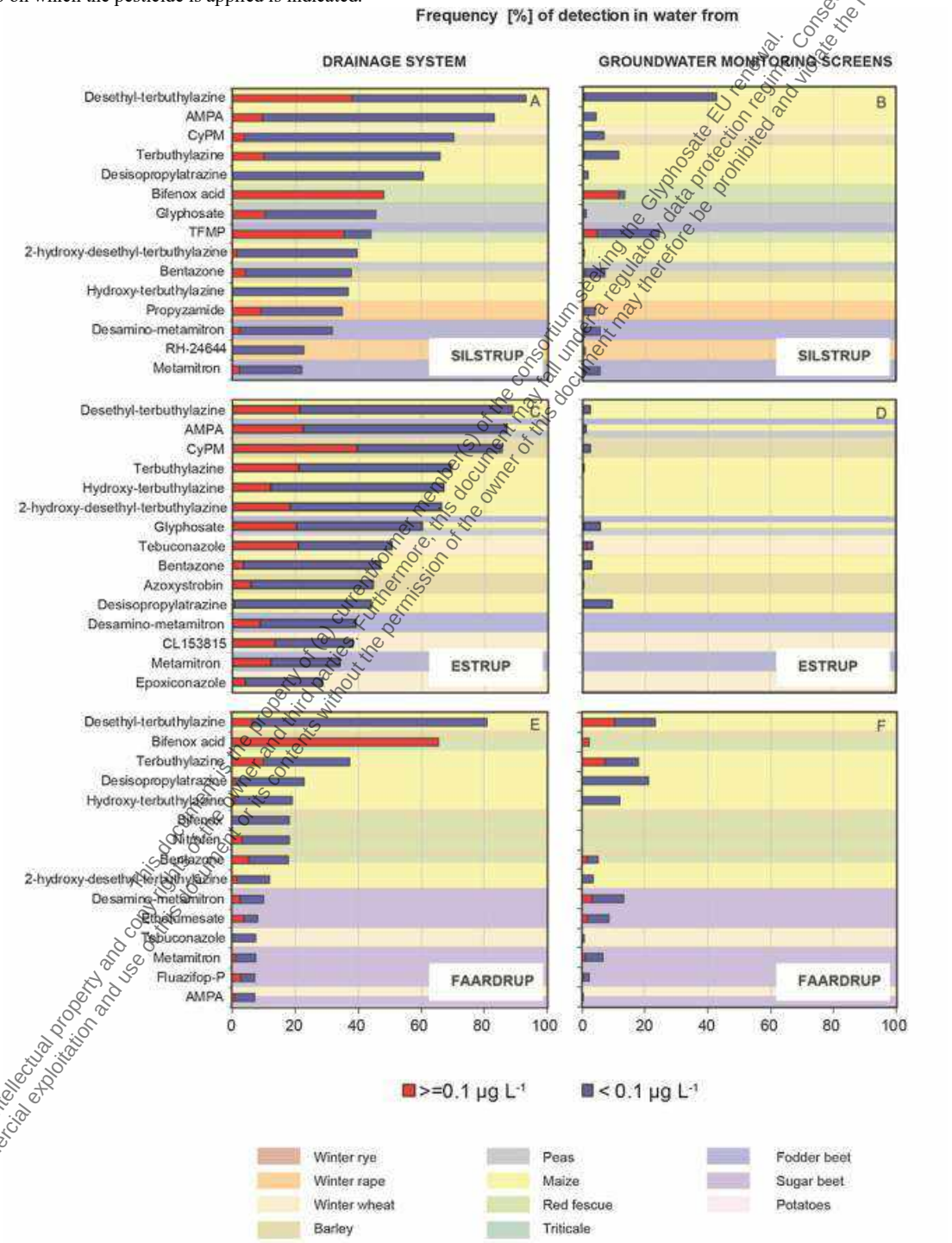


Figure 7.5-51: Frequency of detection in water samples from the drainage system (left) and groundwater monitoring screens (both vertical and horizontal) located deeper than the drainage system (right) in the loamy soil fields: Silstrup (A, B), Estrup (C, D), and Faardrup (E, F).

Frequency is estimated for the entire monitoring period that the different pesticides and/or degradation products have been included in the PLAP program. When pesticides are applied several times or result in long-term leaching, the entire monitoring period can consist of multiple monitoring periods of at least two years or long-term monitoring of up to nine years. The number of analyzed samples therefore varies considerably among the different pesticides and/or degradation products. The figure includes only the 15 most frequently detected pesticides and degradation products. Compounds monitored for less than one year are not included. The crop on which the pesticide is applied is indicated.



Impact of fate processes and hydrogeological setting on leaching

In the sandy fields, the long-term leaching of degradation products following pesticide application in the early summer or summer months seems to be the primary leaching scenario of concern. The high frequency of leaching in the loamy fields demonstrates the dominance of effective transport processes in these soils. It is well documented that the effective porosity in loamy soil corresponding to connected discontinuities, such as wormholes and fractures, is low compared to that of sandy soil, with the result that climatic conditions have a greater impact on the pore system. The consequences are: (i) immediate response to a precipitation or snowmelt event causing rapid flow and transport through discontinuities, which may sometimes be directly connected to drains; and (ii) seasonal fluctuations of up to 4 m in the groundwater table with resultant drainage, primarily in periods when the groundwater table is located above the drains (above 1.1 - 1.2 m b.g.s.). This very dynamic hydrogeological setting enables a larger variety of pesticides and their degradation products to reach groundwater in intense pulses, before being diluted or retarded to varying degrees. Glyphosate is an example of a pesticide suddenly appearing in intense pulses of high concentrations more than two years after application and following several pronounced rain events (more than 50 mm/day) during the late summer. Such leaching scenarios can only be a result of very slow degradation and strong adsorption in the topsoil, which is supported by, who found half-life DT_{50}-values greater than 100 days for soils with strong adsorption and as short as 10 days for soils with weak adsorption. The PLAP results demonstrate that pesticide leaching occurs after both early summer and autumn application. Analysis of the data in Table 7.5-67 for the three loamy fields reveals notable leaching of 23 % of the pesticides applied in early summer, and 60 % of the pesticides applied in the autumn. Pesticides applied in summer (April - August) accounted for 7 of the 13 pesticides having a high degree of leaching, 11 of the 15 pesticides having a medium degree of leaching, and all nine of the pesticides having in a low degree of leaching. Based on fate studies, the first seven pesticides can be grouped as follows:

- slightly mobile hydrophobic + persistent (azoxystrobin) or non-persistent (fluazifop-P-butyl). Even though the persistent pesticide azoxystrobin was detected in water from the drainage system, it was primarily the long-term leaching of R234886 (degradation product of azoxystrobin) in the Silstrup and Estrup fields and TFMP (degradation product of fluazifop-P-butyl) in the Silstrup field that was of concern.

- moderately mobile + persistent and hydrophobic (ethofumesate and terbuthylazine) or non-persistent (metamitron (log P = 0.85) and pirimicarb (log P = 1.7)). Notable leaching of these pesticides was unexpected due to their fate properties. All four of the pesticides were detected in water from the saturated zone, especially at the Silstrup and Faardrup fields (Table 7.5-69 and Table 7.5-70), and leached to 1 m b.g.s. in average concentrations exceeding 0.1 µg/L within the first season following application, primarily at the Estrup field (Table 7.5-67 and Table 7.5-68).

- mobile hydrophilic + non-persistent (bentazone). The fact that bentazone is found to be non-persistent (DT_{50} <30 days) in the plow layer apparently does not play a role in its retardation in the topsoil. Bentazone was detected in water from the saturated zone in the three loamy fields, especially the Silstrup field (Table 7.5-69 and Table 7.5-70), and leached to 1 m b.g.s. in average concentrations exceeding 0.1 µg/L within the first season after application, primarily in the Estrup field (Table 7.5-67 and Table 7.5-68).

In Denmark, most pesticides authorized for autumn application are strongly adsorbing due to the increased leaching risk caused by periods of intense precipitation. Hence, the pesticides applied in the autumn and causing unpredicted leaching scenarios were in fate studies found to be:

- non-mobile hydrophobic + non-persistent (bifenox) or moderately persistent (pendimethalin and picolinafen) and

- slightly mobile + non-persistent (glyphosate) or moderately persistent and hydrophobic (propyzamide and tebuconazole).

Pesticides that strongly sorb to minerals and organic sorption sites were nevertheless detected in water samples, primarily from the drains and occasionally from the saturated zone. The more frequent detection

in water from drains is attributable to the groundwater table often being above drain depth during the autumn and winter periods. Applying pesticides under these conditions allows their drain-facilitated direct transfer from the uppermost groundwater to the surface water. The difference in the hydrogeological setting in early summer and autumn is evident in relation to the risk of leaching. During early summer, the drier soil profile leaves the plow layer and dead-end discontinuities more open to entry by the applied pesticides and retardation compared to autumn when the pore space will be close to full saturation, allowing rapid preferential transport through the well-connected discontinuities. The leaching scenarios in the three loamy fields indicate hydrogeological settings dominated by rapid, preferential transport and low retardation capacity of the plow layer.

Crop related effects on leaching
Of the eleven crops included in PLAP, leaching seems to be greatest with potatoes, maize and beet crops (Figure 7.5-51). One common attribute of these crops is that they are all cultivated in systems with wide row spacing (maize 0.5 - 0.75 m; beet 0.5 - 0.63 m; potato 0.75 m).

Pesticide detection in the different types of installation
A comparison of the monthly sum of samples containing pesticide/degradation products in concentrations either below, equal to or exceeding MAC in water from installations approximately 1 m b.g.s. (cups or drains) and in the saturated zone (vertical or horizontal wells) reveals that (Figure 7.5-52):

- The percentage of water samples containing pesticide/degradation products is higher in water from the suction cups than from the vertical wells in the sandy fields, which can be explained by the dilution or retardation of solute mass on its way through the soil profile.

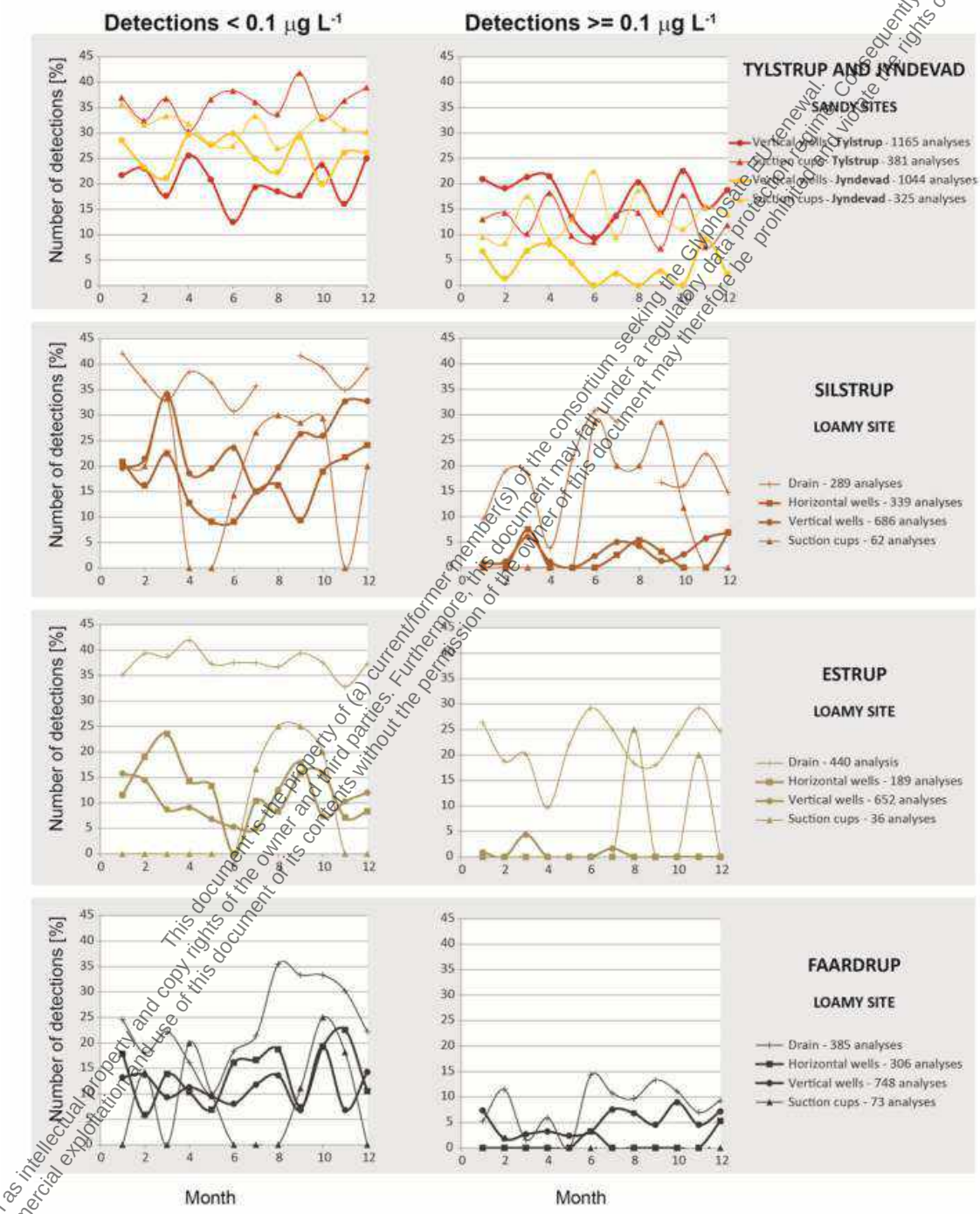
- The percentage of pesticide-containing samples from the vertical and horizontal wells each month during the 12-year period at each of the loamy fields is more or less identical for both <MAC and ≥MAC. A small difference between detections ≥MAC in vertical and horizontal detections is, however, to be found in Faardrup.

- The percentage of pesticide-containing water samples from the drainage system was fairly similar at the Silstrup and Estrup fields, but much lower at the Faardrup field for concentrations both <MAC and ≥MAC.

- The percentage of water samples with detections from the variably saturated zone (drainage system and suction cups) and saturated zone (vertical and horizontal wells) respectively was very similar at Faardrup, but less similar at Silstrup and Estrup.

- There does not seem to be any relationship between the percentage of samples that contain pesticides and the month of the year, i.e. the percentage is not significantly higher in any particular month or months.

Figure 7.5-52: **Percentage of samples containing pesticides for each month of the year subdivided into samples with concentrations either below (left) or equal/exceeding 0.1 mg/L (right) shown for each field (sandy fields: Tylstrup and Jyndevad; loamy fields: Silstrup, Estrup, and Faardrup) and installation type (suction cups, drain, vertical and horizontal wells). The total number of analyses is shown for each installation type and field.**



Conclusions

The PLAP monitoring results show that fewer pesticides and/or their degradation products are detected in water samples collected both 1 m b.g.s. (Table 7.5-67 and Table 7.5-68) and from groundwater (Table 7.5-69 and Table 7.5-70) in the sandy fields than in the loamy fields. A number of groundwater leaching scenarios do not seem to be adequately described by the European Union pesticide authorization procedure, including long-term leaching of degradation products of pesticides applied to potato crops on sandy fields and leaching of a variety of pesticides and degradation products following pesticide application to loamy fields. Leaching exceeding MAC in yearly averages at 1 m depth and/or in detections in the groundwater was seen with 32 % of the pesticides applied to the loamy fields in early summer and with 60 % of those applied in the autumn. Based on the insight into the fields' hydrogeological setting, the compound properties and crop development, these findings indicate that rapid preferential transport through well-connected discontinuities such as wormholes and fractures enable the pesticides to bypass the otherwise retarding plow layer. This seems to be triggered by the soil profile being close to saturation following autumn application and by possible sealing of the soil surface following the early summer application of pesticides. The physics behind this rapid preferential transport is not fully understood and hence not fully accounted for in the EU pesticide authorization procedure. Furthermore, agricultural practice seems to enhance leaching in the case of pesticides applied to crops with widely-spaced rows, such as potatoes, maize, and beet. Comparison of pesticide detection frequency in water from the installations in the variably saturated and saturated zones provides a good indication of the hydraulic contact between the zones and whether the quality of the water in the variably saturated zone can serve as an early warning of the trend in water quality in the saturated zone or surface waters. Pesticide detection frequency does not appear to depend on the month of the year, but monthly variation in detection frequency is higher in the loamy fields than in the sandy fields, primarily due to the dominant effect of spatial and temporal variation in preferential transport in the variably saturated zone of the loamy soils.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes results from the Danish pesticide leaching program. Analytics are not well described, but there is a statement of careful selection and strong quality control of the laboratories. The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/020
Report author	McManus, S. *et al*.
Report year	2014
Report title	Pesticide occurrence in groundwater and the physical characteristics in association with these detections in Ireland
Document No	Environmental Monit Assess (2014) 186:7819–7836
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (EPA Ireland)
Acceptability/Reliability:	Reliable

2. Full summary

This study explores the associations of pesticide occurrence in groundwater to geological characteristics of the monitoring points (MPs) contributing area. Pesticide analyses were undertaken during a 2-year groundwater monitoring campaign, which generated 845 samples. MCPA and mecoprop were the most frequently detected pesticides in groundwater. Each MP (n = 158) had a specifically delineated zone of contribution (ZOC) and the dominant physical characteristics present from nine national datasets were recorded for each ZOC. Associations between detections in groundwater and the dominant physical characteristic in each MPs ZOC tested were then statistically analyzed using Fisher's exact test, logistic regression, and multiple logistic regression. The original physical characteristic datasets used that were associated with detections in groundwater were the type of MP, aquifer type, and Quaternary deposit type. Logistic regression revealed that springs, regionally important aquifer types, aquifers with a karstic flow regime, and alkaline Quaternary deposits in existence above karst aquifers in a MP's ZOC were more likely to have a pesticide detection in groundwater. Multiple regression from this exploratory work showed some mutual dependency between soil association, aquifer type, and the Geological Survey of Ireland groundwater vulnerability map. The combination of national monitoring data and physical attribute datasets can be used to explore key areas where groundwater is more vulnerable to pesticide contamination.

Materials and Methods

Groundwater monitoring network summary and analytical methods

Many of the monitoring points (MPs) in the monitoring network are public or private drinking water supplies with relatively large abstraction rates (7,000 m^3/day) or springs with relatively large discharges (12,000 m^3/day). These MPs were targeted because the water quality from their relatively large ZOC is less likely to be impacted by localized pressures (Craig *et al*. 2005). This study focuses on a period between 2007 and 2008. During this period, 158 MPs were sampled between one and nine times (average 5; median 6). Representative samples were collected using standard methods (ISO 5667).

For solid phase extraction (SPE), groundwater was loaded through a Biotage C18 (Uppsala, Sweden) SPE cartridge conditioned using liquid chromatography (LC)-grade dichloromethane, left to dry, then further conditioned using LC-grade methanol. Compounds were eluted from the cartridge using dichloromethane and blown down under argon gas. Quantification was carried out using an Agilent gas chromatogram mass spectrometer (GC-MS) in electron ionization (EI) mode. The method was validated in accordance with criteria provided in SANCO guidelines (SANCO/825/00 and SANCO/10232/2006). The 13 pesticides analyzed are detailed in Table 7.5-71 along with their current registration status in Ireland.

Zones of contribution

A ZOC is defined as the catchment area that contributes water to an abstraction or a spring (Misstear *et al*. 2006) which supports the abstraction point, monitoring point, or spring discharge (hereafter referred to as MPs) from long-term groundwater recharge. Hydrogeologically mapped ZOCs are preferred over arbitrary radii around a MP, since the latter generally do not adequately characterize the pressures and pathways contributing to any concentrations measured at a MP (Franzetti and Guadagnini 1996; Lim *et al*. 2010), especially in heterogeneous environments. The ZOC polygons were manually aligned to honor hydrogeological controls on the orientation, with scaling to match abstraction with recharge. As such, the ZOCs represent a relatively robust, but rapidly derived, spatial estimate of the region contributing groundwater flow to a MP (Kelly 2010).

Dataset assembly for statistical analysis with corresponding ZOCs

The dataset was categorized into three levels based on the detected pesticide concentration found at each MP. This approach was required because of the extremely high level of censoring in the dataset due to analytical limits of detection. In our study, non-detects did not have values substituted by another numerical value as it may lead to abnormalities in the statistical conclusions, and because of this, standard methods for continuous data could not be applied. For every compound, the limit of detection was 0.01 µg/L and this was unadjusted for all data analysis.

The concentration category levels that MPs were grouped into were as follows: 1. MPs which never had a detection greater than or equal to the analytical detection limit of 0.01 µg/L during the 2-year monitoring period. 2. MPs with at least one detection greater than the analytical limit of detection but less than the DWS 3. MPs with at least one detection greater than or equal to the DWS. The concentration level detected at each MP explained above and the most widespread category for each physical characteristic in the MP's ZOC (explained below) were then collated to produce a combined dataset. This was used to generate count tables for statistical analysis in SAS (2004).

Table 7.5-71: Pesticides (a i) quantified during monitoring, their registration status in Ireland 2017 and use

Name	CAS name	CAS number	Registration status in Ireland	Use
2,4-D	(2,4-dichlorophenoxy)acetic acid	94-75-7	active	herbicide
atrazine	6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine	1912-24-9	expired 2007	herbicide
bentazone	3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide	25057-89-0	active	herbicide
chlorotoluron	N'-(3-chloro-4-methylphenyl)-N,N-dimethylurea	15545-48-9	expired 2006	herbicide
cypermethrin	cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate	52315-07-8	Active	insecticide, veterinary treatment
DDT	1,1'-(2,2,2-trichloroethylidene)bis[4-chlorobenzene]	50-29-3	expired 1981	insecticide
dieldrin	(1aR,2R,2aS,3S,6R,6aR,7S,7aS)-rel-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3-b]oxirene	60-57-1	expired [illegible]	insecticide
diuron	N'-(3,4-dichlorophenyl)-N,N-dimethylurea	330-54-1	expired 2009	herbicide, biocide
glyphosate	N-(phosphonomethyl)glycine	1071-83-6	active	herbicide
IPU	N,N-dimethyl-N'-[4-(1-methylethyl)phenyl]urea	34123-59-6	active	herbicide
lindane	(1α,2α,3β,4α,5α,6β)-1,2,3,4,5,6-hexachlorocyclohexane	58-89-9	expired 2001	insecticide, acaricide
MCPA	(4-chloro-2-methylphenoxy)acetic acid	94-74-6	active	herbicide
mecoprop	2-(4-chloro-2-methylphenoxy)propanoic acid	7085-19-0	active	herbicide

CAS = Chemical Abstracts Service

Physical characteristic national dataset summary

The most prevalent ZOC physical characteristics for each MP were assigned from national datasets in Table 7.5-72. MP type was classified by the Irish EPA as springs, drilled boreholes, and wells. (Wells include sites known to be dug wells and sites where the well construction method is unknown). ZOC size corresponding to each MP was placed into one of the following seven categories: 0-4.9, 5-9.9, 10-19.9, 20-199, 200-399, 400-699, 700-799 km^2.

Land use was taken from the Corine land cover dataset in 2006 (European Environment Agency 2011) and categories within the entire Corine land cover dataset were amalgamated into a fewer number of categories (Table 7.5-72) so (1) there were enough observations for statistical analysis and (2) there were fewer categories to assist logistic regression.

Two national soil datasets are currently available, and both were examined. Nine amalgamated categories were created from each of the two datasets. These are listed in Table 7.5-72. Quaternary deposit (subsoil) type was subdivided using two methods: the first according to the type of Quaternary deposit (genesis) and the second (Quaternary deposit acid/base) based on its reaction with 10 % v/v hydrochloric acid to determine the calcium carbonate ($CaCO_3$) content. Subsoil permeability was determined by the GSI using the British Standards Institution BS 5930 system (1981) (Swartz *et al*. 2003). Textural descriptions were made of each subsoil (Quaternary deposit) using plasticity, dilatency, density, compactness, and the presence of discontinuities (Misstear and Daly 2008). Bedrock geology was obtained from the GSI (1999) and contains 27 bedrock units created by grouping over 1,200 bedrock Formations and Members based on their hydrogeological properties and other factors from the original bedrock geology file. Some of the most commonly occurring bedrock geologies are listed. The bedrock geology map was also the foundation for the national aquifer type map which produced 11 aquifer types across Ireland of which two groupings were used for statistical analysis.

Groundwater vulnerability in Ireland is determined primarily according to the thickness and permeability of the Quaternary deposits. Categories listed in Table 7.5-72 are in order of decreasing vulnerability. Subsoil deposits 0-3 m thick are classified as extreme (E) with a subset of the "extreme" category termed the "X-extreme" category, relating to areas of bedrock outcrop or subcrop, or within 30 m of a location of

point recharge (Daly 2004). Areas with deposits greater than 3 m thick are classified as high, moderate, or low vulnerability based on subsoil thickness and permeability after (Daly 2004).

Table 7.5-72: National datasets for physical characteristics selected for the prediction of groundwater pesticide occurrence and categories for each characteristics subsequent association within each category

MP type	Corine Land Cover 2006[1]		Soil Type		Quaternary deposits (subsoil)			Bedrock geology[6]	Aquifer type[7]		Gr vul
	Land use II	Land use III	Soil association[2,3]	IFS soil type II[2,3]	Quaternary deposit genesis[4]	Quaternary deposit acid/base[4]	Subsoil permeability[5]		GSI aquifer importance	WFD flow regime	
Spring	Agriculture (non-irrigated arable land and pasture)	Arable (non-irrigated arable land)	Acid brown earth	Deep well drained mineral	Alluvium	Acidic	High	Granites and other igneous intrusive rocks	Poorly productive aquifers	Poorly productive fissured bedrock	Ex
Drilled borehole	Forestry[8]	Non-arable (pasture land)	Blanket peat	Shallow well drained mineral	Peat	Alkaline	Low	Dinantian pure bedded limestones	Locally important aquifers	Karstic	Ex
Well	Other land use[9,10]	Forestry[8]	Brown podzolic	Deep poorly drained mineral	Gravels		Low-high	Dinantian upper impure limestones	Regionally important aquifers	Productive fissured bedrock	Hi
		Other land use[9,10]	Degraded brown podzolic	Shallow poorly drained mineral	Irish sea till		Low-low	Dinantian pure massive limestones		Intergranular	Hi
			Gley	Poorly drained mineral soils with peaty topsoil	Karstified rock		Mod-high	Dinantian upper impure limestones			Mo
			Grey brown podzolic	Shallow lithosolic or podzolic type soils potentially with peaty topsoil	Till		Moderate	Dinantian (early) sandstones, shales, and limestones			Lo
			Minimal grey brown podzolic	Alluvium			Moderate-low	Ordovician metasediments			
			Rendzina and outcropping rock	Peats			Mix.*	Devonian old red sandstones			
			Shallow brown earth	Miscellaneous				Ordovician volcanics			

[1] European Environment Agency (2011)
[2] Gardiner and Radford (1980)
[3] Bulfin et al. (2002)
[4] Fealy et al. (2009); GSI (2011)
[5] GSI (2011)
[6] GSI (1999)
[7] Dominant soil type for each association in each ZOC
[8] Amalgamation of categories listed by Bulfin et al. (2002) into a new number for input into statistical analyses
[9] Where incomplete vulnerability or permeability was assigned as mixed classification
[10] Forestry includes broad leaved forest, coniferous forest, mixed forest, and transitional woodland scrub
[11] Other land use includes natural vegetation, urban land use, bare rocks, mineral extraction sites, and peat bogs

Statistical methods

For each MP, the most prevalent category for each physical characteristic within its ZOC was recorded to generate count tables for input into SAS (2004). These tables of observational data were analyzed using the categorical data analysis procedures of SAS (2004). As the analysis is exploratory in spirit, both marginal tests of one factor at a time and multiple regression were used to assess the association of ZOC characteristics with pesticide detections.

For the marginal tests, tables were generated using the three concentration level categories listed above with an emphasis on answering a priori questions. All statistical results have been given with 95 % confidence intervals ($p \leq 0.05$).

Fisher's exact test (Agresti 2002), a non-parametric test, was used for the analysis of the summary data tables as the low counts caused by non-detects in a large proportion of the cells made a chi-square test unreliable. The analysis was carried out using the Proc Freq procedure in SAS (2004) using the Monte-Carlo approximation for larger tables. Physical characteristics were deemed to be significantly associated with pesticide occurrence if the Fisher's exact test p value was <0.05. Any significant associations were then further examined using logistic regression (Agresti 2002) to determine which categories within the physical characteristic differed and which had the greatest likelihood of a pesticide detection. Odds ratios from logistic regression were the main tool for assessing this detail. Statistical tests of various sub-groupings of the data set are intended to be interpreted in an exploratory sense and are not adjusted for the multiplicity

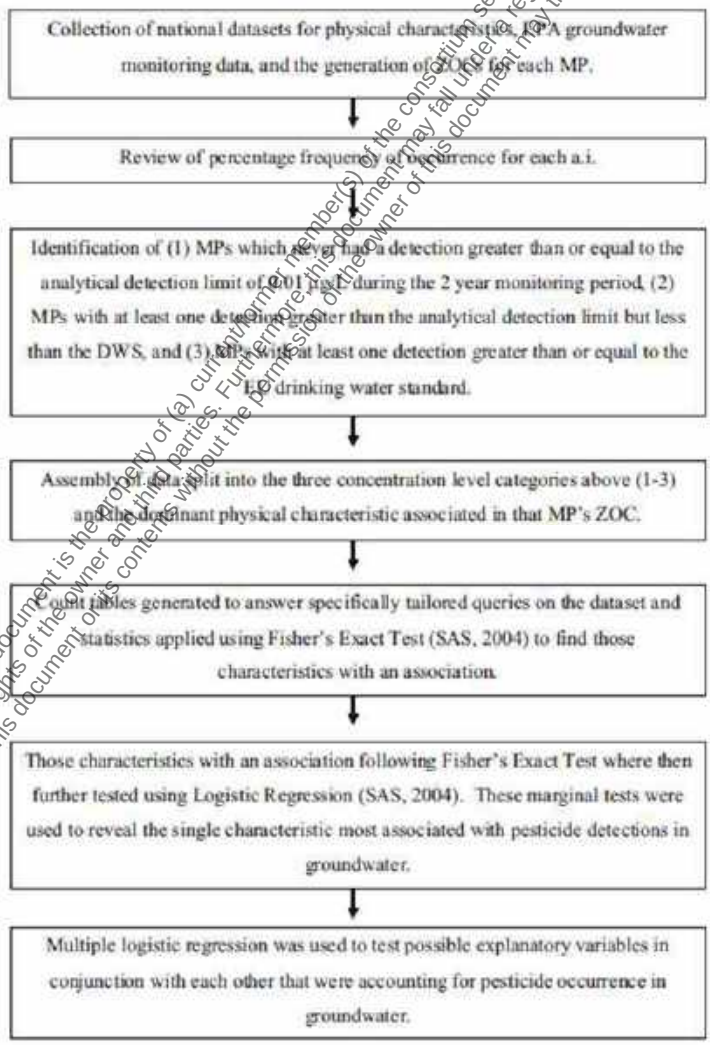
of tests carried out. The sequence of method steps for identification of physical characteristics most associated with pesticide detections in groundwater is summarized in Figure 7.5-53. A second analysis approach used multiple logistic regression and automatic variable selection procedures (SAS 2004) to determine which factors from the marginal tests were jointly associated with pesticide detections.

Results

National pesticide occurrence

Of the 845 samples analyzed, 73 % had no detection of any pesticide, 24 % had detections greater than the analytical limit of detection but less than the DWS of 0.1 µg/L, and 3 % of samples had at least one detection greater than or equal to the DWS. The percentage frequency of occurrence from the national groundwater pesticide dataset (Figure 7.5-54) shows pesticide occurrence during monitoring on one sampling occasion, with the number of detections for each pesticide expressed as a percentage of the total number of samples analyzed for that particular pesticide. Nationally, MCPA and mecoprop were the most frequently observed pesticides in groundwater, being found in 8.7 and 8 % of samples, respectively.

Figure 7.5-53: Sequence of work undertaken to allow for the assessment of ZOC physical characteristics and pesticide detections in groundwater



The DWS was exceeded for mecoprop in one sample. Five priority substances: atrazine, isoproturon (IPU), DDT, dieldrin, and diuron, were tested during monitoring. Atrazine was detected in five samples and IPU

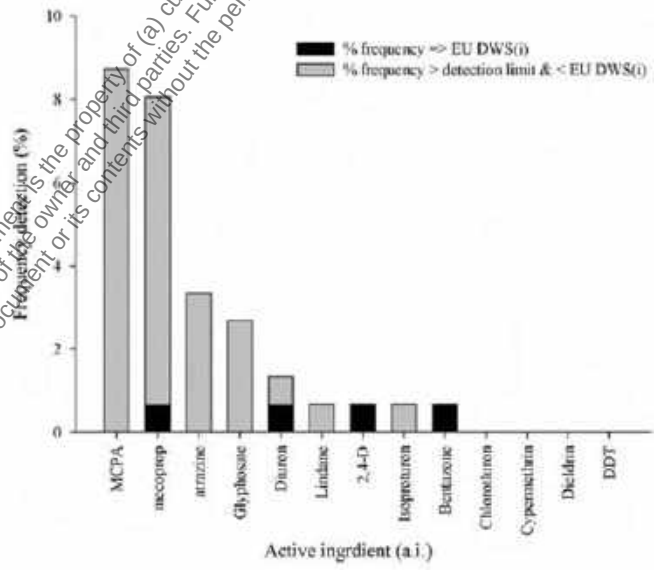
was detected in one sample, but none were observed greater than or equal to the DWS. Diuron was detected in two samples, with one having a detection greater than the DWS. Lindane, glyphosate, bentazone, and 2,4-D were also included for analysis and found in detectable concentrations with only bentazone and 2,4-D exceeding the DWS. Samples were analyzed for chlorotoluron, dieldrin, DDT, and cypermethrin but no detections greater than the analytical detection limit were observed for these four compounds.

When positive detections were expressed as a function of the total number of MPs sampled during the campaign, 47.5 % of MPs had no detection, 40.5 % of MPs had one or more detections greater than the analytical detection limit but less than the DWS, and 12 % of MPs had a detection greater than or equal to the DWS on at least one sampling occasion. No samples processed during the monitoring campaign exceeded the 0.5 µg/L DWS limit set for total pesticides in any one sample. Figure 7.5-55 indicates the spatial distribution of MPs and the concentration category level throughout the course of monitoring. Figure 7.5-56 shows the geographic distribution of the four most commonly detected pesticides, with a cluster of MPs in the West of Ireland with frequent detections. Mecoprop and MCPA were the most commonly encountered pesticides at MPs with 36 and 39 % of MPs having at least one detection of these compounds, respectively. Lindane was detected in at least at 17 % of MPs and glyphosate at 8 %. The percentage of MPs with detections for each of the other individual compounds not shown in Figure 7.5-56 was 6 % for atrazine, 4 % for 2,4-D, 3 % for bentazone and diuron, and 0.6 % for IPU.

Statistical analysis of occurrence with ZOC properties

This dataset is a sample of convenience since samples were not collected in a truly random fashion. Thus, there may be some unquantifiable bias associated with it. However, given the large size and wide coverage of the dataset, it is the authors' opinion that it is informative and can be used effectively to examine relationships in a purely exploratory manner.

Figure 7.5-54: Frequency of pesticide detections as a % of the total number of samples analyzed for a particular pesticide compound in 1 month during the sampling campaign in 2007-2008. Values are adjusted to each compound's analytical detection threshold of 0.01 µg/L. The Council Directive 98/83/EC drinking water standard (DWS) is 0.1 µg/L



Fisher's exact test

Pesticide detections in groundwater were found to be significantly related to seven of the physical characteristics present in each MPs ZOC (Table 7.5-73). Some of the original physical characteristic national datasets were further amalgamated into a smaller number of categories (Table 7.5-72).

Logistic regression
Following Fisher's exact test, the five physical characteristics with a significant degree of association were tested using logistic regression. The results indicate that springs are more likely to have a pesticide detection followed by wells, and then closely followed by boreholes (p = 0.0028) (Figure 7.5-57a).

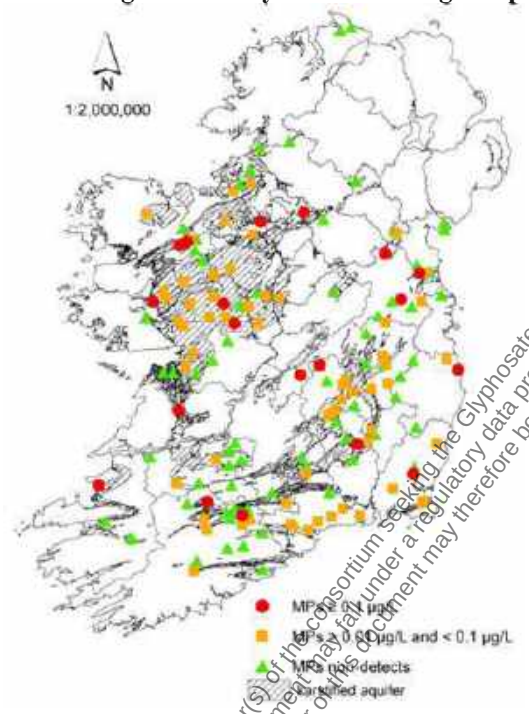
With aquifer types split according to their GSI classification based on aquifer importance, the output revealed (Figure 7.5-57b) that there is a greater likelihood of a detection in a regionally important aquifer compared to a locally important aquifer or a poor aquifer (p = 0.0007).

The 11 aquifer types were also classified using the Irish system of flow regime used for the WFD (Table 7.5-72; Figure 7.5-57c). In descending order of probability of a pesticide detection in groundwater were karstic aquifers >intergranular >productive fissured bedrock >poorly productive fissured bedrock aquifer types (p = 0.0002).
Pesticide detections at MPs were tested in relation to aquifer transmissivity, using aquifer importance as a surrogate (GSI aquifer importance, Table 7.5-72). Higher yielding regionally productive and potentially higher transmissivity aquifers were more likely to have a pesticide detection in groundwater than poor - or locally important - aquifer types (p = 0.0007, Figure 7.5-57b). Karstic aquifer types (Rkc and Rkd) were removed from the dataset so they did not influence the outcome. The count tables were reanalyzed and Fisher's exact test revealed there was still an association between pesticide detections in groundwater and regionally important aquifer types (p = 0.0013) (Table 7.5-73). Figure 7.5-57 shows the SAS output for logistic regression.

An association was found between Quaternary deposits and groundwater pesticide detections using Fisher's exact test (p = 0.0260). Splitting Quaternary deposit types into six categories (Quaternary deposit genesis; Table 7.5-72) revealed no association (p = 0.1820) using Fisher's exact test; thus, no further logistic regression was performed.

Logistic regression on Quaternary deposit chemistry classification revealed that there was a significantly (p = 0.0048) greater chance of a pesticide detection in an alkaline Quaternary deposit compared to an acidic Quaternary deposit (Figure 7.5-57d). Although Fisher's exact test indicated an association between pesticide detections in groundwater and IFS soil type II using Fisher's exact test (p = 0.0095), logistic regression on this classification indicated that there was no further statistically significant relationship within the nine categories listed in Table 7.5-72 (p = 0.1069) due to sparseness in the table (detections vs. non-detections among the categories listed in Table 7.5-72 for IFS soil type).

Figure 7.5-55: Spatial distribution of MPs with detections exceeding the EU DWS, MPS with detectable detections of pesticides, and MPs which never had a pesticide detection throughout the 2-year monitoring campaign



Multiple logistic regression

Severe numerical problems during multiple logistic regression prevented satisfactory modeling of the three-level multinomial response for detection but it was possible to fit a binary response for detect/non-detect using the logistic procedure in SAS with penalized likelihood using the Firth option. There were difficulties in this process too because of multicollinearity but Table 7.5-75 contains the outcome for this dataset. It is of interest to contrast the factors found to be useful in the multiple regression model with those in the marginal tests. Only GSI aquifer type and Quaternary deposit acid/base were significant in marginal tests.

While it is difficult to interpret all of these changes in detail because of sparseness in the tables results in some extreme odd ratios, it appears from simple effect testing within the interactions that there was an impact of GSI aquifer type at the extremes of groundwater vulnerability, i.e., high or greater ($p < 0.0001$) and moderate or lower ($p = 0.0007$) but not for H-L ($p = 0.45$). For the soil association × groundwater vulnerability interaction, there was an impact of groundwater vulnerability for shallow soils ($p = 0.0002$) and acid brown earth soils ($p = 0.0013$).

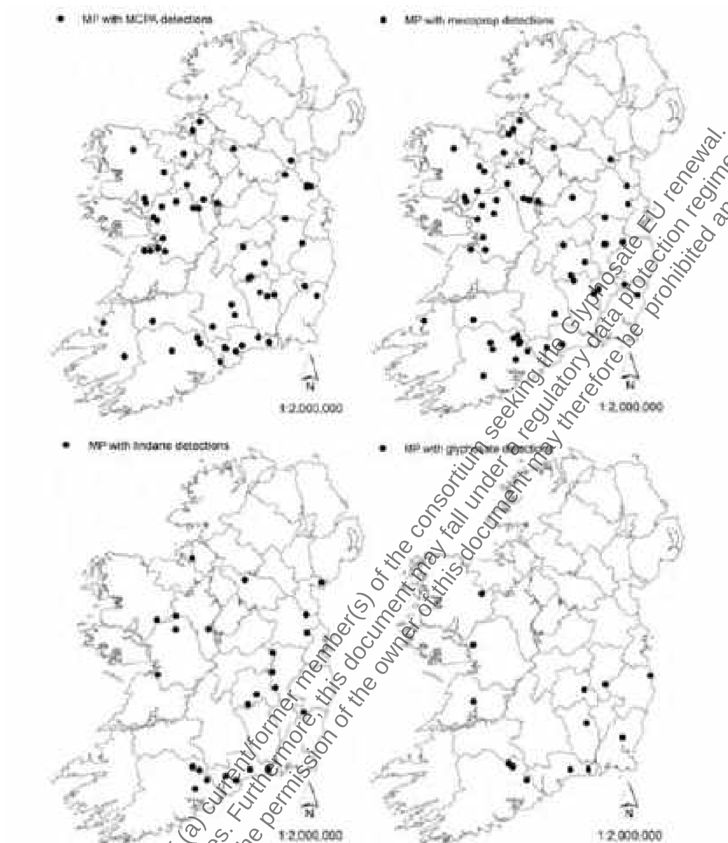
Discussion

Frequency of pesticides

From the whole monitoring campaign, MCPA and mecoprop were the most frequently detected pesticides to exceed the DWS with detections in less than 1 % of samples collected between 2007 and 2008 (Figure 7.5-54). MCPA and mecoprop are extensively used in varying land uses across Ireland. IPU never exceeded the DWS during monitoring while bentazone was detected in samples at concentrations equal to the DWS. From Table 7.5-74, less bentazone was applied in comparison to IPU, yet all four detections of bentazone exceeded the DWS. IPU had approximately 191 times the amount applied in Ireland in comparison to bentazone (Table 7.5-74) yet IPU was rarely detected. Three compounds (atrazine, lindane, and diuron), banned between 2000 and 2008 (Table 7.5-71), were detected in groundwater. Neither chlorotoluron, cypermethrin, dieldrin nor DDT were detected in groundwater between 2007 and 2008. Transformation products should be considered for future monitoring campaigns not just in Ireland but

across the world, to help further understand their fate, transport, persistence, and ecological significance in the environment.

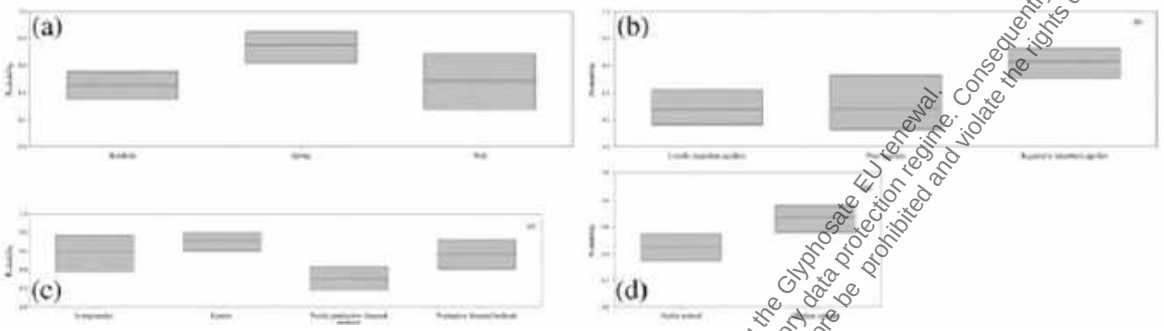
Figure 7.5-56: Spatial distribution of MPs with detections of each individual pesticide:MCPA, mecoprop, lindane and glyphosate on at least one occasion



Frequency of detections at monitoring points

The cluster of detections in the West of Ireland corresponds to an area dominated by karst geology, alkaline Quaternary deposits, and where many of the MPs are springs - also highlighted during statistical analysis (Figure 7.5-57). Detections for individual compounds (Figure 7.5-56) are not located in any one particular County of Ireland although no glyphosate was detected in the North East. For the four most frequently detected compounds, their detections, as with Figure 7.5-55, center in karst areas in the west and south.

Figure 7.5-57: **Results from logistic regression based on (a) The type of monitoring point (p=0.0028); (b) Aquifer type classified using the GSI classification system based on aquifer importance (p=0.0007); (c) Aquifer type classified using the Irish system of flow regime adopted for the WFD (p=0.0002); and (d) Acid versus Quaternary deposits as identified by their reaction (RxN) with 10 *v/v* hydrochloric acid (p=0.0048)**



Physical characteristics associated with pesticide detections

Aquifer type

Regionally important aquifers are capable of yielding water on a regional scale owing to a greater number, size, and connectivity of fractures and fissures within their lithology compared to locally important - and poor aquifers. Even with regionally important karst aquifers removed from the dataset, the statistical outcome remained that regionally important aquifer types were more associated with pesticide detections. Within the WFD aquifer flow regime classification, karstic aquifers had the highest probability of having a pesticide detection (p = 0.0002). Karst systems are very heterogeneous with many having solution features that can act as easy access points for water containing pesticides to enter the groundwater below. Karst aquifers are mainly found in the west and north west of Ireland (Figure 7.5-55 and Figure 7.5-56).

Karstic, intergranular, and productive fissured flow regime aquifers all had a greater association with pesticide detection in groundwater compared to poorly productive bedrock flow regime aquifers (Figure 7.5-57c) since regionally important aquifers with flow through fissures can act as fluid pathways. The fractures are larger and more connected than those present in poorly productive fissured aquifers potentially allowing more movement of pesticides within aquifers. Inter-granular flow aquifers, which in Ireland are generally composed of fluvioglacial sands and gravels, ranked second in their association with pesticide occurrence in groundwater (Figure 7.5-57c). Multiple logistic regression revealed that groundwater vulnerability in conjunction with GSI aquifer type can be used to indicate which areas are more associated with pesticide occurrence (Table 7.5-75). The groundwater vulnerability map assesses areas based on the depth of overburden material (soil and subsoil above bedrock). The shallower this protective layer above bedrock, in conjunction with a regionally productive aquifer was an area identified to be more associated with pesticide detections in groundwater across Ireland.

Table 7.5-73: Statistical analysis *p* values for Fisher's exact test and logistic regression, $p<0.05$ infers an association between pesticide occurrence in groundwater and the physical characteristic or amalgamated category tested

Physical characteristic	*p* value
Fisher's exact test	
Aquifer type[a]	0.0001
GSI aquifer importance excluding karst aquifer types	0.0013
Monitoring point type[a]	0.0080
IFS soil type II	0.0095
Quaternary deposit acid/base[a]	0.0101
IFS soil type	0.0116
Quaternary deposit type[a]	0.0260
Soil association (soil type)[a]	0.1061
ZOC size	0.1148
Land use III excluding karst aquifers	0.1771
Bedrock geology[a]	0.1793
Land use III	0.1820
Quaternary deposit genesis[a]	0.1820
Land use III excluding springs as MPs	0.2689
Groundwater vulnerability only for karst aquifers	0.2766
Land use III	0.2822
Quaternary deposit type excluding karst aquifers	0.3074
Groundwater vulnerability[a]	0.4214
Subsoil permeability[a]	0.4634
Land use[a]	0.5489
Logistic regression	
WFD flow regime aquifer type	0.0002
GSI aquifer importance aquifer type	0.0007
GSI aquifer importance aquifer type excluding karst	0.0030
Quaternary deposit acid/base[a]	0.0048
Monitoring point type[a]	0.0080
IFS soil type II	0.1069

[a] Original physical characteristic datasets which were not amalgamated into fewer number of categories

Soil type

The majority of MPs were located on well-drained soils with 32.3 % of these MPs having a detection less than the DWS and 12 % of MPs on well-drained soils had a detection greater than the DWS. Well-drained soils appear more likely to have a detection but this finding cannot be confirmed statistically. Our marginal test results indicate that the IFS soil type classification is more influential than soil association (Table 7.5-72) when using large robust datasets in an exploratory manner to predict the physical characteristics which affect pesticide leaching to groundwater in a MP's particular ZOC. The lack of a statistically significant marginal relationship between soil type derived from soil associations and groundwater pesticide occurrence may be due to the contrasting properties of the 13 pesticide compounds tested, with differences in their solubility and adsorption properties. The multiple regression results (Table 7.5-75) revealed some of the interactions with soil association that may be important to examine in future surveys. Multiple logistic regression revealed that soil association in conjunction with groundwater vulnerability had explanatory power to pesticide occurrence in groundwater.

Table 7.5-74: Total amount of a.i. applied to arable, and grassland and fodder crops in Ireland

Pesticide (a.i.)	Grassland and fodder crops 2003 (PCS 2006)	Arable crops 2004 (PCS 2007)	Total (kgs a.i.)
MCPA	221,883	10,012	231,895
Glyphosate	93,056	116,731	209,787
Mecoprop-p	74,598	112,058	186,656
IPU	349	107,852	108,201
Mecoprop	21,761	8,992	30,753
Atrazine	24,152	0	24,152
2,4-D	23,458	0	23,458
Cypermethrin	73	2,274	2,347
Bentazone	566	0	566
Total	459,896	357,919	817,815

Table 7.5-75: Statistical analysis p values for Multiple Logistic Regression. $p<0.05$ infers an association between pesticide occurrence in groundwater and the physical characteristic or amalgamated category tested

Physical characteristic	p value
GSI Aquifer Type	<.0001
Land Use III	0.0002
Quaternary Deposit acid/base	0.0083
Quaternary deposit Type	0.047
Groundwater Vulnerability B	0.0036
GSI aquifer type * Groundwater Vulnerability B	0.016
Soil Association	0.094
Soil Association* Groundwater Vulnerability B	0.0003

Conclusion

Using simple logistic regression on a 2-year national groundwater monitoring campaign revealed several physical characteristics were more associated with pesticide detections in groundwater. These were springs, karstic flow regime aquifer types, regionally important aquifers, and alkaline Quaternary deposits in existence with karst aquifers. There was some evidence from multiple regression that there was mutual dependency between some of these factors and that they interacted with soil association and the GSI groundwater vulnerability dataset. The geographic distribution of monitoring points with exceedances coincides with mapped karst areas, which was also confirmed statistically. Of monitoring points, 47.5 % never had a pesticide detection greater than the limit of detection, while 12 % of monitoring points had a detection greater than the European Union Drinking Water Standard of 0.1 µg/L on at least one occasion.

Of the 13 pesticides monitored, MCPA and mecoprop were the most frequently detected, although banned compounds such as lindane and atrazine were still detected but not exceeding the EU drinking water standard. Provided a large sample size is available, the methods used here can highlight geographical areas more susceptible to groundwater contamination. Future monitoring programs should analyze for each parent active ingredient along with any relevant transformation products to assess their depletion in the environment. It is hoped this study will improve conceptual understanding and assist in the assessment of groundwater chemistry through the interpretation of groundwater quality data: a fundamental requirement of the WFD.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports the evaluation of a two-years national groundwater monitoring campaign in Ireland.
Methods and results are sufficiently described.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/021
Report author	Norgaard, T. *et al*.
Report year	2014
Report title	Leaching of Glyphosate and Aminomethylphosphonic Acid from an Agricultural Field over a Twelve-Year Period
Document No	Vadose Zone J. doi:10.2136/vzj2014.05.0054
Guidelines followed in study	None
Deviations from current test guideline	Not applicable.
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The globally used herbicide glyphosate [*N*-(phosphonomethyl)glycine] and its most frequently detected metabolite, aminomethylphosphonic acid (AMPA), were studied in a unique 12-yr field-scale monitoring program. The leaching of glyphosate, AMPA, and soil particles was studied in a shallow drainage system beneath a 1.26-ha field. Five annual glyphosate applications were applied with different autumn application dates. Solute mass flux from the drain system following the five glyphosate applications were compared to determine how different factors affect the leaching of glyphosate, AMPA, and particles. Glyphosate and AMPA leaching were highly event driven, controlled by the time and intensity of the first rainfall event after glyphosate application. A high similarity in cumulative drainage and leached pesticide masses with time suggests near-constant drainage and leaching rates. There was no clear relationship between particle-facilitated transport and the transport of glyphosate or AMPA. However, soil particles, glyphosate, and AMPA all showed distinct, simultaneous concentration curves, indicating common dominant transport mechanisms. Also, soil-water content at the time of application and the level of the groundwater table relative to the drain depth exerted clear controls on detection of solutes in the drainage water. To summarize our findings, we present a leaching risk chart to illustrate the dependence of glyphosate, AMPA, and soil particle leaching based on rainfall intensity and the timing of rainfall events after glyphosate application.

Materials and methods
This study analyzed the results from a unique 12-yr field-scale monitoring program measuring the leaching of glyphosate and AMPA. Measurements were made in a shallow drainage system beneath a 1.26-ha field. The study compared five glyphosate applications with different autumn application dates. The overall objective was to determine which climatic conditions and soil properties affected the leaching of glyphosate, AMPA, and soil particles. Based on previous studies, we hypothesized that the timing of rainfall events in relation to glyphosate application, the applied glyphosate dose, and location-specific conditions promoting particle-facilitated transport would be important factors controlling the leaching of glyphosate

and AMPA. Based on this high-resolution, long-term monitoring data set, we developed a risk chart and propose directions for further development in research on glyphosate and AMPA leaching.

Leaching of glyphosate and its degradation product AMPA was studied at a Danish experimental field site in Estrup, southern Jutland.

The field site in Estrup is a loamy, highly heterogeneous soil with considerable variations in both topsoil and aquifer characteristics. The field is located on glacial till and covers an area of 1.8 ha, where 1.26 ha is cultivated. The field is virtually flat, with a slope of 0 to 1° toward the northeast. The soil is heavily fractured and bioturbated to the 1-m depth, with a plow layer containing 100 to 1000 biopores m^{-2}. The Estrup field site has a relatively shallow water table located approximately 1 to 3 m below ground surface (bgs), and the field site is systematically tile drained at an average depth of about 1.1 m.

Tile-drainage water from the cultivated area is directed to a monitoring well with a Thomson weir (30° V-notch) at the outlet of the drainage system in the northeastern corner of the field. The water height behind the Thomson weir is measured automatically using a pressure transducer (PDCR1830, Druck) coupled to a CR10X datalogger (Campbell Scientific), and the drainage is sampled using flow-proportional sampling. A sample of 200 mL is taken for every 3000 L (0.24 mm) of drainage flow from September to April and 1500 L (0.12 mm) from May to August; therefore, the sampling rate depends on the intensity of drainage flow. Analyses of pesticides and inorganic chemicals were performed weekly on the pooled 200-mL subsamples, such that the reported concentrations represent the weekly average concentrations in the drainage water collected. Because the samples were pooled, they do not represent peak concentrations that may occur during the week. Before July 2004, drainage was sampled both time proportionally and flow proportionally.

However, this study used only the flow-proportional sampling, which was collected consistently throughout the 12-yr monitoring period. Glyphosate has been applied to the agricultural field site in Estrup five times, including the first application on 10 Oct. 2000. Table 7.5-76 shows the glyphosate application dates and the amount of active ingredient applied.

Table 7.5-76: Glyphosate application dates and conditions during application

Date	Type of glyphosate [1]	Application rate	Amount of active ingredient (g/ha)	Cover before application	Air temperature at application (°C)
13 Oct. 2000	Roundup Bio	4.0 L/ha	1440	Stubble, 10 cm	9.5
2 Sept. 2002	Roundup Bio	4.0 L/ha	1440	Stubble, 14 cm	20.1
9 Nov. 2005	Roundup Bio	4.0 L/ha	1440	Stubble, 20 cm	10.0
24 Sept. 2007	Roundup Max	1.5 L/ha	1020	Stubble, 12 cm, and shredded straw	15.2
3 Oct. 2011	Roundup Max	2.0 L/ha	1360	Stubble, 12 cm, and shredded straw	17.5

[1] The active glyphosate ingredient in Roundup Bio is the isopropylamine salt of glyphosate in a liquid form, whereas the active ingredient in Roundup Max is an ammonium salt of glyphosate in a granular form

From 31 Oct. 2000 to 17 Mar. 2011, the particle concentrations were measured as the amount of suspended matter determined by filtration through a 1.6-mm Whatman glass fiber filter (DS/EN 872:2005). From 22 Sept. 2010 and onward, the particle concentrations were also determined from the turbidity of the sample using a Hach 2100AN turbidimeter. These results were converted to a particle concentration (mg/L) according to the procedure of Schelde *et al*. (2002). For a period of 2 yr, whenever enough water could be sampled, both methods were used simultaneously. From this period, a regression was obtained between the particle concentrations determined from the suspended matter method and the turbidity method (suspended matter concentration [mg L^{-1}] = 0.51 × turbidity concentration [mg L^{-1}] + 1.51, $R2$ = 0.7987) to allow for consistent analyses throughout the monitoring period.

Until July 2007, glyphosate and AMPA were analyzed according to Method 2275 (Eurofins Environment Denmark, Internal Method 76 542275, Glyphosate and AMPA in water by GC/MS). After July 2007, this method was replaced by Method 8270 (Eurofins Environment Denmark, Internal Method 76 548270, Glyphosate and AMPA in water by LC/MS/MS). Unfortunately, field-site control samples showed an underestimation of glyphosate using the newer method. This underestimation was assumed to be caused by a complex formed between glyphosate and potential multivalent cations (like Ca, Zn, Cu, Fe, Ni, and Cd) in the samples. This was discovered in 2010, and from 1 July 2010, an extended version of Method 8270 with acid-shock treatment was applied. Glyphosate concentrations analyzed in the period from 1 July 2007 to 1 July 2010 have been corrected to allow for the underestimation by multiplying by a factor of two following the procedure of Kjær *et al*. (2011b).

Results

For Applications 1, 2, and 3, the active glyphosate ingredient in Roundup Bio was the isopropylamine salt of glyphosate in a liquid form, whereas for Applications 4 and 5, the active glyphosate ingredient in Roundup Max was an ammonium salt of glyphosate in a granular form. It is unclear whether this difference in formulation would affect the fate properties of glyphosate and hence leaching, but the concentrations of AMPA were slightly lower after Applications 4 and 5.

Application 4 stands out from the remaining applications because the concentrations of glyphosate and AMPA were considerably smaller after Application 4 and, in contrast to the pesticide concentrations after Applications 1, 2, and 3, these concentrations with time curves were bell shaped. The smaller concentrations could be due to the smaller dose in Application 4 (1020 g ha^{-1}) compared with Applications 1, 2, and 3 (1440 g ha^{-1}, Table 7.5-76) or attributed to the shredded straw left on the field before glyphosate application. The straw might have retained some of the applied glyphosate, providing a slower release to the soil. This, however, contradicts the findings of Gjettermann *et al*. (2009), where the adsorption coefficient for glyphosate to straw (Kd <1 L/kg) was smaller than to soil (Kd = 503 L kg^{-1}). Similar to Application 4, straw was shredded on the field before Application 5. However, this did not result in the same low concentrations as after Application 4. The applied dose in Application 5 was almost the same as in Applications 1, 2, and 3, and therefore we assume that the low glyphosate concentrations detected after Application 4 were the result of a lower applied dose rather than the shredded straw.

It is difficult to generalize about the direct effects of tillage operations on glyphosate and AMPA leaching due to differences in weather conditions among the five glyphosate applications. In some cases, the potential direct effects of tillage operations are easily confounded by the effects of high-intensity rain events. Although similarities in the responses of particle, glyphosate, and AMPA leaching suggest common underlying dominant transport processes, there were no direct correlations between glyphosate, AMPA, and particle concentrations (data not shown), probably due to the complex interactions of flow processes, transport, degradation, climate conditions, and GWT fluctuations. The particle concentration curve has the same shape as the glyphosate and AMPA concentration curves, suggesting that the dominant processes controlling particle leaching are the same as those for glyphosate and AMPA. In summary, although we hypothesized that particle-facilitated transport would be an essential driver for glyphosate and AMPA leaching, this was not evident from our long-term field results.

The largest amounts of precipitation and drainage within the first 150 d after application occurred after Applications 1 and 5, whereas the largest amount of glyphosate (11.26 g) was leached after Application 3. The main contributor to this high glyphosate mass was the concentration detected on 17 Nov. 2005 of 31 µg L^{-1}, which contributed 53 % (5.95 g) to the total leached glyphosate mass. Glyphosate was applied on 9 Nov. 2005, and on 15 November there was a rainfall of 21 mm. The second-largest amount of glyphosate (3.16 g) was leached within the first 150 d after Application 5. This glyphosate mass mainly originated from the glyphosate leached on 19 Oct. 2011, which contributed 51 % (1.62 g) to the total glyphosate mass leached-most likely as a consequence of the high-intensity rain event on 18 October (36 mm). In our study, for Applications 3 and 5, the main contributing rain events fell within the first 6 and 15 d following glyphosate application.

Although the amount of AMPA leached within the first 150 d is larger after Application 3, the ratio between leached glyphosate and AMPA is also considerably higher after Application 3 than after the other

applications. The high glyphosate/AMPA ratio probably reflects rapid glyphosate transport shortly after glyphosate application with insufficient time for degradation. Rain events and SWC definitely had an influence on the glyphosate/AMPA ratio. The daily SWC in the first month after Application 3 was the second highest of the five applications. Application 3 also had the latest application time in the year and a low outside air temperature at application (10°C, Table 7.5-76), which possibly led to less microbial activity and hence limited glyphosate degradation. Applications 1 and 3 had the same low air temperatures at application of 9.5 and 10.0°C, respectively. Nevertheless, the leached concentrations of glyphosate and AMPA for the two applications were of completely different magnitudes and thus it is reasonable to assume that the outside air temperature at the time of application was less essential for the leached concentrations than the application timing.

Application 3 had the smallest amount of precipitation within the first 150 d and the cumulative drainage was not considerably different from the other applications. Rather, the highest loss of glyphosate took place in the period after Application 3, suggesting that more likely the timing of the application in relation to the next high-intensity rain event is crucial.

This event-driven transport mechanism explains the first detected concentrations of glyphosate, AMPA, and soil particles on 31 Oct. 2000 (Application 1, 13 October) after a long-duration rain event where the rain intensity peaked on 30 October. Also, it is more likely that the occasional glyphosate, AMPA, and particle concentrations detected between 12 Sept. 2001 and March 2002, after Application 1, was a consequence of event-driven leaching rather than glyphosate, AMPA, and particle mobilization due to harvest.

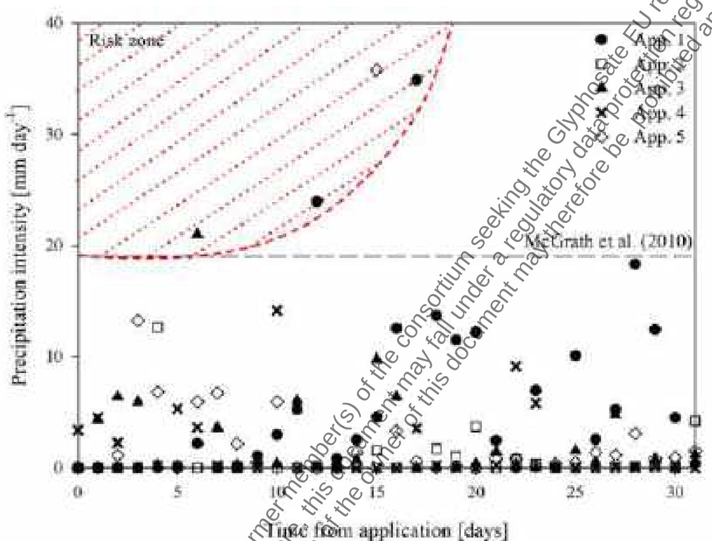
After Application 2, we measured increased particle, glyphosate, and AMPA concentrations in the drainage water collected from the end of November 2003 to April 2004, which was probably also the effect of increased rain frequency and intensities. It is possible that harvest on 29 Aug. 2004 mobilized soil particles and AMPA, but the continuous detections for almost 1 yr could only have originated from event-driven leaching because there were no other influencing processes in this time period. The high concentrations of glyphosate and AMPA on 17 Nov. 2005 (8 d after Application 3 on 9 November) following a rain event on 15 November emphasizes the event-driven leaching mechanism. Similarly, multiple periods of continuous concentration detections after Application 4 indicate event-driven leaching, possibly also a result of more intensified tillage procedures. Finally, the concentrations of glyphosate and AMPA detected on 19 Oct. 2011, 16 d after Application 5 (3 October) were also possibly the effect of a high-intensity rain event of 35 mm on 18 October.

The results of our long-term observations, over five applications, strongly suggest that precipitation intensity following application represents a major control on particle and pesticide leaching. To summarize this finding, Figure 7.5-58 presents a risk chart of the daily precipitation intensity within the first 31 d after each of the five glyphosate applications. McGrath *et al*. (2010) identified a minimum threshold for a single rainfall event of 19 mm as an indicator for a high likelihood of rapid herbicide transport. We suspect that the 19-mm threshold arises from interplay of evaporation, rainfall timing and intensities, and soil hydraulic properties valid for a specific soil type, different climate conditions, and the experimental fieldscale setup. Therefore, this threshold might not necessarily be universally applicable. Rainfall depths <19 mm might also trigger preferential flow events if the water content of the soil is already near the critical infiltration capacity. Thus, more frequent but less intense rainfall events might also contribute to preferential flow (McGrath *et al*., 2010).

All of the rain events observed in the first month after the five applications in our study were below the 19-mm threshold except for four events. The first event was on Day 6 after Application 3, another event was from Day 15 after Application 5, and the last two events were from Days 12 and 17 after Application 1.We have added an approximate potential elevated-risk zone to build on the threshold concept of McGrath *et al*. (2010). This zone includes the 19-mm threshold of McGrath *et al*. (2010) and captures the four mentioned events. Strikingly, the four events within the elevated-risk zone are from the three applications that led to the highest leached masses of glyphosate and AMPA.

As noted above, the event on Day 6 after Application 3 contributed 5.95 g (53 %) to the total glyphosate leaching loss within the first 150 d after application. The event after Application 5 contributed 1.62 g (51 %) to the total leached glyphosate within the first 150 d. The two events after Application 1 contributed 0.48 g (23 %) of the total leached glyphosate mass within the first 150 d after application.

Figure 7.5-58: Risk chart showing rain intensity each day within the first 31 d after each of the five glyphosate applications. The dashed horizontal line is the 19-mm threshold for rapid herbicide transport events (McGrath *et al.*, 2010). This study suggests an elevated-risk zone for rapid glyphosate and aminomethylphosphonic acid (AMPA) leaching as emphasized by the red area.



The smaller contribution of the two events after Application 1 to the total leached glyphosate within the first 150 d is probably due to the delay in precipitation after glyphosate application compared with Applications 3 and 5. Thus, there was no precipitation until Day 6 and the SWC on the day of Application 1 was the lowest of the three applications. These results indicate that precipitation intensity and timing of rain events after glyphosate application are decisive for glyphosate leaching.

Assuming that particle leaching is controlled by the same factors as the leaching of glyphosate and AMPA, namely the timing of high-intensity rain events after soil disturbance could equally well cover particle leaching dynamics. The boundaries of the elevated-risk zone are only approximately defined by this study. The non-constant risk zone is defined based on the assumption that higher intensities are required to trigger enhanced glyphosate leaching with longer times between glyphosate application and the next intense rainfall event. Still, the boundaries of the risk zone will depend on, e.g., soil type, drain depth, and climate conditions. We would suggest that future work should be focused on identifying the soil properties and field conditions that define the limits of the risk zone with the hope of developing a universally applicable guideline for leaching risk assessment.

Conclusion

We have presented an extensive data series of glyphosate, AMPA, and particle leaching collected over 12 yr, including five glyphosate applications. In this initial examination of the data, we have examined previous hypotheses about particle and pesticide transport in light of this new data set. Our ultimate objectives are to examine these hypotheses with an eye toward guiding the responsible use of glyphosate. We have also made efforts to identify the remaining questions that are not resolved by this data set, thereby suggesting possible future research priorities. We specifically examined two hypotheses. First, that the timing of precipitation in relation to glyphosate application is a controlling factor for glyphosate and AMPA leaching. This hypothesis was supported by our field observations, which showed that the leaching of these two compounds was highly event driven. Taken together, these findings suggest that care should be taken to avoid the application of glyphosate in periods when the leaching potential is relatively high. Particle leaching was also seen to be event driven; however, it was controlled by the timing and intensity of the precipitation event in relation to the most recent soil disturbance, not to the timing of glyphosate application. Our second hypothesis was that particle-facilitated leaching controls the leaching of glyphosate and AMPA. This hypothesis is not supported by our observations.

Specifically, while there were clear similarities in the concentration vs. time curves for the particles and pesticides, there was no direct correlation between their leached concentrations. In addition, to decrease the likelihood of particle-facilitated transport, management procedures that cause intensified soil disturbance should be separated in time from glyphosate application.

Our results also highlight complications in relating the flux of pesticides to the groundwater based on measurements made in drain systems. The soil-water content at the time of application and the elevation of the water table in relation to the drain depth are critical factors for determining whether solutes are captured by the drains or bypass the drain system. This has clear implications for the representativeness of drainage water for recharge water. We suggest a risk concept that relates precipitation intensity and timing in relation to glyphosate application to the likelihood of glyphosate and AMPA leaching into drains. A risk chart that is suggested to illustrate the risk and results of this monitoring series was compared with the results of previous work in this context.

Despite the extensive data set presented here, there are still significant uncertainties regarding pesticide transport. Factors such as soil tillage should be considered further to see if intensified soil disturbance creates a higher risk for particle-facilitated leaching of glyphosate. More studies should be conducted in areas that experience high-intensity precipitation during pesticide application periods to define the suggested elevated-risk zone more clearly. It is our hope that this new data set will lead to improved understanding of pesticide leaching, leading to improved guidance for responsible pesticide application.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports the results of a 12-year field-scale monitoring program on the leaching of glyphosate and AMPA in Denmark. The analytical method 8270 applied between 2007 and 2010 showed insufficient recovery. The correction procedure by a constant factor of 2 is considered not appropriate in the context of the active substance approval under Regulation (EC) No 1107/2009. Further, it is discussed that measurements in the drainflow may origin from drainage of surface water as well as from groundwater, i.e. a clear conclusion about drained substance amounts cannot be drawn. As the overall results of the article may add valuable supplementary information to the data set.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/022 CA 7.5/023 (Translation)
Report author	Martin, J. *et al*.
Report year	2013
Report title	Sugar Cane, Herbicides And water Pollution in Reunion Island: Achievements and Perspectives at the End of the First Decade of monitoring
Document No	Conference paper: 22nd Conference of COLUMA, International Days on Weed Control, Dijon, France, December 10-12, 2013 pp.641-651 ref.13
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

BRGM, the French geological and hydrogeological survey, compiled data from three local agencies involved in water quality. The compilation included data from 247 sites monitored between May 1999 and January 2010. Positive results were found in 55 % of the sites. Among the 398 substances checked, 73 were found and 65 were quantified: 35 of them were herbicides or herbicide degradates, and 17, were involved in sugarcane weeding. 1,811 results were positive, 1,407 results were quantified and 251 results had concentrations over the drinking water threshold (0.1 ppb). Sugarcane herbicides, including glyphosate, were responsible for 80 % of the results over 0.1 ppb. Two sugarcane herbicides widely used in the past, atrazine and diuron, were responsible for 80 % of the quantified results and 67 % of the results over 0.1 ppb. Four herbicides currently in use have been found at concentrations over 0.1 ppb. They are being tested in the laboratory BRGM in order to assess the pollution risks for groundwater when applied on some native soils.

Materials and methods

OLE initiated the tracking of pesticides in freshwater in May 1999, the physico-chemical analyzes campaigns started in 1992 on 49 water points. The ARS, concerned about the sanitary quality of water intended for human consumption, did the same from the end of 2000 on 203 water points. The DEAL concerned with the quality of the surface water of 13 particular sites is the third actor.

The 'Phytosanitary transfer' compilation is ultimately based on a total of 384,627 analysis results of pesticide detection. This number includes all the OLE-DEAL analyzes and some of the ARS analyzes, those giving a positive result. The absence of ARS analyzes negative results preclude calculating absolute rates of positivity to a given substance. The period covered up to 2009 inclusive for OLE and DEAL with around a quarter of analyzes and until January 2010 for the ARS with about three quarters of the analyzes compiled.

With 18 water points common to ARS and OLE, the total number of sampling points amounts to 247, about three quarters ARS and a quarter OLE-DEAL. Those of the ARS relate for almost three quarters to ESO, while those of OLE-DEAL almost half relate to ESU. Overall, two thirds of the water points and analyzes concern ESOs. When compiling, the limits of quantification (LQ) of the AS, likely to change during the decade or between laboratories, have not been exhaustively identified. Some substances have been

'detected' without quantification, whereas within the meaning of the DCE a substance is considered 'present' if and only if it has been quantified: 'proven' pollution. Subsequently, the term 'positive' analyzes and 'detected' substances includes all the quantified and detected substances without quantification. Some ASs have unquantified detection rates for this period much more important than the average.

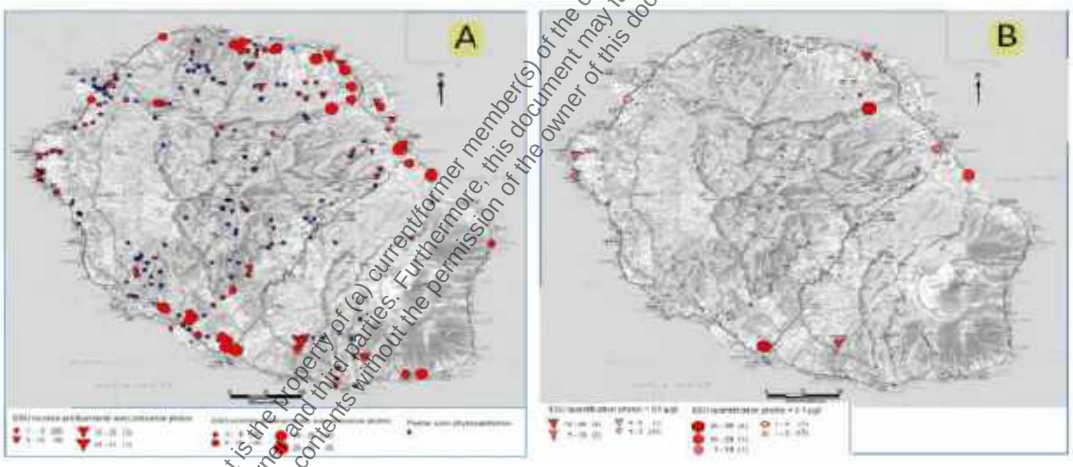
Results

Regarding ARS, 43.8 % of the water points were positive at least once to a pesticide (or metabolite) detected or quantified, while for the OLE-DEAL, this rate rises to 80.6 % (55 % globally).

Table 7.5-77 lists the 72 substances detected (including their metabolites) - 65 of which are present according to the meaning of the DCE - after 89,675 analyzes, values to be compared with the cumulation of 398 detected substances in more than 384,627 analyzes grouped together in this first decennial compilation (the negative analyzes of the ARS do not appear there). Noted are 1,811 positive analyzes, including 1,407 cases of proven pesticide presence and 251 case of exceeding the potability threshold of 0.1 µg/l. These results are graphically rendered, all substances combined, by the maps in Figure 7.5-59-A for detections, Figure 7.5-59-B for exceedances of the threshold of 0.1 µg/L (thereafter 'exceedances').

Figure 7.5-59: Monitoring pollution of freshwater by pesticides in Reunion Island, ARS + OLE + DEAL, period 1999-2010. ESU: surface water, ESO: groundwater.

A: Positive samples, B: > 0.1 µg/L results.
Monitoring of water pollution by pesticides, Reunion Island, 1999-2010. ESU: surface water, ESO: groundwater; A: positive results; B: results > 0.1 ppb.



Whether for ESO or ESU, these maps show the sites where pollution cases are recurring and those where they are occasional. The map of detections clearly shows the most exposed areas: the Northeast border (from Sainte-Rose to Sainte-Marie), and a part of the South-East border (between Saint- Leu and Saint-Pierre).

The map of exceedances reports about thirty sites victims of occasional pollution, as well as the ten or so sites that are victims of more frequent or even chronic pollution especially North of Saint- Pierre (BAC of Salette) and Sainte-Suzanne (BAC of Sainte-Vivienne) with more than 20 exceedances over the decade (for a frequency of 4 annual sampling in principle), and to a lesser extent Sainte-Anne (less than 20 exceedances over the decade).

Table 7.5-77: Pesticides in fresh waters (ESU + ESO) in Reunion, 1999-2010 period. Pesticides found in water, Reunion Island, 1999-2010.

#S	#H	CAS#	Phytosanitary Substances Detected or Quantified	Number of Analyzes	Number of Detections not Quantified	Number of Quantifications ≤ 0.1 µg	Number of Quantifications > 0.1 µg/l	For herbicides, main uses	status or year of withdrawal(*)
1	1	1	desethylatrazine (DEA)	1748	66	525	159	Metabolite of atrazine	
2	2	2	diuron	2012	45	136	15	ex CAS	2008
3	3	3	glyphosate	1054	0	1	9	TTG, CAS et al	Authorized
4	4	4	aminomethylphosphonic acid (AMPA)	1054	0	2	8	Metabolite of glyphosate	
5	5	5	hexazinone	1765	24	28	7	ex CAS	2007
6			DDE-4,4'	1148	0	0	6		
7			diazinon	1852	3	9	5		
8			DDD-4,4'	1150	0	0	5		
9			DDT-4,4'	1505	0	0	5		
10	6	6	bentazone	1549	14	13	4	Corn, meadow, peas, herbs, CAS tested	Authorized
11			endosulfan alpha	1901	0	0	4		
12	7	>>	2,4-MCPA	1417	0	0	3	TTG, meadow, lawn	Authorized
13			endosulfan beta	1902	0	0	3		
14	8	7	atrazine	1984	79	271	2	ex CAS	2003
15	9	8	metolachlore / S-metolachlore	1780	18	50	2	(ex CAS), in CAS	Authorized
16	10	9	bromacil	1417	25	5	2	ex CAS	2003
17	11	10	2,4-D	1270	3	13	2	CAS	Authorized
18	12		metazachlore	1503	2	4	2	Cabbage, sunflowers, CAS tested	Authorized
19	13		aminotriazole	364	0	5	2	TTG, fruit trees, trees and bushes, corn	Authorized
20	14	11	metribuzine	1059	0	0	1	ex + in CAS	Authorized
21			oxadixyle	1769	4	20	1		
22	15		triclopyr	1547	1	2	1	TTG meadows (brush killer)	Authorized
23			cyprodinil	1149	1	3	1		
24	16		monuron	1061	5	5	1	Asparagus, vine	1994
25	17		isoproturon	1635	1	3	1	Cereals, lavender	Authorized
26			piperonyl butoxide	1095	1	1	1		
27			chlorpyriphos ethyl	1906	2	3	1		
28	18		terbutryne	1069	0	0	1	Potatoes, peas, corn	2003
29			tebuconazole	356	2	[illegible]	1		
30			nonylphenols	250	1	0	1		
31	19	13	paraquat	718	0	0	1	TTG, ex CAS et al	2007
32	20		bromoxynil	1060	0	0	1	Meadows, corn, CAS tested	
33	21		prometryne	1063	0	0	1	Lentils, carrots, leeks, celery	2007
34	22	14	1-(3,4-dichlophenyl)-3-methylurea	1054	20	30	0	Diuron metabolite	
35	23	(15)	alachlore	1559	3	2	0	Corn, CAS tested	2008
36	24		mecoprop	1414	2	5	0	Lawns	Authorized
37			anthraquinone	977	1	5	0		
38			carbendazime	1500	[illegible]	4	0		2009
39	25		oxadiazon	1794	0	4	0	TTG, fruit trees, wine, horticulture	Authorized
40	26		dichlorprop-p (DCP)	1060	[illegible]	3	0	TTG, forests, meadows, lawns, CAS tested	Authorized
41			hch gamma	935	0	3	0		
42			imidaclopride	1056	[illegible]	2	0		
43	27	16	desisopropylatrazine (DIA)	1097	[illegible]	2	0	Metabolite of atrazine	
44	28		propyzamide	1194	1	2	0	Fruit trees, wine, forest, vegetables, horticulture	Authorized
45			mepiquat chlorure	326	1	2	0		
46			deltamethrine	1474	0	2	0		
47			fenitrothion	[illegible]	0	2	0		
48			Fipronil	1645	0	2	0		
49			permethrine	1030	0	2	0		
50	29	17	2,4,5-T	1667	0	2	0	Ex CAS	2003
51			thiabendazole	368	0	2	0		
52			chloromequat chlorure	356	0	2	0		
53	30		acetochlore	976	4	1	0	Corn, CAS tested	(2007) 2003
54	31		ethofumesate	1062	1	1	0	Meadows, herbs	Authorized
55	32		imazamethabenz	1057	1	1	0	Cereals	2007
56	33	>>	dicamba	976	1	1	0	TTG, meadows, lawns, corn	Authorized
57			folpel	1663	0	1	0		
58			dinitrocresol	1572	0	1	0		
59			pentachlorophenol	1515	0	1	0		
60			DDT-2.4'	1151	0	1	0		
61			tolyfluanide	1062	0	1	0		
62			metalaxyle	1061	0	1	0		
63	34		dinoterbe	1058	0	1	0	Meadows, protein crops, corn	1997 (France)
64			picoxystrobine	974	0	1	0		
65			endosulfan	35	0	1	0		
66			metamidophos	8	0	1	0		
67			propoxur	1061	2	0	0		
68			temephos	1661	1	0	0		
69			fenpropidine	1416	1	0	0		
70			procymidone	1061	1	0	0		
71	35		metoxuron	1060	1	0	0	carrots	
72			propiconazole	1059	1	0	0		
73			spiroxamine	634	1	0	0		
			CUMULATED	89675	409	1176	251		
398	number of substances detected								

#S number of detected substances:, #H: herbicide-related, reported shaded, #HCAS: sugarcane-related, (x): non-approved CAS use, >>: post-2010 CAS use; TTG: general treatments; CAS: sugar cane, ex CAS: formerly CAS, in CAS: currently CAS, CAS et al. : use partly related to the CAS; (*) authorized is meant on 25 July 2013
N.B.: the number of analyzes includes all the OLE and DEAL analyzes but only the positive analyzes of the ARS.

Some of these ESO points are polluted by several substances at once with some cases of exceeding the threshold of 0.5 µg/L for the sum of the concentrations: 4 occurrences in Saint-Benoît and Sainte Anne. Still on the east coast, between Bras-Panon and Sainte-Suzanne, the ESU experienced in 2007 very intense pollution peaks, with sums of concentrations reaching 10 to 20 times the threshold value. These exceptional

levels of pollution are to be linked to repeated heavy rains in the first half of 2007, probably erosive rains. That year, the cyclone Gamède, more active on the west coast, caused two occurrences of sums of concentrations 5 and 6 times the threshold of 0.5 µg/L. In the South, there are three cases of exceeding this threshold, two of which in very permeable terrain (very recent volcanic formations of the volcano of the Fournaise).

Table 7.5-77 indicates that among the 73 substances detected in the Reunion Island's fresh waters, 35 are herbicides or their metabolites, of which 17 (or 23.3 %) are predominantly or partially related, currently or in the past to the weeding of cane. Table 7.5-78 details the impact of the 19 herbicides that have directly or via their metabolites exceeded the threshold of 0.1 µg/L; they alone are responsible for the 94 % of proven pollution and 87 % of exceedances. As a result, the remaining 6 % of the remaining pollution is caused by other pesticides in larger number (52 substances). Cane or partially cane herbicides caused 91 % of proven pollution and 80 % of exceedances.

Table 7.5-78: Herbicides quantified above the threshold of potability (0.1 µg/L). Water pollution (ESU + ESO) in Reunion, period 1999-2010. Herbicides found in water at concentrations> 0.1 ppb, Reunion Island, 1999-2010.

H #	HCAS #		Quantification (Qt)		Quantifications> 0.1 µg/l		Qs/Qt
			Number of cases	%	Number of cases	%	%
		Reminder: All pesticides	1407	100.00%	251	100.00%	18%
		Among which herbicides	1320	93.8%	217	86.5%	16%
		Among which those linked to weeding of cane	1276	90.7%	201	80.1%	16%
1	1	Atrazine + DEA + DIA	950	67.5%	152	60.6%	16%
2	2	Diuron + its metabolite	181	12.9%	15	6%	8%
		Subtotal atrazine + diuron	1131	80.4%	167	66.5%	15%
3	3	Glyphosate + its metabolite (partially CAS)	20	1.4%	17	6.8%	85%
4	4	**Hexazinone (ex CAS)**	35	2.5%	7	2.8%	20%
5		Bentazone	17	1.2%	4	1.6%	24%
6		2.4-MCPA (meadows and soon CAS)	3	0.2%	3	1.2%	100%
7	5	**Metolachlore + S-metolachlore (CAS + miscellaneous)**	32	2.3%	2	0.8%	6%
8	6	**Bromacil (ex CAS)**	32	2.3%	2	0 8%	6%
9	7	**2.4-D (CAS + corn + meadows)**	15	1 1%	2	0.8%	13%
10		Metazachlore	6	0.4%	2	0.8%	33%
11		Aminotrazole	5	0.4%	2	0.8%	40%
12	8	**Metribuzine (CAS + tomatoes + potatoes)**	2	0.1%	2	0.8%	100%
		Subtotal (hexazinone + metibruzine	147	10.4%	26	10.4%	18%
13	9	Triclopyr (brush killer, borders CAS)	8	0.6%	1	0.4%	13%
14		Monuron	4	0.3%	1	0 4%	25%
15		Isoproturon	4	0.3%	1	0.4%	25%
16		Terbutryne	1	0.1%	1	0.4%	100%
17	10	Paraquat (partially ex CAS)	1	0 1%	1	0.4%	100%
18		Bromoxynil	1	0.1%	1	0.4%	100%
19		Prometryne	1	0.1%	1	0.4%	100%
		Sub-total (triclopyr – prometryne)	20	1.4%	7	2.8%	35%

CAS Sugar cane, #H Number of herbicides #HCAS: Number of CAS herbicides

Aftereffects of some Old Fashioned Cane Sugar Herbicides…

The two most frequent cases of exceedances concern two old herbicides massively used with sugar cane, atrazine - withdrawn in 2003 - and diuron - withdrawn in 2008; with their metabolites, they are responsible for 80 % of cases of proven pollution or 66.5 % of cases of exceedances of the potability threshold (0.1 µg/L) (Table 7.5-78). Glyphosate, for which we consider lacking better estimate that it would be used for half on sugar cane, comes in third position with a 1.4 % contribution to proven pollution which amounts to 6.8 % of cases exceeding the thresholds. These 3 herbicides are therefore responsible in their own right for 82 % of proven pollution and 73 % of cases of exceeding the threshold of potability during the decade.

The cases of exceedances of the threshold of potability related to the cases of proven presence give an average ratio of 18 % for all pesticides and 16 % for all herbicides or cane herbicides (Table 7.5-78). This ratio hides nevertheless significant variations: 6 % for the metolachlor, 8 % for diuron, 16 % for atrazine, 20 % for hexazinone and 85 % for glyphosate.

Of the 7 herbicides that exceeded the threshold one time, there is no herbicide dedicated to cane weeding. Two herbicides could nevertheless be used by cane growers: paraquat (banned since 2007) on glyphosate-type uses (preparation of the ground before planting, or associated with pre-emergence herbicides, or in treatment directed at the foot of the cane post-emergence): triclopyr, brush cutter approved on meadows or in general treatments of paths and borders, totally selective of grasses, sometimes used to devitalize the perennials at the edge of the fields, or sometimes even within localized treatments.

Particularly Persistent

As part of the Phytos Transfer Project, BRGM has undertaken to characterize the risks of diffuse pollution from the study of two of the most polluted priority BACs, that of Sainte- Vivienne, on the eastern very rainy slope (> 3m/year), and that of La Salette, on the western less rainy slope (<1m/year). Risks will be assessed from field surveys of agricultural practices and laboratory tests. These are aimed at determining the adsorption dynamics and degrading four cane herbicide, and ultimately their GUS (Groundwater ubiquity score) from soil samples that are agriculturally representative. These four herbicides are 2.4-D, S-metolachlor, metribuzin and glyphosate; historical herbicides still in use, they are widely used and have some instances of exceedances to their liabilities (respectively 2 + 2 + 2 + 17, Table 7.5-78). In this context, specific water samples were collected monthly from both BACs to track 106 pesticides or their metabolites (mainly herbicides) between September 2011 and April 2012. The list of 106 is not a strict subset of the list of 398 because it includes for example both metabolites metolachlor , absent from the list of 398.

Twelve substances were quantified, all related to herbicides, mainly cane herbicides (with the exception of dinoterbe, and partially glyphosate). Table 7.5-79 reports a single case of pollution with AMPA (glyphosate metabolite) in St. Vivienne ESU. Glyphosate is like metolachlor and metribuzin already an aged herbicide in terms of use, although at the time of atrazine it was probably less used than today, paraquat being available.

Table 7.5-79: The 12 pesticides present in the waters taken from the BACs of Sainte-Vivienne (municipality of Sainte-Suzanne) and La Salette (municipality of Saint-Pierre) between September 2011 and April 2012 as part of the BRGM Phyto Transfer Project (monthly remittances).

		BAC of Sainte Vivienne		BAC of Salette			
		F1 drilling (ESO)		Downstream of F1 (ESU)		F5 Drilling (ESO)	
	LQ	Number of analyzes	% of presence	Number of analyzes	% of presence	Number of analyzes	% of presence
	(µg/l)		(> LQ)		(> LQ)		(> LQ)
1 atrazine	0.005	7	0%	7	100%	7	100%
2 desethylatrazine	0.005	7	100%	7	100%	7	100%
3 desisopropylatrazine	0.005	7	0%	7	100%	7	100%
4 hexazinone	0.005	7	43%	7	100%	7	0%
5 metolachlor	0.005	7	14%	7	100%	7	14%
6 metolachlor EAS	0.01	7	0%	7	71%	6	0%
7 diuron	0.01	7	0%	7	57%	7	0%
8 metribuzin	0.005	7	0%	7	29%	7	0%
9 amtretryne	0.005	4	0%	4	25%	4	0%
10 dinoterb	0.1	4	0%	5	20%	4	0%
11 AMPA	0.05	6	0%	6	17%	6	0%
12 bromacil	0.01	4	0%	4	0%	4	25%
glyphosate	0.05	6	0%	6	0%	6	0%
2.4-D	0.01	6	0%	6	0%	6	0%
93 other substances			0%		0%		0%

BAC: Catchment Supply Basin; ESO: Groundwater; ESU: Surface Water. LQ: Limit of Quantification

Conclusion

The high inertia of pesticide pollution phenomena affecting freshwater resources can be explained by the average age of the groundwater, between their entry into the soil, their percolation in the unsaturated zone via draining rains and their removal from a source or borehole. As part of the Phytos Transfer Project, the BRGM estimated the average stay times of several situations in Reunion Island to often several decades. The case of the delayed impact of old sugarcane herbicides in Reunion Island is particularly demonstrative, in particular for atrazine and diuron, responsible for 67 % of exceedances and 73 % of detections.

However, the average age of water affects all herbicides in the same way. The risks of diffuse pollution are linked on the one hand to the quantities applied and on the other hand to the characteristics intrinsic properties of cross-products with the properties of surface soils and the conditions of application, including the prevailing weather conditions during the preceding days and the days following the spreading.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article summarizes the results of monitoring pesticides in groundwater and surface water conducted by the responsible authorities of Reunion Island. As the data were generated by authorities, it is assumed to be quality assured (even though no details on sample collection and analytical methods are reported). Application of herbicides to sugar cane on Reunion Island is considered only limited representative for European conditions.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/024
Report author	Mörtl, M. *et al*.
Report year	2013
Report title	Determination of glyphosate residues in Hungarian water samples by immunoassay
Document No	Microchemical Journal 107 (2013) 143–151
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (Central Food Research Institute, Hungary)
Acceptability/Reliability:	Reliable

2. Full summary

An enzyme-linked immunosorbent assay (ELISA) for the detection of glyphosate was investigated for assay performance characteristics and was applied for determination of glyphosate contamination levels in selected surface and ground water resources in Hungary in 2010 and 2011. Advantages of the method include its simplicity (no laborious extraction) and specificity (cross-reactivity is below 0.1 % for related compounds, e.g. aminomethyl-phosphonic acid, glufosinate). On the basis of our experiments, the practical limit of detection (LOD) ranged between 0.05 and 0.12 ng/mL. The standard curve was of sigmoid (logistic)

characteristics, and it co-occurred with curves obtained for spiked surface water samples. Matrix effects were observed in tap water, possibly due to chlorination and/or heavy metal ions, e.g. copper and zinc. The method was applied for the analysis of 42 surface and ground water samples collected from Békés county in Hungary at 14 sampling sites in 2010 and 18 surface water samples collected from the Danube River and Lake Velencei in Hungary at 12 sampling sites in 2011. Exceedingly high glyphosate levels (nearly 1 ng/mL) were measured in 5 samples, and significant concentrations were determined in 16 cases (0.54-0.76 ng/mL) in 2010, while practically no contamination was found in 2011. The great contrast between the two sampling regimes is explained by differing agricultural locations, natural precipitation and, to a greater extent, catchment area characteristics, resulting in varying leaching or run-off of glyphosate to surface waters.

Materials and Methods

Reagents

The glyphosate analytical standard (Pestanal grade), Amberlite IR 120 strongly acidic cation exchange resin and all reagents were purchased commercially.

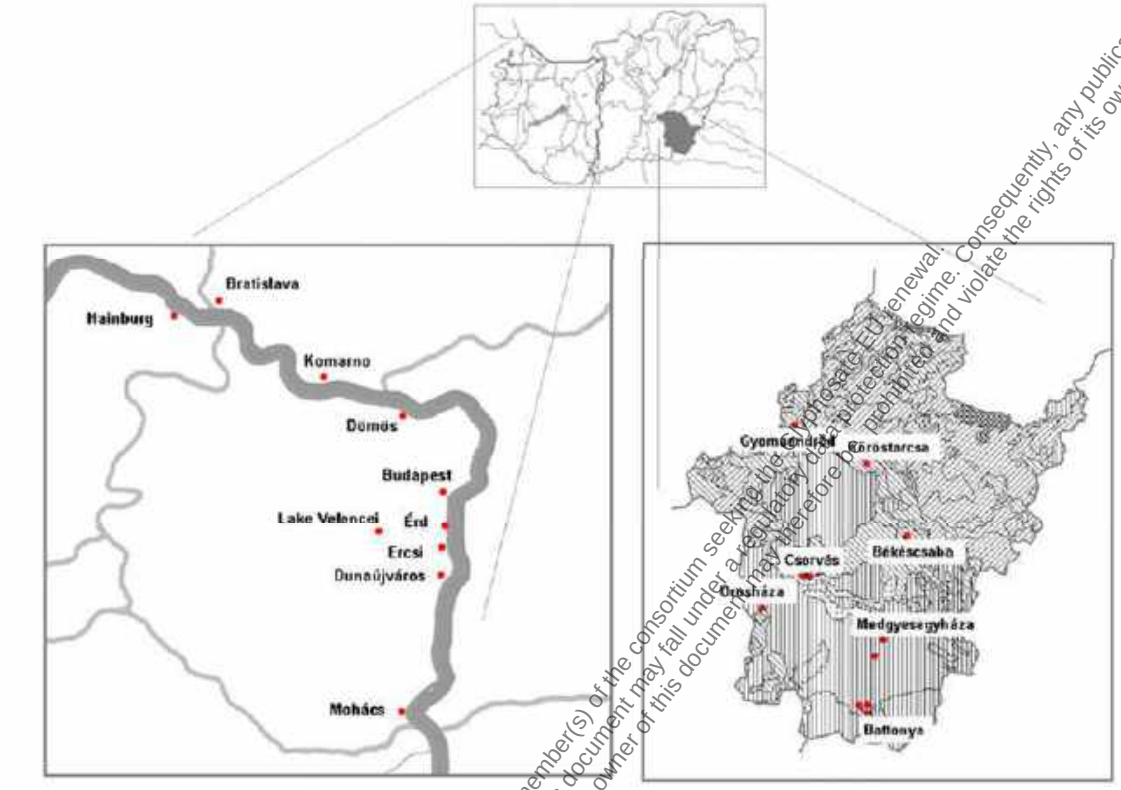
ELISA

For immunoanalytical detection of glyphosate, the commercially available ELISA method by Abraxis LLC was used.

Calibration, limits of detection and matrix effects

Calibration curves were established with standard solutions provided by Abraxis at five concentration levels between 0.075 and 4.0 ng/mL (0, 0.075, 0.2, 0.5, 1.0, 4.0 ng/mL), two replicates each. An analytical quality control solution (0.75 ng/mL) was also used. LODs, defined as glyphosate concentration causing 10 % decrease in the optical assay signal, i.e. 90 % B/B_0 (where B/B_0 is the signal obtained with the given sample divided by the maximum signal obtained with a sample containing no glyphosate), were determined in all experiments. For investigation of matrix effects, a stock solution of glyphosate (1.0 mg/ml) was prepared in MilliQ water. This solution was diluted to 0.1 µg/mL (spike solution). Solutions containing glyphosate at final concentrations (typically 0.075, 0.2, 0.5, 1.0, 4.0 ng/mL) were made by addition of appropriate amounts of spike solution to different water matrices.

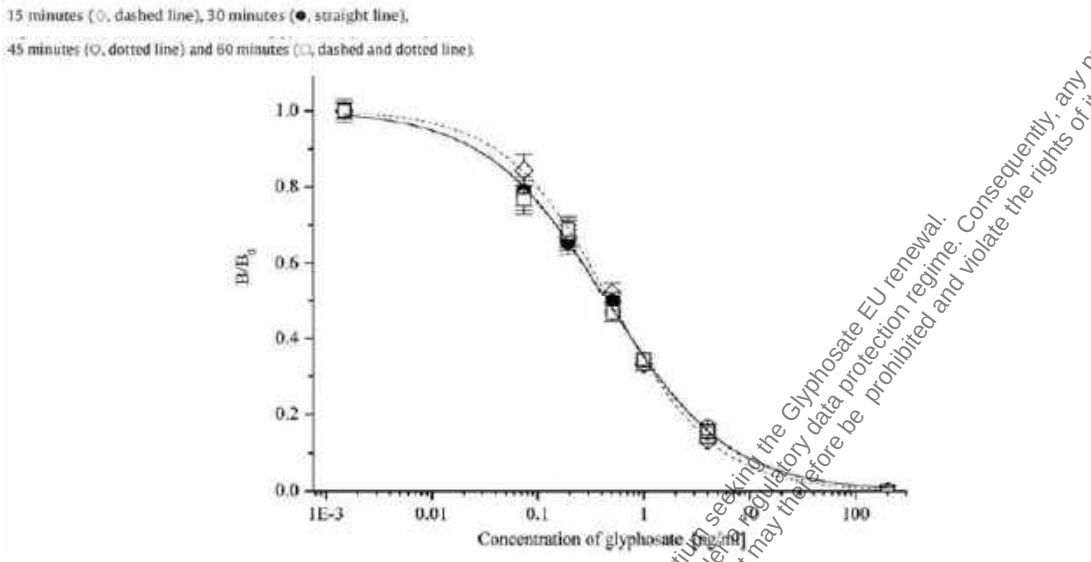
Figure 7.5-60: Sampling sites in Hungary along the Danube and in Békés county



Influence of sample preparation

The influence of different sample preparation steps proposed by Küsters *et al*. for drinking water has also been investigated. Briefly, for the cleanup of spiked water samples, the cation exchange resin Amberlite IR 120 was converted to sodium form. This, and all subsequent column regeneration steps after each sample, was carried out with a 4 M sodium chloride solution. Then, each sample was passed through the cation exchange column, followed by washing with deionized water. All eluates were collected in round bottom flasks and then evaporated to dryness. The residues obtained were dissolved in deionized water. After each sample preparation step, concentrations of glyphosate were determined by ELISA, with concentrations obtained corrected according to the volume change.

Figure 7.5-61: Standard calibration curve in the glyphosate-specific competitive ELISA, and the effect of preincubation



Sample collection

Municipal water at the laboratory site (II. District, Budapest, Hungary) was used as tap water. Field samples were collected in amber glass bottles previously washed with aqueous hydrochloric acid (pH 2) and repeatedly rinsed with deionized water. During sampling, the bottles were rinsed twice with the water sampled, then filled and tightly capped. Samples were stored at 4°C in the dark. In the scope of a national environmental survey, 42 water samples (6 surface water and 36 ground water samples) were obtained on September 7-8, 2010, from 14 sampling sites in Békés county, Hungary. In addition, 18 surface water samples were collected on October 1, 2011, from 11 sampling sites along the Danube River and one site at Lake Velencei, Hungary. The sampling sites are depicted on Figure 7.5-60.

Figure 7.5-62: Matrix effects in the glyphosate-specific competitive ELSA indicated by standard calibration curves obtained in assay matrix

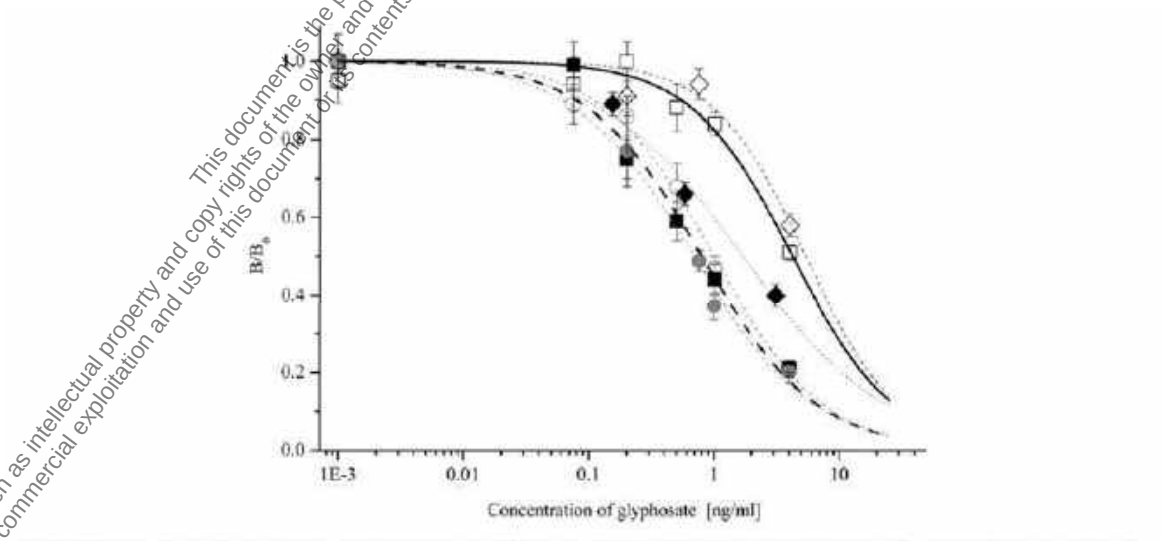


Fig. 3. Matrix effects in the glyphosate-specific competitive ELISA indicated by standard calibration curves obtained in assay matrix (○, gray dotted line), distilled water (●, gray dashed line), tap water (□, thick solid line), tap water treated by cation exchange and spiked (■, thick dashed line), tap water spiked and treated by cation exchange (◆, dotted line) and tap water spiked, treated by cation exchange, evaporated and resolved in distilled water (○, dashed line).

Table 7.5-80: Detected glyphosate concentrations and corresponding recoveries in unspiked and spiked water samples as a measure of matrix effects on ELISA performance

Sample Type	Glyphosate concentration detected [ng/ml]		Spike recovery [%]
	Unspiked	Spiked with glyphosate at 0.5 ng/ml	
Water matrix effect			
Deionized water	<0.075	0.579	115.8
Tap water	<0.075	0.141	28.2
Surface water	<0.075	0.636	127.2
Tap water treated with ascorbic acid (0.125 mg/ml)	<0.075	0.501	100.3
Tap water treated with sodium nitrite (0.005 mg/ml)	<0.075	0.383	76.6
Solvent effect by methanol			
Methanol content [%]			
0	<0.075	0.620	124.0
20	<0.075	0.656	131.2
40	<0.075	0.550	110.0
60	<0.075	0.446	89.2
80	<0.075	0.581	116.2
100	<0.075	0.450	90.0

Table 7.5-81: Compositional characteristics of the water types studied[a]

	pH	Ca^{2+}	K^+	Mg^{2+}	Na^+	HCO_3^-	Cl^-	SO_4^{2-}	Ba	Co	Cr	Cu	Fe	Mn	Mo	Ni	P	Pb	Sr	Zn
		mg/l							µg/l											
Tap water	7.44	56.6	2.84	13.8	12.3	208	12.0	37.2	45.6	<LOD	0.5	52.6	6.4	14.1	2.0	1.2	32.3	4.1	242	16.5
Tap water treated by ion exchange	7.75	0.62	0.0	0.17	143	160	114	28.2	16.7	0.4	0.7	8.8	<LOD	4.8	1.4	<LOD	80.1	<LOD	2.7	<LOD
Surface water (BA2G/FV)	7.22	133	24.2	39.2	31.3	648	143	61.2	106	0.3	2.4	12.8	10.5	15.3	0.8	2.6	1842	<LOD	651	7.4
LOD									1.0	0.2	0.5	2.0	0.2	1.0	0.2	1.0	3.0	2.0	0.1	2.0

[a] Characteristic anion and cation concentrations were detected by titration or inductively coupled plasma atomic emission spectroscopy according to the corresponding national standard procedures (pH [55], cations, SO_4^{2-} [57], PO_4^{3-} [58], HCO_3^- [58], Cl^- [59]). Carbonate anions (CO_3^{2-}) were not detected in any of the three types of water samples. Microelement concentrations were determined by atomic absorption spectroscopy. Concentrations of Al, As, Cd, Hg and Se were below the corresponding LODs (6.0, 2.0, 0.1, 0.6, 2.0 µg/l, respectively) in all water samples.

Results and Discussion

Assay performance

The Abraxis glyphosate ELISA kit applies the principle of the competitive immunoassay, with prior sample derivatization by acetic anhydride. A unique feature of the ELISA is that two key steps of the protocol are carried out simultaneously: the derivatized analyte is preincubated with glyphosate-specific antibodies, and the latter are bound to IgG-specific antibodies immobilized on the solid surface of the microwells of the ELISA plate. The competitive ELISA provides a sigmoid (logistic) standard curve downward with increasing glyphosate concentration (Figure 7.5-61). Typical analytical parameters of the immunoassay carried out in buffer were analyte concentration resulting in 50 % inhibition of the assay signal (IC_{50}) at 0.66 ± 0.16 ng/mL, slope of the standard curve at the IC_{50} at 1.52 ± 0.76 ng/mL, and LOD at 0.05 ng/mL. This LOD value is the 90 % B/B_0, commonly used to indicate sensitivity, which is the estimated minimum detectable concentration based on 90 % binding (10 % inhibition) in the assay. The concentration of the first calibration standard was 0.075 ng/mL. Although levels between 0.05 ng/mL and 0.075 ng/mL are within the detectable range of the assay, as with any analytical technique (ELISA, GC, etc.), there must be valid calibration points on either side of a sample value to be considered a legally defensible, valid sample result. As the results for these samples were all below the first standard (0.075 ng/mL), Table 7.5-80 lists the results for the unspiked samples (with no glyphosate detected) as <0.075 ng/mL, rather than giving a (less exact) value below the calibration range of the assay. The ELISA is highly specific for glyphosate:

cross-reactivities of related compounds, including main metabolites AMPA and glycine; glufosinate, an herbicide active ingredient of related chemical structure; and glyphosine, a withdrawn fungicide active ingredient of related chemical structure, were all below 0.1 % as calculated at both the LOD and at the IC_{50} of each compound.

Table 7.5-82: Detected glyphosate concentrations in surface and groundwater samples collected in Hungary in 2010 and 2011

Sample code	Type of water	Sampling site	Glyphosate concentration detected (ng/ml)
2010 sampling regime			
BA1F/TV	Surface water	Battonya	0.12 ± 0.085
BA1G/TV	Surface water	Battonya	0.17 ± 0.013
BA2F/TV	Surface water	Battonya	0.27 ± 0.131
BA2G/TV	Surface water	Battonya	0.68 ± 0.090
BA3F/TV	Surface water	Battonya	0.66 ± 0.150
BA3G/TV	Surface water	Battonya	0.63 ± 0.070
CSF1/TV	Ground water	Csorvás	0.65 ± 0.130
CSF2/TV	Ground water	Csorvás	0.82 ± 0.040
CS1F/TV	Ground water	Csorvás	0.68 ± 0.120
KT2F/TV	Ground water	Kőröstarcsa	0.76 ± 0.040
KT2G/TV	Ground water	Kőröstarcsa	0.41 ± 0.043
MH2F/TV	Ground water	Medgyesegyháza	0.75 ± 0.000
BSZ1A/TV	Ground water	Békéscsaba	0.93 ± 0.[illegible]
BSZ1B/TV	Ground water	Békéscsaba	0.60 ± 0.[illegible]
BSZ1C/TV	Ground water	Békéscsaba	0.44 ± 0.[illegible]
BSZ1D/TV	Ground water	Békéscsaba	0.[illegible] ± 0.037
BSZ1E/TV	Ground water	Békéscsaba	0.[illegible] ± 0.100
BSZ1F/TV	Ground water	Békéscsaba	0.[illegible] ± 0.009
GYN1A/TV	Ground water	Gyomaendrőd	0.[illegible] ± 0.066
GYN1B/TV	Ground water	Gyomaendrőd	0.[illegible] ± 0.069
GYN1C/TV	Ground water	Gyomaendrőd	0.98 ± 0.003
GYN1D/TV	Ground water	Gyomaendrőd	0.56 ± 0.260
GYN1E/TV	Ground water	Gyomaendrőd	0.33 ± 0.055
GYN1F/TV	Ground water	Gyomaendrőd	0.35 ± 0.012
GYN1G/TV	Ground water	Gyomaendrőd	0.63 ± 0.040
GYN1H/TV	Ground water	Gyomaendrőd	0.59 ± 0.040
GYN1I/TV	Ground water	Gyomaendrőd	0.25 ± 0.009
GYN1J/TV	Ground water	Gyomaendrőd	0.59 ± 0.110
GYN1K/TV	Ground water	Gyomaendrőd	0.87 ± 0.080
OR1A/TV	Ground water	Orosháza	0.39 ± 0.063
OK1A/TV	Ground water	Orosháza	0.39 ± 0.07
OK1D/TV	Ground water	Orosháza	0.38 ± 0.011
OK1E/TV	Ground water	Orosháza	0.33 ± 0.186
OK1F/TV	Ground water	Orosháza	0.35 ± 0.122
OK1H/TV	Ground water	Orosháza	0.31 ± 0.031
OK1G/TV	Ground water	Orosháza	0.66 ± 0.040
OK1I/TV	Ground water	Orosháza	0.96 ± 0.100
OK1B/TV/c	Ground water	Orosháza	0.58 ± 0.060
OK1C/TV/b	Ground water	Orosháza	0.30 ± 0.019
OK1K/TV	Ground water	Orosháza	0.33 ± 0.009
OK1L/TV	Ground water	Orosháza	0.33 ± 0.046
OK1M/TV	Ground water	Orosháza	0.54 ± 0.003
2011 sampling regime			
DH1/FV	Surface water	Hainburg	<LOD[a]
DB1/FV	Surface water	Bratislava	<LOD
DKP1/FV	Surface water	Komarno-bridge	<LOD
DKP2/FV	Surface water	Komarno-bridge	<LOD
DD1/FV	Surface water	Dömös-river bank	<LOD
DD2/FV	Surface water	Dömös-river bank	0.043 ± 0.009[b]
DL1/FV	Surface water	Luppa-island	<LOD
DL2/FV	Surface water	Luppa-island	<LOD
DDU1/FV	Surface water	Dunaújváros	<LOD
DKP1/FV	Surface water	Kopaszi gát-dam	<LOD
DKP2/FV	Surface water	Kopaszi gát-dam	0.035 ± 0.017
DED1/FV	Surface water	Érd	<LOD
DED2/FV	Surface water	Érd	<LOD
DER1/FV	Surface water	Ercsi	<LOD
DER2/FV	Surface water	Ercsi	<LOD
DDU2/FV	Surface water	Tökölbackwater	<LOD
DM1/FV	Surface water	Mohács	<LOD
V1/FV	Surface water	Lake Velencei	0.064 ± 0.021

[a] LOD is estimated to be 0.05 ng/ml (on the basis of 90% B/B_0) and 0.12 ng/ml (on the basis of the value and standard deviation of the upper plateau of the sigmoid standard curve).
[b] Detected values are near the LOD (on the basis of 90% B/B_0).

The effect of preincubation

Preincubation of the sample with the specific antibody is a key element in the achievable analytical sensitivity of the immunoassay. Longer preincubation of the antibodies with the free analyte (sample) allows antibody binding to approach equilibrium and should therefore favorably affect assay sensitivity. Results from the present study carried out to determine whether increasing the preincubation, up to 60 minutes, would provide an increase in sensitivity which would justify the additional analysis time. Thus, the effect of preincubation time with glyphosate at various concentrations (between 0.075 and 4.0 ng/mL) was tested. Experiments were carried out using four preincubation durations (ranging from 15 to 60 minutes) and are depicted in Figure 7.5-61. As seen on the resulting sigmoid curves, the duration of preincubation resulted in a moderate improvement in the reproducibility of the analytical standard curves, as those obtained with 30-60 minute preincubation were practically identical to each other. In consequence, the very slight improvement noted with the 45 minute preincubation, as opposed to the 30 minute preincubation, was not considered significant enough to justify the additional analysis time. Other assay parameters did not show dependence on the preincubation time of 15-60 minutes, i.e. the IC_{50} values were found to be 0.59, 0.46, 0.45 and 0.45 ng/mL at preincubation times of 15, 30, 45 and 60 minutes, respectively. Corresponding LOD values, calculated at 90 % of the upper plateau of the sigmoid curve, were 0.069, 0.032, 0.025 and 0.022 ng/ml, respectively. The 30 minute preincubation resulted in a significant improvement in IC_{50} and LOD when compared to the 15 minute preincubation.

Solvent effect

In the present study, solutions containing various concentrations of methanol in deionized water were analyzed unspiked and spiked with 0.5 ng/ml of glyphosate, and achievable recoveries were recorded. Methanol (applied at 0 %, 20 %, 40 %, 60 %, 80 % and 100 %) did not produce false positive results up to 100 % in the ELISA (Table 7.5-80). This is due to the beneficial buffering effect of the assay medium allowing the use of sample solutions even in pure methanol, as the final solvent content is diluted to 20 % in this case. Spiked samples with methanol concentrations up to 100 % showed spike recoveries of 89.2-131.2 %, with overestimation (recoveries above 100 %) at low methanol content.

Matrix effects

In this study, no matrix effects were seen for surface water, but considerable matrix effects were observed for spiked tap water, with a curve shift towards higher concentrations (Figure 7.5-62). This matrix effect, however, was eliminated if tap water was processed by distillation or ion exchange, changing all cations to sodium ions, and then spiked. This indicated that the component(s) causing the matrix effect in tap water can be removed. Moreover, as the standard curve in distilled water runs closely to those in ion exchanged water and assay buffer, the assay is insensitive to ionic strengths between 0 (distilled water) and 0.41 M (phosphate buffered saline), due to the buffering effect seen for high organic solvent (methanol) tolerance as well. In contrast, applying ion exchange after spiking could not fully eliminate the matrix effect and resulted in a standard curve with an approximately 30 % lower slope than that of the standard curve in assay buffer. After evaporation of water to dryness and solution of residue the curve obtained was practically the same as for tap water itself indicating that the component possibly causing the matrix effect is non-volatile, e.g. partly non-volatile disinfection by-products of chlorination.

To evaluate the possible effects of chlorine applied in water treatment, various water samples (deionized water, tap water, and surface water) were analyzed unspiked and spiked with 0.5 ng/mL of glyphosate, and achievable recoveries were recorded (Table 7.5-80). No false positives were detected in any of the water samples. Good spike recoveries were seen in deionized water and surface water samples. The spike recovery for tap water showed a biased low recovery (28.2 %), due to matrix interference, possibly from chlorine. To test possible involvement of chlorine used for tap water purification, unspiked and spiked tap water samples were then treated with either ascorbic acid or with sodium nitrite (commonly used dechlorinating agents) at final concentrations of 0.125 and 0.005 mg/mL, respectively. The treated water samples were vortexed thoroughly and were then derivatized and analyzed by the ELISA protocol. As seen from the resultant data, treatment with ascorbic acid prior to analysis neutralized the matrix interferences from chlorinated tap water samples, allowing accurate analyte recovery. To exclude possible matrix interferences by ascorbic acid, unspiked samples were treated along with the glyphosate-spiked tap waters. These unspiked ascorbic acid treated tap water samples did not show any recovery. Treatment with sodium nitrite also resulted in the elimination of the matrix interference and improved recovery, although to a lesser extent.

To further differentiate between chemical and mineral composition of the water samples used, characteristics of tap water, ion exchanged tap water, and surface water are summarized in Table 7.5-81. There is only a slight difference in the composition of surface and tap water; concentrations of copper and zinc were higher in the tap waters than in the surface waters examined. Since spiked surface water samples did not show any matrix effects, the interference observed in tap water may arise from complex formation by glyphosate with the copper or zinc content of tap water. Organic matter content in water must also be taken into account, as it may act as a limiting factor of complex formation. In principle, ascorbic acid, used as a dechlorinating agent to eliminate matrix effects by chlorine (see above), could interact with the copper content in tap water (e.g. reducing Cu(II) to Cu(I), or forming chelates with Cu(II)) as established in quantitative antioxidant capacity assays. Biochemically important amino acids however, inhibit this catalytic autoxidation of ascorbic acid due to the high conditional stability constant of their Cu-complexes. Being a phosphonate derivative of glycine, glyphosate also shows higher affinity to Cu(II) ions than ascorbic acid. Therefore, an eliminatory effect of ascorbic acid on matrix effects caused by Cu(II) is not expected.

Analysis in field samples

The study area in the case of contamination of agricultural origin covered four settlements in Békés county (Kőröstarcsa, Medgyesegyháza, Csorvás, and Battonya). Both intensive and organic parcels were chosen in all four settlements (4 organic and 4 intensive), and the pasture was designated in Csorvás. Contamination of industrial origin was examined in three settlements in Békés county (Orosháza, Gyomaendrőd, and Békéscsaba) at five sites (Orosháza-Linamar, Orosháza-Közútkezelő, Orosháza-Üveggyár, Gyomaendrőd-Nagylapos, and Békéscsaba-Szennyvíztelep). The subsequent 2011 sampling regime focused on the Danube River and its catchment area. Altogether, 17 surface water samples were collected from the Danube River in the Middle and Lower Danube region from the Austrian-Slovakian border to the Hungarian-Croatian border, and one standing water sample from Lake Velencei.

Glyphosate content was determined in all surface and ground waters collected using the Abraxis ELISA method. The practical LOD was found to be 0.12 ng/mL as calculated from the value and standard deviation of the upper plateau of the sigmoid standard curve (as opposed to the 0.05 ng/ml LOD value determined from the 90 % B/B_0 value and the concentration of the lowest analytical standard). A stunning difference between the results of the two sampling regimes in 2010 and 2011 was that while all samples collected in the first year contained detectable levels of glyphosate, only a slight proportion of the samples obtained in the second year had detectable glyphosate concentrations (Table 7.5-82). In 2010, severely or significantly contaminated samples represent half of the surface water samples obtained in the given sampling regime. In contrast, in 2011 glyphosate concentrations detected in the Danube River samples remained, in the vast majority, below the LOD of the assay (0.05 ppb) specified by the manufacturer on the basis of 90 % B/B_0. Only the sample from Lake Velencei showed a concentration higher than the LOD (0.064 ng/ml), while two other samples from the Danube River (Dömös, Kopaszi gát) were near the LOD (0.043 and 0.035 ng/mL, respectively). There are at least two characteristic differences between the two sampling regimes in 2010 and 2011: sampling location and meteorological characteristics prior to and during sampling. Findings in the 2010 campaign of the present survey did not indicate a statistically significant difference in detected glyphosate concentrations in surface and ground water: detected glyphosate concentrations in surface water were 0.422 ± 0.271 ng/mL (with average concentrations in individual samples ranging between 0.12 and 0.68 ng/mL), while corresponding concentrations in ground water were found to be 0.537 ± 0.224 ng/mL (0.5 - 0.98 ng/ml). In our survey in 2011, in contrast to 2010, due to the drought period and the lack of rain events prior to sampling, glyphosate applied in September most likely remained bound to soil particles and was not leached from the fields by the date of sampling (Oct 1, 2011).

The sharp contrast between the contamination rates found in the two campaigns is likely largely due to regional differences (different catchment areas and agricultural circumstances), and partly by meteorological differences between the two years (a major difference in natural precipitation). The 2010 samples were collected in early autumn after a rainy summer. These findings are in agreement with glyphosate contamination reported in environmental water contamination studies. In the United States, surface water contamination has been reported due to run-off from agricultural areas or pesticide drift.

Conclusion

In conclusion, ELISA is a suitable and convenient method for glyphosate detection and has been successfully applied to surface and ground water samples. Although the lack of cross-reactivity with AMPA and the cost may hinder its widespread application, ELISA is still more cost-effective for routine analysis, especially in monitoring programs, as compared with traditional wet chemistry methods, if all sample preparation/measurement steps and the instrumental demand are all considered. In order to obtain more accurate results and eliminate matrix effects, characteristics of the water sample to be analyzed must be taken into account. As matrix effects were not experienced at all with surface water, the ELISA method appears to be readily applicable to surface water samples. Significant matrix effects were, however, experienced with tap water, indicating that the chlorine content of drinking water and/or the presence of multivalent cations may cause a considerable bias resulting in lower glyphosate content measured. Such effect was not eliminated by evaporation and subsequent resolution in water, yet was successfully eliminated by reducing agents such as ascorbic acid.

The level of glyphosate pollution in surface water detected in environmental studies may vary tremendously among locations and years of sampling, as glyphosate is strongly influenced by precipitation. Rain events result in the leaching of glyphosate from soil, due to its high-water solubility. In this way, glyphosate may contaminate surface water and locations distant from its application site. This effect was seen in the current study. In spite of the fact that cultivation of GT crops is prohibited in Hungary, glyphosate was found at significant concentrations in surface water and ground water samples after a rainy period in 2010. In contrast, samples from a different catchment area, the Danube River, after a dry period in 2011 were found not to be contaminated by this target analyte.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a monitoring study where immunoassay analytical method was used. Several findings in different compartments (surface waters, ground water) were reported. Methods and results are sufficiently described.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/025
Report author	Sanchís, J. *et al*.
Report year	2012
Report title	Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry
Document No	Anal Bioanal Chem (2012) 402:2335–2345
Data point:	CA 7.5/026
Report author	Sanchís, J. *et al*.
Report year	2012
Report title	Erratum to: Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry
Document No	Anal Bioanal Chem (2012) 404:617
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Reliable

2. Full summary

Despite having been the focus of much attention from the scientific community during recent years, glyphosate is still a challenging compound from an analytical point of view because of its physicochemical properties: relatively low molecular weight, high polarity, high water solubility, low organic solvent solubility, amphoteric behaviour and ease to form metal complexes. Large efforts have been directed towards developing suitable, sensitive and robust methods for the routine analysis of this widely used herbicide. In the present work, a magnetic particle immunoassay (IA) has been evaluated for fast, reliable and accurate part-per-trillion monitoring of glyphosate in water matrixes, in combination with a new analytical method based on solid-phase extraction (SPE), followed by liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS), for the confirmatory analysis of positive samples. The magnetic particle IA has been applied to the analysis of about 140 samples of groundwater from Catalonia (NE Spain) collected during four sampling campaigns. Glyphosate was present above limit of quantification levels in 41 % of the samples with concentrations as high as 2.5 μg/L and a mean concentration of 200 ng/L. Good agreement was obtained when comparing the results from IA and on-line SPE-LC-MS/MS analyses. In addition, no false negatives were obtained by the use of the rapid IA. This is one of the few works related to the analysis of glyphosate in real groundwater samples and the presented data confirm that, although it has low mobility in soils, glyphosate is capable of reaching groundwater.

Methods

Sample collection

Groundwater samples were collected by the Catalan Water Agency between May and September in 2007, 2008, 2009 and 2010. The samples were collected in 500-mL amber glass bottles. Then, 20-mL aliquot of each sample were separated and frozen during the transport to the laboratory and analysed immediately

after sampling by the IA. The rest of the samples were frozen and stored in the dark in order to inhibit the degradation mechanism. A total of 139 samples from 69 wells located in 11 different sampling sites (water bodies) in Catalonia (Spain) were analysed. The number of samples varied between different campaigns: 18 samples from five different areas, 19 samples from eight areas, 37 samples from eight areas and 55 samples from ten different areas were collected during 2007, 2008, 2009 and 2010, respectively. The main characteristics of the sampling areas are summarised in Table 7.5-83. With the exception of one, all the areas studied presented a high impact from intensive agriculture and they were qualified as of high risk areas.

Chemicals

Analytical standards of glyphosate (reference 45521) and glyphosate-2-^{13}C (99 % isotopic purity and reference 606502) were purchased from Sigma-Aldrich (Steinheim, Germany). The derivatisation agent FMOC-Cl (≥99.0 % purity and reference 23814) and auxiliary reagents ethylenediaminetetraacetic acid (EDTA; 99.4–100.6 % purity and reference E9884), sodium tetraborate ($Na_2B_4O_7$; 99 % purity and reference 221732) and potassium hydroxide (KOH pellets, ≥85 % purity and reference 221473) were also purchased from Sigma-Aldrich. HPLC-grade methanol, acetonitrile (ACN), ultra-pure water, dimethyl sulfoxide (DMSO) and formic acid and hydrochloric acid for analysis (25 %) were supplied by Merck (Darmstadt, Germany). FMOC-Cl stock solution of 650 µM was prepared by dilution of 0.0168 g of FMOC-Cl in 100 mL of ACN. Tetraborate buffer was prepared by diluting 4 g of $Na_2B_4O_7$ in 500 mL of ultra-pure water. EDTA oversaturated solution was prepared by diluting 41.6 g of EDTA in 100 mL of ultra-pure water. All stock solutions were prepared weekly and stored at 4°C, with exception of FMOC-Cl stock solution, which was prepared daily.

Magnetic particle immunoassay

The glyphosate IA was developed and supplied by Abraxis LLC. This IA is based on polyclonal antibodies attached to paramagnetic particles, and the competitive reaction between derivatized glyphosate and derivatized enzyme labelled glyphosate for the antibody binding sites on the magnetic particles. The analysis procedure was performed in accordance with the operating manual accompanying the glyphosate kit. Very briefly, an aliquot of 250 µL of each sample was thoroughly mixed with 100 µL of diluted DMSO that served as derivatisation agent and incubated at room temperature for 10 min. After this period, 300 µL of derivatised sample and 500 µL suspended glyphosate antibody-coupled paramagnetic particles were mixed in a glass test tube and incubated for 30 additional minutes at room temperature. Incubation of another 30 min at room temperature followed after the addition of 250 µL of glyphosate enzyme conjugate. A magnetic field separator was then applied in order to separate any reagents unbound to the magnetic particles and keep hold of the bound reagents. Decanting of unwanted material took place after three washing cycles with deionised water; 500 µL of colour solution, containing the enzyme substrate (hydrogen peroxide) and the chromogen (3,3′,5,5′-tetramethylbenzidine), were added to the particles, and the mixture was incubated for 20 min at room temperature. The colour development reaction was stopped and stabilised by the addition of 500 µL of 2 % sulphuric acid solution, and absorbance was then read at 450 nm using a photometer *Photometric Analyzer II* (Abraxis LLD, Warminster, PA) within 15 min after adding the stopping solution. Colour development was inversely proportional to glyphosate concentration. Standard calibration curves were prepared testing nine levels of increasing concentrations of glyphosate from 0.1 to 5 µg/L. The standard sigmoidal curves were fitted to a four-parameter equation according to the following formula:

$$A = B + \frac{T - B}{1 + 10^{(\mathrm{LogEC_{50}} - \mathrm{LogC}) \times \mathrm{HS}}}$$

Where A is absorbance, T is the maximum absorbance value, B is the minimum absorbance value, EC_{50} is the concentration producing 50 % of the maximum absorbance, C is the concentration and HS is the slope at the inflection point of the sigmoid curve. A standard curve was prepared with each set of samples analysed and two-matrix blank samples were analysed along with each sample set to determine possible interferences. No interferences were detected above the LOQ during the samples analysis. The average of at least three replicates was calculated and presented in this work.

Immunoassay evaluation

The recoveries and the matrix effects on the IA were previously studied and reported. Nevertheless, the matrix interference can be quite variable depending on the different types of water. For this reason, the first step of this work was to evaluate the suitability of the IA for the different types of ground water and river water selected in this study. Therefore, the different types of water as well as ultra-pure water, and tap water, free on glyphosate were fortified with glyphosate in a wide range of concentrations covering from 25 to 10 µg/L, were assayed after derivatization using the IA procedure described above, and the standard curves were fitted for the different types of water.

Table 7.5-83: General characteristics of sampling areas

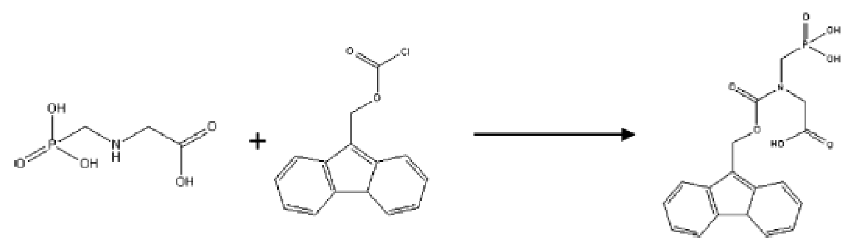
Sampling site	Dominant lithology	Total surface (km^2)	Multilayer	Permeability (m/day)	Transmissivity (m^2/day)	Dependency with surface waters	Intensive agriculture risk	Monitoring campaigns
1	Alluvial	165	No	40–300	100–4,000	Yes	Moderate	2009 and 2010
2	Granite and Palaeozoic	444	No	0.1–4 (granite); 10–20 (quaternaries)	20 (granite); 100–400 (quaternaries)	Yes	High	2010
3	Detritus not alluvial	72	Yes	No data	90–360	No	Nule	2008, 2009 and 2010
4	Detritus not alluvial	179	Yes	100–2,500	10–50 (clay); 2,000–3,000 (gravels)	Yes	High	2008, 2009 and 2010
5	Detritus not alluvial	265	Yes	100–2,500	10–50 (argyles); 2,000–3,000 (grayes)	Yes	High	2008, 2009 and 2010
6	Alluvial	184	Yes	No data	100–1,500 (deep layers); 200–30,000 (surface layers)	Yes	High	2007 and 2008
7	Alluvial	165	Yes	100–1,000	2,500–11,000	Yes	High	2007, 2008, 2009 and 2010
8	Alluvial	18	No	No data	No data	Yes	High	2007, 2008, 2009 and 2010
9	Alluvial	191	No	No data	No data	Yes	High	2007, 2008, 2009 and 2010
10	Alluvial	275	No	350–4,200	No data	Yes	High	2007, 2008 and 2010
11	Alluvial	328	No	No data	500	Yes	High	2009 and 2010

In a previous work, the possible interference of structurally related compounds was evaluated. In the present work, this study was extended and the possible cross reactivity of other organic pollutants commonly found in groundwater from these sampling areas was studied. The compounds included here were triazine compounds (atrazine, desethyl atrazine and terbuthylazine), phenylurea compounds (diuron and linuron) and organophosphates (fenitrothion, diazinon, malathion and dimethoate) and measured with the IA. The cross-reactivity values were calculated according to the equation:

Immunoreactivity equivalents = (IC_{50} glyphosate/IC_{50} tested compounds) x 100

In addition, 30 blind prepared samples in assay buffer and 30 blind prepared samples in groundwater free of glyphosate were evaluated in triplicates, in order to assess the accuracy, precision and possible false negative and positive detected by the IA.

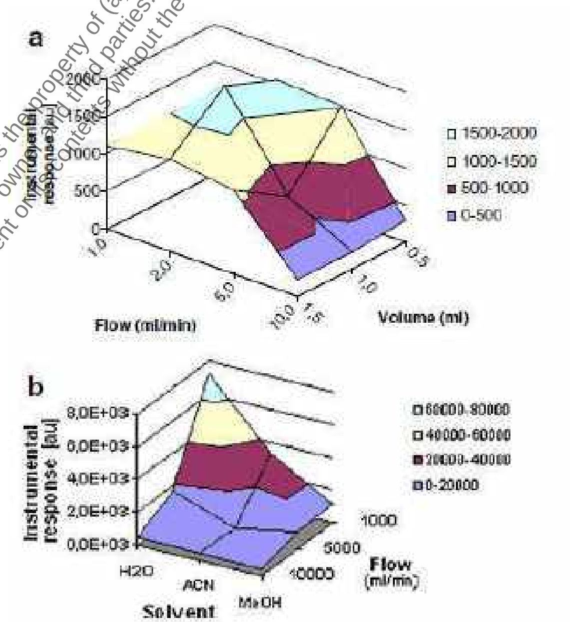
Figure 7.5-63: Chemical reaction between glyphosate and FMOC-Cl



Sample preparation for the instrumental analysis

Four millilitres of water samples were placed in an amber vials, were spiked with ^{13}C-glyphosate subrogate standard and were acidified with HCl 6 M to pH = 1.0. The acidified samples were stirred during 1 h in order to break the metal-glyphosate complexes that may happen under real environmental conditions. After this time, the presence of glyphosate is assumed to be in free form and the samples were neutralised with KOH 6 M. Derivatisation of the samples was performed according to the method previously described by Hanke *et al*. Very briefly, 1 mL of FMOC-Cl 650 μM in ACN and borate buffer (1:1) were added to the samples, and the mixture was stirred during 2 h at room temperature. Then the samples were acidified to pH 3 with formic acid, and 0.5 mL of aqueous EDTA (1.1 M) was added in order to prevent further metal complexation of glyphosate. The derivatised glyphosate (gly-FMOC) incorporates a fluorenylmethyloxycarbonyl group bounded to the glyphosate's amine group (Figure 7.5-63). The stability of gly-FMOC stored at 4°C during 12 h was proved. However, drastic loses of signal were detected when derivatized samples were stored overnight. Therefore, instrumental analysis was always carried out within the 12 h after derivatization.

Figure 7.5-64: Instrumental signals (in arbitrary units) obtained during the optimization of the on-line extraction: (a) Extraction step with three volumes of CAN with formic acid at four different flow rates; (b) Washing step with three solvents at three different flow rates



On-line extraction procedure
Derivatised water samples were loaded onto C18EC (Spark Holland, Emmen, The Netherlands) SPE cartridges previously conditioned with 2 mL of methanol and equilibrated with 1 mL of water at 2 mL/min. Derivatised samples (2 mL) were loaded at a slower flow rate (2 mL/min) with 1 mL ACN (0.1 % formic acid) as transfer solvent. SPE cartridges were then washed with 0.5 mL of water at 1 mL/min flow rate. Elution was carried out using the mobile phase solvents. Following the elution step, and in order to avoid sample carry over, multiple valve and clamp washes were carried out with water.

Liquid chromatography coupled to tandem mass spectrometry
LC was performed using the Symbiosis Pico system (Spark Holland, Emmen, The Netherlands) equipped with a 5-mL sample loop. The chromatographic separation was achieved with a LC column Synergy 4 µ Hydro-RP 50×2.0 mm, 4 µm (Phenomenex, reference 00B- 4375-B0). Mobile phase composition consisted of (A) ammonium acetate (2.5 mM, pH=9.0) and (B) methanol. The elution gradient conditions for the LC mobile phase started with 10 % eluent B, maintained isocratic during 1 min, increasing to 90 % of eluent B in 1 min and holding for 1 min more. Initial conditions were reached in 1 min and re-equilibration was achieved in 2 min. The flow rate was kept at 0.2 mL/min through the total chromatographic run. As pointed elsewhere, the presence of ammonium acetate and pH = 9 are needed in order to obtain a good chromatographic shape of gly-FMOC although high concentrations of the modifier decreased the S/N ratio.

The Symbiosis Pico LC system was coupled to a 4000QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer equipped with a Turbo Ion Spray source from Applied Biosystems-Sciex (Foster City, California, USA), employed in the negative electrospray ionisation mode (ESI (−)).

Simple reaction monitoring was used in order to obtain the required quantification points for confirmation. Quantification was performed with the Analyst software, version 1.5. Optimal instrumental were set as follows: curtain gas (CUR)=40; collision gas (CAD): high; ion spray (IS)=−4,500 V; source temperature (TEM): 390; ion source gas 1 (GS1): 60; ion source gas 2 (GS2): 50.

Results

Optimisation of LC-MS/MS
Due to the previous experience in our group, a Synergy Hydro-RP (50 ×2 mm, 4 µm) analytical column was selected. For the mobile phase, different compositions and solvents were tested including water, methanol, acetonitrile and ammonium acetate (2.5 mM, pH = 9.0). Solvents used for the mobile phase were methanol and ammonium acetate, and the elution gradient was optimised by varying the percentage of organic solvent throughout the run. The optimised gradient was selected in order to obtain the best signal-to-noise ratio. The use of ammonium acetate was crucial for the gly-FMOC peak shape and retention time.

For the optimization of MS/MS conditions, a solution of gly-FMOC at a concentration of 1 mg/L was infused in order to select the two most relevant transitions of product ions. Once identification of the most abundant fragment ions was achieved, as well as the ionisation parameters for each transition, full-scan chromatograms were obtained, indicating the retention of derivatised glyphosate. Flow injection analysis was then used, in order to optimise the ion source conditions in the mass spectrometer, namely the ion source TEM, IS voltage, CUR, GS1 and GS2 and CAD.

Optimization of on-line SPE
The type of sorbent, injection volume, sample loading and wash solvent were investigated in order to improve the on-line extraction process. Different sorbent types were studied; C_{18}EC, C_{18}HD, HLB, Hysphere Resin GP and Varian polymer phase PLRPs. Best recovery was achieved with C_{18}EC with a mean value of 89 % being slightly better than C_{18}-HP cartridges (mean value, 68 %), and Resin GP cartridges (mean value, 62 %).

Injection volume tests were performed with partial injections on a 5-mL sample loop in order to check for breakthrough in the range of 20–2,500 µL. No break- through volume was found at 2,500 µL, which was the maximum admitted amount using partial loop injection. Therefore, 2.5 mL was set as injection volume. Cartridge activation, sample loading and cartridge washing steps were also optimised. Different volumes

and flow rates of methanol were tested to optimise cartridge activation and final conditions were 2 mL of methanol at 2 mL/min flow rate. Six different solvents methanol, ACN, water, ammonium acetate 2.5 mM at pH=9.0, ACN (0.1 % formic acid) and water (0.1 % formic acid) were tested in order to select the optimal elution solvent. Different volumes of ACN (0.1 % formic acid) were evaluated at different flow rates. As can be seen in Figure 7.5-64a, the highest signal was obtained when the transfer solvent was 2 mL of acidified ACN at 2 mL/min followed by 1 mL of ACN at 2 mL/min for equilibration. Finally, the washing step was also optimised using different solvents and flow rates, obtaining the maximum instrumental response using 0.5 mL of water at a flow rate of 1 mL/min. Finally, cartridge elution was performed by the gradient elution. The recovery of gly-FMOC was calculated from the peak area obtained for the most intense transition.

On-line SPE-LC-MS/MS method validation
The method was validated according to the EU Decision 2002/657/EC. Blank groundwater was spiked at three concentrations levels: 80.0, 200 and 400 ng/L. Six replicates of each concentration were analysed at each concentration levels. The intraday reproducibility was calculated resulting in 15 %, 12 % and 8 %, respectively.

Criteria for the LOQ was established as the lowest concentration fulfilling all of the following criteria: (1) bias from the calibration curve less than 25 %, (2) relative standard deviation of four replicates below 19 %, (3) peak shapes acceptable and (4) signal-to-noise ratio at least 10. Method limit of detection and method limit of quantification (MLOQ) were found to be 3.2 and 9.6 ng/L, respectively. The decision limit (CCα) was defined as the lowest concentration level at which the method is able to discriminate the gly-FMOC presence, with a statistical certainty of 99 %. By analysing 20 blanks, CCα was estimated as 1.6 ng/L. The detection capability (CCβ) was defined as the smallest concentration of gly-FMOC that may be detected, identified and/or quantified in a sample with an error probability of β. By analysing 20 samples spiked at CCα, CCβ was established as 3.1 ng/L.

Linearity was assessed by constructing a seven-point calibration curve (ranging between 50 and 500 ng/L) in triplicate. Least-square linear regression analysis was performed by plotting the peak area of the analyte over the analyte concentration. R^2 of 0.99925 was achieved.

In order to assess the possible carryover of the method blank samples were analysed after analysis of groundwater samples fortified at 5 µg/L. In all these cases, blank samples showed values for glyphosate under the LOQ. Therefore, carryover could be considered negligible.

Immunoassay performance and specificity
The IA intra-assay precision was evaluated by determining the variation (CV %) between replicates assayed at various concentrations on the standard curves; as can be seen, good precision was shown by the IA with CV % of 13.4. Good agreement was found between fortified blank natural waters and the standard curve prepared in assay buffer and no significant changes on slopes were found. The recovery percentages range from 93 % to 105 % and 92 % to 102 % for groundwater and river water, respectively.

Very low cross reactivity was found for glyphosine and glufosinate, and no cross reactivity was found with other related compounds such as AMPA, in agreement with previous studies. No interference was found with other organic pollutants studied here, including other organophosphate compounds.

Sixty blind samples were prepared spiking glyphosate concentrations in the range between 0 and 4 µg/L. Thirty of these samples were prepared in assay buffer, and 30 samples more were prepared in a real groundwater samples free in glyphosate. The samples were analysed by magnetic particle immunoassay. The results of this test showed that no false negatives or false positives were obtained by the IA, very good correlation was obtained between the results obtained using the IA and the concentrations of fortification with coefficient of correlation $R^2 = 0.9907$ in assay buffer and $R^2 = 0.9816$ in groundwater. In addition, slight tendency to overestimation was observed in groundwater.

Table 7.5-84: Summary of glyphosate concentrations in groundwater samples analysed during four sampling campaigns

Sampling site	Number of analysed samples (no samples over MLOQ)					Median (ng/L)					Average (ng/L)					Range (ng/L)				
	2007	2008	2009	2010	Total	2007	2008	2009	2010	Total	2007	2008	2009	2010	Total	2007	2008	2009	2010	Total
1	0	0	6 (2)	7 (5)	13 (7)	–	–	<MLOQ	154	126	–	–	102	581	360	–	–	<MLOQ-304	<MLOQ-2560	<MLOQ-2560
2	0	0	0	7 (5)	7 (6)	–	–	–	186	186	–	–	–	212	212	–	–	–	<MLOQ-524	<MLOQ-524
3	0	2 (0)	6 (1)	5 (1)	13 (2)	–	<MLOQ	<MLOQ	<MLOQ	<MLOQ	–	<MLOQ	77	145	97	–	<MLOQ	<MLOQ-288	<MLOQ-624	<MLOQ-624
4	0	1 (0)	4 (2)	4 (2)	9 (4)	–	<MLOQ	86	137	<MLOQ	–	<MLOQ	76	143	108	–	<MLOQ	<MLOQ-118	<MLOQ-273	<MLOQ-273
5	0	2 (2)	7 (2)	6 (1)	15 (5)	–	204	<MLOQ	<MLOQ	<MLOQ	–	204	109	97	117	–	198-209	<MLOQ-514	<MLOQ-358	<MLOQ-514
6	3 (0)	3 (3)	0	0	6 (3)	<MLOQ	620	–	–	219	<MLOQ	603	–	–	514	<MLOQ	544-646	–	–	<MLOQ-646
7	6 (2)	3 (1)	8 (5)	9 (5)	26 (13)	<MLOQ	<MLOQ	140	126	76	124	202	189	146	171	<MLOQ-345	<MLOQ-527	<MLOQ-492	<MLOQ-444	<MLOQ-527
8	1 (0)	1 (1)	1 (0)	1 (1)	4 (2)	<MLOQ	480	<MLOQ	107	66	<MLOQ	480	<MLOQ	107	138	<MLOQ	480	<MLOQ	107	<MLOQ-480
9	3 (1)	3 (3)	4 (2)	5 (3)	15 (9)	<MLOQ	749	72	283	243	122	741	137	286	304	<MLOQ-315	717-756	<MLOQ-378	<MLOQ-729	<MLOQ-756
10	3 (1)	4 (0)	0	9 (7)	16 (8)	<MLOQ	<MLOQ	–	282	<MLOQ	47	<MLOQ	–	364	171	<MLOQ-137	<MLOQ	–	<MLOQ-919	<MLOQ-919
11	0	0	1 (1)	2 (2)	3 (3)	–	–	366	451	366	–	–	366	433	409	–	–	366	88-781	88-781
Total	18 (4)	19 (10)	37 (15)	55 (32)	129 (61)	<MLOQ	198	<MLOQ	126	<MLOQ	80	304	129	252	202	<MLOQ-345	<MLOQ-827	<MLOQ-514	<MLOQ-2560	<MLOQ-2560

Finally, all the samples of the last sampling campaign were analysed in parallel by means of the magnetic particle IA and on-line SPE-LC-MS/MS. The average relative error between the IA analyses and the confirmation method was lower than 12 %.

Applicability of the method

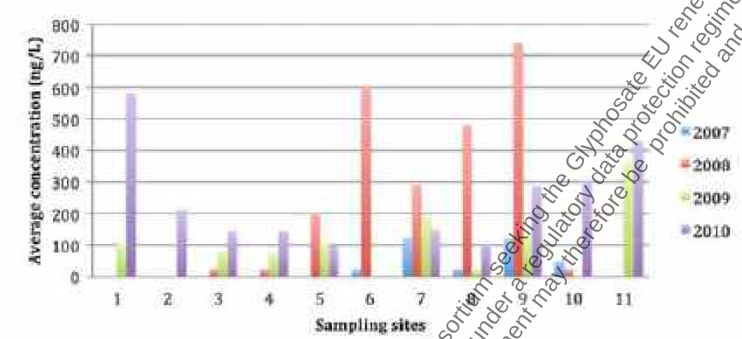
Glyphosate was investigated in 139 samples, and it was detected at quantifiable levels in 61 samples (47 %). Table 7.5-84 summarises the median concentration, average and range of concentrations along the different campaigns. All samples were analysed using the magnetic particle immunoassay, and positive samples were confirmed by instrumental analysis. No false negatives were found using the immunoassay. The concentrations of glyphosate range from MLOQ to 2.6 µg/L, and the average was 202 ng/ L (samples under limit of quantification were computed as half the MLOQ for the average calculation). Mean concentrations of glyphosate are presented in Figure 7.5-65. In general, in terms of average concentrations, slight differences were obtained along the sampling campaigns, which range from 97 ng/L for the cleanest site to 409 ng/L. As it was expected, more contaminated areas (sites 6, 9 and 11) were found in those regions of thriving agriculture activity. However, the higher value was achieved in 2010, in site no. 1, which corresponds to an area with moderate agricultural activity. In addition, a significant difference was obtained compared with the same site during 2009 campaign. In this case, the presence of glyphosate can be related to their increasing use as herbicide for non-agricultural applications, such as, the control of weeds on margins or streams and drains, around buildings, railways, roads and industrial areas.

All sampling campaigns were carried out during the application season but, in some of the sampling areas (1, 3, 4 and 11), an increasing trend was observed along the different campaigns, and in others, such as, 5, 7, 8 and 9, the higher average concentrations were obtained during the first sampling campaign in 2008. In this sense, it should be mentioned that the degradation of glyphosate is highly variable according to the environmental conditions. The degradation of glyphosate in surface water has been reported to be very fast. Whereas, in groundwater glyphosate is rapidly adsorbed to organic matter, precipitated and then can be retained in the soil where half-life can be longer than 2 years. In addition, the mobility and leaching capability of glyphosate also depend on the type of soil. Borggaard *et al*. reported that the different glyphosate forms can be moved by leaching through uniform gravelly soils and in structured soils with macro-pores, being determinant other factors such as rain precipitations, timing, tillage and vegetation. Therefore, the results showing the higher concentrations can be associated to sites where the sampling was carried out immediately after glyphosate application in the area. In addition, glyphosate can be accumulated in soil leaching by precipitation. This fact can partially explain high concentrations in some areas during 2008, such as sites 5 and 7, which coincides with the onset of spring rains in 2008 after 3 years of heavy drought that could have favoured the dissolution of glyphosate retained in the soil. After these high levels in the 2008 campaign, during the 2009 and 2010, campaigns registered a progressive decrease.

The presence of glyphosate in groundwater has been exiguously reported, and very few works have been carried out to study this presence. In most of previous studies, no quantifiable levels of glyphosate were found in groundwater, even in areas where surface water is found to contain the herbicide. However, it

should be pointed out that these studies were carried out with analytical methods presenting LOQ in the range of micrograms per litre, and the present study use a, IA capable to detect glyphosate at pictogram-per-millilitre range without sample pre-treatment, just derivatisation, and an on-line SPE-LC-MS/MS method for confirmation of the glyphosate at nanogram-per-litre range. Second, in this study the sampling campaigns were carried out during the peak season of glyphosate application in those areas, in order to investigate main areas susceptible of glyphosate accumulation in soils. These areas should be determined and controlled in order to follow the behaviour and dissolution of this herbicide under certain environmental conditions as after rains.

Figure 7.5-65: Average concentrations of the sampled areas during four sampling campaigns



Conclusion

The magnetic particle IA for glyphosate analysis from Abraxis LLC was proved to be a suitable, sensitive and cost-effective method for the fast ultra-trace screening analysis of a large number of real groundwater samples. The here presented IA is the most sensitive in the literature for the analysis of glyphosate. In addition, a new methods based on on-line SPE-LC-MS/MS was developed and validated as rapid confirmatory analytical method for glyphosate analysis at ultra-trace level.

The good performance of these analytical approaches, as well as, the applicability of the combined methodology for the analysis of glyphosate in groundwater has been proved using the approach for the analysis of groundwater from 11 different areas in Catalonia. The results showed a 41 % of the samples presenting quantifiable concentrations of glyphosate when were sampled. In addition, the results of this study corroborate the hypothesis of previous studies pointing that glyphosate may exhibit certain grade of mobility in soils. This is the first that experimental data about glyphosate reaching groundwater provided. Despite the tendency of glyphosate of being immobilised in soils, aquifer contamination with glyphosate has been demonstrated to happen because of its intensive use. Higher concentrations for 2008 were registered and it was linked to 2008 spring precipitations finishing with a 3-year drought period. Since the environmental source of glyphosate is certainly related to agricultural practices, runoff to surface waters is very likely to occur. Therefore, the potential ecological impact of this contamination should be taken in consideration in a more global view. Although the levels reported in this work are relatively low, their variability is significant through space and time, and an increase tendency has been observed in some sampling points, underpinning the importance of further analysis of glyphosate and their degradation products in groundwater samples.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article presents an analytical method to determine glyphosate in groundwater samples from Catalonia / Spain. Glyphosate findings in the respective groundwater samples are presented. Methods and results are well described. Maximum concentration of glyphosate measured at 2560 ng/L in 2010. The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/027
Report author	Bruchet, A. *et al.*
Report year	2011
Report title	Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge
Document No	European Journal of Water Quality 42 (2011) 123-133
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary

The fate of various emerging contaminants as well as priority pollutants from the European Union Water Framework directive was examined along a complex combination of natural and engineered processes used to produce drinking water downstream of a major metropolitan area. The sampling points examined comprised Seine river water downstream of the Paris area, water from a primary well after bank filtration, water from a secondary well influenced by an artificial recharge process and water from the mixture of secondary wells after drinkingwater treatment. More than 80 organic contaminants includingglyphosate and AMPA, were monitored during five campaigns. River bank filtration and to a lesser extent artificial recharge clearly decreased the variety of contaminants, in particular glyphosate and AMPA were reduced from <0.1 – 0.12 µg/L and 0.25 – 0.65 µg/L, respectively, in the river to <0.1 µg/L in both the primary and secondary wells.

Materials and methods

Study site
The aquifer studied (Figure 7.5-66) is located along the Seine river, downstream of Paris and its urban wastewater plants. In particular, it is located downstream of a wastewater plant that treats the effluents from 6.5 million people at a rate of 2 million m^3/day. This aquifer covers an area of 40 km^2 and comprises 36 primary and secondary wells. The primary wells are located mostly along the river, naturally re-supplied under anoxic conditions through river bank filtration. The primary wells output is pumped and re-infiltrated through a sand-gravel artificial basin (under slightly aerobic conditions) to recharge secondary production wells. Water from the secondary wells is further treated in a drinking water plant that comprises settling with addition of powdered activated carbon, sand filtration, ozonation and final disinfection with chlorine. The plant production is equal to 144 000 m^3/day.

The following points were sampled (grab samples) on five occasions during September and October 2008:

(1) the Seine raw water,

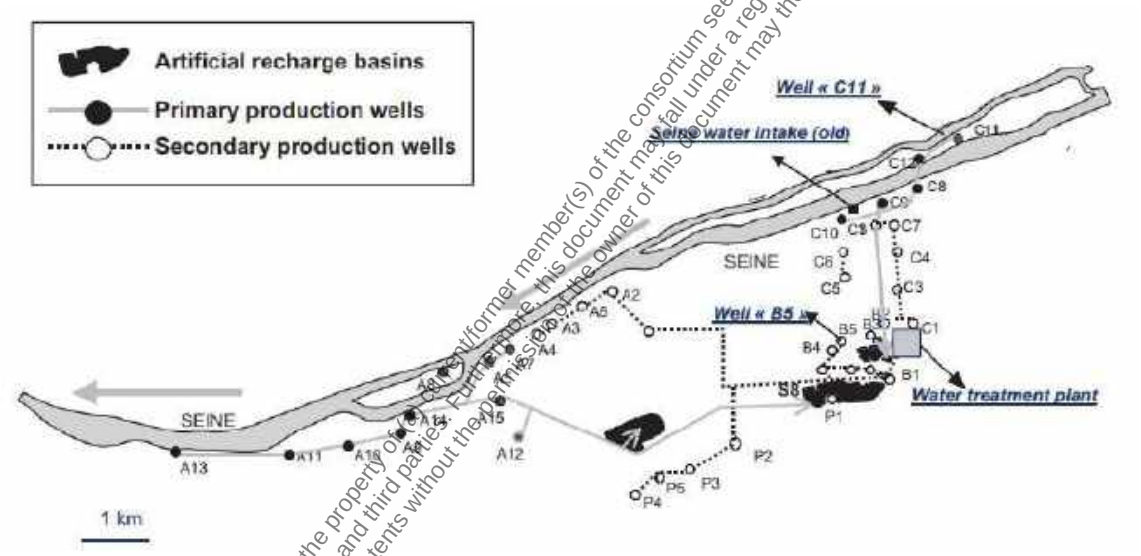
(2) primary production well C11 (one of the C wells in Figure 7.5-66) which is located on a small island on the Seine river and hence directly influenced by the river after bank filtration,

(3) secondary well B5 (one of the B wells in Figure 7.5-66) which is influenced by the main artificial recharge basin. However, due to the direction of underground flows, this well is also influenced by other areas of the aquifer and,

(4) the treated water at the outlet of the drinking water plant.

The sampling period covered both low flow conditions (220 m^3/s) and higher flow rates (up to 343 m^3/s).

Figure 7.5-66: Description of study site showing the four sampling points. Flow of the river is from right to left.



Analytical methods

A wide array of analytical methods was used to cover most priority pollutants and emerging contaminants. Volatile organic compounds (VOC's) were determined by Purge and Trap gas chromatography-mass spectrometry (GC/MS). Glyphosate and AMPA were determined by FMOC derivatization-HPLC-fluorescence.

Results and Discussion

Although the sampling point on the Seine river is located downstream of a metropolitan area with 11 million people, most EU priority compounds were never detected.

The only pesticide or degradate found at a level exceeding 0.1 µg/L in the Seine river is **glyphosate** (on one occasion) and its degradate **AMPA** (systematically in the range 0.25-0.65 µg/L). AMPA can also be present as a wastewater contaminant, from household detergent use. These two compounds are totally removed by bank filtration, in accordance with previous observations and do not reappear in the aquifer.

Table 7.5-85: Fate of priority and emerging contaminants during bank filtration (C11), artificial recharge (B5) and drinking water treatment.

Parameter	Unit	Seine river $n = 5$	C11 well $n = 5$	B5 well $n = 5$	Drinking water $n = 5$
Semi-volatiles compounds					
Fluoranthene	ng/L	9–14	<10	<10	<10
DEHP	ng/L	191–675	367–509	320–2013	243–521
Atrazine	ng/L	17–23	19–23	34–50	3–9
Diuron	ng/L	32–40	58–73	26–52	<1
Isoproturon	ng/L	<1–3	24–35	15–31	<1
Simazine	ng/L	3–9	6–10	7–15	<1
Glyphosate	µg/L	<0.1–0.12	<0.1	<0.1	<0.1
AMPA	µg/L	0.25–0.65	<0.1	<0.1	<0.1
Alkylphenols					
4-NP (Nonylphenols)	µg/L	0.06–0.21	0.45–1.71	0.06–0.25	0.05–0.18
4-t-OP (Octylphenols)	µg/L	<0.01–0.05	0.05–0.57	<0.01–0.02	<0.01–0.03
4-NP1EO	µg/L	0.02–0.11	0.04–0.19	0.02–0.06	0.002–0.04
4-NP2EO	µg/L	0.01–0.13	0.03–011	0.03–0.04	0.01–0.08
4-NP1EC	µg/L	0.08	0.19	0.03–0.01	<0.001–0.003
Beta-blockers					
Atenolol	ng/L	99.5–155.2	<LoQ-0.6	0.7–2.5	<LoQ-1.6
Sotalol	ng/L	65.9–117.1	3.3–12.1	<LoQ-3.8	<LoQ-0.6
Nadolol	ng/L	1–4	<LoQ	<LoQ	<LoQ
Timolol	ng/L	<LoQ-0.5	<LoQ	<LoQ	<LoQ
Acetobutolol	ng/L	32.9–75.6	0.6–1.6	3.3–6.2	<LoQ-1.2
Metoprolol	ng/L	8.5–13.5	<LoQ-0.9	<LoQ-0.4	<LoQ-0.4
Oxprenolol	ng/L	<LoQ-0.7	<LoQ	<LoQ	<LoQ
Propanolol	ng/L	8.8–30.0	<LoQ	<LoQ-0.6	<LoQ-0.5
Betaxolol	ng/L	<LoQ-1.7	<LoQ	<LoQ-0.5	<LoQ
Bisoprolol	ng/L	9.5–12.5	<LoQ-0.3	<LoQ-0.3	<LoQ-0.5

In the river, glyphosate was found at <0.1 – 0.12 µg/L, and AMPA at 0.25 – 0.65 µg/L: but, in both the primary well and the secondary well, concentrations of both substances were <0.1 µg/L, as they were in the drinking water samples. (It is worth noting that "<0.1 µg/L" indicates LOQ, and not an absolute concentration – using it as a basis for determining the removal rate for AMPA would give a removal rate of 85 %, and 17 % for glyphosate; whereas, it is clear from the context that removal is more likely to be 100 %. Indeed, the authors state that "both these compounds are totally removed by bank filtration" in this case.

Conclusion

The present study allowed most priority substances from the EU Water Framework Directive to be measured, and also a wide variety of emerging substances in a surface water downstream of a major metropolitan area that treats the majority of its urban wastewaters (the Seine river downstream of Paris). The study site selected allowed the fate of the substances detected to be observed, during their infiltration into an aquifer primarily re-supplied by natural bank filtration. The fate of the substances reaching the aquifer was monitored along a natural recharge process and at the outlet of a drinking water plant treating a mixture of boreholes from this aquifer.

In a system influenced by urban wastewaters downstream of a major metropolitan area, a drinking water produced by a complex combination of natural bank filtration, artificial recharge, clarification with powdered activated carbon addition, ozonation and chlorination, complies with the current legislation. In particular, glyphosate and AMPA were reduced, by the bank filtration process, from <0.1 – 0.12 µg/L and 0.25 – 0.65 µg/L, respectively, in the river, to <0.1 µg/L in the primary and secondary wells. It is also worth noting that "<0.1 µg/L" indicates LOQ, and not an absolute concentration – using it as a basis for determining the removal rate for AMPA would give a removal rate of 85 %, and 17 % for glyphosate; whereas, it is clear from the context that removal is more likely to be 100 %. Indeed, the authors state that

"both these compounds are totally removed by bank filtration" in this case.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a monitoring experiment with glyphosate and AMPA among different other substances from Seine river and a drinking water production area downstream of the Paris urban area. The study is well described, the analytical methods used are sufficient.

With respect to glyphosate and AMPA, the study sheds light on the effectiveness of the water treatment train employed for a major surface water to drinking water plant, where the primary treatment process is bank filtration. In this case, it is clear that bank filtration has been shown to be an effective process to remove glyphosate and AMPA to <0.1 µg/L from water destined to be drinking water.

The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/028
Report author	[redacted]
Report year	2011
Report title	Investigation of the potential glyphosate groundwater contamination in Lombardia region (North Italy)
Report No	BVL No. 2310280
Document No	
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Glyphosate concentrations above the drinking water limit were detected in five groundwater monitoring sites from the Lombardia region (North Italy), all collected during the May 2007 monitoring campaign, and all located in the South East part of the region. In order to investigate these groundwater detects, all accessible information (including information on methods and tools implemented or used by ARPA Lombardia for planning monitoring programs and also information on the analytical method) was requested from the responsible authorities (ARPA-Lombardia) and checked. Detailed information on soil characteristics, technical and hydrogeological information was also gathered, and a site inspection carried out. In addition, parallel samples from contaminated sites, and from surrounding areas where piezometers were available, were collected to assess residues levels, characterize the water, and to investigate possible different analytical methods. In four of the sites, the glyphosate content of the additional samples taken more than 3 years after the reported detections did confirm the findings, highlighting the persistence of the groundwater contamination. Site inspections have revealed that findings could be attributed to artificial influences, as inflow of surface water and mud/sediment or point source contamination. For one of the five wells, investigations are still ongoing to confirm some assumptions of possible contamination such as infiltration from a stream or infiltrating wastewater.

Generally, it can be noted that the conditions of the wells were not suitable for the collection of groundwater quality samples for the assessment of a possible contamination of plant protection products at trace concentrations

Materials and Methods

The clarification of the reported glyphosate findings was done in stepwise procedure. In the first part of the study, all accessible information (including information on methods and tools implemented or used by ARPA Lombardia for planning monitoring programs and also information on the analytical method) was requested from the responsible authorities (ARPA Lombardia) and checked. As this information was insufficient to clarify the findings, detailed information on soil characteristics, technical and hydrogeological information was gathered, and a site inspection carried out to verify the well status and inspect the well surrounding. Local authorities or the owners of the wells were also contacted.

As the available information did not allow to assess the quality of the analyses and the water sampling method, parallel samples from contaminated sites, and from surrounding areas where piezometers were available, were collected to assess residues levels, characterize the water, and to investigate the impact of a different analytical method: the analytical method used by ARPA Lombardia (FMOC-Cl derivatization with HPLC:fluorescence detector) was implemented in the laboratories of the University of Piacenza and compared with method using FMOC-Cl derivatization followed by LC/MS/MS (method LOD of 0.02 µg/L).

Results and discussion

The table below provides an overview of the findings and the result of the assessment

Table 7.5-86: Overview on findings of glyphosate and results of the assessments

Site	Date of findings	Glyphosate level (µg/L)	Cause of the findings	Date of re-sampling	Glyphosate level (µg/L)
Pandiono (CR)	10 May 2007	0.9	Inflow of surface water	28 Nov 2010	< LOQ
Trigolo (CR)	22 May 2007	0.2	Point source contamination	28 Nov 2010	0.252
Caselle Lurani (LO)	08 May 2007	0.2	Point source contamination	30 Nov 2010	0.163
Asola (MN)	05 June 2007	0.7	Investigation still ongoing	16 Oct 2010	0.525
San Benedetto (MN)	06 June 2007	1.2	Point source contamination	12 Jan 2011	1.375

Pandino: The inspection of the monitoring well showed that the piezometer was not sealed and that surface water and mud from the adjacent areas were standing between the cast iron manhole and the piezometer, and could thus flow into the well.

Trigolo: The owner of the site explained that during the investigation period he weeded the stretch of land around the piezometer with a glyphosate containing herbicide to facilitate access. A careful inspection of the site showed that the base of the piezometer was not well isolated and could thus lead to preferential flows.

Asola: Investigations are ongoing to confirm hypotheses of possible contamination such as infiltration of rainwater from paved surfaces and drainages

Casselle Lurani: The old well (more than one century) is located in a private courtyard of a farm, and is not completely sealed. Surface water from the courtyard can enter directly into the well from the manhole. The farmer uses the well to wash the spray equipment and tractor next to the well.

San Benedetto: the well is located in the courtyard in front of the product and sprayer storage of a company applying herbicides on railways. The area next to the well is used to clean and maintain the trucks used for herbicide application.

The results of the analysis of the samples taken more than 3 years after the reported detect did confirm the levels observed in 2007 in the wells from Trigolo, Caselle Lurani, Asola and San Benedetto, demonstrating the persistence of the contamination.

Conclusion
The detailed investigation has allowed plausible explanations of the origin of the glyphosate findings at 4 of the 5 locations. In one location, the investigation is still ongoing. In all cases, the origin of the glyphosate concentrations could be allocated to surface inflow or to point source contamination. Up until now, there was not a single case for which the findings could be correlated with the normal and proper use of glyphosate in the field.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article elucidates reported glyphosate concentrations above the drinking water limit (0.1 µg/L) detected in May 2007 in North Italy (Lombardia region). Methods and results are sufficiently described. The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/029 CA 7.5/030 (Translation)
Report author	[redacted]
Report year	2010
Report title	Evaluatie van metingen van glyfosaat en AMPA in grondwater in Nederland (Evaluation of glyphosate and AMPA measurements in groundwater in The Netherlands)
Report No	Report 354
Document No	BVL No. 2310284
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No (no experimental work performed)
Acceptability/Reliability:	Reliable

2. Full summary

Glyphosate and AMPA detects were mentioned in two reports (RIVM, 2007 and Royal Haskoning, 2008), summarising the residues of plant protection products in Dutch groundwater. Before conducting an on-site investigation, information about the site characteristics, analytical method and data processing were gathered remotely and evaluated. The investigation showed that the protection of the well was poor at 2 sites and medium at 5 sites, and that surface water inflow or contamination by spray drift cannot be excluded at these locations. Uncertainty about sample processing was observed for 2 sites. For 6 (out of the 10) glyphosate detects, no plausible explanation could be found based on this investigation. It should be noted that no special attention was given to the AMPA detects, as this compound is considered as a non-relevant metabolite in The Netherlands, and the 0.1 µg/L trigger does thus not apply.

Materials and Methods

The clarification of the reported glyphosate and AMPA findings was done in stepwise procedure. In a first step information on the analytical method, sampling and data processing, and on the characteristics well surroundings were gathered and evaluated. The second step involved an on-site investigation with a special focus on the protection of the well.

Results and discussion

Glyphosate was found in 6 out of the 189 measurements (3.71 % of all measurements) carried out in one report (2008, Royal Haskoning), and 4 out of 691 measurements (0.58 % of all measurements) carried out in the second report (2007, RIVM). Table 7.5-87 summarizes available information about the different locations investigated. The locations 1-10 related to the 2008 report, locations 11 to 14 to the 2007 report.

Table 7.5-87: Overview on findings of glyphosate/AMPA and results of the assessments

Location	Concen-tration (µg/L)	Date	Description	Ground-water body	Land use in surrounding area	Well protection[1]	Sample processing[2]
1. South Limburg, Vaals	0.16 glyphosate	8th October 2007	Spring near Sinselbeek	Chalk South Limburg	Agriculture / Nature (forest)	+/-	+
2. South Limburg, Valkenburg	0.13 glyphosate	11th October 2007	Spring on plateau near Geul	Chalk South Limburg	Agriculture / Nature (forest)	+/-	+
3. Central Limburg, Maasbracht	0.20 glyphosate 0.12 AMPA	11th July 2007	Semi-deep groundwater (6-8 m)	Maas Deep Channel	Agriculture	-	+
4. North Limburg, Tegelen,	0.12 glyphosate	19th Sept. 2007	Semi-deep groundwater (13-15 m)	Maas Sand	Agriculture	+/-	+
5. North Limburg, Nuland,	0.62 glyphosate 0.23 AMPA	1st October 2007	Shallow groundwater (2 m)	Maas Deep Channel	Agriculture/ Groundwater protection area	+	+
6. North Limburg, Laarbeek,	0.13 glyphosate	23rd October 2007	Shallow (phreatic) groundwater (2-3 m)	Maas Sand	Agriculture	+	+
7 Central Limburg, Grathem,	0.17 AMPA	17th October 2007	Semi-deep groundwater (3 m)	Maas Deep Channel	Recreation (bungalow park)	+/-	+
8. North Limburg, Broekhuizn	0.17 AMPA	24th Sept. 2007	Semi-deep groundwater (8-10 m)	Maas Sand	Agriculture	+/-	+
9. North Limburg, Gennep,	0.17 AMPA	15th August 2007	Semi-deep groundwater (8-10 m)	Maas Sand	Urban area	-	-

Table 7.5-87: Overview on findings of glyphosate/AMPA and results of the assessments

	0.13 AMPA		Deep groundwater (18-20 m)				
10 North Brabant, Eindhoven,	0.29 AMPA	3rd October 2007	Semi-deep groundwater (3 m)	Maas Sand	Car park / sports grounds	+	Uncertain bottle code
11. South Holland, Noordwijkerhout	0.99 glyphosate	2003-04	Shallow (< 7 m)		Agricultural (bulbs), residential district	+	
12. North Holland, Texel	4.74 glyphosate	2006	Shallow (< 7 m)		Agricultural	+	
13. Groningen, Hoogezand	0.47 glyphosate	2006	10 m deep		Agricultural	+	
14. Groningen, Winschoten	0.32 glyphosate	2006	10 m deep		Agricultural	+	

[1] On the basis of inspection on location, + signifies good protection of the well, +- signifies that the appearance of surface translocation or drift is unlikely but cannot be excluded, - signifies a likely chance of drift or surface translocation.
[2] On the basis of insights into possible errors which occurred during the processing of the samples and the analysis of the data, + signifies no indication that errors have occurred, - signifies that possible errors have occurred.

The results show that at least 2 sites (Maasbracht and Gennep) showed a poor well protection that surface water influx cannot be excluded. At five other sites, the well wasn't fully protected either (2 springs, and 3 sites where the covers did not fully close) and it is conceivable that contact with surface water my occur. Uncertainty related to the data processing was evidenced at 2 sites. For 6 of the 10 sites at which glyphosate was detected, no explanation could be found during this investigation.

It should be noted that numerous AMPA detects were reported from the 2007 studies, but not investigated in his report. AMPA is considered as a non-relevant metabolite in The Netherlands and the 0.1 µg/L trigger does thus not apply.

Conclusion
This has evidenced a potential contact between ground and surface water due to a bad sealing of the well cover for 7 wells, and uncertainty related to data processing in 2 sites. No explanation for 6 out of the 10 glyphosate detects could be found within the remit of this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article elucidates findings of glyphosate and AMPA in groundwater in The Netherlands. The methods and results are sufficiently described.
The article is considered reliable.

Assessment and conclusion by RMS:

B. Water

B.2a Surface water

Concentrations of glyphosate (GLY), AMPA and HMPA in surface water arising from public monitoring datasets collected from regional/national environment agencies as well as published peer reviewed publications from literature searches rated as potentially relevant/reliable are reported in this section.

There are nine new applicant studies presented on surface water. [redacted] (2020, CA 7.5/001) describes the collection of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA. [redacted] (2020, CA 7.5/002) assesses the data collected by [redacted] (2020, CA 7.5/001). These two recent studies were designed to be the more comprehensive than previous studies by considering additional metabolites, compartments and time periods. [redacted] (2020, CA 7.5/002) covers a range of environmental compartments, however, the study summary below only includes the results relevant to this environmental compartment. [redacted] (2016, CA 7.5/010) updates a previous investigation period described by an existing study ([redacted] 2012, CA 7.5/013). The study by [redacted] (2012, CA 7.5/013) is presented for completeness. Studies specific to France include [redacted] (2019a, CA 7.5/008, and 2019b, CA 7.5/032) which focus on a more recent (2008-2014) period of the same dataset investigated by [redacted] (2016, CA 7.5/009) which considered 1997-2013. [redacted] (2018a, CA 7.5/033 and 2018b, CA 7.5/034) investigate sites with elevated GLY and AMPA detections. There is a large degree of overlap between the datasets used in these various studies, for example the French dataset is common to all studies and given its size often comprises the majority of compiled European datasets. [redacted] (2019, CA 7.5/031) details a multi-year investigation of farmer engagement strategies on water quality for a catchment in Belgium.

Several publications found in the literature research are presented outlining concentrations in surface water. These include studies in Member States of the EU, its overseas territories as well as Norway and Switzerland:

- National studies presented by Schreiner *et al*. (2016, CA 7.5/046) considered FR, NL and four regions of DE but were essentially focussed on pesticide mixtures detected in surface water and as such are limited as they do not present a detailed assessment of measured concentrations.
- Regional studies are presented by Di Guardo and Finizio (2018, CA 7.5/036) for Lombardy IT, Masiol *et al*. (2018, CA 7.5/038) for North East IT, Poiger *et al*. (2017, CA 7.5/017) for specific regions of Switzerland, Székács *et al*. (2014, CA 7.5/006 and 2015, CA 7.5/048) for different parts of HU and Busetto *et al*. (2010, CA 7.5/066) for the Brianza region in Lombardy IT. These datasets may wholly or in part overlap with each other and the applicant studies. In addition, Stenrød (2015, CA 7.5/047) covers 6 catchments in Norway, Mottes *et al*. (2017, CA 7.5/042) target Martinique while Martin *et al*. (2013, CA 7.5/022) consider concentrations in Reunion.
- River studies, focussing on runoff events, are presented by Reoyo-Prats *et al*. (2017, CA 7.5/043) for the River Tet in FR, Petersen *et al*. (2012, CA 7.5/059) for 3 catchments in DK, Meyer *et al*. (2011, CA 7.5/065) for a single catchment in Luxembourg, Gregoire *et al*. (2010, CA 7.5/068) for a vineyard catchment in France, Peschka *et al*. (2006, CA 7.5/072) for the Rhine and two tributaries in Hesse DE and Augustin (2003, CA 7.5/073) for the river Selz DE.
- River studies, focussing on sources, are presented by Desmet *et al*. (2016, CA 7.5/044) for the river Meuse NL, Daouk *et al*. (2013a, CA 7.5/053) for the river Lutrive and Lake Geneva Switzerland, Houtman *et al*. (2013, CA 7.5/054) for the river Meuse NL and Litz *et al*. (2011, CA 7.5/063) for the river Havel Berlin DE.
- Urban dominated catchment concentrations are reported by Botta *et al*. (2012, CA 7.5/057), Hanke *et al*. (2010, CA 7.5/069) and Botta *et al*. (2009, CA 7.5/070).
- A river study focussing on river bank filtration, but that reports raw surface water concentrations, is presented by Bruchet *et al*. (2011, CA 7.5/027).

- Several studies, including Maillard and Imfeld (2014, CA 7.5/051), Imfeld *et al.* (2013, CA 7.5/055), Coupe *et al.* (2012, CA 7.5/058) and Maillard *et al.* (2011, CA 7.5/064), detail the attenuation of runoff carrying pesticides by a constructed wetland. The wetland is located at the outlet of a small vineyard dominated catchment with no permanent waterbody and as such essentially details surface runoff responses from large rainfall events in vineyards.
- Surface runoff concentrations prior to entry into surface water bodies are reported by Lefrancq *et al*. (2017, CA 7.5/040), Lerch *et al*. (2017, CA 7.5/041), Larsbo *et al*. (2016, CA 7.5/045), Napoli *et al*. (2016, CA 7.5/005) and Daouk *et al*. (2013b, CA 7.5/007). These are presented for completeness and context.
- Drainflow concentrations prior to entry into surface water bodies are reported by Dairon *et al*. (2017, CA 7.5/039) and Norgaard *et al*. (2014, CA 7.5/021). These are presented for completeness and context.
- Urban runoff concentrations prior to entry into surface water bodies are reported by Tang *et al*. (2015, CA 7.5/049), Gasperi *et al*. (2014, CA 7.5/050), Ramwell *et al*. (2014, CA 7.5/052), Vialle *et al*. (2013, CA 7.5/056), Zgheib *et al*. (2012, CA 7.5/060), Birch *et al*. (2011, CA 7.5/061) and Lamprea and Ruban (2011, CA 7.5/062). These are presented for completeness and context.

A summary of maximum concentrations of glyphosate (GLY) and AMPA in surface water reported by these studies is presented in Table 7.5-88 while the maximum reported rates of exceedance of various thresholds by these datasets are summarised in Table 7.5-89. Studies that did not assess or report values are not reported in these tables.

Glyphosate
Several maximum measured concentrations of GLY, greater than the regulatory acceptable concentration (RAC), up to 91 600 µg/L are reported, however, these extreme values are likely erroneous as they would be difficult to generate from GLY containing products in real world water bodies short of a major pollution incident having occurred and gone unreported. [redacted] (2019, CA 7.5/031) present evidence of point source pollution concentrations up to 153 µg/L for a small catchment in Belgium. Several studies indicate that concentrations in raw runoff prior to entering a surface water body and undergoing dilution approach the RAC, for example [redacted] (2017, CA 7.5/040) report up to 387 µg/L in surface runoff from a steep vineyard and Zgheib *et al*. (2012, CA 7.5/060) report up to 232 µg/L in urban storm runoff. [redacted] (2020, CA 7.5/002) identified 58 outliers in their dataset, including the maximum value of 91 600 µg/L, which when removed refined the maximum value to 57.0 µg/L which is well below the RAC of 400 µg/L.

Several of the applicant studies provide statistical summaries of the GLY concentration datasets investigated and these shed additional light on the few extreme values that influence the maximum values in these datasets: [redacted] (2020, CA 7.5/002) calculate that the RAC of 400 µg/L represents the 99.987th percentile GLY concentration in their dataset, including these extreme/anomalous values. They also determined that the 99th percentile concentration for their European dataset was 2.3 µg/L while [redacted] (2019a, CA 7.5/008) calculate the 99th percentile for their French dataset as 2.1 µg/L. Surface water GLY concentrations below 5-10 µg/L are consistent with the broader literature values for catchment studies, including during runoff events e.g. Reoyo-Prats *et al*. (2017, CA 7.5/043).

Assessment of rates of exceedance of thresholds requires the dataset to be large enough to capture a range of agronomic, geographical, pedoclimatic and hydrological situations. The dataset analysed by [redacted] (2020, CA 7.5/002) best meets this criterium and in addition is the only study that considers compliance with the RAC. Similarly, [redacted] (2020, CA 7.5/002) and Stenrød (2015, CA 7.5/047) are the only publications that consider compliance with Environmental Quality Standards (EQS) for countries where such a threshold has been defined or proposed.

Nevertheless, [redacted] (2020, CA 7.5/002) report that compliance with the GLY RAC of 400 µg/L for their European dataset (including outlier values) was extremely high (99.994 % of samples; 99.90 % of sites). The compliance rate would be 100 % if the outliers were excluded. They report that these results are consistent with reported national assessments against predicted no effect concentrations of 60 µg/L in

France and 100 µg/L in Germany. Their results are also in line with Di Guardo and Finizio (2018, CA 7.5/036) who report 100 % compliance against a PNEC of 112 µg/L for sites in Lombardy, Italy. [redacted] (2020, CA 7.5/002) also demonstrated that compliance for GLY with EQS values for a number of countries where an EQS was defined was at least 99.987 % which they found was in line with other studies from BE and FR. Their results are also in line with that of Stenrød (2015, CA 7.5/047) who reports 100 % compliance with an EQS-MAC of 28 µg/L for 6 catchments in Norway.

AMPA

Several maximum measured concentrations of AMPA, greater than the regulatory acceptable concentration (RAC) of 1200 µg/L, up to 230 000 µg/L are reported, however, these extreme values are likely erroneous or derived from other sources, like detergents emitted from waste water treatment plants. Several studies present evidence of such urban sources e.g. Desmet *et al*. (2016, CA 7.5/044) report concentrations of up to 130 µg/L for the river Ur, which is dominated by emissions from a waste water treatment plant while [redacted] (2019, CA 7.5/031) report 264 µg/L in sewage being emitted into the Cicindria catchment. In addition, others also indicate that concentrations in raw runoff from fields prior to entering a surface water body and undergoing dilution are below the RAC, for example Napoli *et al*. (2016, CA 7.5/005) report up to 151.9 µg/L in surface runoff from a steep vineyard. [redacted] (2020, CA 7.5/002) identified 3 outliers in their European dataset, including the maximum value of 230 000 µg/L, which when removed refined the maximum value to 224.4 µg/L which is well below the RAC.

Several of the applicant studies provide statistical summaries of the AMPA concentration datasets investigated and these shed additional light on the few extreme values that influence the maximum values in these datasets: [redacted] (2020, CA 7.5/002) calculate that the RAC of 1200 µg/L represents the 99.999th percentile GLY concentration in their dataset, including these extreme/anomalous values. They also determined that the 99th percentile concentration for their European dataset was 5.81 µg/L while [redacted] (2019a, CA 7.5/008) calculate the 99th percentile for their French dataset as 4.2 µg/L. Surface water AMPA concentrations below 5-10 µg/L are consistent with the broader literature values for catchment studies, including during runoff events in urban dominated catchments e.g. Hanke *et al*. (2010, CA 7.5/069) and Botta *et al*. (2009, CA 7.5/070).

Nevertheless, [redacted] (2020, CA 7.5/002) report that compliance with the AMPA RAC of 1200 µg/L for their European dataset (including extreme values) was near complete (99.999 % of samples; 99.976 % of sites). The compliance rate would be 100 % if the outliers were excluded. They also demonstrated that compliance for AMPA with EQS values for a number of countries was 100 % which they found was in line with other studies from BE and FR.

Conclusions

[redacted] (2019, CA 7.5/031) highlight the difficulty of interpreting monitoring data in the absence of a detailed understanding of the monitoring location and practices in the upstream catchment, identifying several point source pollution events during their monitoring campaign as well as raw sewage and industrial emissions in their catchment. The literature highlights that some uses in some situations, for example use on urban hard surfaces or vines on extreme slopes, may present a risk of elevated losses to surface water bodies that may need to be managed. However, the several papers focussing on a constructed wetland in FR targeting vineyard runoff highlight that the best way of managing local risk is through gaining a better understanding of the issue and designing and implementing local solutions, like a constructed wetland. Similarly, [redacted] (2019, CA 7.5/031) evidence a reduction in the number and strength of peak concentrations through farmer engagement and awareness raising.

The data presented in this section demonstrate that the environmental concentrations typically encountered in this environmental compartment likely associated with typical agricultural and urban usage do not pose a risk for ecosystems. Safe use with respect to surface water is demonstrated for the vast majority of use environments in Europe.

Table 7.5-88: Summary of reported maximum concentrations of glyphosate (GLY) and AMPA in surface water

Reference	Context	Maximum Concentration (µg/L)	
		GLY	AMPA
[redacted] 2020, CA 7.5/002	EU Summary	91600 57.0[1]	230000 224.4[1]
[redacted] 2019, CA 7.5/031	BE Catchment Study	153.0[7]	218.0[7]
[redacted] 2019a, CA 7.5/008	FR Summary	2237 2.1[4]	3369 4.2[4]
[redacted] 2019b, CA 7.5/032	FR Summary – SW associated with vineyards	21	106
[redacted] 2018a, CA 7.5/033 and 2018b, CA 7.5/034	FR Summary – Site investigations	13.1	106
[redacted] 2016, CA 7.5/010	EU Summary	393	3400
[redacted] 2016, CA 7.5/009	FR Summary	3257 2.8[4]	3369 4.6[4]
[redacted] 2012, CA 7.5/013	EU Summary	370	>200
Di Guardo and Finizio, 2018, CA 7.5/036	Italy case study Lombardy	108	NA
Huntscha, S. *et al.* 2018, CA 7.5/037	CH Lake case study - Tributaries	1.43	0.42
	CH Lake case study - Lake	0.15	0.10
Masiol, M. *et al.* 2018, CA 7.5/038	North East Italy – SW	1.4	1.4
	North East Italy – TW (Venice lagoon)?	2.1	1.4
Dairon, R. *et al*. 2017, CA 7.5/039	FR Raw drainflow before entering SW	12	NR
Lefrancq, M. *et al.* 2017, CA 7.5/040	FR Vineyard surface runoff before entering SW	386.9	47.0
Lerch, R.N. *et al*., 2017, CA 7.5/041	Runoff buffer study - field surface runoff study before entering SW	Expressed as input normalised loads	NA
Poiger, T. *et al.*, 2017, CA 7.5/017	Various regions of CH e.g. Zurich and Vaud	<50[2] 2.1[5]	<10[2] 2.6[5]
Reoyo-Prats, B. *et al*., 2017, CA 7.5/043	River Tet FR – rainfall event streamflows	1.7	1.1
Desmet, N. *et al.*, 2016, CA 7.5/044	NL Meuse river modelling – validation data	12.0	130.0[8]
Larsbo, M. *et al*., 2016, CA 7.5/045	SE Field runoff before entering a SW	7.4[6] 2.7[9]	2.7[6] 0.85[9]
Napoli, M. *et al.* 2016, CA 7.5/005	IT Field runoff before entering SW	128.9	151.9
Stenrød, M., 2015, CA 7.5/047	NO catchment study 6	4.0	NA
Székács, A., *et al*., 2015, CA 7.5/048	HU site investigations	1.0	NA
Tang, T. *et al.*, 2015, CA 7.5/049	BE Urban runoff before to entry into SW	6.1	5.8
Gasperi, J. *et al*., 2014, CA 7.5/050	FR Urban stormwater before entry SW	0.2[3]	0.47[3]
Maillard E., Imfeld G., 2014, CA 7.5/051	FR Constructed wetland for vineyard catchment	Expressed as loads	Expressed as loads
Norgaard, T. *et al*., 2014, CA 7.5/021	Danish PLAP - Drainflow before entry into SW	31.0	~1.6[2]
Ramwell, C. *et al*., 2014, CA 7.5/052	UK Urban runoff – before entering SW	8.99	1.15

Table 7.5-88: Summary of reported maximum concentrations of glyphosate (GLY) and AMPA in surface water

Reference	Context	Maximum Concentration (µg/L)	
		GLY	AMPA
Székács, A., *et al*., 2014, CA 7.5/006	HU site investigations	0.98	NA
Daouk, S. *et al.*, 2013a, CA 7.5/053	CH SW site associated with vineyards - River	0.80	0.30
	CH SW site associated with vineyards - Lake	<LOQ (10 ng/L)	0.067
Daouk, S. *et al.*, 2013b, CA 7.5/007	CH vines raw surface runoff before entry into SW	110.0	14.0
Houtman, C. *et al.*, 2013, CA 7.5/054	NL River Meuse	0.21	2.28
Imfeld G. *et al*., 2013, CA 7.5/055	FR Constructed wetland for vineyard catchment	150	19.0
Vialle, C. *et al*., 2013, CA 7.5/056	FR Roof runoff prior to entering SW	6.0	0.9
Botta F. *et al*., 2012, CA 7.5/057	FR Urban dominated catchment	NR	5.1
Coupe, R. *et al.*, 2012, CA 7.5/058	FR Constructed wetland vineyard catchment	86	44
Petersen, J. *et al*., 2012, CA 7.5/059	Runoff event sampling in 3 DK catchments	2.80	0.54
Zgheib, S. *et al*., 2012, CA 7.5/060	FR Urban storm water Paris, before entering SW	232	9.37
Birch H. *et al*., 2011, CA 7.5/061	DK Urban stormwater runoff, before entering SW	1.3	1.3
Bruchet, A. *et al*. 2011, CA 7.5/027	Bank filtration – River water	0.12	0.65
Lamprea, K., Ruban, V., 2011, CA 7.5/062	FR Urban storm wastewater, before entry in SW	71.0	1.45
Litz, N.T. *et al.*, 2011, CA 7.5/063	River Havel, Berlin, DE	5.0	NR
Maillard, E. *et al*., 2011, CA 7.5/064	FR Constructed wetland vineyard catchment	15.0	21.0
Meyer, B. *et al.*, 2011, CA 7.5/065	Small catchment Luxembourg, runoff events	6.22	1.12
Busetto, M. *et al*., 2010, CA 7.5/066	Brianza region, Lombardy IT	2.20	16.0
Gregoire, C. *et al*., 2010, CA 7.5/068	Vineyard catchment, FR, Runoff events	86.0	44.0
Hanke I. *et al*., 2010, CA 7.5/069	Urban dominated catchments, CH	4.2	1.11
Botta, F. *et al*., 2009, CA 7.5/070	Urban dominated catchments, Paris, FR	1.7	1.93
Peschka, M. *et al*., 2006, CA 7.5/072	Rhine and 2 tributaries, Hesse, DE	0.4	NR
Augustin, B., 2003, CA 7.5/073	Selz river, DE	1.8	NA

‡ - Maximum annual value of 7 years
[1] - Excluding outliers
[2] - Inferred from graph
[3] – 80th Percentile
[4] - 99th Percentile
[5] – 95th percentile value
[6] – Aqueous phase
NR – Not reported;
[7] - Point source pollution
[8] – Highly influenced by waste water emission
[9] – Sediment phase
NA – Not applicable/analysed

Table 7.5-89: Summary of reported rates of concentrations of various thresholds for measured concentrations of glyphosate (GLY) and AMPA in surface water

Reference	Context	Exceedance threshold and rates		
		Threshold (µg/L)	GLY (%)	AMPA (%)
[redacted] 2020, CA 7.5/002	EU Summary	400	0.006	NA
		1200	NA	0.001
		0.1	23.0	47.5
[redacted] 2019a, CA 7.5/008	FR Summary	0.1	21.7[1]	53.6[1]
		2	0.4[1]	2.5[1]
[redacted] 2019b, CA 7.5/032	FR Summary – SW associated with vineyards	0.1	39[1]	51[1]
		2	2[1]	3[1]
[redacted] CA 7.5/010	EU Summary	0.1	21.0	41.0
[redacted] 2016, CA 7.5/009	FR Summary	0.1	76.5[3] 36.8[4]	58.3[2]
		2	3.9[3]	5[2]
[redacted] 2012, CA 7.5/013	EU Summary	0.1	≥23	≥46
Di Guardo and Finizio, 2018, CA 7.5/036	Italy case study Lombardy	0.1	78.7[5]	NA
		112	0.0[5]	NA
Mottes, C. *et al.*, 2017, CA 7.5/042	Martinique 121ha tropical volcanic soils	0.1	6.4[6]	21.3[6]
Stenrød, M., 2015, CA 7.5/047	NO study 6 catchments	28[7]	0.0	NA
Martin, J. *et al*., 2013, CA 7.5/022	Reunion island (GW + SW)	0.1	0.85	0.76

[1] Maximum annual value of 7 years
[2] Maximum annual value of 15 years
[3] Maximum annual value of 17 years
[4] Excluding small sample sizes of <1000 samples
[5] % of sites where annual 95th percentile exceeds PNEC of 112 µg/L or threshold of 0.1 µg/L
[6] Concentrations of weekly flow proportional samples spanning 11/10/2011 to 01/02/2013
[7] EQS-MAC
NR – Not reported;
NA – Not applicable/analysed

Applicant studies

New studies/assessments

1. Information on the study

Data point:	CA 7.5/001
Report author	[redacted]
Report year	2020
Report title	Collection of public monitoring data for European countries for the compartments soil, water, sediment and air for Glyphosate, AMPA and HMPA
Document No	110057-1
Guidelines followed in study	Methodology is based on the Groundwater Monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations') Minimum quality criteria of monitoring data described by the FOCUS Ground Water Work Group chapter 9.5 (European Commission, 2014)
Deviations from current test guideline	None

Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The report provides information about the outcome of a search for readily accessible and available monitoring data in European countries at a regional/national level for the time period 1995-2019. The main focus was on the time period 2012-2019 while earlier years are already covered by existing data. The search included raw data, requested from regional/national authorities or downloadable from their websites, as well as aggregated data extracted from reports compiled by authorities.

Data from 14 European countries were considered: Austria, Belgium, Denmark, France, Germany, Hungary, Ireland, Italy, The Netherlands, Poland, Romania, Spain, Sweden and the United Kingdom. The countries represent the major markets of products containing glyphosate sold in the EU. The data compilation included the active substance glyphosate and its metabolites AMPA and HMPA, in the soil, groundwater, surface water, tidal water, drinking water, sediment and air environmental compartments.

As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities and other bodies of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were not actually included in any of the monitoring programs.

Surface Water Compartment Conclusion

A large surface water public monitoring dataset was compiled, comprising raw datasets from 10 countries (AT, BE, DK, ES, FR, IE, IT, NL, SE and UK) and aggregated datasets from published reports for 8 countries (AT, DE, DK, ES, HU, IE, IT and NL). Collectively these cover a wide range of pedoclimatic and hydrological settings typically spanning more than a decade.

I. MATERIALS AND METHODS

The general methodology of data collection of public monitoring data and minimum quality criteria is based on existing guideline documents for groundwater monitoring programs. The underlying principles have been applied to all environmental compartments, especially where no specific guidance is at hand. Data search, acquisition and processing approaches are described below. The same approach was applied for each country, compartment and substance. Country specific adaptations to the general procedure were made in order to generate a harmonized database. The data collected for this report refers to third party organization data regarding all environmental compartments (SOIL, GW, SW, TD, DW, SD, AIR) and was further differentiated into the two different data types, i.e. raw data and aggregated data. Aggregated data refers to information provided in publicly available reports, e.g. from environmental agencies or research institutes. Such reports might hold only summary information on substance findings over space and time and may intersect with the raw data. Raw data refers to mid to long term time series of data that are provided on request by e-mail or by database from governmental authorities and are therefore recognized as official monitoring data. These datasets hold the information of sampling values, quality information (sampling, treatment, limit of detection - LOD, limit of quantification - LOQ) as well as information of location and time of sampling.

The following data source types were investigated in order to collect monitoring data:

• E-mail requests: a general e-mail was sent to the national responsible authorities with regard to the required information.

• Governmental webpages: the official webpages of the national responsible authorities were searched for information regarding available reports and datasets.

• Public online databases: available data from online databases were downloaded as provided by the webpages of governmental authorities and other institutions.

The data search resulted in a very heterogeneous collection of tabular data and reports in different formats and structure. Data were processed into a harmonized tabular format by selecting relevant information and adapting data organisation. In general, the complete datasets were included in the final harmonized database as provided by the authorities, but obvious duplicates were deleted. In general, all entries for the digital database were checked for consistency and plausibility. For the raw data it was assumed that information was already subjected to critical scrutiny by the respective organization. For the aggregated data the same assumption was made with quality assurance of the data (mostly summaries) being the responsibility of the authors of the respective reports.

II. RESULTS AND DISCUSSION

The final data collection of raw data and aggregated data is summarised for each compartment and each country in Table 7.5-90.

Surface water

- Austria (AT)
  - Raw monitoring data from national authorities for surface water were downloaded from the H2OFachdatenbank.
  - Aggregated monitoring data from reports published by national authorities for surface water were downloaded from several sources.

- Belgium (BE)
  - Raw monitoring data for surface water for both Flanders and Wallonia compiled by the Belgian association for the plant protection products industry were received *via* e-mail.
  - An additional dataset by the Flemish EPA was received for surface water in Flanders.
  - No aggregated monitoring data from reports published by national authorities were considered in case of the compartment surface water, because of the good data availability by raw data.

- Germany (DE)
  - Raw monitoring data from national authorities for surface water were provided by the regional authorities of Brandenburg, Bavaria, Bremen, Mecklenburg-Vorpommern, North Rhine – Westphalia, Rhineland-Palatinate, Schleswig-Holstein, Saxony-Anhalt, Hesse, the state of Baden-Württemberg, and the state of Saxony.
  - Additionally, data were received for the large river systems Elbe and Rhine.
  - The regional authority in Mecklenburg-Vorpommern also provided data on tide waters.
  - Aggregated monitoring data from reports published by national authorities for surface water were downloaded from the German EPA, the LAWA, the environmental authority of Mecklenburg-Vorpommern, and the states of Rhineland-Palatinate, Schleswig-Holstein and Thuringia.

- Denmark (DK)
  - No raw monitoring data from national authorities for surface water in Denmark were identified.
    - Aggregated monitoring data from reports published by national authorities for surface water were downloaded from the National Center for Environment and Energy (DCE).

- Spain (ES)

- Raw monitoring data from national authorities for surface water were provided from the Ministry of Agriculture, Fisheries and Food after contacting the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) *via* e-mail.
- Aggregated monitoring data from reports published by national authorities refers to two reports from Madrid Polytechnical University and a non-governmental organization (Ecologistas en Acción).

- Europe (EU)
  - Raw monitoring data from on the European level were found from the International Commission for the Protection of the Rhine and Danube River.
  - Aggregated monitoring data from reports were found from RIWA-Maas and the World Health Organization as well as from the International Commissions for the Protection of the Rhine.

- France (FR)
  - In France, monitoring data for surface water are published by the Public Water Information Service (eaufrance). Raw monitoring data from national authorities for surface water were downloaded from NAIADES.
  - No aggregated monitoring data from reports published by national authorities were considered, because of the very good data cover by the raw monitoring data.

- Hungary (HU)
  - Raw monitoring data from national authorities for surface water were not available.
  - Aggregated monitoring data from reports published by national authorities for surface water were obtained in form of a peer-reviewed paper of the National Agricultural Research and Innovation Centre published in Journal of Chemistry.

- Ireland (IE)
  - Raw monitoring data from national authorities for surface water were provided by the Irish EPA *via* e-mail.
  - Aggregated monitoring data from reports published by national authorities for surface water were downloaded from the Irish EPA.

- Italy (IT)
  - Raw monitoring data from national authorities for surface water were downloaded from the regional environment agencies (ARPA) of the regions of Lombardia, Toscana, Veneto and Umbria.
  - The provincial environmental agency (APPA) of the province of Trento and the regional environment agencies of the regions of Emilia-Romagna, Marche, Venetia and the region of Pimento provide raw data for measurements in surface water, but no explicit data on glyphosate.
  - Aggregated monitoring data from reports published by national authorities for surface water were downloaded from ISPRA.

- The Netherlands (NL)
  - Raw monitoring data from national authorities for surface water were downloaded from the Water Dutch Quality Portal. Raw monitoring data for surface water in the Netherlands were also provided by RIWA Rhine *via* e-mail.
  - Aggregated monitoring data from reports published by national authorities for surface water were downloaded from RIVM and VROM. Aggregated monitoring date from reports for surface water were also provided by and downloaded from the Association of River Waterworks RIWA.

- Poland (PL)
  - The responsible authorities for monitoring data in Poland are the Polish Geological Institute and the Chief Inspectorate Of Environmental Protection. The latter authority confirmed by e-mail that in Poland there is currently no public monitoring of glyphosate or its metabolites in surface water.

- Romania (RO)
  - The responsible authority for monitoring data is the Ministry of Water and Forests. The Water Resources Management Directorate confirmed on behalf of the Ministry of Water and Forests that no public monitoring of glyphosate or its metabolites is carried out in any water compartment in Romania.

- Sweden (SE)
  - Raw monitoring data from national authorities in Sweden for surface water were provided by SLU *via* e-mail. Additional raw monitoring data for surface water were directly downloaded from the SLU homepage. Moreover, SLU provided another database containing raw data for surface water issued from other sources than national monitoring, e.g. regional monitoring and private wells. This dataset was separately processed.
  - Aggregated monitoring data from reports published by national authorities for surface water were not identified. However, aggregated national monitoring data in tabular form for surface water were downloaded from the SLU homepage.

- United Kingdom (UK)
  - Raw monitoring data from national authorities for surface water were downloaded from the Environment Agency for England, and were provided *via* e-mail by the Scottish EPA for Scotland. For tide waters, data were available for England from UK's EPA webpage.
  - No aggregated monitoring data from reports were provided.

Table 7.5-90: Overview of public monitoring data availability of raw data (R) and aggregated data (A)

Country	Soil	Water				Sediment	Air
		Ground	Surface	Tidal	Drinking		
Austria	-	R, A	R, A	-	A	-	-
Belgium	-	R	R	-	A (Flanders)	-	-
Denmark	-	R, A	A	-	A	-	-
France	-	R	R	-	A	R	-
Germany	R (Brandenburg)	R, A	R, A	R	R (Schleswig-Holstein), A	-	-
Hungary	-	A (one research article)	A (one research article)	-	-	-	-
Ireland	-	R, A	R, A	-	R, A	-	-
Italy	-	R (Lombardia), A	R, A	-	-	-	-
The Netherlands	-	R, A	R, A	-	R	-	-
Poland	Confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Romania	Confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Spain	-	R, A	R, A	-	A	-	-
Sweden	-	R, A	R	-	R, A	R	-
UK England	-	R	R	R	A	-	-
UK Northern Ireland	-	R	-	-	-	-	-
UK Scotland	-	-	R	-	-	-	-

Table 7.5-90: Overview of public monitoring data availability of raw data (R) and aggregated data (A)

UK Wales	-	-	R	-	A	-	-

R: Raw data available; A: Aggregated data from reports available; -: No raw or aggregated data available

III. CONCLUSIONS

The collection of public monitoring data for glyphosate, AMPA and HMPA in soil, groundwater, surface water, drinking water, tide water, sediment and air resulted in a comprehensive database of 'raw monitoring data from national authorities' and 'aggregated monitoring data from reports published by national authorities'. As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were actually not included in any of the monitoring programs.

A large surface water public monitoring dataset was compiled, comprising raw datasets from 10 countries (AT, BE, DK, ES, FR, IE, IT, NL, SE and UK) and aggregated datasets from published reports for 8 countries (AT, DE, DK, ES, HU, IE, IT and NL). Collectively these cover a wide range of pedoclimatic and hydrological settings typically spanning more than a decade.

3. Assessment and conclusion

Assessment and conclusion by applicant: The study describes the collection process of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA. The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Document No	-
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al.*, 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The report provides information about the outcome of an analysis of public monitoring data comprising environmental concentrations of glyphosate (GLY) and its primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA) collated from readily available public monitoring databases held by national/regional environment agencies. This data collection and analysis was designed to expand previous reviews to include other compartments and supplement them for surface water, groundwater and drinking water. Public monitoring data from the following Member States (MS) were assessed for the water, sediment and soil compartments: Austria (AT), Belgium (BE), Denmark (DK), France (FR), Germany (DE), Ireland (IE), Italy (IT), Netherlands (NL), Spain (ES), Sweden (SE) and the United Kingdom (UK). Three MS, namely Poland (PL), Hungary (HU), and Romania (RO) confirmed that they do not conduct analyses for GLY, AMPA and HMPA in any environmental compartment. No data for HMPA was identified for any MS or compartment. Note that at the time the study was started the UK was a Member State and is referred to as a Member State throughout the report.

Analyses of the large spatial and temporal dataset of measured concentrations occurring in several environmental compartments, namely surface water, groundwater, drinking water, tidal water, sediment and soil, were conducted to assess their state. This analysis not only sought to assess the state of the environmental compartment but also to consider the potential impacts this might have on biota, ecosystems and human health by using regulatory endpoints and thresholds from a range of European (EU) Directives. These included the Water Framework Directive (Directive 2000/60/EC) and associated Groundwater (2006/118/EC), Drinking Water (1998/83/EC) and Priority Substances (2008/105/EC28) Directives in addition to the Plant Protection Products Directive (1107/2009/EC).

<u>Surface water</u>

Surface water (SW) data from AT, BE, DE, ES, FR, IT, SE, UK and two large transboundary catchments relating to the Rhine and Danube river basins were analysed for compliance with a range of regulatory endpoints and thresholds. The SW data were assessed against a RAC of 400 µg/L for GLY and 1200 µg/L

for AMPA. Additional analyses against MS specific annual average (AA) and Maximum Allowable Concentration (MAC) EQS values were also undertaken.

Glyphosate

The large GLY public monitoring dataset (>291 000 samples collected from >13 800 sampling sites) was dominated by data sourced from France (~65 %) with smaller contributions from Belgium (9 %), Germany (~8.5 %), the Netherlands (~5.6 %) and Spain (~4.9 %). Detection of GLY above the limit of quantification (>LOQ) in SW samples was ~40 % which compares well with the ~31 % of samples from the previous data collection, with the apparent increase likely a function of improving LOQs. Compliance with the GLY RAC of 400 µg/L was extremely high (99.994 % of samples; 99.90 % of sites) and the very occasional exceedances (0.006 % of samples; 0.10 % of sites) were largely on separate non-consecutive occasions (0.003 % of samples being consecutive). MS results for DE and FR are consistent with other published examples. A small number of high maximum concentrations in the dataset were confirmed to be outliers and once excluded indicated a maximum concentration of 57 µg/L, which is well below the RAC. Assessment of the spatial distribution of locations that exceed the GLY RAC did not indicate any specific pattern or bias. No EU-wide EQS values, annual average (AA) or maximum allowable concentration (MAC), were available for assessment as broader ecosystem endpoints. Consideration of the MS GLY surface water data against MS EQS values indicates that the presence of GLY is not expected to have any adverse impacts on ecosystems with a near total compliance (99.987 %) across the large EQS-MAC dataset (~228 000 samples from ~9 000 sites) with very few exceedances (0.013 % of samples; 0.22 % of sites) identified. Similarly, 100 % compliance for the large EQS-AA dataset (~11 000 years from ~1 600 sites) is indicated with no exceedances identified. These EQS results are consistent with national and regional published results for France, and Flanders in Belgium.

AMPA

The large AMPA public monitoring dataset (>269 000 samples collected from >12 400 sampling sites) was dominated by French data (~68.3 %) with smaller contributions from Belgium (~9.6 %), Germany (~9.0 %) and the Netherlands (~5.9 %). Detection of AMPA >LOQ in SW samples was ~64 % which compares well with the ~50 % of samples in the previous data collection, likely a function of improving LOQs. Compliance with the AMPA RAC of 1200 µg/L was very high (99.999 % of samples; 99.976 % of sites) with infrequent exceedances (0.001 % of samples from 0.024 % of sites) occurring on 3 separate non-consecutive occasions. MS results for FR are consistent with other published examples. A small number of high maximum concentrations were confirmed to be outliers and once excluded indicated a maximum concentration of 224.4 µg/L, which is well below the RAC. Assessment of the spatial distribution of locations of AMPA exceedance of the RAC did not indicate any specific pattern or bias. It should be borne in mind that AMPA may originate from sources other than GLY, for example detergents. No EU-wide EQS values, AA or MAC, were available for assessment as broader ecosystem endpoints. Consideration of the MS AMPA surface water data against MS EQS values indicates that the presence of AMPA, from GLY or other sources, is not expected to have any impacts with 100 % compliance for the large EQS-MAC (~218 000 samples from ~9 000 sites) and EQS-AA (~10 000 years from ~1 400 sites) datasets. The EQS results are consistent with national and regional published results for France, and Flanders in Belgium.

HMPA

No monitoring data were available for HMPA.

Understanding Sources of Exposure

In order to gain a better understanding of the sources and drivers of current residues in the environment further attention was paid to the surface water compartment given the richness of the dataset available and the fact that residues in this compartment may arise from several use environments, for example urban, railway and arable.

Regression tree models (RTM) were developed for a case study focusing on France to predict the number (total and consecutive) and rate of exceedance (%) of 0.1 µg/L in surface waters using predictor variables describing sources of GLY/AMPA and factors affecting emission and detection, for example the extent of different landcovers, GLY sales and extent of Urban Waste Water Treatment emissions in the catchment of the monitoring points. These RTMs indicate that urban areas and urban waste water treatment works

emissions were the most important drivers in the rate of exceedance as well as the number of exceedances, total and consecutive. They also demonstrated that arable (to a lesser extent) and permanent crops (to even smaller extent than arable) were important factors in GLY and AMPA detection.

Consideration of published relevant literature which explores the source apportionment of GLY and AMPA in aquatic environments reinforced the conclusions drawn from assessment of the public monitoring data. GLY and AMPA concentrations appeared to be generally larger from urban sources than from diffuse agricultural ones. With respect to urban sources, use on railways/roads seemed to result in the highest residues, while garden use resulted in lower residues in comparison to amenity use. In addition, from urban sources AMPA concentrations were often greater than glyphosate and likely to be derived from other compounds like detergents. Storm events often gave rise to large spikes in concentration in agricultural settings, and even more so where there was an urban contribution. If the sampling location was downstream of urban, or major infrastructure (rail or roads) then the GLY and AMPA residues were mostly likely not to have come from agricultural uses. These observations mirror that of the RPMS.

Surface Water Compartment Conclusion

No information on HMPA was available. Analysis of the large GLY and AMPA surface water datasets indicates they are both frequently detected above the LOQ, however compliance against regulatory endpoints and thresholds is extremely high with the frequency of exceedance being very low. The environmental concentrations typically encountered do not pose a risk for biota or ecosystems.

I. MATERIALS AND METHODS

An integral part of potentially understanding the patterns of exposure highlighted by the public monitoring data is where products containing GLY were used and the extent of usage. Assessment of usage of GLY and other sources of AMPA considered published data and summaries. The dataset analysed comprised individual surface water analysis records as well as existing aggregated analyses extracted from reports sourced from regional/national environment agencies (see [redacted] 2020, CA 7.5/001). The surface waterbodies captured by the dataset included streams, rivers, canals and lakes. They did not include transitional brackish water bodies which were included in a separate section. The approach taken for the data processing was precautionary in that it preserved samples in the analysis where there was any doubt regarding their reliability. As such the number of records excluded from the analysis were small ($n = 8\,672$), especially relative to the total number of samples ($n = 569\,400$) prior to removal. Similarly, no attempt to remove outliers prior to the analysis or calculation of statistics was undertaken despite the presence of extreme values being present in the datasets. In order to explore the extreme nature of some of the values included in the surface water dataset and assess the implications for this analysis, an outlier analysis was performed on the combined EU dataset. The development of Regression Tree models was also undertaken for the French surface water public monitoring dataset. These models sought to predict the exceedance rate (%) of the arbitrarily defined regulatory threshold of 0.1 µg/L as well as the number of exceedances through relating them to descriptors of the catchment upstream of each monitoring location.

Table 7.5-91: Summary of Environmental Quality Standards (EQS), average annual (AA) and maximum allowable concentration (MAC) utilised for the different Member States

Member State	GLY		AMPA	
	EQS-AA	EQS-MAC	EQS-AA	EQS-MAC
	µg/L			
AT - Austria	NA	NA	NA	NA
BE - Belgium (Wallonia)	28	70	450	45200
BE - Belgium (Flanders)	NA	64	NA	800
BE - Belgium (Combined)[1]	28	64	450	800
DK - Denmark	NA	NA	450	45200
EU - Transboundary (Danube/Rhine)	NA	NA	NA	NA

Table 7.5-91: Summary of Environmental Quality Standards (EQS), average annual (AA) and maximum allowable concentration (MAC) utilised for the different Member States

Member State	GLY		AMPA	
	EQS-AA	EQS-MAC	EQS-AA	EQS-MAC
	µg/L			
FR - France	28	70	452	45200
DE - Germany	28	NA	96	NA
IE - Ireland	60	NA	NA	NA
IT - Italy	NA	70[2]	NA	45200[2]
NL - Netherlands	77	NA	79.7	NA
ES- Spain	NA	NA	NA	NA
SE- Sweden	NA	100	NA	500
UK – United Kingdom	196[3]	398[3]	NA	NA
EU Combined Dataset	NA	NA	NA	NA

NA – Not available as not defined

[1] Lowest value taken from each federal region to define a worst case set of national values

[2] Italian law (Legislative Decree 172, 2015) stipulates that where an EQS value has not been set for an individual pesticide a value of 0.1 µg/L should be used for both parent and metabolites unless a scientific justification is presented for an alternate value. In this case alternate values adopted by France have been used as this is the only other southern Zone MS to have set such values

[3] These are 90th percentiles, applied as maximum values in the first instance in a precautionary approach.

Analysis and assessment of the data against thresholds was undertaken using the statistical software R (R Core Team, 2019) and graphs produced with the R package ggplot2 (Wickham, 2009). The average annual (AA) value for comparison against the MS EQS-AA (Environmental Quality Standard), where available, was calculated in accordance with Article 5 of Directive 2009/90/EC, namely technical specifications for chemical analysis and monitoring of water status, whereby values denoted as being below the limit of quantification (LOQ) are set to half the LOQ when calculating the arithmetic mean. This rule was also applied to the data denoted as being below the limit of detection (LOD). An additional rule was also applied whereby 12 values were required for any one year for this calculation. This was considered to be consistent with the monthly sampling intensities laid down in the Water Framework Directive (2000/60/EC) for priority substances (PS). For surface water the monitoring data was evaluated against the following thresholds and endpoints:

- Ecotoxicological endpoint: Regulatory Acceptable Concentration (RAC) of 400 µg/L for GLY and 1200 µg/L for AMPA.
- Ecosystem endpoint: Environmental quality standards (EQS) where these were proposed/available (see Table 7.5-91) at a Member State level, comprising annual average EQS (EQS-AA) and maximum allowable concentration (EQS-MAC). No values for these endpoints have been set at a European level.
- Evaluation threshold: The data were also evaluated against a threshold of 0.1 µg/L for comparative purposes with other published evaluations despite there being no regulatory requirement to present such data.

II. RESULTS AND DISCUSSION

Glyphosate Product Sales/Usage

The data presented in Figure 7.5-67 were derived from annual sales data submitted by registrants to the national authorities, in many instances reported under the Sustainable Use Directive (Directive 2009/128/EC). Sales/usage data (see Figure 7.5-67) indicated that annual amounts in recent years for some MS are very small e.g. DK where these range between ~300 and ~1 900 kg a.s.. For many MS these amounts were below 3 000 tonnes (see Figure 7.5-67b) while in others they were large e.g. ES where >10 000 tonnes were sold annually. The following trends in available sales/usage were evident for the data in the last 5 years:

- Increasing in ES, UK and the combined 11 MS for which monitoring data is available;
- Static or possibly decreasing in AT, BE, DK, FR, NL and SE
- Decreasing in DE
- No data for IE and IT

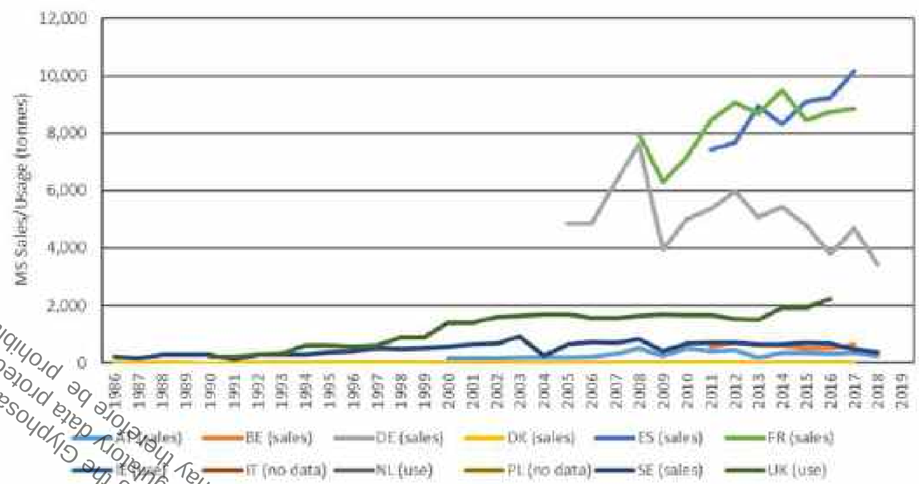
Little data on railway, urban/amenity/amateur usage was identified. Evaluation of the product sales in the FR BNVD dataset for 2017 suggests that approximately 14 % of sales were to the amateur/amenity sector (see Table 7.5-92).

Table 7.5-92: Summary of glyphosate sales volumes in France differentiated by key user groups (ANSES, 2019)

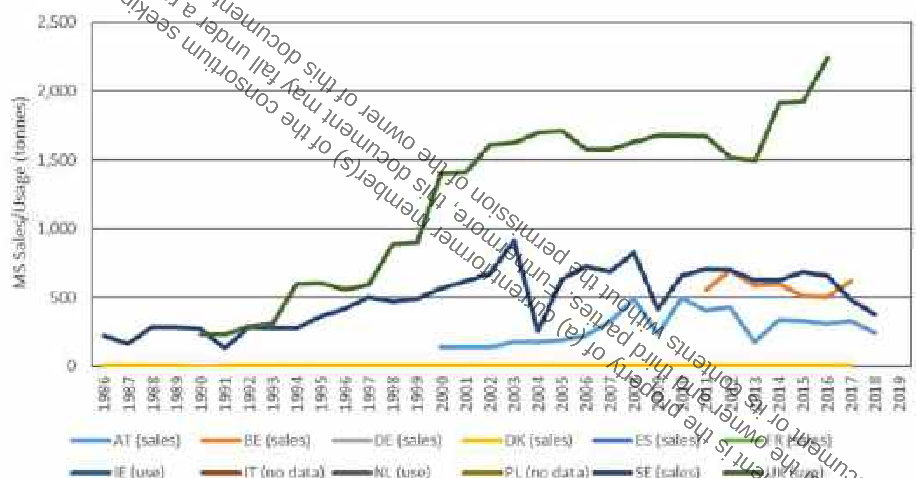
Year	Units	2009	2010	2011	2012	2013	2014	2015	2016	2017
Agricultural	tonnes	5157	5798	6731	7075	6616	7753	6930	7151	6951
	%	80.3	80.5	79.5	78.1	76.3	81.7	82.0	82.0	86.1
Amateur/amenity	tonnes	1264	1407	1739	1987	2057	1733	1522	1570	1125
	%	19.7	19.5	20.5	21.9	23.7	18.3	18.0	18.0	13.9
Total	tonnes	6421	7205	8470	9062	8673	9486	8452	8721	8076

Railway usage was documented for a number of MS for 2017 and indicated that applications of GLY may be as low as ~1 tonne (DK) up to ~60 tonnes (IT), typically representing only a few percent of total GLY sales.

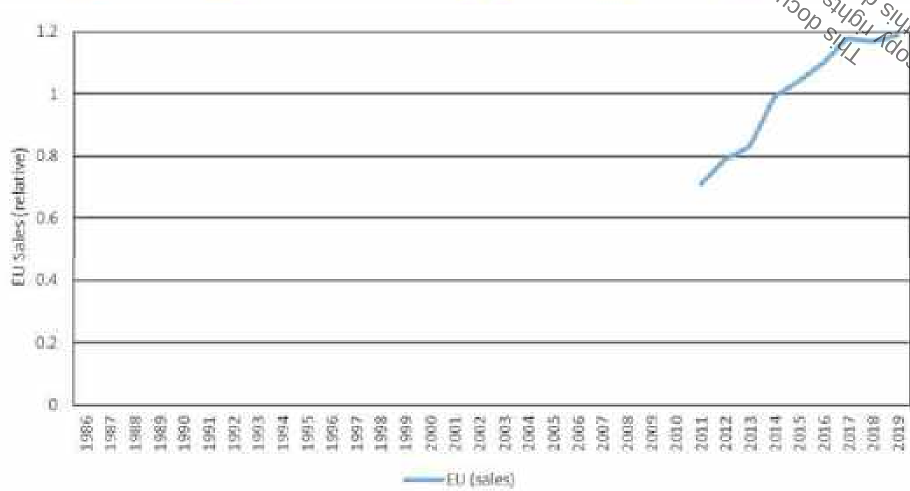
Figure 7.5-67: Illustration of glyphosate (GLY) sales/usage in Member States targeted by the monitoring for (a) all sales/usage tonnage (b) those with sales/usage below 2500 tonnes/annum and (c) all MS for which monitoring data was identified. The European sales figures are expressed relative to the average of those years and as such is a relative value for illustrative purposes only.



(a)



(b)



(c)

Other Sources of AMPA

It was emphasized that AMPA may be derived from other parent compounds used in both industrial and household applications, including detergents, fire retardants, anti-corrosives, anti-scaling agents and complexing agents in the textile industry. ECHA REACh registrations indicated that these compounds may be included in water softeners, polishes and waxes, washing and cleaning products, coating products, cosmetics, personal care products, water treatment products, textile treatment products, dyes, leather treatment products, paper chemicals, amongst others. The usage classes for such compounds (see Table 7.5-93) suggested that these compounds would contribute meaningfully to loads of AMPA in the environment, especially where specific industries, for example paper or textile, emit effluent.

Table 7.5-93: Summary of REACh registration tonnage and published estimates for key phosphonates that break down in the aquatic environment to form AMPA (*after* JRC, 2015b)

Parent Compound Name	Parent Compound ID	Main Use (in Europe)	EU Tonnage Class tonnes/annum[2]	Number AMPA molecules[1]
Amino tris(methylenephosphonate)	ATMP (CAS 6419-19-8)	Industrial boilers/cooling	10000-100000	1
Diethylenetriamine penta(methylenephosphonate)	DTPMP (CAS 15827-60-8)	Detergents	1000-10000	3
Ethylenediamine tetra(methylenephosphonate)	EDTMP (CAS 1429-50-1)	Laundry detergents	10-100	2
Hexamethylenediamine tetra(methylenephosphonate)	HDTMP (CAS 38820-59-6)	Industrial boilers/cooling	No current registration	2

[1] Number of AMPA molecules that can potentially be formed from one molecule of each compound.
[2] https://echa.europa.eu/, accessed March 2020.

The total volume of phosphonates used in Europe was found to not be well documented, but estimated to be in the range of 10,000-50,000 tons/year on an active acid basis, of which 12,000 tons of ATMP, HDTMP and DTPMP were used in household detergents and cleaning products (JRC, 2015b). AMPA is poorly removed in sewage treatment works and consequently household and industrial emissions containing detergents were considered likely to contain AMPA, leading the JRC to conclude that "the AMPA load from detergents should not be underestimated in surface water, if compared to the indirect contamination that could occur following the use of glyphosate" (JRC, 2015b).

<u>*Monitoring Data Assessment*</u>

Glyphosate

Temporally the GLY (see Figure 7.5-68) data indicated some bias at a MS level with fewer samples typically collected in the winter and spring months resulting in a unimodal distribution. In some MS, notably FR and SE, the data had a potentially bimodal distribution with data collection in spring and autumn, during key agricultural and hard surface usage periods, being greater than at other times of the year. The spatial distribution of GLY public monitoring locations for MS where data was collected was biased (see Figure 7.5-70). For some MS, e.g. DE, IT and ES, this was a function of data only arising from some provincial/regional environment agencies while for others, e.g. the UK, this was likely a function of spatial targeting. The input data collated for analysis of GLY residues in SW were dominated by data sourced from France (~65.4 %) with smaller contributions from Belgium (~9 %), Germany (~8.5 %), the Netherlands (~5.6 %) and Spain (~4.9 %).

Across all MS the GLY public monitoring dataset compiled comprised >291 000 samples collected from >13 800 sampling sites (see Table 7.5-94). Detection of GLY above the limit of quantification (LOQ) in SW was ~40 %, ranging from as low as 6.5 % in BE to as high as 67.5 % in SE, relative to a varying LOQ with an average of 0.15 µg/L (min: 0.01 – max: 1000 µg/L). These compared well with the previous data collection (Horth, 2012, CA 7.5/013 and 2016, CA 7.5/010) where ~31 % of samples were found to have detected GLY (see Table 7.5-98).

Compliance with the GLY RAC of 400 µg/L was extremely high (99.994 % of samples; 99.90 % of sites), ranging from 100 % (e.g. in AT) to 99.44 % (UK), with exceedances being extremely rare (16 samples from 23 sites; 0.006 % of samples from 0.10 % of sites). MS results for DE and FR were consistent with other published analyses, using predicted no effect concentration (PNEC) thresholds. When exceedances occurred, they occurred largely on separate non-consecutive occasions (0.003 % of samples; see Table 7.5-99). The spatial distribution of the GLY exceedance locations (see Figure 7.5-70) did not indicate any specific patterns or bias.

Maximum measured concentrations up to 91 600 µg/L were reported, however, these extreme values were considered likely erroneous as they would be difficult to generate from GLY containing products in real world water bodies short of a major pollution incident having occurred and gone unreported. The 99th percentile concentration (see Table 7.5-96), the concentration that 99 % of samples are below, was 2.3 µg/L and the RAC represents the 99.987th percentile value. In line with the precautionary data processing approach adopted in this study possible outliers were not removed from the dataset prior to analysis. However, an additional analysis step was conducted to identify likely outliers in the dataset and the implications of these for the analysis assessed. This identified 58 outliers which if excluded, suggest the maximum concentration would be 57.0 µg/L which is well below the RAC and as such 100 % compliance with the RAC would be expected (see Table 7.5-96).

No EU-wide EQS values, AA or MAC, were available for analysis of the combined EU dataset. Consideration of the MS GLY surface water data against available MS EQS-MAC (see Table 7.5-95) and EQS-AA (see Table 7.5-100) endpoints, indicated that the presence of GLY was not expected to have any impacts with near total compliance (99.987 % of samples) across the large EQS-MAC dataset (~228 000 samples from ~9 000 sites) with very few exceedances (0.013 % of samples; 0.22 % of sites) identified. In all cases the values exceeding the MAC were classed as likely outliers in the combined EU dataset. Similarly, 100 % compliance for the large EQS-AA dataset (~11 000 years from ~1 600 sites) was indicated with no exceedances identified. These results were considered to be consistent with national published results for Flanders in BE, and France, using regional/national EQS values.

In order to compare these detailed GLY results with published aggregated results, assessment against the arbitrarily defined regulatory threshold of 0.1 µg/L was also undertaken. Detection above the threshold of 0.1 µg/L was ~23 % of samples (~54.0 % of sites), ranging from 3.4 % in AT to 57.5 % in BE. These results compared well with the aggregated values extracted from reports (see Table 7.5-97) which ranged from 0.2 % in AT to 22.9 % of samples in DE. Similarly, these results compared well with the previous data collection where ~21 % of samples were found to exceed 0.1 µg/L.

Annual and monthly investigations of sampling effort and compliance were also documented within the report. These have not been summarised as they do not alter the conclusions of the primary study, instead providing additional detail should this be required.

Figure 7.5-68: Bar chart of surface water monthly glyphosate (GLY) sampling effort

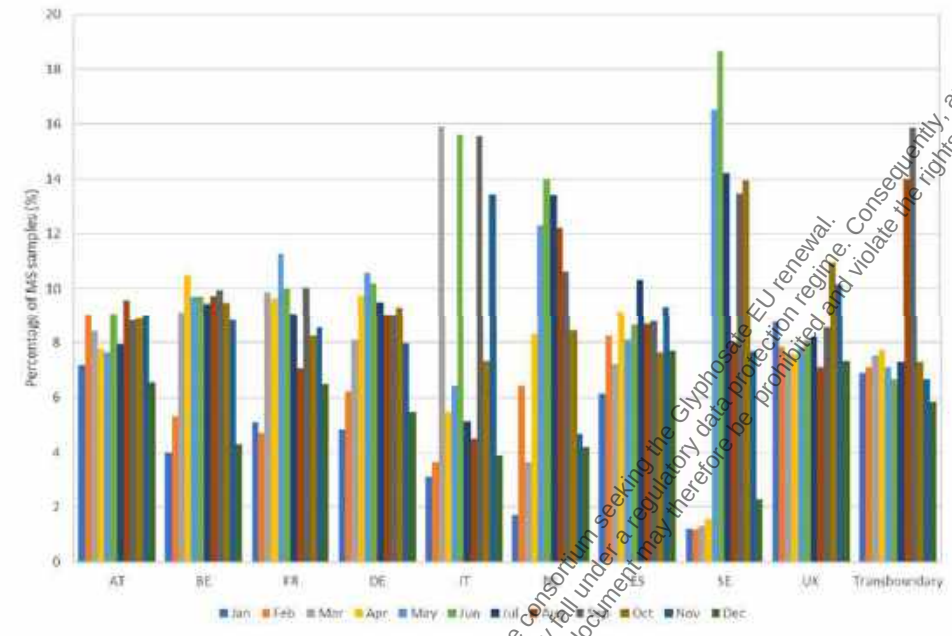


Figure 7.5-69: Bar chart of surface water monthly AMPA sampling effort

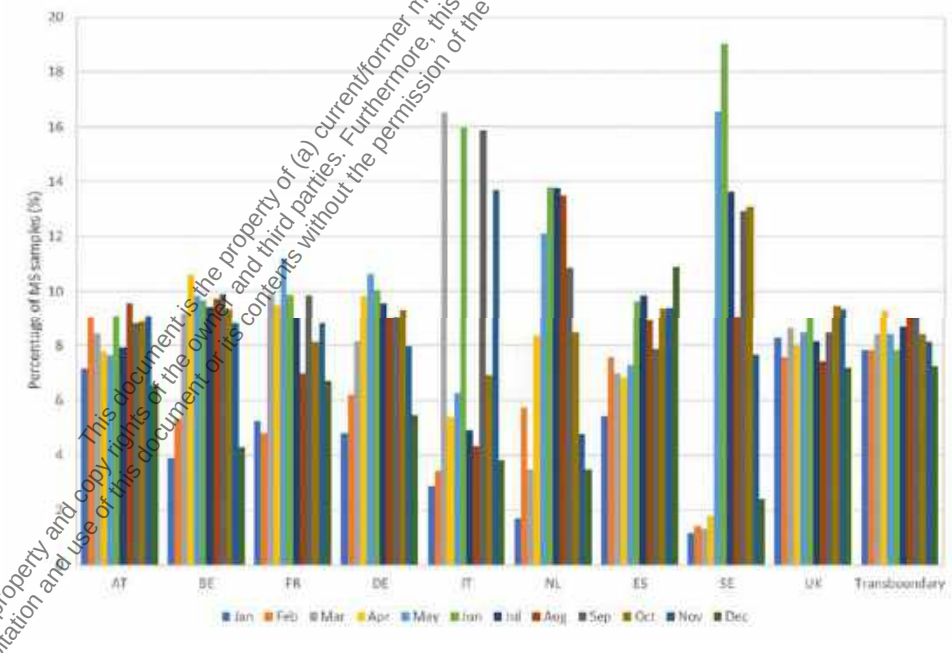


Figure 7.5-70: **Map illustrating the distribution of glyphosate (GLY) surface water (SW) sampling locations. Also illustrated are the number of exceedances of the SW regulatory acceptable concentration (RAC) at each location.**

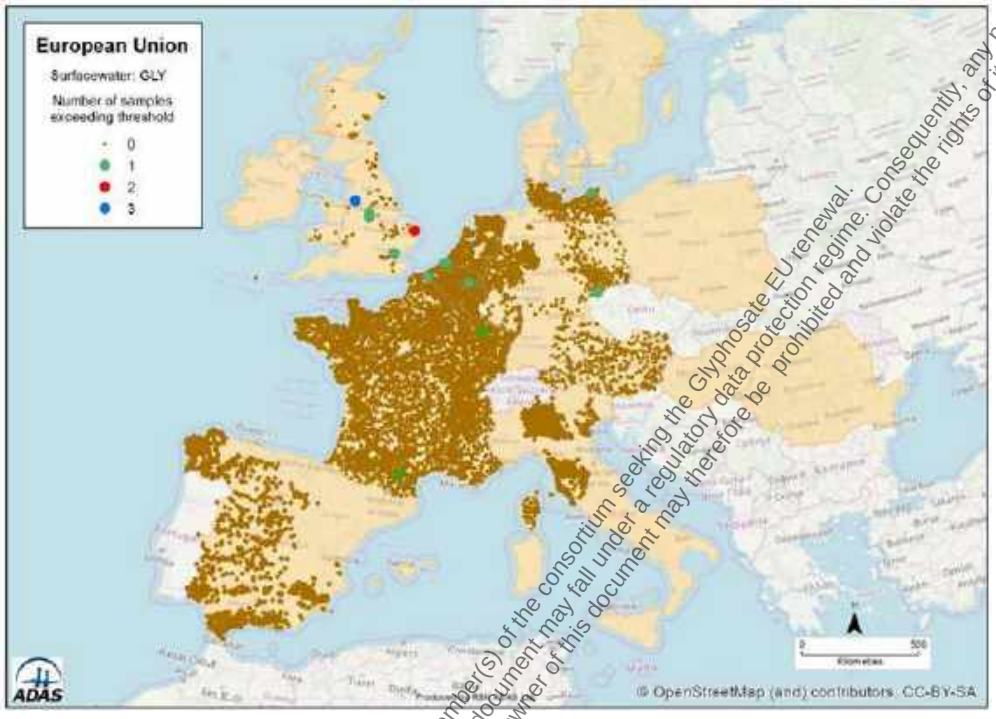
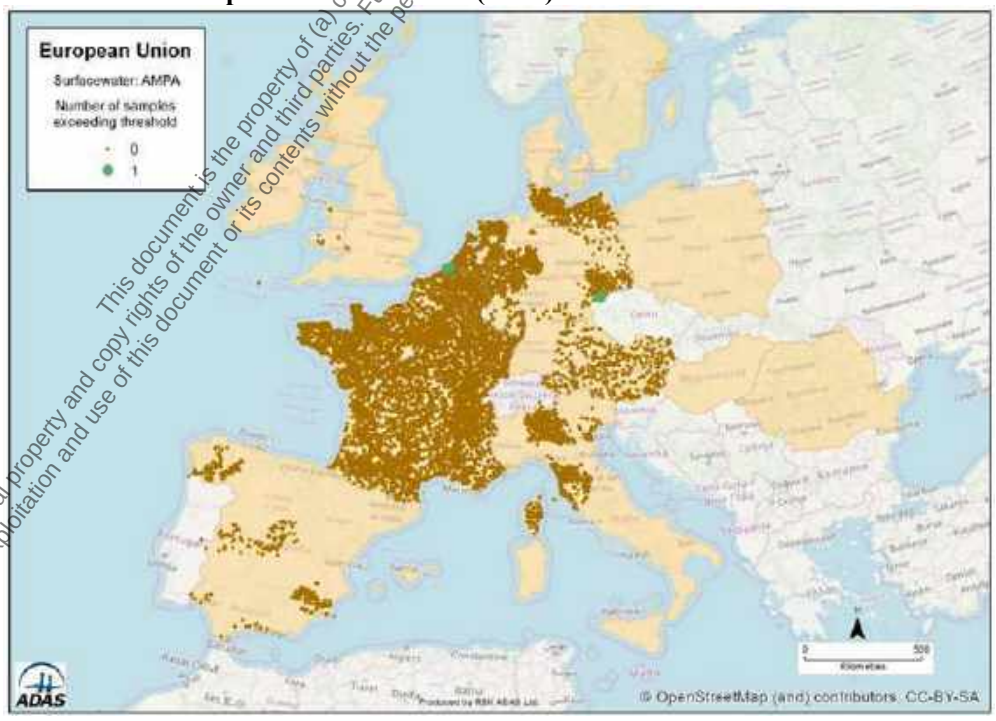


Figure 7.5-71: **Map illustrating the distribution of AMPA surface water (SW) sampling locations. Also illustrated are the number of exceedances of the SW regulatory acceptable concentration (RAC) at each location.**



AMPA

Temporally the AMPA (see Figure 7.5-69) data indicated some bias at a MS level with fewer samples typically collected in the winter and spring months resulting in a unimodal distribution. In some MS, notably FR and SE, the data had a potentially bimodal distribution with data collection in spring and autumn, during key agricultural and hard surface usage periods, being greater than at other times of the year. The spatial distribution of AMPA public monitoring locations for MS where data was collected was biased (see Figure 7.5-71). For some MS, e.g. DE, IT and ES, this was a function of data only arising from some provincial/regional environment agencies while for others this was likely a function of spatial targeting. The French data dominated the combined dataset (~68.3 %) with smaller contributions from Belgium (~9.6 %), Germany (~9.0 %) and the Netherlands (~5.9 %).

Across all MS the AMPA public monitoring dataset compiled comprised >269 000 samples collected from >12 400 sampling sites (see Table 7.5-94). Detection of AMPA >LOQ in all SW was ~64 %, ranging at a MS level from as low as ~24.1 % in AT to as high as ~87.7 % in BE, relative to a varying LOQ with an average of 0.07 µg/L (min: 0.01 – max: 10 µg/L). These results were similar to the previous data collection where ~50 % of samples were found to detect AMPA (see Table 7.5-98).

Compliance with the AMPA RAC of 1200 µg/L was very high (99.999 % of samples; 99.976 % of sites), ranging at a MS level from 100 % (e.g. in AT) to 99.98 % (NL), with infrequent exceedances (3 samples from 3 sites; 0.001 % of samples from 0.024 % of sites) occurring on 3 separate non-consecutive occasions (see Table 7.5-99). This observation was consistent with a published analysis using a PNEC threshold for France. The spatial distribution of the AMPA exceedance locations (see Figure 7.5-71) did not indicate any specific patterns or bias. It was highlighted that AMPA may originate from sources other than GLY, for example detergents.

Maximum measured concentrations up to 230 000 µg/L were reported, however, these extreme values were considered to likely be anomalous. The 99th percentile concentration, the concentration that 99 % of samples are below, was 5.81 µg/L (see Table 7.5-96) while the RAC was the 99.999th percentile concentration. An additional analysis step was conducted to identify likely outliers in the dataset and the implications assessed. This identified 3 outliers in the combined EU dataset which if excluded, indicated the maximum concentration would be 224.4 µg/L which is well below the RAC and as such 100 % compliance with the RAC would be expected (see Table 7.5-96).

No EQS values, AA or MAC, were available for assessment of the combined EU dataset. Consideration of the MS AMPA surface water data against EQS-MAC (see Table 7.5-95) and EQS-AA (see Table 7.5-100) endpoints indicates that the presence of AMPA from GLY or other sources was not expected to have any impacts as there was 100 % compliance with the large EQS-MAC (zero exceedances of ~218 000 samples from ~9000 sites) and EQS-AA (zero exceedances in ~10 000 data years from ~1 400 sites) datasets compiled. These results were considered to be consistent with published results for Flanders in BE, and France, which reported compliance against EQS values.

In order to compare these AMPA results with aggregated results from published reports, assessment against the arbitrarily defined regulatory threshold of 0.1 µg/L was also undertaken. Detection above the threshold of 0.1 µg/L was ~47.5 % of samples (~67.6 % of sites), ranging from 16.3 % in AT to 77.7 % of samples in BE. These results were comparable with aggregated values extracted from reports (see Table 7.5-97) which range from ~44.4 % in IT to ~91.7 % of samples in the NL. Similarly, these results compare well with the previous data collection where ~41 % of samples were found to exceed 0.1 µg/L (see Table 7.5-98).

Annual and monthly investigations of sampling effort and compliance were also documented within the report. These have not been summarised as they do not alter the conclusions of the primary study, instead providing additional detail should this be required.

Table 7.5-94: Member State and combined European dataset public monitoring summaries for glyphosate (GLY) and AMPA in surface water

MS	Substance	Number of Sites	Number of Samples	Years	LOQ (µg/L)	Samples with LOQ≤0.1 µg/L		Detected above LOQ		Detected >0.1 µg/L		Detected >RAC[1]		Measured Concentration (µg/L)[2]
					Mean (min - max)	Sites	Samples	Samples	%	Samples	%	Samples	%	Median (min - max)
AT	AMPA	314	4507	2003 - 2015	0 05 (0 04 - 0 50)	314	4505	1086	24 1	735	16 3	0	0 000	0 03 (0 007 - 3 8)
AT	GLY	316	4508	2003 - 2015	0 05 (0 04 - 0 50)	316	4506	290	6 5	155	3 4	0	0 000	0 03 (0 015 - 3 6)
BE	AMPA	842	25957	2001 - 2019	0 06 (0 02 - 1 0)	842	25836	22653	87 7	20069	77 7	0	0 000	0 59 (0 017 - 199 0)
BE	GLY	849	26364	2001 - 2019	0 06 (0 02 - 1 0)	849	26115	16710	64 0	15012	57 5	0	0 000	0 17 (0 013 - 139 0)
DE	AMPA	1639	24309	1996 - 2019	0 07 (0 01 - 0 41)	1600	23624	18154	76 8	15406	65 2	1	0 004	0 20 (0 000 - 5000 0)
DE	GLY	1714	24898	1996 - 2019	0 05 (0 01 - 0 20)	1643	23731	9011	38 0	4383	18 5	0	0 000	0 05 (0 005 - 171 0)
DK	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DK	GLY	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ES	AMPA	323	2457	2012 - 2018	0 09 (0 03 - 0 20)	323	2457	1521	62 0	1471	60 0	0	0 000	0 24 (0 030 - 81 9)
ES	GLY	1172	14234	2006 - 2018	0 09 (0 00 - 100)	1170	13226	4373	33 1	2960	22 4	0	0 000	0 05 (0 000 - 100 0)
EU Transboundary	AMPA	3	361	2007 - 2017	0 39 (0 03 - 5 0)	3	345	325	94 2	248	71 9	0	0 000	0 20 (0 030 - 5 0)
EU Transboundary	GLY	72	493	2002 - 2017	0 09 (0 01 - 5 0)	72	479	135	28 2	23	4 8	0	0 000	0 05 (0 010 - 5 0)
FR	AMPA	7185	183708	2000 - 2019	0 06 (0 01 - 10 0)	7172	182539	107537	58 9	73679	40 4	0	0 000	0 10 (0 005 - 164 0)
FR	GLY	7410	190660	2000 - 2019	0 06 (0 01 - 10 0)	7338	189879	69589	36 6	33607	17 7	1	0 001	0 05 (0 005 - 558 0)
IE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IE	GLY	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IT	AMPA	688	5349	2011 - 2019	0 08 (0 01 - 0 10)	688	5349	2785	52 1	2178	40 7	0	0 000	0 10 (0 002 - 79 2)
IT	GLY	701	5611	2007 - 2019	0 08 (0 01 - 0 10)	701	5611	1977	35 2	1063	18 9	0	0 000	0 10 (0 000 - 26 0)
NL	AMPA	1014	15984	1995 - 2018	0 13 (0 01 - 1 00)	944	12812	10225	79 8	9239	72 1	2	0 016	0 22 (0 000 - 230000 0)
NL	GLY	1019	16230	1995 - 2018	0 05 (0 01 - 0 50)	904	11082	6169	55 7	4661	42 1	2	0 018	0 20 (0 000 - 40000 0)
SE	AMPA	393	5663	1997 - 2018	0 20 (0 02 - 2 00)	383	4117	2019	49 0	1421	34 5	0	0 000	0 00 (0 000 - 36 0)
SE	GLY	400	5703	1997 - 2018	0 20 (0 02 - 2 00)	395	5658	3817	67 5	2329	41 2	0	0 000	0 08 (0 000 - 370 0)
UK	AMPA	13	850	1996 - 2017	0 1170 19 - 1 00)	13	835	252	30 2	251	30 1	0	0 000	0 10 (0 100 - 4 4)
UK	GLY	151	2882	1996 - 2017	0 (0 05 - 1000)	145	2663	864	32 4	814	30 6	13	0 488	0 10 (0 050 - 91600 0)
EU Combined	AMPA	12414	269145	1995 - 2019	0 07 (0 01 - 10 0)	12282	262415	166557	63 47	124697	47 5	3	0 001	0 10 (0 000 - 230000 0) 0 10 (0 000 – 224 4)[3]
EU Combined	GLY	13804	291583	1995 - 2019	0 15 (0 00 - 1000)	13573	282950	112937	39 91	65007	23 0	16	0 006	0 05 (0 000 - 91600 0) 0 05 (0 000 – 57 0)[3]

[1] RAC = Regulatory Acceptable Concentration of 400 µg/L for glyphosate and 1200 µg/L for AMPA
[2] Values <LOQ and <LOD are treated as equal to LOQ and LOD as a precautionary estimate of the median
[3] Statistics with outliers excluded;
ND = None identified within the timeframe

Table 7.5-95: Summary of Maximum Allowable Concentration (MAC) Environmental Quality Standard (EQS) statistics for those Member States (MS) where such a threshold is available

Substance	GLY					AMPA				
Member State	**BE**	**FR**	**IT**	**SE**	**UK**	**BE**	**FR**	**IT**	**SE**	**UK**
EQS-MAC Threshold (µg/L)	**64**	**70**	**70**	**100**	**398**	**800**	**45200**	**45200**	**500**	**NA**
Number of Sites	849	7378	701	395	145	842	7172	688	383	NA
Number of Samples	26115	189879	5611	5658	2603	25836	182539	5349	4117	NA
Number of samples > threshold	10[1]	3[1]	0	3[1]	8[1]	0	0	0	0	NA
% of samples > threshold	0.04	0.0016	0.00	0.05	0.49	0.00	0.00	0.00	0.00	NA
Number of sites > threshold	5	3	0	3	10	0	0	0	0	NA
% of sites > threshold	0.59	0.041	0.00	0.76	6.9	0.00	0.0	0.0	0.00	NA
Maximum number of samples > threshold at a single site	5	1	0	1	3	0	0	0	0	NA
Maximum number of consecutive samples > threshold at a single site	2	1	0	1	3	0	0	0	0	NA

[1] All of these samples are classed as outliers in the combined EU dataset

Table 7.5-96: Summary statistics for glyphosate (GLY) and AMPA surface water concentration data considering the influence of outliers

Compound	Outlier Status	Concentration (µg/L)										Percentile of RAC	Number of outliers
		Minimum	Mode	25th Percentile	Median	Mean	75th Percentile	90th Percentile	95th Percentile	99th Percentile	Maximum		
GLY	Included	0	0.05	0.048	0.05	1.78	0.105	0.342	0.671	2.3	91600	99.9897	NA
	Excluded	0	0.05	0.048	0.05	0.20	0.105	0.34	0.67	2.23	57	100	58
AMPA	Included	0	0.05	0.05	0.1	1.41	0.34	1	2	5.81	230000	99.9989	NA
	Excluded	0	0.05	0.05	0.1	0.49	0.34	1	2	5.80	224.4	100	3

NA – Not applicable as not considered

Table 7.5-97: Summary of monitoring data aggregated in reports for glyphosate (GLY) and AMPA in surface water

MS	Substance	Number of reports identified	Reports with data relating to threshold						Maximum value (µg/L)
			Number of reports	Date range	Number of samples	Threshold (µg/L)	Samples above threshold	% samples above threshold	
AT	AMPA	2	0	NA	NA	3.0	NS	NS	3.7
	GLY	3	1	2011 - 2013	1852	0.1	4	0.22	8.1
BE	AMPA[7]	1	1	2004-2016	NS	28[4]	0	0.0	NS
	GLY[7]	1	1	2004-2016	NS	28[4]	0	0.0	NS
DE	AMPA[6]	ND	ND	ND	ND	ND	ND	ND	ND
	GLY[6]	1	1	2005-2015	3557	100[2]	1	0.03	NS
	AMPA	9	4	2004-2008	647	0.1	360	55.6	16.2
	GLY	12	4	2004-2008	647	0.1	148	22.9	16
DK	AMPA	1	0	NA	NA	NS	NA	NA	0.12
	GLY	1	0	NA	NA	NS	NA	NA	0.11
ES	AMPA	1	0	NA	NA	0.1	NA	NA	NA
	GLY	2	0	NA	NA	0.1	NA	NA	27.3
EU Transboundary	AMPA	2	0	NA	NA	NS	NA	NA	6.0
	GLY	2	0	NA	NA	NS	NA	NA	1.0
FR	AMPA[5]	1	1	2007-2017	137467	452[4]	0	0.0	NS
	GLY[5]	1	1	2007-2017	137518	28[4]	1	0.001	NS
	AMPA[5]	1	1	2007-2017	137467	45200[3]	0	0.0	NS
	GLY[5]	1	1	2007-2017	137518	70[3]	5	0.004	NS
	AMPA[5]	1	1	2007-2017	137467	60[2]	1[1]	0.001[1]	NS
	GLY[5]	1	1	2007-2017	137518	60[2]	1[1]	0.001[1]	NS
IE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	2	1	2007-2015	790	NS	0	0	NS
IT	AMPA	8	7	2007-2016	9212	0.1	4094	44.4	NS

Table 7.5-97: Summary of monitoring data aggregated in reports for glyphosate (GLY) and AMPA in surface water

MS	Substance	Number of reports identified	Reports with data relating to threshold						Maximum value (µg/L)
			Number of reports	Date range	Number of samples	Threshold (µg/L)	Samples above threshold	% samples above threshold	
NL	GLY	8	8	2007-2016	9340	0.1	1799	19.3	NS
	AMPA	25	7	2007-2018	1497	0.1	1372	91.7	7.9
	GLY	27	8	2007-2018	1576	0.1	305	19.4	1.1
SE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
UK	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND

ND – No data identified
NS – Not specified
[1] - Number/Percentage of sites where the annual average concentration exceeds the threshold
[2] – PNEC
[3] – Maximum allowable concentration (MAC) environmental quality standard (EQS)
[4] – Average annual (AA) concentration EQS
[5] - ANSES (2019) publication
[6] - Szöcs *et al.* (2018) publication
[7] - VMM (2017) publication

Table 7.5-98: Summary of glyphosate (GLY) and AMPA analyses in surface water in Europe (*after* [redacted] 2016, CA 7.5/010)

Country	Compound	Date	Sites	Samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max Conc	LoQ (LoD)
		Range	No.	No.	No.	%	No.	%	µg/L	µg/L
Austria	AMPA	2001-02	?	345	≥90	≥26	90	26	3.4	?
Belgium (Flanders and Wallonia)	GLY F	2007-15	≥131	6802	5510	81	1628	23.9	139	0.02-0.4
	AMPA F	2007-15	≥132	6801	6256	92	3844	56.5	47	0.02-0.4
	GLY W	2001-14	≥171	6118	≥961	≥15.7	961	15.7	15.[illegible]	(0.05)
	AMPA W	2007-14	≥171	5891	≥148	≥86.6	≥148	≥86.6	35.8	(0.025-0.1)
Czech Republic	GLY	2010-14	≥290	6358	2547	40	≤2476	≤38.9	52	0.025-1.0
	AMPA	2010-14	≥236	4845	3185	65.7	≤3020	≤62.3	83	0.05-10
Denmark	GLY	2004-13	≥20	370	281	76	≤281	<76	2.7	0.01-0.1
	AMPA	2004-13	≥20	363	296	81	≤269	<81	0.28	0.01-0.2
Finland	GLY	2007-11	4	82	5	6.1	5	6.1	0.9	0.1
	AMPA	2007-11	4	84	14	16.7	≤13	≤15.5	0.22	0.05
France	GLY	97-2012	≥2003	91044	27999	30.7	19505	21.4	88	0.01-2.5
	AMPA	98-2012	≥2001	80817	42855	53	36053	44.6	106	0.01-0.25
Germany	GLY	97-2013	>204	≥2018	≤831	41	≤712	≤35	4.7	0.02-1.5
	AMPA	97-2013	≥71	≥1362	≤837	61.4	≤719	52.8	1.4	0.05-0.5
Ireland	GLY	2005-12	≥256	≥2544	142	5.6	≤142	≤5.6	186	0.08-0.1/20
	AMPA	2010-12	≥70	870	2	0.2	≥2	≥0.2	>200	20
Italy (Lombardia Region)	GLY	2005-12	≥274	2851	754	26.4	673	23.6	37.6	0.1
	AMPA	2008-12	≥274	2229	1386	62.2	1386	62.2	393	0.1
Norway	GLY	97-2015	12	98	88	89.8	≤71	≤72	0.93	0.01-0.05
	AMPA	97-2015	12	98	90	91.8	≤59	≤60	0.54	0.01-0.05
Slovakia	GLY	2006-14	≥142	5018	835	16.6	775	15.4	4.2	0.05-0.5
Spain	GLY	2009-14	≥343	5418	1847	34	1218	22	3400	0.03-30
	AMPA	2012-14	≥84	830	543	65	534	64	9.2	0.05-0.2
Sweden	GLY	2000-14	≥21	1439	442	30.7	≤433	≤30	370	<0.06-<1
	AMPA	2000-14	≥22	1418	320	22.6	≤312	≤22	36	<0.07-<1
Switzer-land	GLY	2006	5	≥10	≥8	80	1	≤10	0.1	0.0007
	AMPA	2006	5	≥11	≥11	100	≥3	27	0.29	0.0008
Nether-lands	GLY	2006-14	≥373	9316	≥1223	≥13	≤1223	≤13	0.14	?
	AMPA	2006-14	≥373	9270	≥1358	≥15	≤1358	≤15	0.07	?

Table 7.5-98: Summary of glyphosate (GLY) and AMPA analyses in surface water in Europe (*after* [redacted] 2016, CA 7.5/010)

Country	Compound	Date	Sites	Samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max Conc	LoQ (LoD)
		Range	No.	No.	No.	%	No.	%	µg/L	µg/L
UK	GLY	93-2015	≥102	3916	754	19.2	754	19.2	8.2	0.1
Danube	GLY	2013	68	68	5	7.3	0	-	0.07	0.03
	AMPA	2013	68	68	66	97	≤66	≤97	0.96	0.03
Total	**GLY**	**93-2015**	**≥4419**	**≥143470**	**444232**	**31**	**30858**	**21**	**0.07-3400**	**0.01-2.5**
	AMPA	**97-2015**	**≥3543**	**≥115302**	≥57457	50	**≥47876**	**41**	**0.07-393**	**0.01-0.5**

Table 7.5-99: Summary of sites and samples exceeding investigated thresholds for glyphosate (GLY) and AMPA in surface water for the combined EU dataset

Statistic/Threshold	GLY			AMPA		
	RAC: 400 µg/L	EQS-MAC: NA µg/L	Threshold: 0.1 µg/L	RAC: 1200 µg/L	EQS-MAC: NA µg/L	Threshold: 0.1 µg/L
Number of sites	13573	NA	13573	12282	NA	12282
Number of samples	282950	NA	282950	262415	NA	262415
Number of samples > threshold	16	NA	65007	3	NA	124697
% of samples > threshold	0.006	NA	23.0	0.001	NA	47.5
Number of sites > threshold	13	NA	7332	3	NA	8298
% of sites > threshold	0.10	NA	54.0	0.024	NA	67.6
Number of consecutive samples > threshold	8	NA	48663	0	NA	112759
% of samples that are consecutive samples > threshold	0.003	NA	17.2	0.0	NA	42.97
Maximum number of samples > threshold at a single site	4	NA	348	1	NA	399
Maximum number of consecutive samples > threshold at a single site	4	NA	202	1	NA	264

NA – Not available for EU analysis

Table 7.5-100: Summary of Annual Average (AA) Environmental Quality Standard (EQS) statistics for those Member States (MS) where such a threshold is available

MS	Substance	Number of Sites	Number of Years	Number of Sites with 12 Samples per Year	Number of Data Years with 12 Samples per Year	Number of Sites > AA-EQS	Percent of Sites > AA-EQS	Number of Years > AA-EQS	Percent of Years > AA-EQS
BE	AMPA	842	2871	215	1461	0	0.0	0	0.0
	GLY	849	2945	219	1526	0	0.0	0	0.0
FR	AMPA	7172	29143	1049	8147	0	0.0	0	0.0
	GLY	7378	30132	1195	8963	0	0.0	0	0.0
DE	AMPA	1600	3453	273	1001	0	0.0	0	0.0
	GLY	1643	3482	281	1017	0	0.0	0	0.0
NL	AMPA	944	2896	60	286	0	0.0	0	0.0
	GLY	904	2471	65	273	0	0.0	0	0.0
UK	AMPA	NA	NA	NA	NA	NA	NA	NA	NA
	GLY	145	289	39	149	0	0.0	0	0.0

ND – No data identified

Understanding Sources of Exposure

Regression Tree Modelling

The development of Regression Tree models was undertaken for the French surface water public monitoring dataset as this data comprises the majority of the surface water dataset available across the EU and has sub-national GLY sales data available. Regression trees are highly visual means of exploring datasets to assess underlying relationships which makes them good for risk communication purposes. These models sought to predict the exceedance rate (%) of the arbitrarily defined regulatory threshold of 0.1 µg/L as well as the number of exceedances through relating them to descriptors of the catchment upstream of the monitoring location.

Urban areas were identified as the most important predictor variable for both the GLY exceedance rate and the number of consecutive GLY exceedances while the urban wastewater treatment capacity was the most important for the absolute number of GLY failures. Similarly, arable land cover was identified as an important landcover but to a lesser extent, for the exceedance rate (~0.6), the number of exceedances (~0.75) and the number of consecutive exceedances (~0.68).

Urban areas were identified as the most important predictor variable for the AMPA exceedance rate while the urban wastewater treatment capacity was the most important for both the number of consecutive AMPA exceedances and the absolute number of AMPA failures. Similarly, arable land cover was identified as an important landcover but to a lesser extent, for the exceedance rate (~0.53), the number of exceedances (~0.72), and the number of consecutive exceedances (~0.53). It should be borne in mind that AMPA may originate from sources other than GLY, for example detergents.

These models clearly outlined the importance of the urban landcover and urban waste water treatment works capacity in their catchment on high rates of exceedance expressed in diverse ways. The importance of arable landcover was also demonstrated along with permanent crops to a much lesser extent.

Source Apportionment Appraisal
An assessment of source apportionment was conducted through review of peer reviewed literature. With respect to assessing monitoring data for surface water: the glyphosate and AMPA concentrations appeared to be generally larger from urban sources than from diffuse agricultural ones. With respect to urban sources, use on railways/roads seemed to result in higher residues, while garden use resulted in lower residues in comparison to amenity use. In addition, from urban sources AMPA concentration were often greater than glyphosate and likely to be derived from detergents, and phosphonates used in water treatment processes, as well as from glyphosate used in urban environments. Storm events often gave rise to large spikes in concentration in agricultural settings, and even more so where there was an urban contribution. Generally, where the glyphosate route to water bypassed soil, glyphosate residues may be transported into water. If the sampling location was downstream of urban, or major infrastructure (rail or roads) then the glyphosate and AMPA residues were mostly likely not to have come from agricultural uses. These findings echoed that of the regression tree modelling.

III. CONCLUSIONS

The analysis of the large surface water dataset for GLY and AMPA indicated they are both frequently detected above the LOQ in this compartment. However, compliance with regulatory acceptable concentrations and environmental quality standards was very high with few exceedances measured. Most of these exceedances were considered to be anomalous. It should also be borne in mind that AMPA may originate from sources other than GLY, for example detergents. The environmental concentrations typically encountered in this environmental compartment do not pose a risk for biota or ecosystems.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the analysis of public monitoring data for key European countries for the compartments soil, water and sediment for Glyphosate and AMPA. The maximum GLY concentration in SW of 91.6 mg/L was likely anomalous and once outliers were identified and excluded would be 57.0 µg/L. The GLY RAC represented the 99.987th percentile value in the distribution of measured SW GLY concentrations.

The maximum AMPA concentration in SW of 230.0 mg/L was likely anomalous and once outliers were identified and excluded would be 224.4 µg/L. The AMPA RAC represented the 99.999th percentile value in the distribution of measured SW AMPA concentrations.

The available data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in the surface water compartment.

The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/031
Report author	[redacted]
Report year	2019
Report title	Mitigating glyphosate levels in surface waters: Pilot catchment details and monitoring results
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A 5-year mitigation study on glyphosate was performed for the years 2014-2018 to evaluate the effect of mitigation measures to reduce glyphosate loads in surface water. A representative pilot study catchment in Europe, containing mainly agricultural land uses, was monitored two years before (baseline monitoring) and three years after the implementation of mitigation measures.

Basic information such as land-use, connectivity to the river, gross emission of glyphosate, location of other potential sources and erosion mitigating measures was inventoried. Using this data, priority zones within the study area were selected for the implementation of erosion and glyphosate loss mitigating measures.

To discern between runoff sources and point sources, event-based and time-integrated monitoring was established. The monitoring period consists of a baseline period during which no measures were implemented and no communication to farmers was carried out, and a mitigation period after which the mitigation measures were installed. The monitored stretch of river was also just (50 m) downstream of a sewage outlet, serving four villages (2475 inhabitants) and an industrial laundry. A small number of samples were taken within and around this outlet, and it was clear that this was a significant source of glyphosate, and even more so of AMPA.

Communication with farmers in the study area was done by meetings and workshops, and 15 farmers in the priority zones with a significant impact on the pesticide load to surface water were encouraged to enter a voluntary erosion control program supported by the local government. During 2016 and 2017, 11 grassed buffer strips were installed. In addition, 22 biofilters were built by farmers to mitigate point sources of pesticides.

During runoff events, the load intensity was substantially higher compared to baseflow load. After the implementation of the mitigation measures, the loads almost halved from 4-5 g/h to 2-3 g/h. Mitigation measures seemed to have had impact on the event loads. Baseflow loads close to 1 g/h were not influenced by mitigation measures. Over the mitigating period, number and intensity of point sources reduced from 8 to 1 and from 100 µg/L to less than 20 µg/L. Point sources decreased over the period 2014-2018 in number as well as concentration. Influx of glyphosate during rainfall events decreased over the period 2014-2017.

The study strongly suggested that even in a predominantly agricultural area, urban sources of glyphosate and AMPA are still likely to be significant and also that agricultural point losses (point sources) are significant sources of glyphosate/AMPA which can be substantially reduced with appropriate targeted education. Risk profiling and targeted mitigation measures can significantly reduce rain-driven losses of glyphosate/AMPA from treated fields.

I. MATERIALS AND METHODS

Study area

The selection of the study area focused on small agricultural catchments in the region of Haspengouw (Flanders, Belgium). A subcatchment of the Cicindria river was selected as the study area (72 % agriculture, 11 % residential, 6 % airport, 6 % forest/natural) because of its high potential erosion, relatively high measured concentration of glyphosate, its potential for the installation of mitigation measures and the high relative contribution (39 %) of the selected study area to the total gross emission of glyphosate of the Cicindria catchment. These factors point to a high probability that mitigation measures in the study area would have a measurable effect on the levels of glyphosate in surface water.

A map of the gross emission of glyphosate within the catchment area was constructed by combining detailed crop maps with the result of a farmer survey. Emission factors for different transport routes (drift, direct losses, volatilisation, interception, erosion, drainage and leaching) were calculated. Emission to surface water was calculated as the sum of drift, direct losses, erosion and drainage. A 6.5 km stretch of the river was selected (catchment size 1075 ha), and a theoretical risk map was constructed in order to prioritize between fields and farmers to target to reduce glyphosate loads to surface water in the most efficient way. The highest risk for losses of glyphosate was based on the calculated glyphosate gross emission and the connectivity of the field to the Cinidria. This theoretical risk map was validated by the local water board and priority zones for the implementation of erosion and glyphosate loss mitigating measures in the study area were created.

Communication with the relevant stakeholders

In total, 15 farmers in the priority zones with a significant impact on the pesticide load to surface water were encouraged to enter a voluntary erosion control program supported by the government. Starting in 2015, information meetings about the monitoring campaign, the importance of implementing measures, the influence of point sources, and the correct use of pesticides were held.

Analytical conditions

The monitoring set-up consisted of a flow meter to monitor water level and flow velocity at the upstream and the downstream monitoring location of the selected stretch of the Cinidria, a rain gauge at the downstream location and two samplers at both locations. An event-based and time-integrated monitoring was established to monitor the glyphosate concentrations and loads to the river, and to discern between runoff sources and point sources. Time-paced samples are taken every two hours and collected in one bottle for every 24 hours. Event samples are taken once a discharge threshold was exceeded. These were taken every 15 minutes and collected in a sample bottle for every 90 minutes. Samples of the refrigerated time paced samplers were collected on a weekly basis, whole event samples were collected and frozen within 24 hours.

To determine glyphosate and AMPA concentrations in the water samples, a 10 mL sample was spiked with internal standards ($^{13}C_2{}^{15}N$-glyphosate, $^{13}C^{15}N$-AMPA), acidified with 6 M HCL and the mixture was allowed to react for 1 hour. Afterwards it was neutralised with 6 M KOH. Borate buffer (5 % sodiumtetraborate in water) and a solution of FMOC (fluorenylmethyloxycarbonylchloride) in acetonitrile was added for derivatisation. The mixture was allowed to react for 30 minutes and the reaction was stopped by adding formic acid. The mixture was diluted with 12.5 mL water and EDTA solution was added. Analytes were extracted by solid phase extraction using 200 mg Oasis HLB cartridges conditioned with methanol and 0.1 % formic acid. After elution, the cartridge was washed twice with formic acid. A second rinsing was done with methylene chloride. Analytes were eluted with methanol; the extract was evaporated to nearly dryness and reconstituted in 1:9 methanol/mobile phase A.
Analysis was done by UPLC-MS/MS. Limit of quantification was 50 ng/L.

The load of glyphosate was calculated by combining the concentration and discharge measurements, as based on concentration only it is not possible to assess the glyphosate fluxes. Difference between the upstream load and the downstream load was calculated to assess the load that enters the river over the study area.

II. RESULTS AND DISCUSSION

All event and time integrated monitoring results are presented in the appendices. In 2016, eleven new buffer strips were installed in the pilot catchment, all in the priority zones. Most of the buffer strips were 9 m wide, three of them were 21 m wide. For 2017, 4 new buffer strips were planned. From 2017, famers were obliged to use 50 % drift-reducing nozzles. Further changes in agricultural practice (like crop rotation, tillage and cropping techniques) were introduced. Use of glyphosate by residents was prohibited since July 2017.

The tables below provide a summary of the results obtained for 2014-2018 including mean, standard deviation, median and 90th percentile for the upstream and downstream glyphosate load, the upstream and downstream discharge, and the glyphosate influx over the study area.

The results for 2015 correspond rather well with the results obtained for 2014. In both years, the influx under baseflow conditions was clearly lower than the influx under rain event flow conditions. In 2014 the average influx was for both conditions (baseflow and rain event) higher than in 2015.

The results for 2016 were in line with the results obtained in 2014 and 2015. In all years, the influx under baseflow conditions was clearly lower than the influx under rain event flow conditions.

The results for 2017 confirmed that the influx from the catchment was considerably higher during rainfall events compared to baseflow conditions. The results from 2016 indicated a decrease in the loads and influxes compared to the previous years and that decreasing tendency was continued in 2017. The average influx under baseflow conditions was lower in 2017 than in the years before and also the loads in the river (upstream and downstream) were clearly lower than the years before. The lower loads were a combined effect of lower concentrations and lower discharge (less rainfall in 2017). The average influx during rainfall events in 2017 was similar as in 2016, and lower than in 2014-2015. Most of the events in 2017 involved lower fluxes than events in 2016. It was mainly the single high flux rainfall event on the 9th of August that increased the 2017 event flux average to the level of 2016. The median value of the event fluxes in 2017 was lower than the median value for 2016.

In 2018 only 3 events could be analysed for calculating the influx from the study area. The calculated flux during events was lower than in the previous years. The calculated influx during non-event conditions was in line with results from previous years, and higher than in 2017. The results for 2018 should be interpreted carefully because of the extremely dry conditions and the low amount of available data.

Over the 5 years, a decreasing trend can be observed for the influx of glyphosate during rainfall events. The results show a difference between the years in the baseline period (2014-2015) and in the period after measures (2017-2018). This is a combined effect of changes in the management (agricultural practice, mitigation measures), variations in climatic conditions and changes in glyphosate use.

Table 7.5-101: Summary of the results obtained for the upstream and downstream glyphosate load, discharge and the glyphosate influx. All data related to non-event conditions

Non-event conditions		Glyphosate load (g)		Discharge average (m³/s)		Influx (g/h)
		Down-stream	Up-stream	Down-stream	Up-stream	Load Down-Up
2014 (N = 11)	**Mean**	36	14	0.053	0.033	0.9
	StDev	17	5	0.009	0.002	0.5
	Median	33	13	0.051	0.033	0.7
	90th percentile	55	21	0.065	0.036	1.6
2015 (N = 10)	**Mean**	38	22	0.065	0.042	0.6
	StDev	13	7	0.012	0.012	0.4
	Median	36	21	0.062	0.037	0.7
	90th percentile	57	33	0.082	0.053	1.1
2016 (N = 14)	**Mean**	41	25	0.101	0.061	0.9
	StDev	13	13	0.018	0.014	0.5
	Median	44	22	0.093	0.062	0.9
	90th percentile	53	34	0.121	0.079	1.5
2017 (N = 12)	**Mean**	30	21	0.058	0.041	0.4
	StDev	8	9	0.008	0.012	0.3
	Median	29	22	0.059	0.045	0.4
	90th percentile	39	29	0.068	0.047	0.8
2018 (N = 10)	**Mean**	29	10	0.049	0.018	0.8
	StDev	13	6	0.014	0.009	0.5
	Median	26	10	0.045	0.014	0.7
	90th percentile	42	17	0.066	0.029	1.1

Table 7.5-102: Summary of the results obtained for the upstream and downstream glyphosate load, discharge and the glyphosate influx. All data related to event conditions

Event conditions		Glyphosate load (g)		Discharge average (m³/s)		Influx (g/h)
		Down-stream	Up-stream	Down-stream	Up-stream	Load Down-Up
2014 (N = 8)	**Mean**	64	38	0.444	0.184	4.7
	StDev	62	55	0.463	0.122	2.5
	Median	38	11	0.285	0.194	4.3
	90th percentile	147	122	0.836	0.302	7.5
2015 (N = 8)	**Mean**	104	73	0.223	0.136	3.3
	StDev	54	43	0.107	0.084	1.9
	Median	91	61	0.193	0.112	3.1
	90th percentile	163	130	0.308	0.191	5.5
2016 (N = 7)	**Mean**	73	45	0.252	0.139	2.5
	StDev	32	19	0.132	0.077	1.1
	Median	66	48	0.196	0.114	2.6
	90th percentile	112	66	0.378	0.216	3.6
2017 (N = 6)	**Mean**	34	16	0.235	0.095	2.6
	StDev	25	12	0.070	0.020	2.3
	Median	22	13	0.230	0.088	1.8
	90th percentile	66	27	0.306	0.117	4.9
2018 (N = 3)	**Mean**	55	56	0.430	0.351	0.8
	StDev	41	43	0.522	0.507	0.3
	Median	52	47	0.223	0.136	0.7
	90th percentile	97	105	0.918	0.806	1.0

III. CONCLUSIONS

The number of findings of point losses decreased over the years with 6 and 7 possible point loss detections per year in the baseline period (2014-2015) to 2 to 3 per year in the mitigation period (2016-2018). Maximum concentration observed in point losses decreased over the years from over 100 µg/L to less than 20 µg/L. The load intensity of glyphosate decreased with time, and the loads almost halved from 4-5 g/h to 2-3 g/h after the implementation of measures. Mitigation measures did not have impact on baseflow loads close to 1 g/h.

The estimated yearly influx (based on 2014 dataset) of glyphosate under low flow conditions is about 7 kg/year and the influx under rain event flow conditions was about 4 kg/year. This means that about a third of the loads enter the river during events, which occured only 10 % of the time.

The baseline concentrations in the upstream location were on average 5.6 µg/L and in the downstream location on average 6.5 µg/L. Baseline concentration in the downstream location was consistently higher than in the upstream location indicating also an influx during non-event conditions.

The communication to the farmers with information meetings proved to be successful with 11 installations of buffer strips in 2016 and 4 more in 2017.

In order to have a lasting effect on glyphosate load in the river, the interactions with the different stakeholders in the area need to be maintained and strengthened. Communication and sensitisation is crucial to have actor involvement in decreasing the loads to the river.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study investigates the effect of mitigation measures on loads of glyphosate and AMPA in surface water in a small agricultural catchment in the region of Haspengouw (Belgium) over five years. The study strongly suggested that even in a predominantly agricultural area, urban sources of glyphosate and AMPA are still likely to be significant and also that agricultural point losses (point sources) are significant sources of glyphosate/AMPA which can be substantially reduced with appropriate targeted education. Risk profiling and targeted mitigation measures can significantly reduce rain-driven losses of glyphosate/AMPA from treated fields.

The study methods and results as well as the analytical procedures are properly reported.

The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/008
Report author	[redacted]
Report year	2019a
Report title	Phase 1: Traitements et analyses statistiques sur les données SOES UIPP 2008 - 2014 Analyses des données de suivi de glyphosate et de l'AMPA dans les eaux de France Période 2008-2014 (Processing and statistical analysis of the 2008-2014 SOES UIPP data. Analysis of the 2008-2014 water monitoring data for glyphosate and AMPA in France.)
Document No	REA-DOC-026
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, but likely conducted by COFRAC approved testing facilities
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

The study is relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/032
Report author	[redacted]
Report year	2019b
Report title	Phase 3 et 4: Traitements et analyses statistiques sur les données SOES UIPP 2008 - 2014 Analyses des données de surveillances sur 6 territoires témoins. Synthèse des données sur l'ensemble des territoires viticoles. (Phase 3 and 4: Statistical analysis of SOES UIPP data 2008 - 2014 Analysis of surveillance data for 6 control regions. Synthesis of data for all wine production regions.)
Document No	REA-DOC-026
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, but likely conducted by COFRAC approved testing facilities
Acceptability/Reliability:	Valid

Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

This report is an update of a previous report "Analysis of monitoring data for glyphosate and AMPA in French waters in the period 1997-2013" ([redacted] 2016, CA 7.5/009). It includes the 2014 monitoring data for glyphosate and AMPA in ground and surface waters, extracted from the SOES UIPP database in July 2017. The report also looked at the monitoring of AMPA and glyphosate in surface waters associated with six wine growing regions across France.

The study assessed the number of water quality monitoring stations in each area, the regularity of the monitoring (number of samples per year per station) and compared the frequency of quantification and exceedance of drinking water thresholds (0.1 µg/L and 2 µg/L) for both AMPA and glyphosate. Data were also examined in relation to seasonality and trends across the seven-year monitoring period.

The representativeness of stations and associated analysis results presented for the 6 vineyard areas are very limited. There are three areas where four stations monitor the water quality. Estimating water quality of an area from a limited number of sampling points can introduce bias into the interpretation. In addition, the placement of some sampling stations in some areas limits robust estimates of pollution in the area of the vineyard.

For the three other vineyards the number and position of the stations gives a better indication of the level of contamination by glyphosate and AMPA in these areas. However, certain stations should be excluded, because even though they are strictly located in the area, the information generated reflects the conditions and contaminants of much larger areas than the vineyards studied.

In comparison to the analysis made at national level (Phase 1), the frequency of quantification of AMPA was less at the monitoring stations associated with the vineyards (5-20 % less). The quantifications of AMPA and glyphosate generally followed the same overall variations year on year. In the vineyard stations the quantifications >0.1 µg/L represented one third of the data; those greater than 2 µg/L of AMPA represent 1-3 % of data.

I. MATERIAL AND METHODS

Size of database

At national scale

At the national level the entire dataset for surface waters consists of 148561 analyses for AMPA and glyphosate, across the whole of France including Guadeloupe. The number of unique stations is 3006. The present study focusses on analysis of data from mainland France. Therefore, the database selected for the study comprises 148295 analyses (74138 for AMPA and 74157 for glyphosate) from 2980 stations for the study of surface waters.

At the scale of the 6 winegrowing regions studied

Phases 3 and 4 focus on presenting AMPA and glyphosate residues in surface waters associated with 6 vineyards distributed across France, namely:

- Languedoc – Hérault et Picpoul de Pinet;
- Champagne;
- Coteaux de Saumur;
- Entre deux mers;
- Beaujolais village;

- Cognac.

The number of monitoring stations sampling each year is very variable, in part a function of the differing size of the vineyard regions:

- Picpoul de Pinet et Hérault Languedoc – 4-12 stations
- Champagne - 17-20 stations;
- Coteaux de Saumur - 1-3 stations
- Entre deux mers – 1-3 stations
- Beaujolais village - 1 monitoring station
- Cognac – 38-74 stations (except 2008, 14 stations)

Data for glyphosate and AMPA were generated by the same number of monitoring stations for each region. The number of analyses for glyphosate and AMPA being very similar, within one or two values for the 6 vineyards for the 7 years studied, except for the Cognac vineyard in 2012 (290 analyses for glyphosate compared to 315 for AMPA). For most vineyards, the average number of analyses per station year is between 3 and 5. Some stations have only 1 or no analyses in certain years whilst others had more than 7-8 analyses per year in certain years. The overall average for all vineyards is between 5 and 7 analyses per station per year. These analyses include data across all SANDRE codes of reliability (1 = > LOQ; 2 = < LOD; 7 = >LOD but <LOQ – substance present but not possible to quantify accurately; 10= <LOQ [since 2007]).

II. RESULTS AND DISCUSSION

Multi-year continuity analysis

This analysis looked at continuity of analyses within the time period and therefore at the ability to draw conclusions in terms of multi-annual trends, based on the number of years of monitoring. Due to the inter and intra-annual climatic variability and crop rotations, it is necessary to have several years of monitoring to analyse trends.

Multi-year data may not use consecutive years, thus a station monitoring for 5 years may have non-consecutive years e.g. 2008, 2009, 2010, 2013, 2014). These data show that monitoring is very regular at stations located in the vineyards of Beaujolais village, Champagne and Entre deux mers. For the vineyards of Cognac and Picpoul de Pinet, the number of stations is relatively higher compared to other vineyards (except Champagne), however their monitoring is fairly irregular with more than a third of the stations monitoring for 4 years or less. For Coteaux de Saumur, monitoring was less regular with fewer stations present in this area.

- For Beaujolais-Village, there was one station monitoring every year for seven years for both AMPA and glyphosate (100 % of all stations in the area).
- For Champagne, for both AMPA and glyphosate, out of 22 stations: two stations monitored in just one year (9.1 % of all stations in the area); one station monitored for two, three and five years (4.5 %); 17 stations monitored for seven years (77.3 %).
- For Cognac, for both AMPA and glyphosate, out of 77 stations: two stations monitored in just one year (2.6 %); 10 stations monitored for two years (13.0 %); one station monitored for three years (1.3 %), 26 stations monitored for four years (33.8 %); two stations monitored for five years (2.6 %); 22 stations monitored for six years (28.6 %) and 14 stations monitored for seven years (18.2 %).
- For Coteaux de Saumur, for both AMPA and glyphosate, out of four stations: one station monitored in one and three years (25 %); two stations monitored for five years (50 %).
- For Entre deux mers, for both AMPA and glyphosate, out of three stations: two stations monitored for six years (66.7 %) and one station station monitored for seven years (33.3 %).
- For Picpoul de Pinet et Hérault Languedoc, for both AMPA and glyphosate, out of 13 stations: three stations monitored for two years (23.1 %); one station monitored for four years (7.7 %); four stations monitored for five years (30.8 %); one station monitored for six years (7.7 %) and four stations monitored for seven years (30.8 %).

Analysis of the annual number of monitoring data
The examination of the continuity of monitoring across multiple years includes the annual number of monitoring data. The data are presented as seven ranges to reflect the number of monitoring events made per station per year: 1 per annum (p.a.); 2-3 p.a.; 4-5 p.a.; 6-9 p.a.; 10-14 p.a.; 15-49 p.a.; >50 p.a.

In the Beaujolais, Coteaux de Saumur, Champagne and PicPoul de Pinet vineyards, the number of monitoring events for both glyphosate and AMPA by station and by year is generally 6 to 9 per year. At the Entre deux mers and Cognac vineyards, the data are less frequent, mostly between 4 and 5 monitoring events per station per year.

Review of the trend in quantifications
In this section, the results of the analytical results of glyphosate and AMPA >LOQ are assessed. The quantified concentrations are compared against the regulatory values provided for the provision of drinking water: ≥0.1 µg/L for potable water and ≥ 2 µg/L for water which is to be made potable.

Table 7.5-103: Annual summaries of AMPA quantifications for all (a) and individual (b – g) vineyard regions

(a) Combined 120 stations for 6 vineyards							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	189	331	277	521	530	652	662
Number > LOQ	123	152	91	201	162	308	245
% > LOQ	**65 %**	**46 %**	**33 %**	**39 %**	**31 %**	**47 %**	**37 %**
Number ≥ 0.1 µg/L	96	111	74	155	93	181	141
% ≥ 0.1 µg/L	**51 %**	**34 %**	**27 %**	**30 %**	**18 %**	**28 %**	**21 %**
Number ≥ 2 µg/L	2	3	7	8	9	7	7
% ≥ 2 µg/L	**1 %**	**1 %**	**3 %**	**2 %**	**2 %**	**1 %**	**1 %**
(b) Beaujolais village							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	6	6	6	8	6	6	6
Number > LOQ	0	2	4	6	5	5	3
% > LOQ	**0 %**	**33 %**	**67 %**	**75 %**	**83 %**	**83 %**	**50 %**
Number ≥ 0.1 µg/L	0	2	2	5	4	4	2
% ≥ 0.1 µg/L	**0 %**	**33 %**	**33 %**	**63 %**	**67 %**	**67 %**	**33 %**
Number ≥ 2 µg/L	0	0	0	0	0	0	0
% ≥ 2 µg/L	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**
(c) Champagne							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	82	124	114	120	129	126	115
Number > LOQ	52	71	28	49	47	62	38
% > LOQ	**63 %**	**57 %**	**25 %**	**41 %**	**36 %**	**49 %**	**33 %**
Number ≥ 0.1 µg/L	41	50	20	44	23	27	10
% ≥ 0.1 µg/L	**50 %**	**40 %**	**18 %**	**37 %**	**18 %**	**21 %**	**9 %**
Number ≥ 2 µg/L	0	1	1	0	1	0	0
% ≥ 2 µg/L	**0 %**	**1 %**	**1 %**	**0 %**	**1 %**	**0 %**	**0 %**
(d) Coteaux de Saumur							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	12	-	14	26	14	25	41
Number > LOQ	11	-	11	20	12	24	29
% > LOQ	**92 %**	**-**	**79 %**	**7 %**	**86 %**	**96 %**	**71 %**
Number ≥ 0.1 µg/L	11	-	11	20	6	13	22
% ≥ 0.1 µg/L	**92 %**	**-**	**79 %**	**77 %**	**43 %**	**52 %**	**54 %**
Number ≥ 2 µg/L	0	-	0	0	0	0	0
% ≥ 2 µg/L	**0 %**	**-**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**
(e) Cognac							

Table 7.5-103: Annual summaries of AMPA quantifications for all (a) and individual (b – g) vineyard regions

Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	56	164	86	297	315	400	413
Number > LOQ	43	64	25	90	71	157	137
% > LOQ	**77 %**	**39 %**	**29 %**	**30 %**	**23 %**	**39 %**	**33 %**
Number ≥ 0.1 µg/L	27	45	25	63	39	91	83
% ≥ 0.1 µg/L	**48 %**	**27 %**	**29 %**	**21 %**	**12 %**	**23 %**	**20 %**
Number ≥ 2 µg/L	0	2	3	6	6	5	7
% ≥ 2 µg/L	**0 %**	**1 %**	**3 %**	**2 %**	**2 %**	**1 %**	**2 %**
(f) Entre deux mers							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	4	13	6	15	15	24	24
Number > LOQ	4	8	3	10	7	23	20
% > LOQ	**100 %**	**62 %**	**50 %**	**67 %**	**47 %**	**96 %**	**83 %**
Number ≥ 0.1 µg/L	4	7	3	7	6	20	16
% ≥ 0.1 µg/L	**100 %**	**54 %**	**50 %**	**47 %**	**40 %**	**83 %**	**67 %**
Number ≥ 2 µg/L	0	0	0	0	0	0	0
% ≥ 2 µg/L	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**
(g) Picpoul de Pinet et Hérault Languedoc							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	29	24	51	55	51	71	63
Number > LOQ	13	7	20	26	20	37	18
% > LOQ	**45 %**	**29 %**	**39 %**	**47 %**	**39 %**	**52 %**	**29 %**
Number ≥ 0.1 µg/L	13	7	13	16	15	26	8
% ≥ 0.1 µg/L	**45 %**	**29 %**	**25 %**	**29 %**	**29 %**	**37 %**	**13 %**
Number ≥ 2 µg/L	2	0	3	2	2	2	0
% ≥ 2 µg/L	**7 %**	**0 %**	**6 %**	**4 %**	**4 %**	**3 %**	**0 %**

Compared to the analyses at the national scale (Phase 1), the frequencies of quantification of AMPA (Table 7.5-103) are lower at the vineyard monitoring stations (5 % to 20 % less) than nationally, except for 2008 where the frequency of quantification at national scale was 53 % of all the analyses but was 65 % of the analyses across the 120 stations associated with the six vineyards.

For these 120 stations the quantifications ≥0.1 µg/L for AMPA represent a third of the data (except for 2008) against 33 % to 54 % for the national database. The quantifications ≥2 µg/L for AMPA represent 1-3 % of data between 2008 and 2014 (the same order of magnitude as at national scale).

AMPA quantification rates across the different vineyards vary, although for the Beaujolais-Village, Coteaux de Saumur and Entre deux mers vineyards, care should be taken with interpretation as these data are based solely on analyses carried out with just 1 to 3 water quality stations.

For the Beaujolais-Village, Coteaux de Saumur and Entre deux mers vineyards, there is no quantification greater than the 2 µg/L limit. For these three vineyards the percentage of quantification and of quantification greater than 0.1 µg/L are greater than the combined statistics of the 120 stations, probably due to the small number of monitoring stations for these vineyards on which this data is based. This is particularly true for the years 2010 to 2013. For Coteaux de Saumur, the rate of quantification of AMPA is greater than 70 % for all the years (except 2009 where no data are available) and that of the exceedances of the 0.1 µg/L limit is also greater than 70 % for half of the years studied. For Entre deux mers, the level of quantification of AMPA is > 80 % for three years and the rate of quantification greater than 0.1 µg/L is 50 % for five of the seven years.

For the Champagne vineyard, the level of quantification of AMPA is closer to that of the 120 stations taken as a whole, although the frequencies of exceedances of 0.1 and 2 µg/L limits are less (three exceedances of 2 µg/L across the seven years).

For stations situated in Cognac, the water quality data have rates of quantification of AMPA less than 40 % (except in 2008) and the levels of quantification for concentrations > 0.1 µg/L were between 20 and 30 % which are slightly lower than across the 120 stations of all six vineyards.

The Picpoul de Pinet et Hérault Languedoc vineyard is the area with the highest level of quantification of AMPA at concentrations >2 µg/L (levels ≥4 % in four of the studied years).

Table 7.5-104: Annual summaries of glyphosate quantifications for all (a) and individual (b – g) vineyard regions

(a) Combined 120 stations for 6 vineyards							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	189	331	277	521	508	652	662
Number > LOQ	106	113	58	110	132	271	166
% > LOQ	**56 %**	**34 %**	**21 %**	**21 %**	**26 %**	**42 %**	**25 %**
Number ≥ 0.1 µg/L	73	86	44	76	77	134	66
% ≥ 0.1 µg/L	**39 %**	**26 %**	**16 %**	**15 %**	**15 %**	**21 %**	**10 %**
Number ≥ 2 µg/L	3	4	3	8	3	3	2
% ≥ 2 µg/L	**2 %**	**1 %**	**1 %**	**2 %**	**1 %**	**0.5 %**	**0.3 %**
(b) Beaujolais village							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	6	6	6	8	6	6	6
Number > LOQ	2	0	2	2	2	2	5
% > LOQ	**33 %**	**0 %**	**33 %**	**25 %**	**33 %**	**33 %**	**83 %**
Number ≥ 0.1 µg/L	2	0	0	0	1	0	1
% ≥ 0.1 µg/L	**33 %**	**0 %**	**0 %**	**0 %**	**17 %**	**0 %**	**17 %**
Number ≥ 2 µg/L	0	0	0	0	0	0	0
% ≥ 2 µg/L	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**
(c) Champagne							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	82	124	114	120	129	125	115
Number > LOQ	53	55	28	35	68	80	46
% > LOQ	**65 %**	**44 %**	**25 %**	**29 %**	**53 %**	**64 %**	**40 %**
Number ≥ 0.1 µg/L	38	44	21	33	36	32	15
% ≥ 0.1 µg/L	**46 %**	**35 %**	**18 %**	**28 %**	**28 %**	**26 %**	**13 %**
Number ≥ 2 µg/L	3	3	1	3	1	0	0
% ≥ 2 µg/L	**4 %**	**2 %**	**1 %**	**3 %**	**1 %**	**0 %**	**0 %**
(d) Coteaux de Saumur							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	12	-	14	26	14	25	41
Number > LOQ	5	-	8	11	4	16	12
% > LOQ	**42 %**	**-%**	**57 %**	**42 %**	**29 %**	**64 %**	**29 %**
Number ≥ 0.1 µg/L	5	-	6	10	0	2	1
% ≥ 0.1 µg/L	**42 %**	**-%**	**43 %**	**38 %**	**0 %**	**8 %**	**2 %**
Number ≥ 2 µg/L	0	-	0	0	0	0	0
% ≥ 2 µg/L	**0 %**	**-%**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**
(e) Cognac							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	56	164	86	297	293	401	413
Number > LOQ	36	50	9	45	41	137	77
% > LOQ	**64 %**	**30 %**	**10 %**	**15 %**	**14 %**	**34 %**	**19 %**
Number ≥ 0.1 µg/L	19	36	9	24	30	75	42
% ≥ 0.1 µg/L	**34 %**	**22 %**	**10 %**	**8 %**	**10 %**	**19 %**	**10 %**
Number ≥ 2 µg/L	0	1	2	5	2	3	1
% ≥ 2 µg/L	**0 %**	**1 %**	**2 %**	**2 %**	**1 %**	**1 %**	**0 %**
(f) Entre deux mers							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**

Table 7.5-104: Annual summaries of glyphosate quantifications for all (a) and individual (b – g) vineyard regions

Number of analyses	4	13	6	15	15	24	24
Number > LOQ	3	6	0	3	4	12	9
% > LOQ	**75 %**	**46 %**	**0 %**	**20 %**	**27 %**	**50 %**	**38 %**
Number ≥ 0.1 µg/L	2	4	0	3	3	8	3
% ≥ 0.1 µg/L	**50 %**	**31 %**	**0 %**	**20 %**	**20 %**	**33 %**	**13 %**
Number ≥ 2 µg/L	0	0	0	0	0	0	0
% ≥ 2 µg/L	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**
(g) Picpoul de Pinet et Hérault Languedoc							
Years	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
Number of analyses	29	24	51	55	51	71	63
Number > LOQ	7	2	11	14	13	24	17
% > LOQ	**24 %**	**8 %**	**22 %**	**25 %**	**25 %**	**34 %**	**27 %**
Number ≥ 0.1 µg/L	7	2	8	6	7	17	4
% ≥ 0.1 µg/L	**24 %**	**8 %**	**16 %**	**11 %**	**14 %**	**24 %**	**6 %**
Number ≥ 2 µg/L	0	0	0	0	0	0	1
% ≥ 2 µg/L	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**0 %**	**2 %**

For glyphosate (Table 7.5-104), as for AMPA, the frequencies of quantification are less for the vineyard stations (5-20 % less) when compared to the national scale. The exceptions are for 2008 and 2009 where the frequencies of quantification at national scale were 40 % and 26 %, compared to 56 % and 34 %. These findings were the same for the frequencies of quantification of concentrations greater than 0.1 µg/L and 2 µg/L.

As for AMPA, glyphosate quantification rates across the different vineyards vary, although for the Beaujolais-Village, Coteaux de Saumur and Entre deux mers vineyards, care should be taken when interpreting these data as they are based solely on analyses carried out with just 1 to 3 water quality stations.

For the Beaujolais-Village, Coteaux de Saumur and Entre deux mers vineyards, there is no quantification of glyphosate (or AMPA) greater than the 2 µg/L limit. For Beaujolais-Village, the rate of quantification of glyphosate ranged from 25 % to 33 %. There were two atypical years: 2009 with no quantification of glyphosate and 2014 where it was quantified in 5 or 6 samples.

For the stations in Coteaux de Saumur and Entre deux mers, there was a large variation between years in the rates of quantification: between 0 % and 43 % for Coteaux de Saumur and between 0 % and 75 % for Entre deux mers. Again, this was probably due to the small number of monitoring stations for these vineyards on which this data is based. For Entre deux mers, quantifications greater than 0.1 µg/L were observed in one-fifth to one-third of the data, depending on the year.

For Champagne, the rates of quantification of glyphosate are greater than when considering the rates of quantification of the 120 stations as a whole (> 40 % in 2009 and 2014, and > 50 % in 2008, 2012 and 2013). Also, for five of the seven years, more than a quarter of quantifications of glyphosate are greater than 0.1 µg/L. Several exceedances of 2 µg/L were seen.

For Cognac, frequencies of quantification of glyphosate in the water quality data > 0.1 µg/L were comparably less (5 % -10 %) than across the 120 stations studied for the six vineyards. There were some exceedances of the 2 µg/L limit.

The rates of quantification for glyphosate in Picpoul de Pinet et Hérault Languedoc were less than those at the other vineyards. Less than a quarter of the analyses exceeded the 0.1 µg/L drinking water limit and none exceeded 2 µg/L.

Maximum concentrations and 90th, 95th and 99th percentile concentrations

Overall, the maximum concentrations, and 90th, 95th and 99th percentiles are greater for AMPA than for glyphosate except for data associated with the Champagne vineyard. For Beaujolais, Coteaux de Saumur and Entre deux mers, the maximum concentrations never exceed 2 µg/L.

For Cognac, AMPA concentrations in excess of 50 µg/L were observed for four of the seven years and concentrations for glyphosate exceeded 10 µg/L in two years.

In Picpoul de Pinet et Hérault Langeudoc the maximum concentrations below 10 µg/L while in Champagne, the maximum concentrations exceed 10 µg/L.

There appears to be no logical explanation for the maximum concentrations. It is hypothesized that:

- The maximum value is caused by occasional point source pollution events upstream of the monitoring station with no dilution.
- The value is possibly an anomalous value in the database, e.g. a data transcription error, incorrect unit, etc.

Seasonality of quantifications
Quantifications of AMPA and glyphosate were studied according to their distribution by season and application timing to the vines. Autumn is defined as 15th October to 15th December, Winter is 1st February to 15th March, Spring is 15th March to 31st May and summer is 15th June to 31st July. Times not included in these seasonal definitions are defined as "the rest of the year".

Glyphosate is mainly applied between March and June. Analytical quantifications of glyphosate occurred mainly in the Spring. For AMPA, quantifications were mostly seen in the summer and "rest of the year". The fewest quantifications of both glyphosate and AMPA were in winter.

III. CONCLUSIONS

Phases 3 and 4 assess the presence of AMPA and glyphosate in surface waters associated with six vineyards across France in Beaujolais village, Champagne, Cognac, Coteaux de Saumur, Entre deux mers and Picpoul de Pinet et Languedoc-Hérault.

The number of water quality monitoring stations in each area was variable: 1 to 3 stations per year for Beaujolais village, Entre deux mers and Coteaux de Saumur; 10 to 20 stations for Picpoul de Pinet et Languedoc-Hérault and Champagne and more than 30 stations per year for Cognac. The same is true for the regularity of monitoring (number of samples per year per station). Regular monitoring occurred at stations located in Beaujolais Village, Champagne and Entre deux mers. For Cognac and Picpoul de Pinet et Languedoc-Hérault the number of stations is relatively high compared to the other vineyards but the monitoring is more irregular.

In comparison to the analysis made at national level (Phase 1), the frequency of quantification of AMPA was less at the monitoring stations associated with the vineyards (5-20 % less). The quantifications of AMPA and glyphosate generally followed the same overall variations year on year. In the vineyard stations the quantifications >0.1 µg/L represented one third of the data; those greater than 2 µg/L of AMPA represent 1-3 % of data.

The representativeness of stations and the analysis results of the actual vineyards themselves are very limited. For three vineyards there are four stations monitoring the water quality. Estimating the water quality of an area from a limited number of sampling points can introduce bias in the interpretation (point source pollution close to sampling count, inappropriate siting of the station, errors in sampling).

In addition, the placement of some sampling stations in certain areas does not allow good estimates of pollution arising from the vineyard. For example, in Beaujolais (area 393 km^2), the only station of the area is situated on the Saône (which drains many thousands of km^2) upstream from the confluence of the Ardière

which is the only water course that traverses the vineyard. The water quality observed at this sampling point is therefore largely independent of applications made in the Beaujolais village vineyard.

Also, for Coteaux de Saumur, two of the four stations in the area are on the Loire and the two others are downstream of Thouet which drains a basin much bigger than that of the vineyard.

For Entre deux mers, three stations are all sited to the East/South east of the area and one is situated on the Dropt which drains a basin much bigger than the vineyard.

For the three other vineyards the number and position of the stations gives a better estimate of the levels of contamination by glyphosate and AMPA in these areas.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the results of a surface water monitoring study for glyphosate and AMPA for six wine growing areas across France. The work looks at the contextualisation of monitoring data with reference to frequency of quantification and exceedance of regulatory drinking water limits for each vineyard. The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/033
Report author	[redacted]
Report year	2018a
Report title	Etude environnementale du Glyphosate et de l'AMPA à l'échelle des 10 points de surveillance les plus préoccupants pour le Glyphosate et pour l'AMPA. Analyse des suivis du Glyphosate et de l'AMPA en lien avec les bassins versants drainés par les stations de mesures et l'occupation des sols. Etudes des stations sur le glyphosate. (Environmental Study of Glyphosate and AMPA for the 10 most concerning locations for Glyphosate and AMPA. Analysis of Glyphosate and AMPA monitoring data with respect to their drained river areas and land use. Glyphosate Studies.)
Document No	Envilys Report Version 1 (2018)
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, but likely conducted at COFRAC accredited testing facilities
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

This document presents the water quality records of eight surface water quality monitoring stations. These stations were shortlisted from the SOES UIPP 2008-2014 dataset as they have reported the highest median and mean concentration values for glyphosate. These stations are also the only ones that can provide glyphosate data for 5 years between 2008 and 2014 (not necessarily over five consecutive years).

I. MATERIAL AND METHODS

The eight selected glyphosate monitoring stations are listed in Table 7.5-105 and their locations are shown in Figure 7.5-72. Each station record is split and presented over 3 parts, with each describing the regional landscape and hydrology, the rainfall and climate, and the water quality.

Table 7.5-105: List of 8 glyphosate monitoring stations

Station	Station Name	Name of surface water body	Agency
1023000	L'Erclin à Iwuy	L'Erclin	AEAP
3051120	Ru de Courtenain à Fontenailles	L'Almont	AESN
3080025	Yvron à Courpalay	L'Yvron	AESN
3112295	Morbras à Sucy en Brie	Le Morbras	AESN
3113218	Le ruisseau de Cubersault à Coizard-Joches	Le ruisseau de Cubersault	AESN
3167350	Ver sur Launette	La Launette	AESN
5013150	Terrier Raboin	Le Tourtrat	AEAG
5157100	St Caprais	La Sausse	AEAG

Figure 7.5-72: Location of the glyphosate monitoring stations

II. RESULTS AND DISCUSSION

Station 1 - L'Erclin à Iwuy (01023000)

River	L'Erclin	Number of water quality stations in the catchment	1
Catchment size (km^2)	161.598	Length of river (km)	69.97
Number of municipalities	38	Region	Nord

Landscape
The catchment is mostly agricultural (~88 %) with 80 % of the area comprised of arable land and 7 % grassland (but no vineyards). The remaining ~11 % are urban areas.

Rainfall
According to the meteorological data from the Météo France station at St Quentin, the average annual rainfall is 702.6 mm recorded over 122.5 rain days. The climate is temperate and rainfall occurs uniformly spread throughout the year, even in the summer months when the heaviest downpours occur.

Water quality
There were 35 glyphosate measurements taken between 13/02/2008 and 15/12/2014 (Table 7.5-106). The mean and median concentrations of glyphosate were 0.9 µg/L and 0.7 µg/L, respectively. The measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 7.2 µg/L) on two occasions. Approximately 92 % glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 41 AMPA measurements taken between 13/02/2008 and 15/12/2014 with the maximum concentration reported being 8.5 µg/L. The mean and median concentrations of AMPA were 5.6 µg/L and 2.4 µg/L, respectively. There were 7 analyses for AMPA that exceeded the threshold of 10 µg/L (20 % of measurements), 17 that exceeded 5 µg/L (17 % of measurements) and 22 measurements exceeded the threshold of 2 µg/L (54 % of measurements).

Table 7.5-106: Summary of glyphosate and AMPA concentration data at L'Erclin à Iwuy

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	0102300	35	13/02/2008	15/12/2008	0.932	0.672	7 22	1	32	1	1	na	na
AMPA	0102300	41	13/02/2008	15/12/2008	2.75	2.07	8 5	na	19	15	7	na	na

Ave = Average; Med = median; Max = maximum; NA – No Data

Station 2 - Ru de Courtenain à Fontenailles (03051120)

River	L'Almont	Number of stations	1
Area covered (km^2)	71.66	Length of river (km)	42.209
Number of municipalities	10	Region	Seine et Marne

Landscape
The catchment is mostly agricultural with ~63 % of the area comprised of arable land (but no vineyards), 1 % grassland, 29 % natural areas and the remaining 6 % urban areas.

Rainfall
According to the meteorological data from the Météo France station at Melun, the average annual rainfall is 676.9 mm recorded over 117.2 rain days. The climate is temperate and rainfall is uniformly distributed throughout the year. The least rainy month is February and the rainiest period is between May and October.

Water quality
There were 26 glyphosate and AMPA measurements taken between 17/09/2008 and 11/04/2014 (Table 7.5-107). The mean and median concentrations of glyphosate were 1.2 µg/L and 0.5 µg/L, respectively. The measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of

4.2 µg/L) on seven occasions. Approximately 69 % glyphosate measurements were between 0.1 µg/L and 2 µg/L.

The mean and median concentrations of AMPA were 10.8 µg/L and 4 µg/L, respectively. There were ten analyses for AMPA that exceeded the threshold of 5 µg/L (38 % of measurements) and 21 records exceeded 2 µg/L (81 % of measurements). Two measurements exceeded 50 µg/L. The AMPA concentrations seemed to increase during the 2013-2014 period.

Table 7.5-107: Summary of glyphosate and AMPA concentration data at Ru de Courtenain à Fontenailles

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	03051120	26	17/09/2008	04/11/2014	1.17	0.531	4.18	1	18	7	na	na	na
AMPA	03051120	26	17/09/2008	04/11/2014	10.8	4.04	61.4	na	5	11	3	5	2

na – no data; Ave – average; Med – Median; Max – Maximum

Station 3 – Yvron à Courpalay (03080025)

River	L'Yvron	Number of stations	1
Area covered (km^2)	156.986	Length of river (km)	85.358
Number of municipalities	24	Region	Seine-et-Marne

Landscape
The catchment is mostly agricultural with ~90 % of the area comprised of arable land (but no vineyards). Natural areas cover 8 % of the total area with urban areas making up the remaining 2 %.

Rainfall
According to the meteorological data from the Météo France station at Melun, the average annual rainfall is 676.9 mm recorded over 117.2 rain days. The climate is temperate with rainfall spread quite homogeneously throughout the year. The least rainy month is February while May and October register the most rainfall.

Water quality
There were 31 glyphosate measurements taken between 15/07/2008 and 11/11/2014 (Table 7.5-108). The mean and median concentrations of glyphosate were 2 µg/L and 0.8 µg/L, respectively. The measured concentrations exceeded the threshold of 10 µg/L (maximum concentration of 13.1 µg/L) twice and the threshold of 2 µg/L eleven times. Approximately 83 % glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 30 AMPA measurements taken between 15/07/2008 and 11/11/2014. The mean and median concentrations of AMPA were 3.1 µg/L and 1.1 µg/L, respectively. There were seven analyses of AMPA that exceeded the threshold of 5 µg/L (23 % of measurements). Approximately 60 % of AMPA measurements were between 0.1 µg/L and 2 µg/L. The concentrations of AMPA seemed to decrease during the 2013-2014 period.

Table 7.5-108: Summary of glyphosate and AMPA concentration data at Yvron à Courpalay

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0 1 – 2	2-5	5-10	10-50	>50
GLY	03080025	31	15/07/2008	11/11/2014	1.96	0.752	13.1	3	17	9	na	2	na
AMPA	03080025	30	15/07/2008	11/11/2014	3.14	1.13	13	3	18	2	4	3	3

na – no data; Ave – average; Med – Median; Max – Maximum

Station 4 – Morbras à Sucy en Brie (03112295)

River	Le Morbras	Number of stations	1
Area covered (km^2)	50.06	Length of river (km)	30.637
Number of municipalities	17	Region	Seine-et-Marne, Seine-St-Denis, Val-de-Marne

Landscape
The catchment is characterized by a high coverage (49 %) of urban areas plus 6 % parks and gardens. Natural areas (26 %) are at the head of the catchment. Agricultural area (19 %) extend over the whole catchment and include 15 % cropped arable land.

Rainfall
According to the meteorological data from the Météo France station at d'Orly, the average annual rainfall is 616.6 mm recorded over 109.7 rain days. The climate is temperate and rainfall occurrence is homogeneous throughout the year. The least rainy month is February while the months of May, August and October register the most rainfall.

Water quality
There were 34 glyphosate measurements taken between 16/07/2008 and 13/05/2014 (Table 7.5-109). The mean and median concentrations of glyphosate were 0.9 µg/L and 0.5 µg/L, respectively. The measured concentrations exceeded the thresholds of 5 µg/L (maximum concentration of 9.9 µg/L) and 2 µg/L on one and two occasions, respectively. Approximately 85 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 36 AMPA measurements taken between 16/07/2008 and 07/07/2014. The mean and median concentrations of AMPA were both 1.3 µg/L. There were 6 analyses of AMPA that exceeded the threshold of 5 µg/L (17 % of measurements) and 14 records exceeded 2 µg/L (17 % of measurements). Approximately 81 % of AMPA measurements were between 0.1 µg/L and 2 µg/L.

Table 7.5-109: Summary of glyphosate and AMPA concentration data at Morbras à Sucy en Brie

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	03112295	34	16/07/2008	13/05/2014	0.879	0.474	9.88	3	29	1	1	na	na
AMPA	03112295	36	16/07/2008	07/07/2014	1.3	1.31	3.6	1	29	6	na	na	na

na – no data; Ave – average; Med – Median; Max – Maximum

Station 5 – Le ruisseau de Cubersault à Coizard-Joches (03113218)

River	Le ruisseau de Cubersault	Number of stations	1
Area covered (km^2)	29.992	Length of river (km)	14.188
Number of municipalities	9	Region	Marne

Landscape
The catchment is predominantly agricultural with ~66 % of the area comprised of arable land, 1 % grassland and 4 % mixed agricultural and natural areas. Vineyard coverage is 14 %, natural areas 10 % and urban areas make up 4 % of the remaining catchment area.

Rainfall
According to the meteorological data from the Météo France station at Troyes, the average annual rainfall is 644.8 mm recorded over 114.5 rain days. The climate is temperate coastal, with considerable rainfall during the spring and autumn. Summer is the least rainy season but thunderstorm and hailstone events can occur.

Water quality
There were 31 glyphosate measurements taken between 06/08/2008 and 06/04/2014 (Table 7.5-110). The mean and median concentrations of glyphosate were 1.1 µg/L and 0.8 µg/L, respectively. The measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 4.6 µg/L) on four occasions. Approximately 81 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 27 AMPA measurements taken between 06/08/2008 and 06/04/2014. The mean and median concentrations of AMPA were 0.8 µg/L and 0.7 µg/L, respectively. There was one analysis of AMPA that exceeded the threshold of 2 µg/L. Approximately 89 % of measurements were between 0.1 µg/L and 2 µg/L. The concentrations of AMPA and glyphosate seemed to decrease during the 2011-2014 period.

Table 7.5-110: Summary of glyphosate and AMPA concentration data at *Le ruisseau de Cubersault à Coizard-Joches*

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	03113218	31	06/08/2008	06/04/2014	1.14	0.831	4.59	2	25	4	na	na	na
AMPA	03113218	27	06/08/2008	06/04/2014	0.816	0.747	2.59	2	24	1	na	na	na

na – no data; Ave – average; Med – Median; Max – Maximum

Station 6 – Ver sur Launette (03167350)

River	La Launette	Number of stations	1
Area covered (km^2)	39.949	Length of river (km)	28.013
Number of municipalities	12	Region	Oise, Seine-et-Marne

Landscape
The catchment is mostly agricultural with ~71 % of the area comprised of arable land (but no vineyards) and 2 % grasslands. Natural areas cover 29 % of the total area and urban areas make up the remaining ~19 % of the catchment area.

Rainfall
According to the meteorological data from the Météo France station at Roissy-en-France, the average annual rainfall is 693.6 mm recorded over 116.8 rain days. The climate is temperate with rainfall spread homogeneously during the year. The least rainy month is February while the months of May, October and December register the most rainfall.

Water quality
There were 38 glyphosate measurements taken between 21/07/2008 and 24/11/2014 (Table 7.5-111). The mean and median concentrations of glyphosate were 1 µg/L and 0.9 µg/L, respectively. The measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 2.9 µg/L) on four occasions. Approximately 87 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 35 AMPA measurements taken between 21/07/2008 and 24/11/2014. The mean and median concentrations of AMPA were 4.1 µg/L and 3.7 µg/L, respectively. There were 10 analyses of AMPA that exceeded the threshold of 5 µg/L (29 % of measurements) and 28 records exceeded 2 µg/L (80 % of measurements). The highest AMPA concentrations were measured during the 2009-2012 period.

Table 7.5-111: Summary of glyphosate and AMPA concentration data at Ver sur Launette

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	03167350	38	21/07/2008	24/11/2014	0 964	0 923	2.86	1	33	4	na	na	na
AMPA	03167350	35	21/07/2008	24/11/2014	4.11	3.69	15.9	NA	7	18	9	1	na

na – no data; Ave – average; Med – Median; Max – Maximum

Station 7 – Terrier Raboin (05013150)

River	Le Tourtrat	Number of stations	2
Area covered (km^2)	68.498 km^2	Length of river (km)	24.286
Number of municipalities	12	Region	Charente, Charente-Maritime

Landscape

The catchment is mostly agricultural with ~88 % of the area comprised of arable land, 27 % mixed arable and natural areas and 24 % vineyards interspersed with and surrounded by arable land. Urban areas make up the remaining 3 % of the catchment area.

Rainfall

According to the meteorological data from the Météo France station at Cognac, the average annual rainfall is 777.1 mm recorded over 117 rain days. The climate is oceanic "Aquitaine" with considerably more rainfall between October and January than the summer which is the least rainy season.

Water quality

There were 25 glyphosate measurements taken between 13/05/2008 and 24/11/2014 (Table 7.5-112). The mean and median concentrations of glyphosate were 2.2 µg/L and 0.8 µg/L, respectively. The measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 11 µg/L) on nine occasion (36 % of measurements). Approximately 52 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 29 AMPA measurements taken between 18/03/2008 and 24/11/2014. The mean and median concentrations of AMPA were 28.7 µg/L and 5.7 µg/L, respectively. There were 19 analyses of AMPA that exceeded the threshold of 2 µg/L and the maximum concentration of AMPA recorded was 106 µg/L in 2010.

Table 7.5-112: Summary of glyphosate and AMPA concentration data at Terrier Raboin

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	05013150	25	13/05/2009	24/11/2014	2.22	0.81	11	3	13	4	4	1	na
AMPA	05013150	29	18/03/2009	24/11/2014	28.7	5.7	106	na	10	4	3	4	8

na – no data; Ave – average; Med – Median; Max – Maximum

Station 8 – St Caprais (05157100)

River	La Sausse	Number of stations	1
Area covered (km^2)	114.937	Length of river (km)	152.196
Number of municipalities	24	Region	Haute-Garonne

Landscape

The catchment is mostly agricultural with ~74 % of the area comprised of arable land (no vineyards), 11 % mixed arable and natural areas and ~4 % natural areas. Urban areas make up the remaining ~11 % of the catchment area.

Rainfall
According to the meteorological data from the Météo France station at Toulouse-Blagnac, the average annual rainfall is 638.3 mm recorded over 95.7 rain days. The climate is temperate akin to a Mediterranean climate whereby spring is wettest and summer the driest seasons.

Water quality
There were 25 glyphosate measurements taken between 16/03/2008 and 27/11/2014 (Table 7.5-113). The mean and median concentrations of glyphosate were 0.9 µg/L and 0.5 µg/L, respectively. The measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 3.6 µg/L) on four occasions. Approximately 76 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 27 AMPA measurements taken between 16/03/2008 and 27/11/2014. The mean and median concentrations of AMPA were 2.6 µg/L and 1.5 µg/L, respectively. There were eleven analyses of AMPA that exceeded the threshold of 2 µg/L (40 % of measurements) and 16 measurements were between 0.1 µg/L and 2 µg/L (60 % measurements).

Table 7.5-113: Summary of glyphosate and AMPA concentration data at St Caprais

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	05157100	25	16/03/2009	27/11/2014	0.866	0.53	3.6	2	19	4	na	na	na
AMPA	05157100	27	16/03/2009	27/11/2014	2.65	1.5	11	na	16	7	3	1	na

na – no data; Ave – average; Med – Median; Max – Maximum

III. CONCLUSIONS

This document presents the water quality records of eight surface water quality monitoring stations. These stations were shortlisted from the SOES UIPP 2008-2014 dataset as they have reported the highest median and mean concentration values for glyphosate. These stations are also the only ones that can provide glyphosate data for 5 years between 2008 and 2014 (not necessarily over five consecutive years).

Analytics are not described but the analyses were likely conducted by COFRAC accredited laboratories.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes results from analyses of 8 water quality monitoring stations with elevated glyphosate concentrations. Analytics are not described but the analyses were likely conducted by COFRAC accredited laboratories.

The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/034
Report author	[redacted]
Report year	2018b
Report title	Etude environnementale du Glyphosate et de l'AMPA à l'échelle des 10 points de surveillance les plus préoccupants pour le Glyphosate et pour l'AMPA. Analyse des suivis du Glyphosate et de l'AMPA en lien avec les bassins versants drainés par les stations de mesures et l'occupation des sols. Etudes des stations sur l'AMPA. Environmental Study of Glyphosate and AMPA for the 10 most concerning locations for Glyphosate and AMPA. Analysis of Glyphosate and AMPA monitoring data with respect to their drained river areas and land use. AMPA Studies.
Document No	Envilys Report Version 1 (2018)
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, but likely conducted at COFRAC accredited testing facilities.
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

This document presents the water quality records of ten surface water quality monitoring stations. These stations were shortlisted from the SOES UIPP 2008-2014 dataset as they have reported the highest median and mean concentration values for AMPA. These stations are also the only ones that can provide AMPA data for 5 years between 2008 and 2014 (not necessarily over five consecutive years).

I. MATERIAL AND METHODS

The 10 selected AMPA monitoring stations are listed in Table 7.5-114 and their locations are shown in Figure 7.5-73. Each station record is split and presented over 3 parts, with each describing the regional landscape and hydrology, the rainfall and climate, and the water quality.

Table 7.5-114: List of 10 AMPA monitoring stations

Station	Station Name	River Name	Agency
1075000	La Becque de Steenwerck à Steenwerck	La Becque de Steenwerck	AEAP
1089000	L'Yser à Bambecque	L'Yser	AEAP
3051120	Ru de Courtenain à Fontenailles	L'Almont	AESN
3051250	Ru d'Ancoeur à St Ouen en Brie	L'Almont	AESN
3129440	Boué	Le Morteau	AESN
3167350	Ver sur Launette	La Launette	AESN
4143150	Sangueze à Le Pallet	La Sanguèze	AELB
5013150	Terrier Raboin	Le Tourtrat	AEAG
6169050	Alenya	L'Agulla de la Mar	AERMC
6196948	Raumartin	Le Raumartin	AERMC

Figure 7.5-73: Location of the AMPA monitoring stations

II. RESULTS AND DISCUSSION

Station 1 - La Becque de Steenwerck à Steenwerck (01075000)

River	La Becque de Steenwerck	Number of stations	1
Area covered (km^2)	69.908	Length of river (km)	63.795
Number of Municipalities	9	County/Region	Nord

Landscape

The catchment is mostly agricultural with ~90 % of the area comprised of arable land (but no vineyards) and urban areas (10 %).

Rainfall
According to the meteorological data from the Météo France station at Lille-Lesquin, the average annual rainfall is 742.5 mm recorded over 127.4 rain days. The climate is temperate oceanic and downpours are a regular occurrence all year.

Water quality
There were 36 glyphosate measurements taken between 22/07/2008 and 23/12/2014 (Table 7.5-115). The mean and median concentrations of glyphosate were 0.6 µg/L and 0.5 µg/L, respectively. Measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 2.2 µg/L) on one occasion. Approximately 92 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 41 AMPA measurements taken between 18/02/2008 and 23/12/2014. The mean and median concentrations of AMPA were 5.6 µg/L and 2.4 µg/L, respectively. There were 8 analyses of AMPA that exceeded the threshold of 10 µg/L (20 % of measurements) and 17 records exceeded 5 µg/L (41 % of measurements).

Table 7.5-115: Summary of glyphosate and AMPA concentration data at La Becque de Steenwerck à Steenwerck

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	01075000	36	22/07/2008	23/12/2014	0.553	0.477	2.22	2	33	1	na	na	na
AMPA	01075000	41	18/02/2008	23/12/2014	5.57	2.42	40.3	4	16	4	9	8	na

na – no data; Ave – average; Med – Median; Max - Maximum

Station 2 - L'Yser à Bambecque (01089000)

River	L'Yser	Number of stations	1
Area covered (km^2)	378.628	Length of river (km)	277.353
Number of Municipalities	46	County/Region	Nord

Landscape
The region is mostly agricultural with ~97 % of the area comprised of arable land (but no vineyards) with 1 % grasslands and the remaining areas being urban areas.

Rainfall
According to the meteorological data from Météo France station at Dunkerque, the average annual rainfall is 697.8 mm recorded over 121.6 rain days. The climate is temperate oceanic and heavy rainfall is a regular occurrence during autumn and the beginning of winter.

Water quality
There were 30 glyphosate measurements taken between 20/02/2008 and 17/10/2014 (Table 7.5-116). The mean and median concentrations of glyphosate were 0.7 µg/L and 0.4 µg/L, respectively. Measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 2.2 µg/L) on one occasion. Approximately 55 % glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 30 AMPA measurements taken between 20/02/2008 and 17/06/2014. The mean and median concentrations of AMPA were 4.3 µg/L and 1.6 µg/L, respectively. There were 6 analyses of AMPA that exceeded the threshold of 10 µg/L (20 % of measurements) and 14 that exceeded 5 µg/L (46 % of measurements).

Table 7.5-116: Summary of glyphosate and AMPA concentration data at L'Yser à Bambecque

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	01089000	30	20/02/2008	17/10/2014	0.671	0.41	5.03	4	25	na	1	na	na
AMPA	01089000	30	20/02/2008	17/06/2014	4 25	1.57	18.4	na	16	5	3	6	na

na – no data; Ave – average; Med – Median; Max - Maximum

Station 3 - Ru de Courtenain à Fontenailles (03051120)

River	L'Almont	Number of stations	1
Area covered (km^2)	71.66	Length of river (km)	42.209
Number of Municipalities	10	County/Region	Seine-et-Marne

Landscape

The catchment is mostly agricultural with ~63 % of the area comprised of arable land (but no vineyards) and 1 % grasslands. Natural landcover accounts for 29 % of the total area and urban areas make up the remaining 6 %.

Rainfall

According to the meteorological data from the Météo France station at Melun, the average annual rainfall is 676.9 mm recorded over 117.2 rain days. The climate is warm and temperate with rainfall spread quite homogeneously throughout the year. The least rainy month is February while May and October register the most rainfall.

Water quality

There were 26 glyphosate and AMPA measurements taken between 17/09/2008 and 04/11/2014 (Table 7.5-117). The mean and median concentrations of glyphosate were 1.2 µg/L and 0.5 µg/L, respectively. Measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 4.2 µg/L) on seven occasions. Approximately 69 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

The mean and median concentrations of AMPA were 10.8 µg/L and 4 µg/L, respectively. There were 10 analyses of AMPA that exceeded the threshold of 5 µg/L (38 % of measurements) and 21 records exceeded 2 µg/L (81 % of measurements). Two measurements exceeded 50 µg/L. The concentrations of AMPA seemed to increase during the 2013-2014 period.

Table 7.5-117: Summary of glyphosate and AMPA concentration data at Ru de Courtenain à Fontenailles

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	03051120	26	17/09/2008	04/11/2014	1.17	0.531	4.18	1	18	7	na	na	na
AMPA	03051120	26	17/09/2008	04/11/2014	10.8	4.04	61.4	na	5	11	3	5	2

na – no data; Ave – average; Med – Median; Max - Maximum

Station 4 - Ru d'Anoeur à St Ouen en Brie (03051250)

River	L'Almont	Number of stations	2
Area covered (km^2)	101.391	Length of river (km)	60.622
Number of Municipalities	16	County/Region	Seine-et-Marne

Landscape
The catchment is comprised predominantly of agricultural and natural vegetation with 67 % and 27 % of the area, respectively (but not vineyards). The urban areas comprise 9 % of the catchment area.

Rainfall
According to the meteorological data from Météo France station at Dunkerque, the average annual rainfall is 697.8 mm recorded over 121.6 rain days. The climate is temperate oceanic and heavy rainfall is a regular occurrence during autumn and the beginning of winter.

Water quality
There were 33 glyphosate measurements taken between 17/09/2008 and 04/11/2014 (Table 7.5-118). The mean and median concentrations of glyphosate were 0.6 µg/L and 0.5 µg/L, respectively. Measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 2.6 µg/L) on one occasion. Approximately 88 % glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 34 AMPA measurements taken between 17/09/2008 and 04/11/2014. The mean and median concentrations of AMPA were 3.4 µg/L and 1.8 µg/L, respectively. There were 6 analyses of AMPA that exceeded the threshold of 5 µg/L (17 % of measurements) and 14 records exceeded 2 µg/L (41 % of measurements). The concentrations of AMPA seemed to increase during the 2013-2014 period.

Table 7.5-118: Summary of glyphosate and AMPA concentration data at Ru d'Ancoeur à St Ouen en Brie

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	03051250	33	17/09/2008	04/11/2014	0.592	0.5	2.56	3	29	1	na	na	na
AMPA	03051250	34	17/09/2008	04/11/2014	3.36	1.79	16.9	1	16	8	2	4	na

na – no data; Ave – average; Med – Median; Max - Maximum

Station 5 - Boué (03129440)

River	Le Marteau	Number of stations	1
Area covered (km^2)	37.31	Length of river (km)	51.283
Number of Municipalities	8	County/Region	Aisne

Landscape
The catchment is covered with grasslands (47 %) on the right river bank and with woodland and natural vegetation (46 %) on the left river bank (but not vineyards). Urban areas make up 9 % of the catchment, of which 3 % are gardens and parks.

Rainfall
According to the meteorological data from the Météo France station at Cognac, the average annual rainfall is 702.6 mm recorded over 122.5 rain days. The climate is warm and temperate, with abundant rainfall uniformly distributed throughout the year.

Water quality
There were 28 glyphosate measurements taken between 09/07/2008 and 18/05/2014 (Table 7.5-119). The mean and median concentrations of glyphosate were 0.3 µg/L and 0.2 µg/L, respectively. Measured concentrations never exceeded the threshold of 2 µg/L while 75 % of glyphosate measurements were between 0.1 and 2 µg/L.

There were 33 AMPA measurements taken between 09/07/2008 and 18/05/2014. The mean and median concentrations of AMPA were 7 µg/L and 3.8 µg/L, respectively. There were 13 analyses of AMPA that exceeded the threshold of 5 µg/L (39 % of measurements) and 25 records exceeded 2 µg/L (76 % of measurements). The concentrations of AMPA seemed to decrease during the 2012-2014 period.

Table 7.5-119: Summary of glyphosate and AMPA concentration data at Boué

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	03129440	28	09/07/2008	18/05/2014	0 265	0 185	0.83	7	21	na	na	na	na
AMPA	03129440	33	09/07/2008	18/05/2014	6.98	3.77	24	4	4	12	5	8	na

na – no data; Ave – average; Med – Median; Max - Maximum

Station 6 - Ver sur Launette (03167350)

River	La Launette	Number of stations	1
Area covered (km²)	39.949	Length of river	28.013
Number of Municipalities	12	County/Region	Oise, Seine-et-Marne

Landscape

The catchment is mostly agricultural with ~71 % of the area comprised of arable land (but no vineyards) and with 2 % grasslands. Natural vegetation covers 29 % of the total area and urban areas make up the remaining ~19 %.

Rainfall

According to the meteorological data from the Météo France station at Roissy-en-France, the average annual rainfall is 693.6 mm recorded over 116.8 rain days. The climate is warm and temperate with rainfall spread quite homogeneously throughout the year. The least rainy month is February while May and October register the most rainfall.

Water quality

There were 38 glyphosate measurements taken between 21/07/2008 and 24/11/2014 (Table 7.5-120). The mean and median concentrations of glyphosate were 1 µg/L and 0.9 µg/L, respectively. Measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 2.9 µg/L) on four occasions. Approximately 87 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 35 AMPA measurements taken between 21/07/2008 and 24/11/2014. The mean and median concentrations of AMPA were 4.1 µg/L and 3.7 µg/L, respectively. There were 10 analyses of AMPA that exceeded the threshold of 5 µg/L (29 % of measurements) and 28 records exceeded 2 µg/L (80 % of measurements). The highest concentrations of AMPA were measured during the 2009-2012 period. The concentrations of AMPA seemed to increase during the 2013-2014 period.

Table 7.5-120: Summary of glyphosate and AMPA concentration data at Ver sur Launette

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	03167350	38	21/07/2008	24/11/2014	0 964	0 923	2.86	1	33	4	na	na	na
AMPA	03167350	35	21/07/2008	24/11/2014	4.11	3.69	15.9	na	7	18	9	1	na

na – no data; Ave – average; Med – Median; Max - Maximum

Station 7 - Sangueze à Le Pallet (04143150)

River	La Sangueze	Number of stations	1
Area covered (km²)	159.643	Length of river (km)	138.416
Number of Municipalities	8	County/Region	Loire-Atlantique, Maine-et-Loire

Landscape

The catchment is predominantly agricultural with ~92 % of the area comprised of arable land of which 32 % are field crops, 23 % are areas of mixed arable and natural landcover, 20 % improved grass and 17 % vineyards. Urban areas make up 4 % of the remaining catchment area.

Rainfall
According to the meteorological data from the Météo France station at Nantes, the average annual rainfall is 819.5 mm recorded over 119.1 rain days. The climate is temperate oceanic with frequent rainfall and occasional heavy storm events. The rainiest period is winter.

Water quality
There were 34 glyphosate measurements taken between 14/04/2008 and 03/12/2014 (Table 7.5-121). The mean and median concentrations of glyphosate were 0.7 µg/L and 0.3 µg/L, respectively. Measured concentrations exceeded the threshold of 5 µg/L (maximum concentration of 6.1 µg/L) on one occasion. Approximately 67 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 40 AMPA measurements taken between 10/03/2008 and 03/12/2014. The mean and median concentrations of AMPA were 12.1 µg/L and 5.1 µg/L, respectively. There were 14 analyses of AMPA that exceeded the threshold of 10 µg/L (35 % of measurements) and 27 records exceeded 2 µg/L (67 % of measurements).

Table 7.5-121: Summary of glyphosate and AMPA concentration data at Sangueze à Le Pallet

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	04143150	34	14/04/2010	03/12/2014	0.69	0.27	6.07	8	23	2	1	na	na
AMPA	04143150	40	10/03/2010	03/12/2014	12.1	5.12	48.4	na	13	7	6	14	na

na – no data; Ave – average; Med – Median; Max - Maximum

Station 8 - Terrier Raboin (05013150)

River	Le Tourtrat	Number of stations	2
Area covered (km^2)	68.498	Length of river (km)	24.286
Number of Municipalities	12	County/Region	Charente, Charente-Maritime

Landscape
The catchment is mostly agricultural with ~88 % of the area comprised of arable land, of which 27 % is mixed arable land and natural areas and 24 % vineyards interspersed between and surrounded by arable land. Urban areas make up 3 % of the remaining catchment area.

Rainfall
According to the meteorological data from the Météo France station at Cognac, the average annual rainfall is 777.1 mm recorded over 117 rain days. The climate is of the oceanic "Aquitaine" type with frequent rainfall spread between October and January while summer is the least rainy season.

Water quality
There were 25 glyphosate measurements taken between 13/05/2008 and 24/11/2014 (Table 7.5-122). The mean and median concentrations of glyphosate were 2.2 µg/L and 0.8 µg/L, respectively. Measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 11 µg/L) on nine occasion (36 % of measurements). Approximately 52 % glyphosate measurements were between 0.1 µg/L and 2 µg/L.

There were 29 AMPA measurements taken between 18/03/2009 and 24/11/2014. The mean and median concentrations of AMPA were 28.7 µg/L and 5.7 µg/L, respectively. There were19 analyses for AMPA

that exceeded the threshold of 2 µg/L and the maximum concentration of AMPA recorded was 106 µg/L in 2010.

Table 7.5-122: Summary of glyphosate and AMPA concentration data at Terrier Raboin

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	05013150	25	13/05/2009	24/11/2014	2.22	0.81	11	3	13	4	4	1	na
AMPA	05013150	29	18/03/2009	24/11/2014	28.7	5.7	106	na	10	4	3	4	8

na – no data; Ave – average; Med – Median; Max - Maximum

Station 9 - Alenya (06169050)

River	L'Agulla de la Mar	Number of stations	1
Area covered (km^2)	53.852	Length of river (km)	18.879
Number of Municipalities	14	County/Region	Pyrénées-Orientales

Landscape
The majority of the catchment is involved in wine production (44 %) while a further 41 % of the area is used for other agriculture. Urban areas make 10 % of the remaining catchment area.

Rainfall
According to the meteorological data from the Météo France station at Perpignan, the average annual rainfall is 557.6 mm recorded over 54 rain days. The climate is 'Mediterranean' with frequent wet weather in autumn and winter and dryer conditions in the summer, notably August. This region is subject to periodic downpours over just a couple of hours.

Water quality
There were 38 glyphosate measurements taken between 25/02/2008 and 03/12/2014 (Table 7.5-123). The mean and median concentrations of glyphosate were 0.6 µg/L and 0.2 µg/L, respectively. Measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 6.1 µg/L) on two occasions. Approximately 82 % of glyphosate measurements are between 0.1 µg/L and 2 µg/L.

There were 34 AMPA measurements taken between 25/02/2008 and 09/06/214. The mean and median concentrations of AMPA were 4.7 µg/L and 2.9 µg/L, respectively. There were nine analyses of AMPA that exceeded the threshold of 2 µg/L (26 % of measurements; maximum concentration of 24.3 µg/L recorded in 2014). Approximately 50 % of AMPA measurements were between 0.1 µg/L and 2 µg/L.

Table 7.5-123: Summary of glyphosate and AMPA concentration data at Alenya

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	06169050	38	25/02/2008	03/12/2014	0 592	0 231	7.4	5	31	1	1	na	na
AMPA	06169050	34	25/02/2008	09/06/2014	4.69	2.93	24.3	na	8	17	6	3	na

na – no data; Ave – average; Med – Median; Max - Maximum

Station 10 - Raumartin (06196948)

River	La Raumartin	Number of stations	1
Area covered (km^2)	26.369	Length of river (km)	9.829
Number of Municipalities	8	County/Region	Bouches-du-Rhône

Landscape
The catchment is quite diverse in terms of landcover. Urban areas make up 27 % of the total area largely in the lower portions of the catchment. The mid portion of the catchment is dominated by vineyards while natural areas comprise 46 %of the remainder of the catchment.

Rainfall
According to the meteorological data from the Météo France station at Marignane, the average annual rainfall is 515.4 mm recorded over 53.2 rain days. The climate is "Mediterranean" with a very short wet season in autumn and early Winter. A very dry period occurs between June and August.

Water quality
There were 16 glyphosate and AMPA measurements taken between 23/02/2008 and 29/06/2014 (Table 7.5-124). The mean and median concentrations of glyphosate were 0.5 µg/L and 0.3 µg/L, respectively. Measured concentrations exceeded the threshold of 2 µg/L (maximum concentration of 2.2 µg/L) on one occasion. Approximately 81 % of glyphosate measurements were between 0.1 µg/L and 2 µg/L.

The mean and median concentrations of AMPA were 6.3 µg/L and 2.2 µg/L, respectively. There were 9 analyses of AMPA that exceeded the threshold of 2 µg/L (47 % of measurements; maximum concentration of 25.2 µg/L recorded in 2014). None of the records were less than 0.1 µg/L and numerous peaks in the measurements were observed above 5 µg/L between 2012 and 2014.

Table 7.5-124: Summary of glyphosate and AMPA concentration data at Raumartin

Compound	Station	Number analyses	Start date	End date	Ave	Med	Max	Measured concentrations (µg/L)					
								<0.1	0.1 – 2	2-5	5-10	10-50	>50
GLY	06196948	16	23/02/2010	29/06/2014	0.468	0 267	2.22	2	13	1	na	na	na
AMPA	06196948	16	23/02/2010	29/06/2014	6.3	2.22	25.2	na	7	5	1	3	na

na – no data; Ave – average; Med – Median; Max - Maximum

III. CONCLUSIONS

This document presents the water quality records of ten surface water quality monitoring stations. These stations were shortlisted from the SOES UIPP 2008-2014 dataset as they have reported the highest median and mean concentration values for AMPA. These stations are also the only ones that can provide AMPA data for 5 years between 2008 and 2014 (not necessarily over five consecutive years).

Analytics are not described but the analyses were likely conducted by COFRAC accredited laboratories

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes results from analyses of 10 water quality monitoring stations with elevated AMPA concentrations. Analytics are not described but the analyses were likely conducted by COFRAC accredited laboratories.
The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/009
Report author	[redacted]
Report date	2016
Report title	Analyse des données de suivi du glyphosate et de l'AMPA dans les eaux de France - Période 1997-2013 (Analysis of monitoring data for glyphosate and AMPA in French waters – Time period 1997-2013)
Document No	Rapport_AMPA_Glyphosate_1997-2013(V3)
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, but likely conducted by COFRAC approved testing facilities
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

The study is relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/010
Report author	[redacted]
Report year	2016
Report title	Survey of glyphosate and AMPA in groundwaters and surface waters in Europe - 2015/16 update review – final report
Report No	MSL0027535
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

The study is relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

Existing studies/assessments

1. Information on the study

Data point:	CA 7.5/013
Report author	[redacted]
Report year	2012
Report title	Survey of glyphosate and AMPA in groundwaters and surface waters in Europe
Report No	-
Document No	BVL No. 2310291
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (no experimental work performed)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The study is relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/035
Report author	[redacted]
Report year	1972
Report title	Run-off of MON-0573 from Inclined Soil Beds
Report No	AgRR 275
Document No	
Guidelines followed in study	US EPA Guidelines for Registering Pesticides, 2nd draft, 5-1-72, part XI
GLP	No
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Study type: run-off from inclined soil beds Test item: [^{14}C] glyphosate, phosphonomethyl-label (97 % radiochemical purity) Test soil (type): Ray (silt loam), Norfolk (sandy loam), Drummer (silt clay loam) pH: 6.5, 5.7, 7.0 (medium not stated)

	Organic matter: 0.6 %, 0.6 %, 3.5 % Application rate: 1.12 kg a.s./ha; application was made to the upper third of the soil surface with a laboratory sprayer Test design: steel trays (91 x 30 x 15 cm), filled to 11-13 cm; inclined (7.5°) after application, watering of the soil led to unwanted leaching out of the test vessels; for each sampling, artificial rainfall equivalent to 19.05 mm/h was applied until collection of 2 x 50 mL samples of run-off water Sampling: 1, 3 and 7 days after treatment Workup: centrifugation, decantation Analysis of radioactivity: Runoff-water: LSC Runoff-sediment: combustion/LSC
Short description of results:	Radioactivity in run-off samples at day 1 / 3 / 7 (% AR, mean of 2 replicate samples): Ray soil Supernatant: 0.0045 / 0.0010 / 0.0003 Sediment: 0.0019 / 0.0016 / 0.0008 Total: 0.0064 / 0.0026 / 0.0011 Sum after 7 days: 0.0101 Drummer soil Supernatant: 0.0002 / 0.0013 / 0.0008 Sediment: 0.00004 / 0.0001 / 0.00001 Total: 0.0002 / 0.0014 / 0.0008 Sum after 7 days: 0.0042 Norfolk soil Supernatant: 0.0064 / 0.0007 / 0.0002 Sediment: 0.0031 / 0.0002 / 0.0002 Total: 0.0095 / 0.0009 / 0.0004 Sum after 7 days: 0.0108 The results show a maximum total run-off amount of about 0.01 % AR.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered invalid due to the following deficiencies: - Study type is not relevant to the data requirement - No substance-specific analysis performed - Experimental conditions cannot be transferred to field scale and are therefore not relevant for risk assessment - Uncontrolled leaching out of the test vessels
Category study in AIR 5 dossier (L docs)	Category 3b

Relevant literature articles

1. Information on the study

Data point:	CA 7.5/036
Report author	Di Guardo, A., Finizio, A.
Report year	2018

Report title	A new methodology to identify surface water bodies at risk by using pesticide monitoring data: The glyphosate case study in Lombardy Region (Italy)
Document No	*Science of the Total Environment 610–611 (2018) 421–429*
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

In the last decades, several monitoring programs were established as an effect of EU Directives addressing the quality of water resources (drinking water, groundwater and surface water). Plant Protection Products (PPPs) are an obvious target of monitoring activities, since they are directly released into the environment. One of the challenges in managing the risk of pesticides at the territorial scale is identifying the locations in water bodies needing implementation of risk mitigation measures. In this, the national pesticides monitoring plans could be very helpful. However, monitoring of pesticides is a challenging task because of the high number of registered pesticides, cost of analyses, and the periodicity of sampling related to pesticide application and use. Extensive high-quality data-sets are consequently often missing. More in general, the information that can be obtained from monitoring studies are frequently undervalued by risk managers. In this study, we propose a new methodology providing indications about the need to implement mitigation measures in stretches of surface water bodies on a territory by combining historical series of monitoring data and GIS. The methodology is articulated in two distinct phases: a) acquisition of monitoring data and setting-up of informative layers of georeferenced data (phase 1) and b) statistical and expert analysis for the identification of areas where implementation of limitation or mitigation measures are suggested (phase 2). Our methodology identifies potentially vulnerable water bodies, considering temporal contamination trends and relative risk levels at selected monitoring stations. A case study is presented considering glyphosate monitoring data in Lombardy Region (Northern of Italy) for the 2008–2014 period.

Methods

This paper describes a methodology to address the environmental risk analysis for surface water bodies by using pesticide monitoring data as suggested by European regulations and in particular the National Action Plan drafted by Member States in the frame of the Sustainable Use of Pesticides Directive (European Commission, 2009). Its final target is to help risk assessors to identify waterbodies mainly at risk and to prioritise vulnerated areas on the territory. The methodology shall be applied for a single pesticide and foresees two distinct steps (Figure 7.5-74):

Phase 1: acquisition of the available monitoring data (MECs: Measured Environmental Concentrations) and calculation of statistical parameters (MEC_{mean}, MEC_{median} and $MEC_{95th\ percentile}$ for each monitoring station and available year). In addition, the ratios MEC/EQS or MEC/PNEC are calculated, where MEC is one of the above described statistical parameters and EQS and PNEC are the Environmental Quality Standard and the Predicted No Effect Concentration respectively.

Phase2: expert analysis and rules for the identification of areas at risk (Table 7.5-125).

Figure 7.5-74: Flow diagram of the methodology

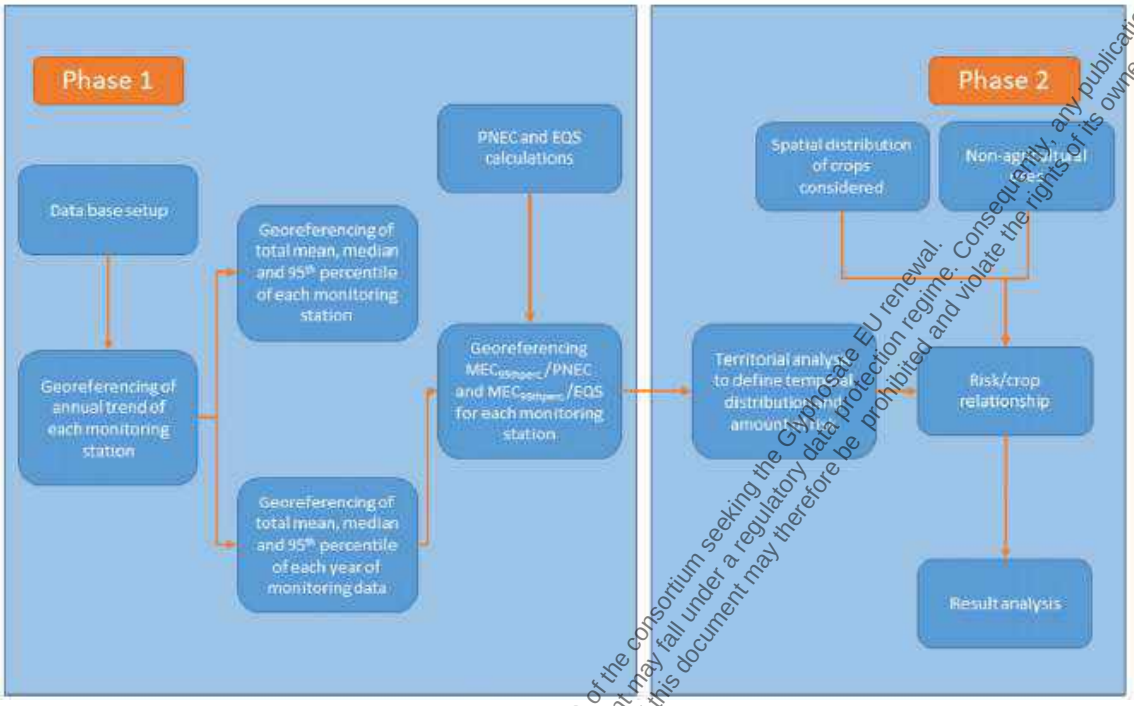


Table 7.5-125: Scheme for the identification of mitigation actions based on temporal trend and risk analysis from surface water monitoring data of pesticides

Trend	Risk	Action
Decreasing	Safe	No action
Decreasing	Low risk	No action
Decreasing	Risk	On-going monitoring
Decreasing	High risk	Mitigation action
Stationary	Safe	No action
Stationary	Low risk	No action
Stationary	Risk	Mitigation action
Stationary	High risk	Mitigation action
Increasing	Safe	No action
Increasing	Low risk	On-going monitoring
Increasing	Risk	Mitigation action
Increasing	High risk	Mitigation action
Random	Safe	No action
Random	Low risk	No action
Random	Risk	Mitigation action
Random	High risk	Mitigation action

Case study

In order to test the methodology, as a case study, we considered the already available historical series of monitoring data (2008–2014) of glyphosate residues in surface water bodies of Lombardy Region in Northern Italy. The data were gathered from the Environmental Protection Agency of the Lombardy Region (ARPA Lombardia).

Glyphosate (*N*-(phosphonomethyl)glycine) is a broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf weeds and grasses known to compete with commercial crops grown around the globe. In Italy, glyphosate has been authorized both for agricultural and non-agricultural uses. According

to the most recent pesticide sales statistics, in 2014 usage of glyphosate in Lombardy Region reached a volume of about 585 Tonnes and because of this important figures we selected glyphosate as test case for our methodology.

For glyphosate, at EU level, there is no an established EQS; since our elaborations are referred to an Italian scenario, we used a value of 0.1 µg/L. This value is suggested by the Italian regulation in absence of an EQS. In addition, for comparative purposes, we have also considered a PNEC value of 112 µg/L as suggested by Lombardy Region in the document implementing the National Action Plan (Giunta regionale della Regione Lombardia, 2015). Using both values (0.1 µg/L and 112 µg/L) allowed us to highlight the importance of setting appropriate EQS values for pesticides to help risk assessor in the decision-making process for risk mitigation measures on the territory.

Lombardy region has an extension of about 23.844 km^2 which almost a half of it is plain (47 %) and the rest consists of hills (12 %) and mountains (41 %). Flat areas extend from West to East, while mountains are located at North (Alps) and in the South-West (Apennine). The last agriculture census reports that arable crops are cultivated in the 92.1 % of the available crop area of the Lombardy plain, while the remaining part is dedicated to woody crops and grasslands; maize is the main crop of the Lombardy region, where it covers almost a half of the total arable area. In Lombardy, there are 669 rivers (520 natural rivers and 149 artificial channels) and 56 lakes (32 natural lakes and 24 artificial reservoirs).

The historical series (2008–2014) of monitoring data for surface water of Lombardy Region was provided by ARPA Lombardia, which oversees the official environmental monitoring for the entire Region. The analytical method utilized for determination of glyphosate was based on the derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl), separation with high performance liquid chromatography (HPLC).

ARPA Lombardia positioned sampling stations considering the most important river courses and the density of the hydrographic network in Lombardy Region. The number of sampling stations of glyphosate increased during the considered period (Table 7.5-126) as more concern about this herbicide arose during last years, passing from an average value of 73 in the 2008–2011 period to 278 in the 2012–2014 period. On the contrary, the number of sampling per year has been fairly constant in all the considered period (4 sampling per year), as well as the Limit of Detection (LOD) which remained set at the value of 0.1 µg/L.

Table 7.5-126: Number of sampling stations in which glyphosate was included in the monitoring programme

	2008	2009	2010	2011	2012	2013	2014
Sampling stations	42	64	88	98	274	279	280
Mean sample number per year	4	4	4	3	4	4	4

Results

Phase 1 of the proposed methodology foresee the development of a georeferenced statistical database. As an example, in our case study, means and 95th percentiles values of MECs for glyphosate were calculated for each sampling station and for all available years. In Table 7.5-127, the annual mean of the herbicide residues (µg/L) measured in surface water bodies of Lombardy Region are summarized. Particularly, the monitoring stations were divided in three different clusters (mean conc. ≤ 0.1; 0.1 b mean conc. ≤ 1; mean conc. > 1). In the same Table, maximum annual means and maximum 95 th percentiles of concentrations are also reported.

Table 7.5-127: Monitoring stations subdivided for class membership of the annual mean and 95th percentile of glyphosate concentration (0.1 µg/L = LOD) and maximum annual mean and 95th percentile detected across all the stations

Year	N. of stations mean conc. ≤ LOD	N. stations 0.1 < mean conc. ≤ 1 (µg/L)	N. stations mean conc. > 1 (µg/L)	Max annual mean conc. (µg/L)	Max annual 95th perc. conc. (µg/L)
2008	13	20	9	9.4	32
2009	26	37	1	1.1	4.2
2010	52	35	1	1.0	2.4
2011	61	34	3	1.7	5.2
2012	189	84	1	1.1	3.5
2013	189	88	2	33	96
2014	212	63	5	1.4	4.5

From Table 7.5-127 and plots of the spatial representation of the 95th percentile of concentrations of glyphosate for 2008, 2010, 2012 and 2014 years (not shown) the following considerations can be made:

- during the considered period, there has been an increase in the number of monitoring stations for glyphosate; however, this did not correspond to a linear increase in contaminated sites where glyphosate has been detected in concentrations above 0.1 µg/L. For example, in 2008 there was 29 contaminated sites and 42 monitoring stations for glyphosate (69 % of contaminated sites) while in 2014 figures were 68 and 280 respectively (24 % of contaminated sites);
- the presence of glyphosate in surface water bodies of Lombardy Region seems to be widespread. Even if the annual mean of MECs are less than the LOD, the residues of this herbicide were measured at least once a year in almost every monitoring stations;
- there is a large spatial and temporal variability of MECs; for example, during different years, even in the same monitoring station, concentrations range from values below the LOD up to tens of µg/L. The highest values of glyphosate concentrations were measured in the areas of Cremona and Mantova (South-Eastern part of the region) which reached annual mean concentrations of 33 µg/L (highest MEC = 108 µg/L) in 2013 and 9.4 µg/L (highest MEC = 38 µg/L) in 2008, respectively. However, in other years, MEC values were more evenly distributed. Consequently, these spike values could be then explained with occasional events such as improper uses of the pesticide.

As a further analysis, we calculated the I(95perc / EQS) index either considering the substance characteristics and in a worst-case perspective. For glyphosate, an EQS of 0.1 µg/L was considered; this represents the regulatory default value in Italy to be used in absence of an EQS at EU level. However, we also considered a PNEC value for glyphosate of 112 µg/L in order to evaluate the importance of EQS in the perception of risk on a territory. If the ratio I(95perc / EQS) (or in alternative I(95perc / PNEC)) is above 1 the water body is considered risk.

It is worth noting the differences when we take into consideration PNEC values instead of the regulatory EQS. The index I(95perc / PNEC) is always <0.1, which is at least an order of magnitude lower than a potential risk for aquatic organisms. In ANNEX VIII of the EU Water Framework Directive (WFD), glyphosate is listed among the so called "Specific Pollutants". They are defined as substances that can have a harmful effect on ecological quality, and which may be identified by Member States as being discharged to water in "significant quantities". Surface water bodies are assigned to one of the Directive's five ecological status classes – High, Good, Moderate, Poor or Bad. The EQS for Specific Pollutants contribute to ecological status classification; in fact, where a standard is failed the water body cannot be classed as Good. In a previous work (Finizio *et al.,* 2011) it was demonstrated that the use of a value of 0.1 µg/L, as a surrogate of EQS cannot be considered appropriate for the evaluation of the effects of pesticides on the aquatic communities, as each pesticide is characterised by its own inherent toxicity for different non-target organisms. In that study, this was clearly evident when the procedures for setting EQS (based on the calculation of PNECs) suggested by the WFD was considered. In fact, the differences in risk characterisation, depending on the approach used, were quite evident. In general, the risk for surface water

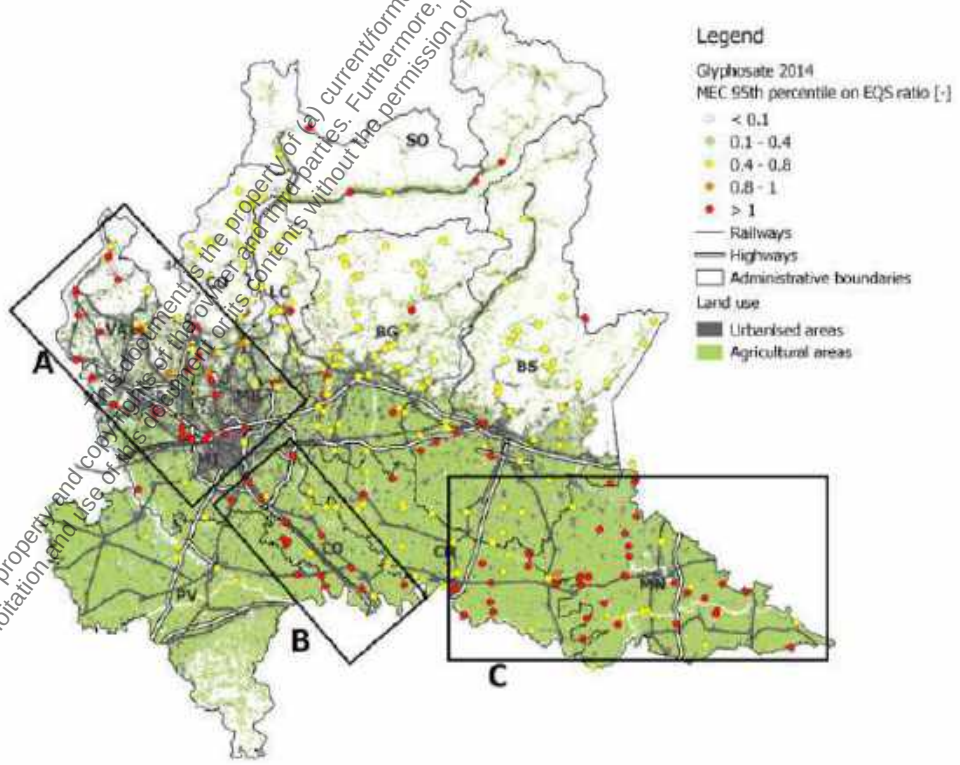
seemed to be higher in the case of insecticides when the PNEC approach was used. On the contrary, the criterion of the 0.1 µg/L cut-off indicated herbicides as the major driver of risk for surface water. These considerations highlight the importance of the availability of well-defined EQS for pesticides both for an appropriate classification of the ecological status of surface water bodies and for a proper action of risk management of these substances.

The second phase of the methodology sets rules for the prioritization of areas where mitigation actions on the territory should be taken in place. It is articulated in three different steps.

Firstly (step 1), a territorial analysis is performed to get a picture of the spatial and temporal distribution of the water bodies at risk on the territory. In our case study, we identified 192 sites in which at least in one year the I(95perc/EQS) was above 1. Particularly, we identified 14 safe sites (I(95 perc / EQS) < 0.8), 27 sites at low risk (0.8 < I(95 perc / EQS) < 1) 54 sites at risk (1 < I(95 perc /EQS) < 2) and 97 sites at high risk (I(95perc / EQS) > 2). Furthermore, the territorial analysis also allowed the identification of the temporal trend of risk for each of the available monitoring sites. Particularly, we identified 12, 34, 30, and 114 sampling sites with a random, decreasing, increasing or stationary temporal trends respectively (2 sites were not classified due to paucity of data).

The second step of phase II links the risk distribution for surface water bodies with the uses of pesticides on the territory. Consequently, it gives precious information about the identification of potential sources of contamination, which should be reduced through risk mitigation actions. In our case, we considered both the agricultural and non-agricultural uses of glyphosate. We used the GIS technique of overlaying the map of I(95 perc / EQS) index with maps of major transportation infrastructures and agricultural land use (all crops). Results are reported in Figure 7.5-75.

Figure 7.5-75: Analysis of areal clusters by overlying [illegible], land use and infrastructural networks maps



In Figure 7.5-75 we identified three main areal clusters where the exceeding of the index threshold is steady during the period (A = Monza-Brianza and North Milan provinces; B = Lodi and Pavia provinces; C = Mantua province). The map in this figure refers to the year 2014, but the same behaviour can be steadily observed in all the available years.

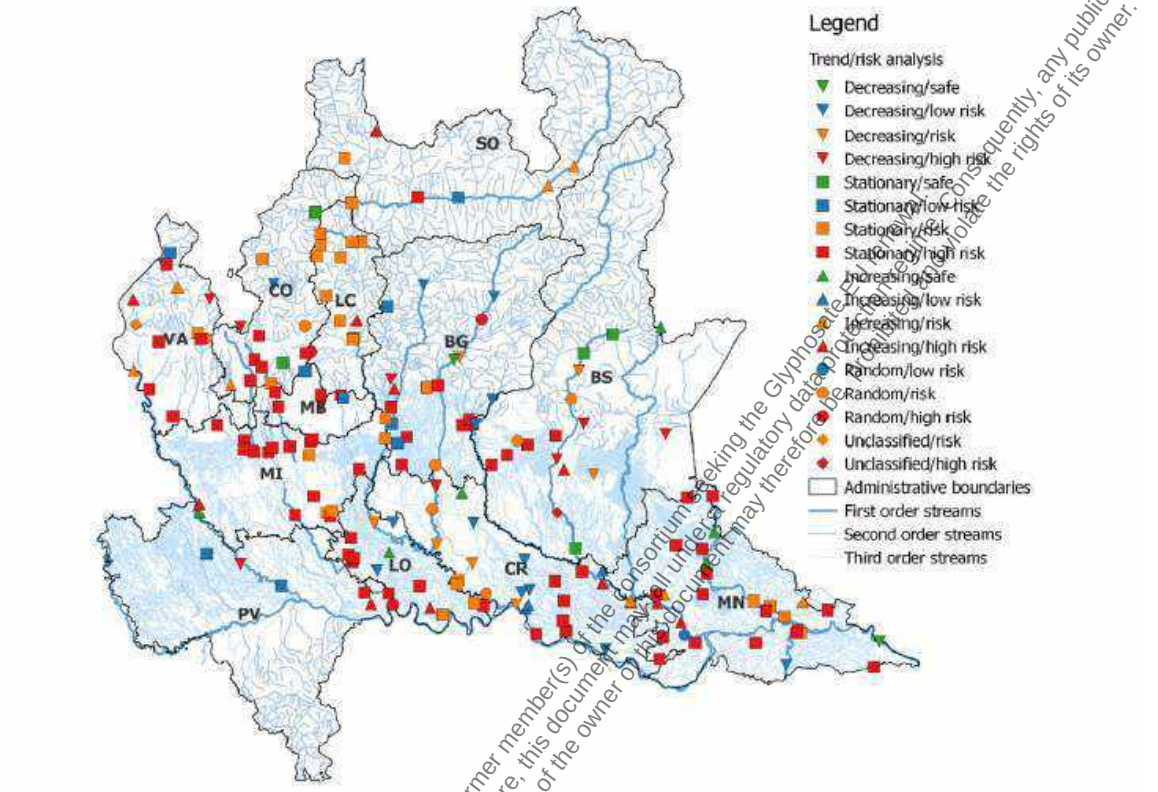
Cluster A is a highly-urbanised area with a strong presence of road infrastructures: in this area glyphosate residues in monitoring stations could be linked to non-agricultural uses. Cluster C is in an area with prevailingly rural activities and therefore the presence of glyphosate in surface water is linked to agricultural uses (particularly maize). The case of cluster B is in the middle of the other two: the area is typically rural, but it is crossed by some of the most important regional rail and road networks.

In the third step of phase II, and following the expert judgement schema reported in Table 7.5-125, risk managers can identify areas where mitigation actions should be undertaken. In Table 7.5-128, the combination of temporal trend and risk analyses (step 1 of phase II) together with the proposed actions for glyphosate are reported. They are also represented in Figure 7.5-76.

Table 7.5-128: Number of sites categorised by trend and risk

Trend	Risk	Action	N. of sites
Decreasing	Safe	No action	2
Decreasing	Low risk	No action	14
Decreasing	Risk	On-going monitoring	7
Decreasing	High risk	Mitigation action	11
Stationary	Safe	No action	6
Stationary	Low risk	No action	9
Stationary	Risk	Mitigation action	29
Stationary	High risk	Mitigation action	70
Increasing	Safe	No action	6
Increasing	Low risk	On-going monitoring	3
Increasing	Risk	Mitigation action	10
Increasing	High risk	Mitigation action	11
Random	Safe	No action	0
Random	Low risk	No action	1
Random	Risk	Mitigation action	7
Random	High risk	Mitigation action	4

Figure 7.5-76: Map of trend and risk analysis on the selected monitoring stations of Lombardy region with details of places where mitigation actions are suggested (following Table 7.5-127)



Conclusion

This study proposes a new methodology for risk managers to implement pesticide risk mitigation measures for surface water bodies at the territorial. The methodology combines GIS techniques and statistical analyses on historical series of monitoring data of PPPs. The latter are derived from national monitoring plans of pesticides residues in surface water. In order to show the proposed approach, the glyphosate in Lombardy region as a case study was proposed. In brief, the analysis highlighted a wide- spread presence of glyphosate in surface water bodies in Lombardy Region; almost the 50 % of the monitoring stations considered in Phase II of the methodology shows a contamination level that should be deepened and seamlessly mitigation actions should be foreseen. In several cases the risk could be attributed to a non-agricultural use of glyphosate. In fact, many monitoring stations classified at risk or high risk are in highly urbanised areas or near railways or major roads. Finally, in this paper, we highlighted that the perception of which substances might present a risk for surface water can be completely different according to the cut-off criteria identified. In fact, the perception of risk posed by glyphosate (or other pesticides) completely changes if the regulatory value of 0.1 µg/L or a more scientifically sound PNEC value is used. This could have significant consequences in the classification of the ecological status of surface water bodies and for implementing appropriate risk mitigation actions on the territory.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant:</u>
The article presents an approach for combining long-term surface water monitoring data from the Lombardy Region of Northern Italy with GIS analysis to identify contamination levels and implement pesticide risk mitigation measures for surface water bodies No experimental or monitoring data were generated. The measured maximum concentration of glyphosate was 108 µg/L in 2013. The article is considered reliable with restrictions.

<u>Assessment and conclusion by RMS</u>:

1. Information on the study

Data point:	CA 7.5/037
Report author	Huntscha, S. *et al.*
Report year	2018
Report title	Seasonal Dynamics of Glyphosate and AMPA in Lake Greifensee: Rapid Microbial Degradation in the Epilimnion During Summer
Document No	Environ. Sci. Technol. 2018, 52, 4641-4649
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Occurrence and fate of glyphosate, a widely used herbicide, and its main metabolite AMPA was investigated in Lake Greifensee, Switzerland. Monthly vertical concentration profiles in the lake showed an increase of glyphosate concentrations in the epilimnion from 15 ng/L in March to 145 ng/L in July, followed by a sharp decline to <5 ng/L in August. A similar pattern was observed for AMPA. Concentrations of glyphosate and AMPA in the two main tributaries generally were much higher than in the lake. Simulations using a numerical lake model indicated that a substantial amount of glyphosate and AMPA dissipated in the epilimnion, mainly in July and August, with half-lives of only ≈2-4 days which is ≫100 times faster than in the preceding months. Fast dissipation coincided with high water temperatures and phytoplankton densities, and low phosphate concentrations. This indicates that glyphosate might have been used as an alternative phosphorus source by bacterio- and phytoplankton. Metagenomic analysis of lake water revealed the presence of organisms known to be capable of degrading glyphosate and AMPA.

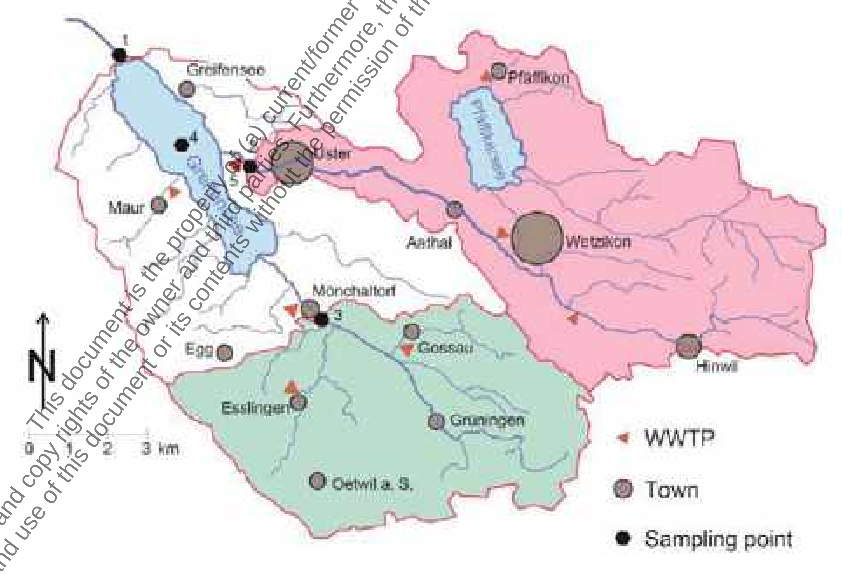
Materials and methods

Field Site: Greifensee and its Catchment Area.

The field study was conducted in the catchment area of Lake Greifensee, a eutrophic lake located near Zurich, Switzerland (47°21′N, 8°41′E). The lake has a surface area of 8.46 km^2 (length: 6.5 km; width: 1.9 km), a maximum depth of 32 m, and is dimictic with vertical mixing from surface to bottom in autumn and spring. During the warmer season (April-November) the lake is stratified into a warmer epilimnion and a cold hypolimnion. Regular sequences of oxic (winter/spring) and anoxic conditions (summer/fall) are observed in the hypolimnion of the lake. It is fed by several tributaries of which the rivers Aa Uster and Aa Mönchaltorf contribute more than 60 % of the total inflow. Its sole outflow is the river Glatt. More details on the hydrology and morphology of the lake are found elsewhere (Ulrich, M. M., 1994).

Lake Greifensee has a catchment area of 160 km^2 of which ≈50 % are used as agricultural land (field crops, grassland, and some orchards). Forests (21 %), urban areas (19 %), water bodies (7 %), and unproductive land (3 %) constitute the other 50 % of the catchment area. Approximately 120 000 inhabitants are living in the catchment area, most of them in that of Aa Uster, which is more urbanized than the Aa Mönchaltorf. Eight wastewater treatment plants (WWTPs) are located in the catchment area, of which two discharge directly into the lake and one into Aa Mönchaltorf downstream of the gauging and sampling station (see Figure 7.5-77).

Figure 7.5-77: Map of the catchment area of Lake Greifensee with sampling points. Weekly flow-proportional composite samples were obtained from the automatic sampling stations at the tributaries to the lake, Aa Uster (2) and Aa Mönchaltorf (3) as well as the outflow from the River Glatt (1). Monthly grab samples from several depths were taken at the deepest point of the lake (4). Daily flow-proportional composite samples of treated wastewater were obtained from WWTP Uster (5). The sub-catchments discharging at the sampling points 2 & 3 are marked in red and green, respectively



Water Sampling and Analysis

To establish a mass balance for glyphosate and its metabolite AMPA in the lake, monthly water samples were taken from 10 different depths (0, 1, 2.5, 5, 7.5, 10, 15, 20, 25, and 30 m) between March and November 2013 by regional authorities (Canton of Zurich), who also measured orthophosphate concentrations. During the same period, weekly flow-proportional composite samples of the rivers Aa Mönchaltorf, Aa Uster, and Glatt were analyzed, allowing determination of input and export loads of the two compounds, based on concentration measurements and river water discharge data. In rare cases (five incidents), when the automated sampling of the tributaries malfunctioned, concentrations were interpolated from values of adjacent weeks.

In WWTP Uster, the largest WWTP in the study area, flow-proportional, 24 h composite samples of treated wastewater were taken every 4-16 days (on average every 8.5 days). The installation operates with a mechanical, biological (activated sludge with an estimated sludge age of 17-20 days, with nitrification and denitrification), and chemical treatment (phosphate precipitation by iron salts, no chlorination), and subsequent sand filtration.

All samples were transferred to the lab in HDPE bottles, fortified with an internal standard solution ($^{13}C_2{}^{15}N$-glyphosate and $^{13}C^{15}ND_2$-AMPA), and kept at 4°C until analysis, typically within 1 week of arrival. Samples were analyzed with a method based on derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl), online-enrichment, reversed-phase liquid chromatography, and tandem mass spectrometry. This method does not include a filtration step so that measured concentrations comprise dissolved and sorbed glyphosate and AMPA. Limits of quantification were 5 ng/L for both compounds.

Lake Model
The software AQUASIM (Version 2.1 g, available from http://www.eawag.ch/en/department/siam/software/) was used to establish a mathematical model for simulation of vertical concentration profiles and mass balances for glyphosate and AMPA in Lake Greifensee with a temporal resolution of 1 day. It considers the morphology and hydrology of the lake as well as fate and vertical transport of chemical compounds. The lake is described by 128 horizontal boxes of 25 cm thickness, for which horizontal mixing within 1 day is assumed. Vertical mixing is described by time- and depth-dependent diffusion coefficients derived from fitting water temperatures to measured vertical temperature profiles.

A water balance was set up with discharge data from gauging stations of the three largest tributaries and the outflow of the lake, lake water levels, evaporation, and precipitation data. The discharge of the remaining nine minor tributaries was calculated by the difference of the above-mentioned. Subsurface water exchange can be neglected (<5 %).

Chemical input of glyphosate and AMPA into the lake was modeled to occur exclusively through the tributaries into the epilimnion of the lake. For the unknown inputs from those tributaries that were not sampled, average concentrations of Aa Mönchaltorf and Aa Uster were used and multiplied with the estimated discharge (see above). Input through the three WWTPs was calculated from the sum of their discharge and the concentrations found in WWTP Uster, which accounts for > 85 % of the treated wastewater directly entering the lake.

The model comprises a degradation process in the lake's epilimnion which was implemented as a (pseudo) first-order degradation in the upper 0.5 m layer of the lake. Through the fast vertical diffusion within the epilimnion, this degradation process affects the concentrations in the whole epilimnion. The degradation rates reported in the Results and Discussion section were thus recalculated using the actual depth of the epilimnion (based on temperature profiles) to refer to the whole epilimnion.

Metagenomic Sequencing
Lake Greifensee water was sampled at three depths (0, 2.5, and 7.5 m) on 7 July 2014. 1 L of lake water per sample was then centrifuged at 5000 rcf for 10 min and the pellet was stored at -20°C until further processing. Total DNA was extracted using the PowerSoil DNA isolation kit (MO BIO, Carlsbad, CA). The integrity of the DNA was assessed on agarose gels and the quantity was measured by the Quant-iT PicoGreen kit (Invitrogen, Carlsbad, CA). Libraries were generated and indexed using the TruSeq DNA library preparation kit (Illumina, San Diego, CA) and sequenced on an Illumina MiSeq generating 300 bp paired end reads available under (https://www.mg-rast.org/linkin.cgi?project=mgp1139). All metagenomic data analyses were performed on the MG-RAST server.

Results
Major Inputs of Glyphosate and AMPA to the Lake from Tributaries and WWTP Uster
Glyphosate concentrations in the weekly composite samples from the two main tributaries ranged from <5 to 1430 ng/L (median, 145 ng/L in Aa Mönchaltorf and 175 ng/L in Aa Uster). The highest concentrations appeared in July and August in Aa Mönchaltorf and in March and July in Aa Uster, which is consistent with the main agricultural uses of glyphosate for treatment of sugar beet and maize fields prior to seeding

in spring and postharvest treatment of cereal fields in summer. Urban use of glyphosate is not so well-defined, but is expected to have a higher impact on the concentrations in Aa Uster, which has a higher percentage of urban land use. Glyphosate concentrations in treated wastewater from WWTP Uster were between 18 and 350 ng/L (median, 106 ng/L) with maximum concentrations in June and September, when they exceeded those in the tributaries.

AMPA concentrations in the two main tributaries ranged from 24 to 415 ng/L (median, 150 ng/L in both rivers). Similar to glyphosate, the highest AMPA concentrations were found in July (Aa Uster) and August (Aa Mönchaltorf). Concentrations in treated wastewater from WWTP Uster reached up to 1680 ng/L (median, 516 ng/L), and were thus higher than those in the tributaries at all sampling times. Maximum concentrations in wastewater were found in August and September. AMPA is also a degradation product of various phosphonates used in industry and degradation of these compounds to AMPA in WWTPs likely is an important source of AMPA in Lake Greifensee.

Spearman's rank correlation analysis indicated fairly strong correlation of glyphosate and AMPA concentrations in the more rural tributary Aa Mönchaltorf ($\sigma = 0.70$, $p < 0.001$) suggesting that the occurrence of AMPA in this stream probably was related to the use of glyphosate in the catchment area. In contrast, in the more urban tributary Aa Uster, there was no apparent correlation ($\sigma = 0.31$, $p = 0.09$) indicating that AMPA may, at least in part, be derived from sources other than glyphosate in the catchment. Even in treated wastewater from WWTP Uster, the correlation between glyphosate and AMPA was higher ($\sigma = 0.62$, $p < 0.001$) than in Aa Uster. The best correlation, however, was found in the outflow of the lake ($\sigma = 0.83$, $p < 0.001$). This is most likely due to the similar fate of the two compounds (see below) rather than similar sources.

Weekly loads of glyphosate into Lake Greifensee (Figure 7.5-78b) were up to 0.97 and 0.63 kg in Aa Mönchaltorf and Aa Uster, respectively. Inputs from the more urbanized catchment area of Aa Uster were highest and quite uniform between March and June. From July on, these inputs decreased to lower levels. Inputs from the agriculturally dominated catchment area of Aa Mönchaltorf started later (mid-April) and fluctuated with a clear maximum during the rainiest week at the end of May. Glyphosate loads from WWTP Uster were generally low and lower than those in the tributaries at all times. Highest loads from WWTPs were found in June.

Weekly loads of AMPA into Lake Greifensee (Figure 7.5-78c) reached their maximum in the week with the highest precipitation, with values of 0.64 and 0.57 kg for Aa Mönchaltorf and Aa Uster, respectively. In other weeks, AMPA loads were generally below 0.3 kg. Median AMPA loads from treated wastewater of WWTP Uster were 0.08 kg/week with a maximum of 0.14 kg in September. Compared with glyphosate, wastewater delivered significant amounts of AMPA, which regularly reached levels similar to those in one of the tributaries and even exceeded the loads in both main tributaries in the first week of September.

Figure 7.5-78: **Daily precipitation in 2013 at a nearby weather station and water discharges at the outflow of Lake Greifensee (Glatt River) and the two main tributaries, Aa Uster and Aa Mönchaltorf (a). Mass loads of glyphosate (b) and AMPA (c) which were transported to and eliminated from the lake, respectively. Concentrations of glyphosate and AMPA (symbols indicate measured values, lines modeled concentrations) as well as phosphate in the uppermost 5 m of Lake Greifensee (d). Chl a was measured either monthly at a depth of 1 m (symbols) or in situ over a depth of 1.5-16 m (the line indicates mean values from 1.5 to 8 m)**

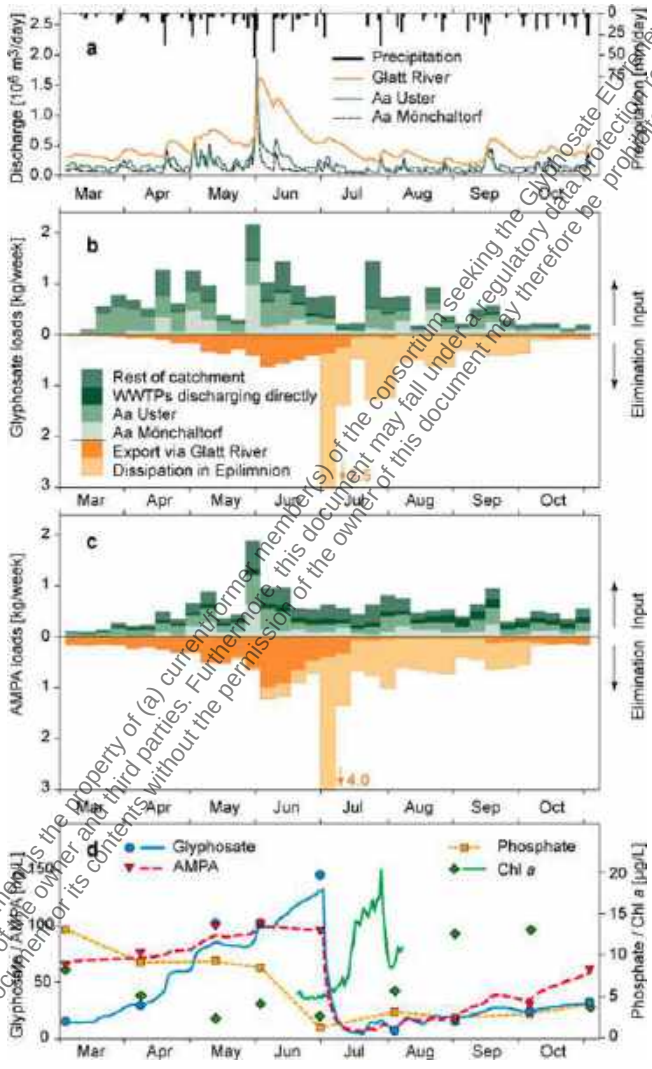
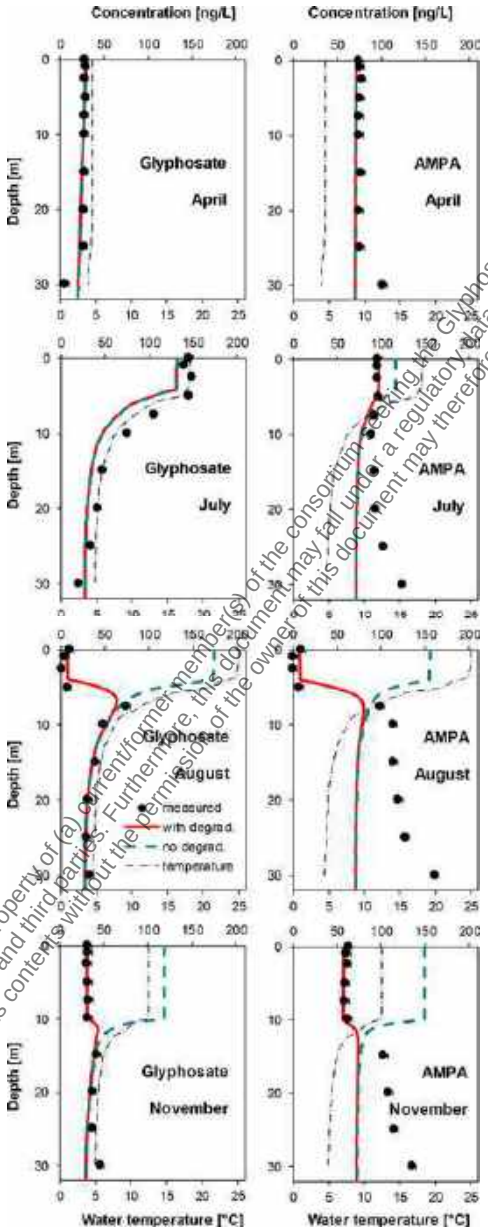


Figure 7.5-79: **Selected vertical concentration profiles of glyphosate (left) and AMPA (right) in Lake Greifensee, 2013. Measured values (circles) are compared to simulated concentrations assuming no degradation (blue dashed lines) or degradation in the epilimnion (red line). Also shown are the measured temperature profiles (dash dotted black lines)**



Concentrations of Glyphosate and AMPA in Lake Greifensee: Rapid Dissipation in the Epilimnion During Summer

Vertical concentration profiles of glyphosate and AMPA in Lake Greifensee were measured monthly between March and November 2013. Selected profiles are shown in Figure 7.5-79. In March and April, glyphosate concentrations were uniform at all depths except for the lowermost sample, which showed lower glyphosate concentrations. Concentrations (slowly) increased from ≈14 ng/L in March to 28 ng/L in April.

Between April and May, rising surface water temperatures initiated the stratification of the lake with the formation of an epilimnion in the upper 4-6 m and a hypolimnion in the lowest 20 m. Both are divided by the metalimnion with a pronounced temperature (and thus density) gradient, which restricts water exchange between the epi- and the hypolimnion. Hence, beginning in May, glyphosate epilimnion concentrations increased steadily to values higher than 100 ng/L due to inputs from the tributaries, whereas hypolimnion concentrations remained constant (≈35 ng/L).

In July, epilimnion concentrations of glyphosate reached a maximum of 145 ng/L (Figure 7.5-79). However, between July and August, a sudden drop of glyphosate concentrations occurred in the epilimnion down to levels below the limit of quantification of 5 ng/L, despite further inputs through the tributaries. This is also illustrated in Figure 7.5-78d, where average epilimnion concentrations are plotted over time. These observations indicate a sudden, rapid dissipation in the epilimnion, which will be discussed in detail below.

From September onward, glyphosate concentrations again slowly increased due to further inputs, but also due to the fact that the depth of the epilimnion was increasing, causing mixing with water from deeper layers containing higher concentrations. Eventually epilimnion concentrations reached 30 ng/L in November (Figure 7.5-79).

For AMPA, a similar temporal pattern was observed as for glyphosate. Initial concentrations of AMPA were higher (70 ng/L) than those of glyphosate (14 ng/L), but they increased to only 100 ng/L until July. Between July and August, the same distinct concentration drop was observed in the epilimnion as for glyphosate, suggesting that the same dissipation process acted on both compounds. In the following months, AMPA epilimnion concentrations recovered to preseason levels of about 60 ng/L.

In contrast to glyphosate, AMPA concentrations in the hypolimnion increased, even after the stratification of the lake starting in April, up to concentrations of 130 ng/L in August (Figure 7.5-79). Since the metalimnion prevents water exchange between epilimnion and hypolimnion, this increase cannot originate from input by the tributaries. Furthermore, in all vertical profiles, AMPA concentrations near the bottom (30 m depth) were higher than in the rest of the hypolimnion. This coincides with slightly lower glyphosate concentrations between March and July in the same depth as mentioned above. Although further evidence is lacking, one could speculate that AMPA may be formed by degradation of phosphonates present in the hypolimnion and in or near the sediment by degradation of glyphosate and/or other phosphonates. Moreover, in analogy to phosphate, AMPA adsorbed to bottom sediment may be released due to reductive dissolution of iron oxides under anaerobic conditions. However, since the focus of this study was to investigate the fate of glyphosate and AMPA in the epilimnion and given the complexity of the matter (numerous possible AMPA precursors, such as nitrilotris-(methylenephosphonic acid) which is used as complexing agent in detergents), formation of AMPA in the hypolimnion was not further studied.

In a less extensive study in 2014, the same glyphosate and AMPA concentration trends were found between June and September.

Mass Balance

Between March and November, the cumulative input loads of both compounds were highest in the more urbanized Aa Uster (7.9 and 6.5 kg of glyphosate and AMPA, respectively, see Figure 7.5-78b, c), followed by the agricultural Aa Mönchaltorf (5.5 and 4.2 kg) and WWTP Uster (0.65 and 2.7 kg). Further input loads from two other WWTPs (0.13 and 0.5 kg) were calculated from the sum of their wastewater discharge and the concentrations found in WWTP Uster. Loads from the tributaries not included in the sampling (7.8 and 5.7 kg) were calculated based on average concentrations of Aa Mönchaltorf and Aa Uster and the estimated discharge from the water balance.

Cumulative glyphosate input loads of about 22 kg were in stark contrast to an export via the Glatt river of only 5.4 kg. In November, about 5.1 kg glyphosate were stored in the lake which was ≈3 kg more than in March (2.1 kg). This results in a dissipated load of 13.6 kg, which was accounted for in the model by the first-order degradation process with the dissipation rates discussed in the next section. Roughly 70 % of the dissipated load (9.5 kg) was disappearing within the 5 weeks between the measurements in July and August.

For AMPA, cumulative input loads of 19.6 kg were similarly contrasted by a relatively low export load of 8.7 kg. Measured storage of AMPA increased from 10.6 kg in March to 12.1 kg in November. However, this increase is largely due to formation of AMPA in the hypolimnion. According to the model calculations (see below), 55 % of the ≈11 kg AMPA which disappeared during the study period were eliminated between the measurements in July and August alone.

Application of the Lake Model: Indication for a Rapid Dissipation Process with a Half-Life of a Few Days
To describe the variation of concentrations over time and depth in the lake, a simple, one-dimensional model was set up including inputs from the various tributaries and WWTPs, export via the Glatt River, and vertical mixing, but, in a first step, excluding any degradation/dissipation processes. This model was able to describe the measured, vertical concentration profiles from March to July (dashed blue lines in Figure 7.5-79). However, in August, modeled concentrations in the epilimnion would have reached levels of 200 ng/L for glyphosate and 160 ng/L for AMPA. Consequently, all measured epilimnion concentrations after August were considerably overestimated by the model.

To account for the rapid elimination of glyphosate and AMPA, the model was refined by inclusion of a first-order dissipation process in the epilimnion (for details see methods section). Average dissipation rates were adjusted for every period between two lake samplings (21-35 days) until measured epilimnion concentrations were adequately represented by the model. Resulting concentration profiles are shown in Figure 7.5-79 (solid red lines).

For glyphosate, this dissipation process was negligible before July with first-order degradation rates <0.001/d, corresponding to half-lives (DT_{50}) >1000 days. In July and the first week of August, a considerably higher (≫100 x) dissipation rate of 0.38/d (DT_{50} = 1.8 days) was determined. Dissipation rates between the samplings in August and September remained high (0.19 d^{-1}; DT_{50} = 3.7 days) and decreased steadily from September (0.05/d; DT_{50} = 13 days) until October (0.002/d; DT_{50} >300 days).

Modeled dissipation rates for AMPA showed the same seasonal trend as those for glyphosate. As for glyphosate, the highest dissipation rate for AMPA was found in July and the first week of August. In general, dissipation rates were very similar to those of glyphosate.

Evaluation of Possible Elimination Processes for Glyphosate and AMPA in the Lake
The modeled dissipation rates represent all processes that may affect glyphosate and AMPA concentrations in the lake's epilimnion, including potential distribution processes between water and air or water and particles/sediment as well as different degradation processes such as hydrolysis, photodegradation, or biological degradation. The importance of these processes will be assessed in the following paragraphs.

Due to their zwitterionic speciation in lake water, glyphosate and AMPA have a very low vapor pressure and a high water solubility and, consequently, low air-water partition coefficients. Therefore, volatilization from the water surface can be ruled out as significant loss process.

Sorption to particles with subsequent sedimentation may lead to a certain loss of glyphosate and AMPA from the epilimnion. However, since the sedimentation of particles is a rather constant process and the sorption to these particles does not change rapidly, this process is unlikely to explain the observed, rapid loss of glyphosate and AMPA from the epilimnion in such a short period.

Both compounds are known to be hydrolytically stable, which excludes abiotic hydrolysis as elimination process.

From experience with other compounds in Lake Greifensee, experimental photolysis half-lives in summer sunlight of ≤1 h would be necessary to have a substantial impact on the concentrations in the epilimnion. Photolysis is thus not expected to contribute significantly to the observed, rapid removal of glyphosate and AMPA in the epilimnion.

This suggests that biodegradation is the most likely main elimination process to reasonably explain the distinct concentration drop of glyphosate and AMPA in Lake Greifensee between July and August. This conclusion is supported by the finding that phytoplankton growth was higher in July and the following months (with a short peak between the samplings in July and August; green line in Figure 7.5-78d) and that water temperatures were higher at the same time. Nevertheless, increasing phytoplankton density and water temperature alone would be expected to promote biodegradation, but still seem unlikely to be the sole cause of the sudden concentration drop, unless the conditions led to rapid growth of organisms, capable of degrading glyphosate and AMPA.

An additional factor enhancing biodegradation of glyphosate and AMPA may be the decreasing free phosphate (orthophosphate) concentration in the epilimnion, which fell below the limit of detection of 2 µg P/L in July (Figure 7.5-78d). As known from the literature, several bacteria, such as cyanobacteria or proteobacteria, are able to take up phosphonates and break the relatively stable C-P bond.

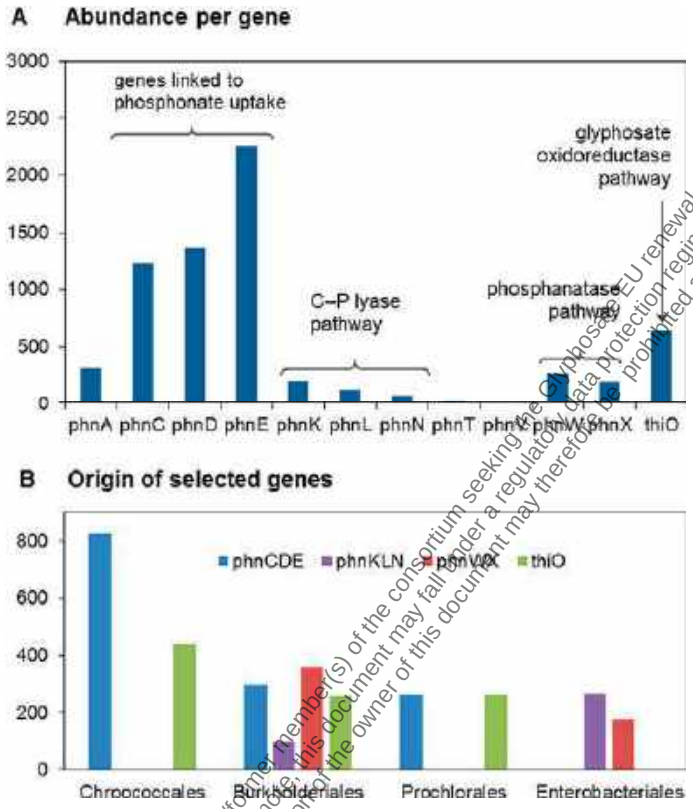
The degradation of glyphosate and AMPA by cyano- and/or proteobacteria is also supported by the observation that measured concentrations in August were lowest in the depths 1 and 2.5 m, where they fell below the limit of quantification of 5 ng/L, whereas in the depths 0 and 5 m, concentrations were between 8 and 11 ng/L despite the rather rapid mixing in the epilimnion. This suggests that degradation took place in a zone below the water surface around 1-2.5 m depth, which was also the zone of maximum primary production.

Metagenomic Sequencing to Identify Organisms Responsible for the Rapid Degradation of Glyphosate
In July 2014, shortly after full depletion of glyphosate and AMPA, water samples for metagenomic analysis were taken from the epilimnion of Lake Greifensee. Sequencing yielded a total of 8.8 Gbp of sequence information. From these data, species abundance was estimated at multiple taxonomic levels. The most abundant phyla were cyanobacteria and proteobacteria. At the genus level, *Synechococcus* showed the highest abundance.

In order to further evaluate possible routes of phosphonate degradation in Lake Greifensee, the abundance of genes linked to phosphonate degradation and their respective species of origin was evaluated using MG-RAST. The *phnCDE* genes previously linked to phosphonate uptake were highly abundant in the sample and were assigned mainly to the genus *Synechococcus* (Chroococcales, Figure 7.5-80). Relatively few DNA reads mapped to selected C-P lyase pathway genes (*phnKLN*, Figure 7.5-80) and thus some evidence for a working C-P lyase pathway was found. These genes were assigned to proteobacteria (Burkholderiales and Enterobacteriales), as were the genes phn*WX* of the phosphonatase pathway. The gene *thiO*, previously reported to catalyze the oxidation reaction from glyphosate to AMPA, was associated with the families of Chroococcales, Burkholderiales and Prochlorales (Figure 7.5-80). No evidence of a glyphosate oxidoreductase gene (*gox*), previously linked to microbial glyphosate oxidation, was found within this study. All these data indicate that microorganisms of multiple genera may be involved in the biodegradation of glyphosate and that the compound is probably degraded via different pathways.

Evidence for Biodegradation from Batch Incubation Experiments
Batch incubation experiments were performed with two cyanobacterial species, *Microcystis aeruginosa* (isolated from Lake Greifensee) and *Synechococcus* (isolated from another Swiss lake). In summary, the experiments with *Microcystis aeruginosa* and *Synechococcus* showed that glyphosate is rapidly degraded and that degradation depends on the depletion of phosphate in the growth medium (no degradation or much slower degradation in the presence of P_i). Extrapolated to a biomass corresponding to 15 µg/L chlorophyll a, as measured in summer 2013 in Lake Greifensee (Figure 7.5-78d), the dissipation rates for P_i-starved *Microcystis aeruginosa* (0.07/d) and *Synechococcus* (0.18/d) were, however, somewhat lower than the rate obtained through modeling, indicating that microorganisms capable of degrading glyphosate and AMPA more efficiently than the two tested species must be present in the lake's epilimnion.

Figure 7.5-80: Functional abundance of selected genes in the Lake Greifensee metagenome (A) and assignment of some of these genes to families of bacteria (B)



Conclusion

This study shows that under certain conditions, degradation of glyphosate and AMPA in large water bodies (i.e. lakes) is orders of magnitude faster than expected. The conditions leading to this phenomenon do not seem to be very specific as they were met in Lake Greifensee at least in the summers of 2006, 2013, and 2014 and in Lake Murten in 2006. Note that in 2006, only a single vertical concentration profile was measured in the two lakes in summer. Nevertheless, as the use pattern was very similar at the time (at least concerning application timing and consequent input to surface waters via surface runoff) it appears likely that the same seasonal changes caused the observed depletion of glyphosate and AMPA in the epilimnion.

A likely explanation for the rapid degradation is a combination of the bloom of cyanobacteria during summer and a depletion of inorganic phosphorus that probably caused increased uptake and metabolism of phosphonates in these organisms. The distinct seasonal dynamics as well as the specific conditions required for efficient degradation of glyphosate and AMPA probably are difficult to reproduce in laboratory degradation experiments as requested in official guidelines for pesticide testing such as the OECD tests for transformation in aquatic sediment systems or aerobic mineralization in surface water. However, this study provides strong evidence, at field scale, for the potential of (cyano)-bacteria in lakes for degradation of glyphosate and AMPA.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the concentrations of glyphosate and AMPA in lake Greifensee in Switzerland representing a catchment with high portion of agricultural land use. The maximum concentration of glyphosate in samples from the two main tributaries of the lake was 1430 ng/L. Maximum glyphosate concentration in treated wastewater discharging into the lake was 350 ng/L. The maximum AMPA concentration in the two main tributaries was 415 ng/L. Concentrations in treated wastewater reached up to 1680 ng/L. For lake Greifensee, concentration of glyphosate reached a maximum of 145 ng/L in the epilimnion, and concentration of AMPA reached a maximum of 130 ng/L in the hypolimnion.

The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/038
Report author	Masiol, M. *et al.*
Report year	2018
Report title	Herbicides in river water across the northeastern Italy: occurrence and spatial patterns of glyphosate, aminomethylphosphonic acid, and glufosinate ammonium
Document No	Environmental Science and Pollution Research (2018) 25:24368-24378
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Glyphosate and glufosinate ammonium are the active ingredients of commonly used herbicides. Active agricultural lands extend over a large part of the Veneto region (Eastern Po Valley, Italy) and glyphosate and glufosinate ammonium are widely used. Consequently, surface waters can be potentially contaminated. This study investigates the occurrence of glyphosate and glufosinate ammonium as well as aminomethylphosphonic acid (AMPA, the degradation product of glyphosate) in river water of Veneto. Eighty-six samples were collected in 2015 at multiple sampling points across the region. Samples were analyzed for the two target herbicides, AMPA as well as for other variables, including water temperature, pH, dissolved oxygen, conductivity, hardness, BOD, COD, inorganic ions, total nitrogen, total phosphorus, total suspended solids, arsenic, and lead. The average concentrations (all samples) were 0.17, 0.18, and 0.10 µg/L for glyphosate, AMPA, and glufosinate ammonium, respectively. The European upper tolerable level for pesticides (annual average 0.1 µg/L) was often exceeded. Chemometric analysis was therefore applied to (i) investigate the relationships among water pollutants, (ii) detect the potential sources of water contamination, (iii) assess the effective water pollution of rivers by identifying river basins with anomalous pollution levels, and (iv) assess the spatial variability of detected sources. Factor analysis identified four factors interpreted as potential sources and processes (use of herbicides, leaching of fertilizers, urban/industrial discharges, and the biological activity on polluted or stagnant waters). A discriminant

analysis revealed that the pollution from anthropogenic discharges is homogeneously present in surface water of Veneto, while biological activity and fertilizers present heterogeneous distributions. This study gives insights into the concentrations of herbicides in rivers flowing through a wide region that has heavy use of these chemicals in agriculture. The study also points out some hot-spots and suggests the future implementation of the current monitoring protocols and network.

Materials and methods

The Veneto region

The Veneto covers an area of ~ 18,000 km^2 and hosts a population of 4.9 million inhabitants. The northwestern part is mainly occupied by mountains (Alps), with a low population density mostly concentrated along narrow valleys. A wide southeastern alluvial plain accounts for most (56 %) of the territory and is affected by heavy anthropogenic pressures due to the presence of major cities, industrial areas, and intensive farming. A belt of hilly environments is located between mountains and the lowland: it hosts rural environments and farming, mostly vineyards and orchards. The alluvial plain is composed of sandy to silty-clay materials deposited by major rivers: the northwestern plain is generally characterized by more permeable soils, while the central and southern plain host heavy soils and waterlogging with shallow groundwater levels (sometimes <2 m). The two areas are separated by a belt of springs called “risorgive”, which generate several streams.

The mountain chains (Alps and Prealps) are mainly composed of sequences of sedimentary rocks (mainly limestone and dolomite) on metamorphic basements with magmatic extrusions. Springs of major rivers (e.g., Piave, Brenta, Adige) are located in the Alps, while other rivers flow (Livenza) or join tributaries (e.g., Brenta) flowing from karstic systems. Other major rivers (e.g., Bacchiglione, Dese, Sile, Zero) born in the “risorgive” area from springs fed by aquifers catching water across the Prealps area. Soils in the plain areas are also characterized by low organic carbon content, especially where intensive agriculture is practiced. The low levels of soil organic matter limit the cation exchange capacity, lower the fertility, and increase the potential mobility of contaminants, including herbicides.

Table 7.5-129: Characteristics of the sampling sites and average (min-max) concentrations of target compounds. GLY glyphosate, GLU glufosinate ammonium, AMPA aminomethylphosphonic acid. Provinces are BL, Belluno; TV, Treviso; VE, Venice; PD, Padua; RO, Rovigo. LOQ limit of quantification

Main drainage basin	Secondary drainage basin	River name	Site no.	Province	No. samples	GLY		AMPA		GLU	
						µg L^{-1}		µg L^{-1}		µg L^{-1}	
						Mean	(min-max)	Mean	(min-max)	Mean	(min-max)
Adige		Adige	206	PD	4	0.04	(<LOQ–0.1)	0.17	(<LOQ–0.6)	0.07	(<LOQ–0.22)
Brenta		Brenta	436	VE	3	0.29	(<LOQ–0.83)	0.13	(<LOQ–0.33)	0.13	(<LOQ–0.33)
Brenta	Bacchiglione	Bacchiglione	181	PD	3	<LOQ	–	<LOQ	–	<LOQ	–
Brenta	Bacchiglione	Cagnola	175	PD	2	<LOQ	–	<LOQ	–	<LOQ	–
Brenta	Bacchiglione	Tesinella	112	PD	3	0.04	(<LOQ–0.06)	<LOQ		<LOQ	–
Brenta	Gorzone	Gorzone	437	VE	2	<LOQ	–	<LOQ		<LOQ	–
Canalbianco		N. Adigetto	223	RO	4	0.40	(<LOQ–1.4)	0.30	(<LOQ–0.75)	0.18	(<LOQ–0.55)
Livenza	Monticano	Monticano	620;1147	TV	5	0.04	(<LOQ–0.1)	0.11	(<LOQ–0.83)	0.08	(<LOQ–0.3)
Livenza	Monticano	Cervada	621	TV	4	0.49	(0.07–1.3)	0.28	(0.07–0.56)	0.11	(<LOQ–0.3)
Livenza	Monticano	Crevada	6008	TV	1	0.45	(0.45–0.45)	<LOQ	–	0.30	(0.3–0.3)
Livenza		Livenza	72;453	TV, VE	4	0.34	(<LOQ–0.85)	0.42	(<LOQ–1.4)	0.05	(<LOQ–0.11)
Livenza		Meschio	23;236	TV	2	0.11	(<LOQ–0.2)	<LOQ	–	0.11	(<LOQ–0.2)
Piave		Anfella	409	BL	4	0.06	(<LOQ–0.1)	0.04	(<LOQ–0.07)	0.07	(<LOQ–0.22)
Piave		Piave	65	VE	5	0.13	(<LOQ–0.66)	0.28	(<LOQ–1.2)	0.06	(<LOQ–0.12)
Piave		Teva	6013	TV	2	0.31	(0.11–0.51)	0.77	(0.72–0.82)	0.42	(<LOQ–0.82)
Piave		Val di Frari	420	BL	4	0.06	(<LOQ–0.17)	<LOQ	–	0.05	(<LOQ–0.12)
Po		Po	227	RO	4	0.05	(<LOQ–0.08)	0.22	(<LOQ–0.54)	<LOQ	–
Sile		Bigonzo	6033	TV	4	0.32	(<LOQ–0.7)	0.16	(0.09–0.27)	0.08	(<LOQ–0.14)
Sile		C.U.A.I.	351	VE	4	0.10	(<LOQ–0.26)	0.06	(<LOQ–0.14)	<LOQ	–
Sile		Melma	333	TV	4	0.13	(<LOQ–0.37)	0.12	(<LOQ–0.26)	0.10	(<LOQ–0.26)
Sile		Sile	238;329	TV, VE	6	0.07	(<LOQ–0.25)	0.10	(<LOQ–0.25)	0.09	(<LOQ–0.25)
Lagoon of Venice		Musoncello	1123	TV	3	0.72	(<LOQ–2.1)	0.48	(<LOQ–1.4)	0.72	(<LOQ–2.1)
Lagoon of Venice		Tergola	212	PD	3	<LOQ	–	<LOQ	–	<LOQ	–
Lagoon of Venice		Zero	448	TV	5	0.04	(<LOQ–0.06)	0.03	(<LOQ–0.05)	0.03	(<LOQ–0.05)
Extra samples		"Risorgive"	NA	TV	3	<LOQ	–	0.05	(<LOQ–0.1)	0.05	(<LOQ–0.1)

Sampling

Sites were selected along 24 major rivers or streams flowing across eight main drainage basins (Table 7.5-129), named Adige, Brenta, Canalbianco, Livenza, Piave, Po, Sile, and the drainage basin of the Lagoon of Venice (DBLV). This latter basin needs special care: it hosts several streams and small rivers flowing directly into a large (~ 500 km^2 wide) coastal lagoon affected by high nutrient and pollutant levels, such as dissolved nitrogen and phosphorous, heavy metals (As, Co, Cd, Cu, Fe, Pb, Zn, Ni, Cr), persistent organic pollutants (polychlorinated biphenyls, organochlorine pesticides), and polycyclic aromatic hydrocarbons in top sediments. Three more samples were collected close to springs in the "risorgive" area. Each site was sampled during 1 year with different frequency (1-5 samples per site). Water was collected near the center of the river or, wherever not possible, at points having flowing water stream (i.e., no samples were collected on stagnant water conditions). Samples were stored in pre-cleaned HDPE bottles and in the dark at + 4°C to prevent sample degradation and photochemical reactions and were analyzed within 6 days (ISO 2014). During the sampling, water temperature was also measured, as well as pH (method APAT-CNR-IRSA-2060) and dissolved oxygen (method APAT-CNR-IRSA-4120).

Experimental
Glyphosate, AMPA, and glufosinate ammonium were analyzed following the method ISO 16308:2014. Briefly, the compounds are derivatized using 9-fluorenylmethylchloroformate (FMOC-Cl) in order to lower their polarity and increase the retention of compound in a separation on a reverse phase column as well as to improve the mass spectrometric detection. The derivatized sample was then purified by liquid/liquid extraction and concentrated by solid phase extraction (SPE). Methanol (≥99.9 %, Sigma Aldrich) was used in SPE extraction. For each sample extraction, ~ 13 mL methanol is used. The analysis is performed by high-performance liquid chromatography coupled with tandem mass spectrometry via an electrospray source (HPLC-ESI-MS/MS), using matrix-matched calibration. Calibration of the instrument was performed for every analytical batch; limit of quantification (LOQs, calculated according to the IUPAC Gold Book) was 0.05 µg/L.

Standards for spikes are dissolved in an aqueous matrix along with internal standards. Spikes are performed from these aqueous solutions. Once prepared, standards are kept at – 20 °C for 6 months max (see ISO 5667-3:2012). Samples were spiked before the derivation step with labeled glyphosate (1,2 $^{13}C2$, ^{15}N) and labeled AMPA (^{13}C, ^{15}N). The range of acceptability for recoveries adopted by ARPAV lab range from 75 to 125 %. The mean recoveries (in the concentration range of analyzed samples) were 103, 103, and 109 % for glyphosate, glufosinate ammonium, and AMPA, respectively. Physicochemical characteristics of water and chemical species were also analyzed using well established analytical protocols.

QA/QC and data handling
Method performance for glyphosate, AMPA, and glufosinate ammonium was tested by participation to LGC proficiency test AQ 492/2015 (LGC Aquacheck 2017) obtaining a satisfactory Z-score (Z <2) among laboratories of European countries and confirming a good accuracy of the adopted analytical protocol. At least two water samples for each batch were spiked with target compounds and then included in the analytical procedure: recoveries were in the range of 80-100 %. Precision was evaluated through analysis of replicated spiked water samples: results showed relative standard deviations <30 %. In this study, all the samples analyzed for herbicides were used for descriptive statistics, but only samples also analyzed for the remaining chemical and physical variables were further used for explorative analysis. Data below the LOQs (see Table 7.5-130) were set as LOQ/2.

Table 7.5-130: LOQs (limits of quantification) for the species/variables analyzed in this study

Species	LOQ	unit
GLY	0.05	µg L^{-1}
GLU	0.05	µg L^{-1}
AMPA	0.05	µg L^{-1}
Conductivity	1	µS cm^{-1}
Dissolved O_2	0.1	%
Hardness	5	mg L^{-1}
TSS	10	mg L^{-1}
BOD	0.5	mg L^{-1}
COD	5	mg L^{-1}
Na^+	0.2	mg L^{-1}
Mg^{2+}	0.5	mg L^{-1}
K^+	0.2	mg L^{-1}
Ca^{2+}	1	mg L^{-1}
F^-	0.1	mg L^{-1}
Cl^-	1	mg L^{-1}
SO_4^{2-}	0.5	mg L^{-1}
N-NH_4	0.02	mg L^{-1}
Total N	1	mg L^{-1}
N-NO_2	0.003	mg L^{-1}
N-NO_3	0.23	mg L^{-1}
P-PO_4^{3-}	0.02	mg L^{-1}
Total P	0.02	mg L^{-1}
As	1	µg L^{-1}
Ni	1	µg L^{-1}
Pb	0.5	µg L^{-1}

Chemometrics

The water samples were collected in rivers with different characteristics and/or affected by different anthropogenic pressures. Rivers also flow over different soil and rock types. This way, the chemical and physical characteristics of water may change according to the strength of natural/anthropogenic sources, the occurrence of biochemical processes in water, the soil characteristics, the flow rate, the closeness to point sources, the spatial distribution of diffuse sources, etc. A factor analysis (FA) was therefore performed to investigate the inter-variable relationships and to identify the most probable sources of water contamination or the ongoing biochemical processes. The principal aim of FA is to reduce the dimensionality of the dataset and to detect the main hidden processes/sources driving most of the variance of the original dataset.

Most of the species analyzed in this study are not normally distributed (Shapiro-Wilks test), with most of the variables exhibiting positive skewness. In addition, most variables have large differences in the units, i.e., the variables exhibits a striking difference in the amount of variability. For these reasons, non-parametric tests and correlations are used.

The Kruskal-Wallis analysis of variance by ranks was applied as a global non-parametric test for depicting statistically significant seasonal variations of analyzed variables. The null hypothesis is rejected for $p < 0.05$, meaning that concentrations are statistically different among seasons.

Since factor analysis is affected by data distribution and data scale, a series of data transformations were applied to obtaining a robust dataset. Firstly, a Box-Cox transformation was applied to approach normal distributions; thus, a standardization (mean zero and unit variance) was applied to scale the data and overcome differences in variation ranges.

In a second step, a discriminant analysis (DA) was applied to the factor score matrix to study the spatial

distribution of identified factors, i.e., to verify whether the sites in a drainage basin are isolated or characterized by a general homogeneity of the sources/processes. DA is typically applied to detect variables which significantly explain differences between two or more groups (drainage basins, in this case). The results of the test of univariate equality of group means can classify variables (factors, in this case) as not discriminant or discriminant: highWilks' Λ (>0.9) and significance >0.3 identify not discriminant variables, i.e., homogeneously present in all drainage basins. On the contrary, significances below 0.05 identify discriminant variables, i.e., having a heterogeneous distribution over the study area.

Results

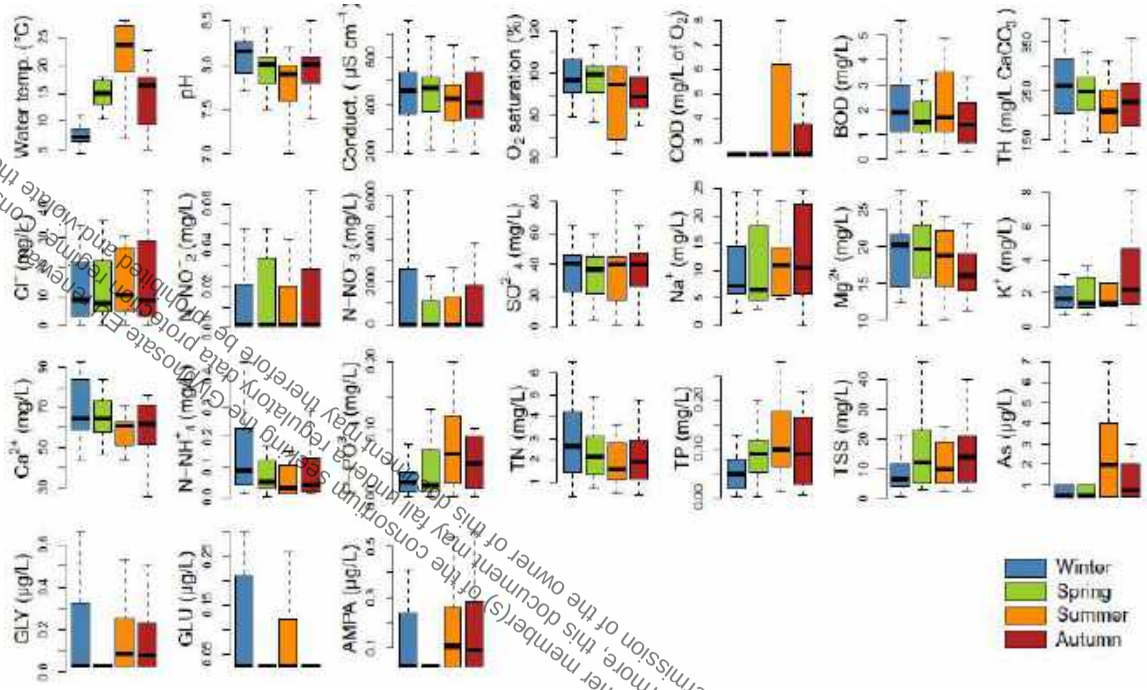
The average concentrations across the Veneto (all seasons, all sites) were 0.17, 0.18, and 0.10 µg/L for glyphosate, AMPA, and glufosinate ammonium, respectively (Table 7.5-129). The higher annual average concentrations of glyphosate were recorded on Musoncello (0.72 µg/L), followed by some sites along Livenza (Cervada, 0.49 µg/L; Livenza 0.45 µg/L) and Canalbianco (Nuovo Adigetto 0.4 µg/L), while AMPA was higher on Teva (0.77 µg/L), Musoncello (0.48 µg/L), and Livenza (0.55 µg/L). The river Musoncello was also affected by the higher annual concentrations of glufosinate ammonium (0.72 µg/L), followed by Teva (0.42 µg/L). Musoncello presents, therefore, the higher annual average concentrations of herbicides: it is affected by substantial loads from the urban sewer of Castelfranco Veneto (~ 33,000 inhabitants) and then flows through agricultural areas by also touching other towns (Resana). Finally, it joins the Dese River and, then, flows into the Lagoon of Venice. Therefore, further investigations and/or sampling campaigns are suggested for those polluted rivers in order to better monitor the sources of herbicides. In addition, more sites should be placed close to the outlets to quantify the load of herbicides flowing into the Lagoon of Venice.

In Europe, the upper tolerable level for all the pesticides in drinking water is administratively set to 0.1 µg/L. This regulatory limit is applied to annual average concentrations. The threshold of 0.1 µg/L was often exceeded in single samples (26, 37, and 22 % of all analyzed samples, respectively); however, only 11, 14, and 7 sites breached the annual upper tolerable level computed over multiple samples for glyphosate, AMPA, and glufosinate ammonium, respectively.

The design of our sampling campaign is not sufficient to accurately represent seasonal concentrations or to identify peak concentrations at single rivers, which can be missed even with a weekly sampling interval. The analysis of seasonal differences was therefore assessed for the whole set of data (Figure 7.5-81): only water temperature, pH, and concentrations of glyphosate and AMPA were statistically different.

The herbicides were rarely detected during spring, while the higher median concentrations for glyphosate and AMPA were measured in summer, followed by autumn and winter. This pattern is likely related to the seasonality of crops. Glyphosate is typically applied after crops and weeds have emerged from the soil, but it can be applied more than once during the growing season: this way, in Northern Italy crops and orchards are mostly treated in late-spring and summer when unwanted plants grow faster. However, herbicides are also applied to vineyards until mid-autumn (grape harvest). In addition, residues of herbicides may remain in the soil for weeks (half-life for glyphosate and glufosinate ammonium are 2-91 and 3-42 days, respectively); therefore, surface runoff and draining to groundwater may continue for months after treatment.

Figure 7.5-81: **Seasonal distributions of the analyzed variables. Data are aggregated to show data collected at all sites during the four seasons. Boxplot lines = medians, boxes = 25th-75th percentile ranges, whiskers = ± 1.5*inter-quartile ranges. Outliers and extremes not shown. *COD* chemical oxygen demand, *BOD* biochemical oxygen demand, *TH* total hardness, *TN* total Kjeldahl nitrogen, *TP* total phosphorus, *TSS* total suspended solids, *GLY* glyphosate, *GLU* glufosinate ammonium, *AMPA* aminomethylphosphonic acid**



Correlations among variables

Glyphosate and glufosinate ammonium exhibit a moderate correlation (0.53) and are also well correlated with AMPA (0.64 and 0.44, respectively). However, they are not well correlated (ρ <0.4) with any other variable. AMPA exhibits a strong correlation with glyphosate (ρ = 0.64), a poor correlation with orthophosphate (ρ = 0.26) and it is uncorrelated to TP. This result suggests that glyphosate degradation is the dominant source of AMPA in river waters of Veneto. However, the lack of a clear correlation with P compounds may be masked by the strong input of P-containing species from other sources, e.g., fertilization and urban and industrial discharges.

Potential sources of river contamination

The transformed dataset (Box-Cox/standardized) was used as input for a Varimax-rotated FA. A first attempt was made by including all the species. However, a pre-selection of variables to be processed in FA was subsequently performed to ensure robust and reliable results and to exclude chemically redundant species: (i) some variables (chemical oxygen demand, Na^+, K^+, Pb)were excluded because their high percentage of missing data (>25 %); (ii) missing data for other variables were substituted with the variable median; (iii) hardness was preferred to Mg^{2+} and Ca^{2+} because of their high correlation and the lower number of missing data; (iv) total phosphorous and total Kjeldahl nitrogen (TKN) were excluded because their strong correlations with orthophosphates and the sum of N-species, respectively; (v) nitrite was excluded because the high associated uncertainty due to its relatively unstable oxidation state; (vi) dissolved O_2 was converted from percent saturation to water concentration by considering the correction factors for water conductivity, water temperature and barometric pressure (USGS DOTABLES); (vii) hydrogen ion activity [H^+] (mEq/L) was calculated from pH to obtain a linear variable. Four factors with eigenvalues >1 were extracted, accounting for ~ 70 % of total variance.

Along with the factor loadings (Table 7.5-131), an $n \times m$ factor score matrix is also extracted: it is composed

of *n* cases (samples collected) and *m* new variables proportional to the daily source impact.

Table 7.5-131: Results of factor analysis (Varimax rotated solution). Variables with factor loadings (> 0.6) are in italics; factor loadings less than 0.35 are not shown; variables are ordered for decreasing absolute loadings. Var (%): percentage of variance explained by each factor; Cum. var. (%): cumulative variance

Factor 1 Fertilizers/salinity	Factor 2 Biological activity/arsenic	Factor 3 Herbicides	Factor 4 Urban/industrial discharges
Hardness (0.85)	*As (0.81)*	*GLY (0.85)*	*BOD (0.77)*
Conductivity (0.81)	*P-PO_4^{3-} (0.69)*	*GLU (0.83)*	*H^+ activity (− 0.73)*
SO_4^{2-} (0.71)	*TSS (0.67)*	*AMPA (0.77)*	Dissolved O_2 (0.55)
Cl^- (0.69)	*Dissolved O_2 (− 0.67)*		N-NH_4^+ (0.4)
N-NO_3^- (0.67)	Cl^- (0.56)		
N-NH_4^+ (0.62)	N-NH_4^+ (0.37)		
P-PO_4^{3-} (0.41)			
Var. = 23%	Var. = 19%	Var. = 15%	Var. = 12%
Cum. var. = 23%	Cum. var. = 42%	Cum. var. = 56%	Cum. var. = 69%

TSS total suspended solids, *BOD* biochemical oxygen demand

Factor 1 (23 % of variance) mainly represents the analyzed ions and, in particular, all the nutrients. It is primarily composed (loading >0.6) of anions (chloride, sulfate, nitrate), ammonium and, secondarily (0.35 <loadings <0.6), orthophosphate (Table 7.5-131). Consequently, the factor also exhibits high loading of hardness (directly linked to Ca and Mg) and water conductivity (0.83), which reflects the ionic activity.

Factor 2 (19 % of variance) is made up of arsenic, orthophosphate, total suspended solids and, secondarily, chloride and ammonium (Table 7.5-131). Under this view, it can be related to a pollution source and/or runoff. However, the temporal frequency of the sampling campaign has not allowed an analysis of the relation with rainfall depth or intensity. Consequently, the effect of runoff in this factor remains unclear.

Factor 2 also shows a strong negative loading with dissolved oxygen (− 0.67), which is indicative of an ongoing aerobic activity. The high loading of TSS further confirms this hypothesis, as the turbidity and the presence of colloids generally increase in more stagnant waters. The poor correlation of factor 2 with biochemical oxygen demand (BOD) (0.23) further suggests that the amount of biodegradable organic material is not a limiting factor for the aerobic activity or may indicate that the biological activity has depleted most of the organic material (i.e., the source does not represent a fresh input to the river).

Factor 3 (15 %) only links glyphosate, AMPA, and glufosinate ammonium (Table 7.5-131). The absence of high loadings with any other analyzed species indicates that the contamination of herbicides is uncorrelated with other pollution sources. The higher scores are found in summer >autumn >winter, and sites in the province of Treviso generally show the higher factor scores throughout the year. Relatively high scores are also recorded during summer in the two more northern sites (Piave drainage basin), which generally show the lower scores for the remaining factors. These rivers (Anfella and Val di Frari) flow in mountain areas and, therefore, are not likely affected by a load of herbicides from agriculture or silviculture. These sites represent an anomaly that should be investigated in more detail.

Factor 4 (12 %) links BOD, dissolved oxygen and, secondarily, ammonium; it also shows a negative correlation with the activity of H^+ (Table 7.5-131), i.e., it is linked to the more alkaline waters. No statistically significant inter-seasonal differences are found, i.e., it is almost constant all the year.

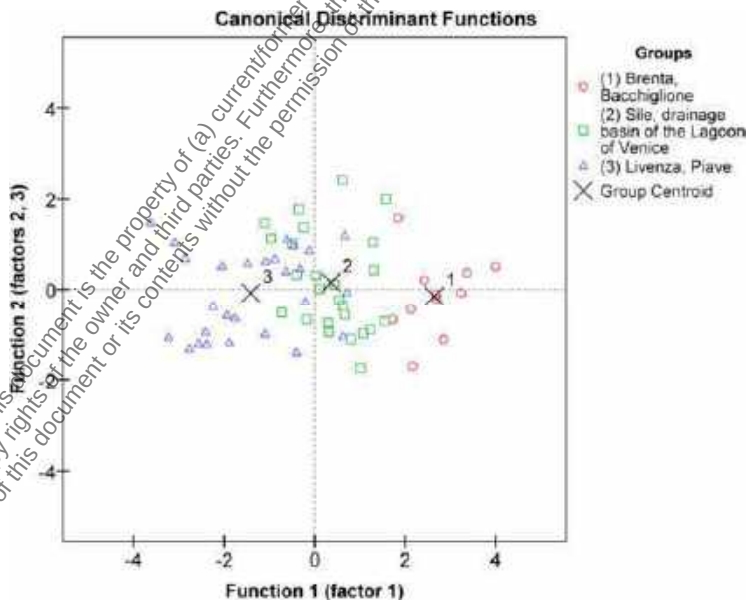
This factor depicts waters with high loads of organic matter (BOD), but it also represents waters with high primary production and/or affected by low aerobic activity (high loading of dissolved O_2). A possible interpretation is the fresh release of anthropogenic discharges of nutrients and effluents and the consequent increased photosynthetic activity.

Spatial distribution of sources

The factor scores were used in DA as independent variables; three areas including five drainage basins having similar characteristics were selected as grouping variable: (1) Brenta and Bacchiglione, i.e., rivers flowing in the center and southern part of Veneto; (2) DBLV and Sile, rivers mostly flowing from "risorgive" springs; (3) Piave and Livenza, rivers flowing in the northern part of Veneto with sources located in the Alps, but also having heavy contributions from "risorgive." Samples collected in Po, Canalbianco, and Adigewere excluded from DA due to the low number of sites and samples. The test of univariate equality of group means shows that only factor 4 is not discriminant, having the highest Wilks' Λ (0.98) and presenting a significance of 0.5. This result indicates that the pollution due to the fresh release of anthropogenic discharges (mostly attributable to urban or industrial sewage effluents) is homogeneously present in all the study area. Since the outputs from urban or industrial sewage effluents are expected to be constant through the year, this result confirms the interpretation of factor 4.

On the contrary, factors 1, 2, and 3 are highly discriminant (significance <0.05), i.e., they present heterogeneous distributions over the three groups of rivers. Two discriminating functions were also extracted and interpreted by analyzing their correlations with the input variables (factors): the first function only presents weak correlations with factor 1 (fertilizers/salinity) and 2 (biological activity and arsenic), while the second one presents the largest absolute correlation with the factors 2 and 3 (herbicides). Figure 7.5-82 shows the bi-dimensional scatterplot of sample scores into the planes defined by the discriminant functions. The plot shows that the samples in the three groups of rivers are generally well differentiated under the discriminant function 1 (weakly correlated with factors 1 and 2), with higher scores for samples collected in the southern area (Brenta-Bacchiglione) and lower for the samples collected to the north (Piave-Livenza). On the contrary, group centroids are not well separated along the discriminant function 2.

Figure 7.5-82: Discriminant scores scatterplot. Group centroids are shown as grey crosses



Conclusion

This study is the first one investigating the occurrence of glyphosate, glufosinate ammonium, and AMPA in river water of the NE Italy. The main findings of this study can be summarized as follows:

- The contamination of herbicides is a critical issue in Veneto: glyphosate, AMPA, and glufosinate ammonium frequently exceeded the European upper tolerable levels for pesticides (annual average 0.1 µg/L) during 2015. However, this tolerable level is based on political consensus, not

ecotoxicological significance and it is very low if compared to the maximum level of glyphosate permitted in the USA (700 µg/L) based on toxicity tests;

- Glyphosate and AMPA showed statistically different seasonal concentrations, with higher medians in summer and autumn and lower in spring. This seasonal pattern agrees with the use of herbicides in agriculture and silviculture;
- The River Musoncello was affected by the higher annual average concentrations of glyphosate and glufosinate ammonium;
- The correlation and factor analyses pointed out the interspecies relationships. Four factors were extracted and interpreted as possible sources/processes affecting the water quality of rivers. Herbicides were identified by a single factor. Two more factors were linked to possible sources: the leaching of fertilizers and the urban/industrial discharges. Another factor was attributed to the biological activity on polluted or stagnant waters;
- A discriminant analysis was performed on the factor scores and over 3 areas representative of 5 drainage basins. Results revealed that the anthropogenic discharges (mostly attributable to urban or industrial sewage effluents) are homogeneously present over all the study area, while biological activity and fertilizers present heterogeneous distributions. However, a clear spatial gradient was not detected.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports measurements of glyphosate and AMPA in surface waters in Northern Italy. Maximum surface water glyphosate concentration measured at 0.72 µg/L, and maximum AMPA concentration at 0.77 µg/L.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/039
Report author	Dairon, R. *et al*.
Report year	2017
Report title	Long-term impact of reduced tillage on water and pesticide flow in a drained context
Document No	Environ Sci Pollut Res (2017) 24:6866-6877
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Influence of more than 20 years (1988–2010) of reduced tillage (RT) practices on water and pesticide balances and dynamics is analyzed and compared to results from a conventional tillage plot (CT). The field study soils are described as silty clay stagnic luvisol, developed on a low permeable schist layer. A drainage network was set up according to French criteria (0.9 m deep, 10 m space) to avoid soil winter waterlogging. Climate is temperate oceanic and drainage generally occurs from November to March. Data were analyzed at yearly, weekly (pesticides) and hourly (water) time steps. Over the long term, cumulated drainage decreases significantly on RT (3999 mm) compared to CT (5100 mm). This differentiation becomes significant from 1999, 10 years after plowing was stopped. Strikingly, hourly drainage peak flows are higher under RT, especially during the second period (2000–2010), associated with low or no base flow. These results suggest a strong influence of the macropore network under RT practice. In particular, drainage peaks are higher at the beginning of the drainage season (mid-October to December). Consistently, pesticides applied in late autumn, which are the most quantified on this site, are often significantly more exported under RT. For atrazine, applied in spring, fluxes are linked to cumulative flow and are de facto higher under CT. For others pesticides, losses appear to be heterogeneous, with generally low or null export rates for spring application. Generally speaking, higher concentrations are measured on RT plot and explain observed exportation rate differences. Finally, there is no clear evidence of correlation between pesticide losses and long-term impacts of RT on hydrodynamics, pointing the importance of studying the short-term effect of tillage on water and especially solute flow.

Materials and methods

Site and plot description

The experimental station of La Jaillière is located in western France (47° 27′ N, 0° 57′ W). Soils, mainly stagnic luvisol are developed on a low permeable schist formation (saturated hydraulic conductivity Ks <0.2 mm/h). Clay content increases from surface layer (22 %) to subsurface (> 40 %), where many hydromorphic features have been observed. Soil structure, fine and sub angular in surface becomes coarse and prismatic with depth. Climate is temperate oceanic, with a mean annual precipitation of 709 mm and a mean annual potential evaporation of 738 mm during the 1988–2011 period. To prevent soil waterlogging and improve crop growth during winter, tiled drainage was implemented in the 80s. The PVC tile drains (54 mm diameter) at this site are 0.9 m deep on average, with a spacing of 10 m, in order to respect French standard. Drain flow, surface runoff, and nitrate and pesticide fluxes have been monitored since 1987, 1989, and 1994, respectively. Historically, the "La Jaillière" site was set up for agronomical purposes to highlight the interest of subsurface drainage on crop yield (1980s). Then environmental issues of water quality in drained conditions rise in the 90s. Among the 11 plots, two were chosen in 1989 to compare RT and CT on the same soil context, climate, and agricultural practices. As previously enounced, this paper focuses on two plots, one conventionally tilled and the other one driven without plowing. Topsoiling and stubble cultivation operations are still performed on the RT plot (Table 7.5-132). These two plots, of 1 ha each, are located on the plateau and are only 200 m far one from the other. Both are hydraulically isolated from other neighbor plots. Slope is gentle on the site for both plots (<2 %).

Soil texture, organic matter (O. M), pH, and C/N ratio were measured in 1987, 1994, 2004, and 2009 in order to investigate temporal modification of main soil characteristics. For soil texture, O.M and pH measurements were performed at 0–10, 10–25, and 25–50 cm for CT and 0–5, 5–10, 10–25, and 25–50 cm for RT. Bulk density was first measured in autumn 1994 (just after plowing). A new set of measurements was performed in 2013, during infiltration measurement campaign (data collected in April, 8 months after any previous tillage operation on CT plots).

Crop rotations and fertilization practices are identical on the two plots throughout the study period. However, pesticide applications slightly differ, because of a more regular use of herbicides on RT (glyphosate). Except for these applications, pesticides are applied at the same dates and rates on two plots.

Hourly precipitation, daily potential evaporation, net radiation, and temperature are recorded on-site. Tiled-drained flow is channeled towards a measurement chamber, where it is hourly recorded thanks to an ultrasound probe once flow has settled. Sampling strategy is based on flux quantification instead of flow

event dynamic. Consequently, flow-weighted mean samples are composed of several subsample taken every 5 m^3/ha of drained water. The weekly samples are then stored at −18 °C for pesticide analysis in order to get representative mean concentrations and to calculate total pollutant export. Pesticides were analyzed on raw water, at INRA Versailles laboratory until 2000. Pesticides were extracted from the liquid phase by dichloromethane and/or by acetone/dichloromethane for pesticides adsorbed on suspended matter. Purifications were performed using solid-phase extraction (styrene divinyl benzene copolymer cartridges). Concentrations of pesticides were determined with gas chromatography equipped with an electron capture detector (GCECD) or with liquid chromatography equipped with a UV detector (HPLC-UV). Since 2000, analyses were performed at GIRPA Angers laboratory with the same extraction method(s). Concentrations were then measured by liquid or gas chromatography coupled with tandem mass spectrometry (MS/MS). For the determination of glyphosate and AMPA, water samples are first extracted with diethylether to remove organic matter then purified with ethylenediaminetetraacetic acid to prevent potential fixing of glyphosate and AMPA on calcium and divalent metals (iron, copper, zinc). The HPLC method used then, consists of sample derivatization, using 9-fluorenylmethylchloroformate (FMOC), followed by HPLC analysis with fluorescence detection (Using the ProStar 363 Fluorescence Detector).

Table 7.5-132: Main physico-chemical soil characteristics on conventional tilled plot

Horizon	Thickness	Clay (%)	Silt (%)	Sand (%)	O. M (%)	Bulk density (g.cm3)	pH (water)	CEC	C/N	Structure
Ap	25	22	46	32	2.2	1.58	6.3	8.7	9	Blocky
E	17	25.9	41.3	32.8	0.77	1.6	7	7.9	8.5	Blocky
Bt	18	49.2	35.3	15.5	0.46	1.7	5.6	9.5	8.5	Prismatic
Bt/C	45	42.7	35.8	21.5	0.36	1.8	4.9	9.5	8.5	Blocky

Results

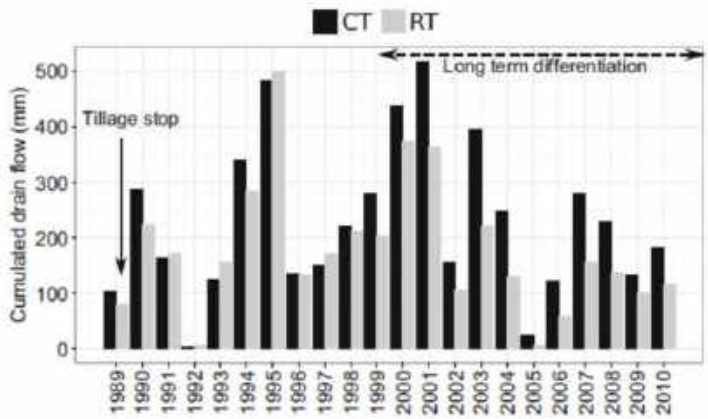
Long-term evolution of soil physico-chemical characteristics

Changes induced by no tillage on the physico-chemical properties of soil are heterogeneous, for organic matter (O. M) has increased from 2.04 to 2.4 % in the top layer (0–25 cm) of CT plot between 1987 and 2009. On RT plot, O. M content has risen in the top layer from 2.04 to 2.54 %. However, in the first case (CT), the increase is uniform over the surface layer (0–25 cm) while for RT, the increase is located in the 0–10 cm with an O. M content of 3.4 %. In the subsoil, O. M remains constant on both plots, around 0.8–0.9 % from 25 to 37 cm and 0.4–0.5 % from 37 to 65 cm. For bulk density, analyzed in 1994, result showed a higher value in the first layer for RT than in CT, with a mean value of 1.58 (±0.03) and 1.48 (±0.05) g/cm^3, respectively. Measurements performed in 2013 highlight bulk density stability on RT (1.59 g/cm^3) while this characteristic has increased in CT plot (1.65 g/cm^3). Soil texture, pH, and C/N ratio show no significant variation during the study period in Ap horizon.

Water balance

Figure 7.5-83 shows annual drain flow for tilled and untilled plots from 1989 to 2010. Over the whole period, there is no significant difference between the two plots if annual data are used (p value 0.164). As illustrated, the two plots behave differently after 1999. So, drain flow becomes significantly lower on RT from 2000 to 2010 (p value 0.037). In the end, in 10 years, 1050 mm more water was drained in CT plot compared to RT plot, which is equivalent to 4 years of annual cumulated drainage (254 mm). We are therefore entitled to wonder how this difference impacts the dynamic of drainage and the consequence on pollutant transfer.

Figure 7.5-83: Annual drain water flow on CT and RT plots from 1989 to 2010



Hourly drainage dynamic

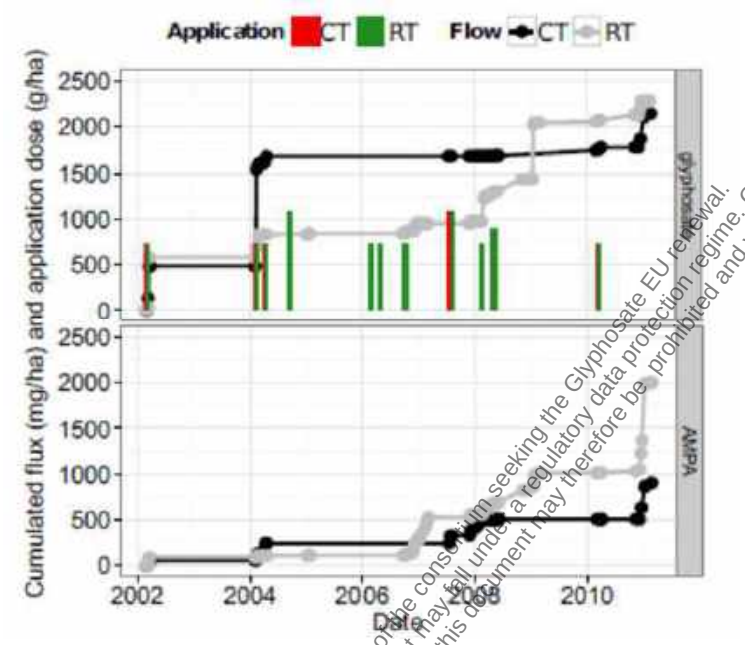
This section presents the results of hourly drainage flow analysis for the two study plots, based on flow duration curves. Analysis performed on the whole study period shows that for short duration events (1 to 6 h), flow values are higher on the untilled plot for most of the return periods. For intermediate duration events (6 to 18 h), both plots show similar flow values. For long duration events (24 to 48 h), flow values are lower on the untilled plot for a majority of the return periods. These two statements indicate that the two plots hydrodynamical behaviors are different. Thus, the unploughed plot, despite a large water deficit, shows higher peak flows than the tilled one.

We previously noticed a long-term differentiation of cumulated drained water between the two soil tillage practices. We will now study if this shift has influence on hourly drain flow dynamic. The goal is to assess how water balance variability affected the drainage hourly dynamic. Results show that for short duration events (1–6 h), ratio is decreasing from the first (1989–1999) to the second period (2000–2010) with values between 0.8–1.0 and 0.60–0.8, respectively. This means that despite a significant decline in the annual flow on RT, drain flow peak intensity has increased. Obviously, long duration events (1–2 days) exhibit higher ratio during the second period (>1.5) to offset water flow deficit. In fact, in most cases, no base flow is observed on RT (i.e, low flow values after rainy periods, corresponding to the drained water table recession). Consequently, drain peak flow on RT can be viewed as a Dirac delta distribution compared to peak flow on CT. Thus, stopping moldboard plow operation had a significant impact on water balance with a decrease of annual drained water on RT after 10 years. Surprisingly, this shift was accompanied by an increase of hourly peak flows. Difference in annual drainage is mainly caused by shorter drain flow recession and by the lack of drainage base flow as observed on the tilled plot.

Pesticides

As outlined previously, greatest applications of glyphosate on RT plot is part and parcel of this system. Thus, we choose to compare glyphosate and AMPA chronicles despite those differences. Quantification rates are higher under RT for glyphosate (58 vs 39 %). Maximum glyphosate concentration and flow were observed on CT 2 days after winter application (11/02/2004) with a value of 12 µg/L and 1058 mg/ha, respectively. Accordingly, as observed in Figure 7.5-84, glyphosate exportations are link to first events following application. Over the long term, 0.052 and 0.025 % of glyphosate applied dose were losses in drainage for CT and RT, respectively. There is no significant flow difference between both systems (p value 0.13) here compared on the overall period and not by application. In contrast, AMPA, which is also more quantified under RT than CT (67 vs 36 %), is significantly more exported on RT plot (p value 0.006) as shown on Figure 7.5-84.

Figure 7.5-84: **Normalized cumulated solute flow (glyphosate or AMPA) versus normalized cumulated water flow from 2002 to 2009 for CT and RT plots. Date and dose of application (in g/ha) of glyphosate are also given**



Conclusion

Despite a strong hydrodynamic differentiation (cumulated drainage, hourly dynamic) after 10 years of no-tillage practices, it is not clear if pesticide flow was or not influenced over the long term. Only periods following moldboard plow operations seem to significantly influence solute flow because tillage induces macropore network destruction, increase of water retention, and disturbance of earthworm activity. So, in this context, pesticides applied in autumn, just after tillage season, are more likely to be exported for no-tilled practices.

In conclusion, this study highlights the importance of very long term studies in tillage research (>10 years) and the interest of drained sites, in particular because of spatial integration and easy data sampling (water and solute). After 20 years without moldboard plow, a gradient of organic matter was observed in the first soil layer. Over the whole period, lower drained water on RT could be beneficial on an environmental point of view, in particular for nitrate ($N–NO_3^-$). In contrast, on RT plot, drainage events are more concentrated, especially during the beginning of the drainage season, leading to increased pollution risk for solute (pesticides, phosphorus) applied during this period. In addition, the absence of mechanical weeding involves an increased use of herbicide (glyphosate here). It therefore induces a possible additional risk to the diffuse pollution risk in agricultural areas, especially for soils where preferential flow are likely to happen. Finally, studying other aspects of farming systems are needed to conciliate economic, social and environmental objectives.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the different long-term drainage behavior of glyphosate among other pesticides under reduced tillage and conventional tillage at the experimental station of La Jaillière located in western France. Influence of more than 20 years (1988–2010) of reduced tillage practices on water and pesticide balances and dynamics is analyzed and compared to results from a conventional tillage plot. The maximum glyphosate concentration in drainflow was observed on the conventional tillage plot 2 days after winter application with a value of 12 µg/L.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/040
Report author	Lefrancq, M. *et al.*
Report year	2017
Report title	High frequency monitoring of pesticides in runoff water to improve understanding of their transport and environmental impacts
Document No	Science of the Total Environment 587-588 (2017) 75-86
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Rainfall-induced peaks in pesticide concentrations can occur rapidly. Low frequency sampling may therefore largely underestimate maximum pesticide concentrations and fluxes. Detailed storm-based sampling of pesticide concentrations in runoff water to better predict pesticide sources, transport pathways and toxicity within the headwater catchments is lacking. High frequency monitoring (2 min) of seven pesticides (Dimetomorph, Fluopicolide, Glyphosate, Iprovalicarb, Tebuconazole, Tetraconazole and Triadimenol) and one degradation product (AMPA) were assessed for 20 runoff events from 2009 to 2012 at the outlet of a vineyard catchment in the Layon catchment in France. The maximum pesticide concentrations were 387 µg/L. Samples from all of the runoff events exceeded the legal limit of 0.1 µg/L for at least one pesticide (European directive 2013/39/EC). High resolution sampling used to detect the peak pesticide levels revealed that Toxic Units (TU) for algae, invertebrates and fish often exceeded the European Uniform principles (25 %). The point and average (time or discharge-weighted) concentrations indicated up to a 30- or 4-fold underestimation of the TU obtained when measuring the maximum concentrations, respectively. This highlights the important role of sampling methods for assessing peak exposure. High resolution sampling combined with concentration-discharge hysteresis analyses revealed that clockwise responses were predominant (52 %), indicating that Hortonian runoff is the prevailing surface runoff trigger mechanism in the study catchment. The hysteresis patterns for suspended solids and pesticides were highly dynamic and storm- and chemical-dependent. Intense rainfall events induced stronger C-Q hysteresis (magnitude). This study provides new insights into the complexity of pesticide

dynamics in runoff water and highlights the ability of hysteresis analysis to improve understanding of pesticide supply and transport.

Materials and Methods

Description of the vineyard catchment

Soils overlay an impermeable Armorican substratum (Namurian Shale, Sandstone and Psammites). The catchment is characterized by three different gradients: (i) The upper catchment has 0-5 % slopes (51 % of the total catchment area); (ii) The middle catchment has 5-15 % slopes (40 %); and (iii) The lower catchment has >15 % slopes (9 %), including agricultural terraces. Soil depths vary from 30 cm in the lower zone to 120 cm in the upper zone. Spatial variability of the soil was characterized using 50 surface soil samples (0-20 cm) taken from across the three areas. Soil characteristics for the catchment are as follows (mean ± SE): sand: 42.3 ± 5.1 %; silt: 36.1 ± 3.0 %; clay: 19.5 ± 2.3 %; OM: 2.1 ± 0.4 %; pH: 7.1 ± 0.4; CEC: 10.4 ± 0.8 meq 100/g; $CaCO_3$: 0.1 %. The structural stability of the soils was measured by immersing soil aggregates in water followed by the separation of the soil fraction using mechanical sieving. Fractions >250 µm were measured and constituted an index of soil stability. Grassed rows were comprised of 38 ± 12 % stable aggregates while weeded rows were comprised of 18 ± 6 % stable aggregates, which indicate a limited risk of soil sealing. Mean annual rainfall is 623 mm (±124 mm) (1985-2014, Beaulieu sur Layon, 3 km from the study site).

Pesticide properties and application

In the studied vineyard, 31 commercial products with 21 different active ingredients were applied in the following amounts: 3.1, 4.8, 2.1 and 3.0 kg in 2009, 2010, 2011 and 2012, respectively. Of those products used, 53, 28, 54 and 32 % were fungicides, respectively. The study focused on 7 pesticides (Dimetomorph (DIM), Fluopicolide (FLU), Glyphosate (GLY), Iprovalicarbe (IPR), Tebuconazole (TEB), Tetraconazole (TET) and Triadimenol (TRI)) and one degradation product (AMPA) because of their detection frequency and their yearly applied mass within the study catchment (Table 7.5-133 and Table 7.5-134). The physical and chemical characteristics of these 8 compounds are provided in Table 7.5-133. The 7 pesticides were mostly applied between March and July. TEB and TRI were generally applied to the upstream section of plot A, while FLU, IPR and TET were only applied to plot B (Figure 7.5-85). The ability of high resolution sampling to improve our knowledge of the pesticide sources, transport pathways and ecological impacts in runoff was assessed using these 8 compounds.

Figure 7.5-85: The study catchment with the experimental setup (Rochefort sur Loire, 47°19″ 19.47″N;0°38″21.39″W)

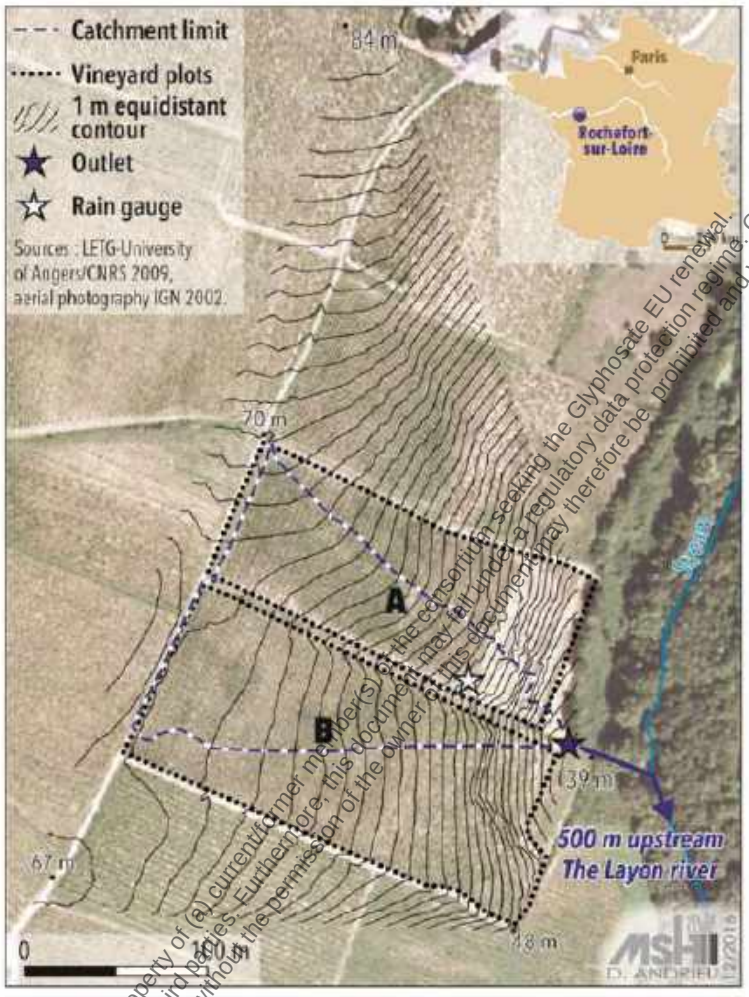


Table 7.5-133: Family, type, commercial formulations, physicochemical properties, toxicity and detection frequency of the 7 pesticides (DIM, FLU, GLY, IPR, TEB, TET, TRI) and degradation product (AMPA)

Compound	Abbrv.	Commercial formulation	Chemical family	Formula	Physico-chemical characteristics of active substances						
					Log K_{ow}[a] [−]	Log K_{oc}[a] [−]	Henry constant[a] (25 °C) [Pa m^3 mol^{-1}]	DT_{50} (aerobic-anaerobic soil)[b] [day]	DT_{50} field[c] [day]	$EC50^{a,c}$ [ppm]	Detection frequency (20 events) [%]
Dimethomorph	DIM	Panthéos/ Arco DTI/ Fastime/ Tulsa	Morpholine	$C_{21}H_{22}ClNO_4$	2.68	2.61	2.04×10^{-05}	75–26	10–61	> 10.6	90.9
Fluopicolide	FLU	Profiler	Benzamide	$C_{14}H_8Cl_3F_3N_2O$	2.90	2.50	4.15×10^{-05}	415–561	132	>1.8	100
Glyphosate	GLY	Roundup/ Vival/ Sirlene	Phosphonoglycine	$C_3H_8NO_5P$	−3.20	3.85	2.10×10^{-07}	96–22	5–21	40	100
AMPA	AMPA	–	Unclassified	CH_6NO_3P	−1.63	–	0.16	n.a.	76–240	n.a.	100
Iprovalicarbe	IPR	Ocarina	Carbamate	$C_{18}H_{28}N_2O_3$	3.20	–	1.40×10^{-06}	15.5–n.a	n.a.	n.a.	40.9
Tebuconazole	TEB	Abilis	Triazole	$C_{16}H_{22}ClN_3O$	3.7	3.00	1.00×10^{-05}	597–1260	20–92	2.79	22.7
Tetraconazole	TET	Eminent	Triazole	$C_{13}H_{11}Cl_2F_4N_3O$	3.56	–	3.60×10^{-04}	364–180	136–1688	3.0	86.3
Triadimenol	TRI	Abilis	Triazole	$C_{14}H_{18}ClN_3O_2$	3.18	2.40	3.5×10^{-06}	250–n.a	110–375	51	31.8

[a] Obtained from the PPDB (2007, 2008, 2009), The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire.
[b] Obtained from the PAN (PAN, 2006) pesticide data base.
[c] Daphnia magna test, 48 h obtained from PPDB data base.
[d] Obtained from AGRITOX.

Table 7.5-134: Application amount [g] and number of applications [-] (in brackets) of the 7 pesticides (DIM, FLU, GLY, IPR, TEB, TET, TRI) in 2009, 2010, 2011 and 2012

	Applied total mass [g] (number of applications [−])				
Compound	2009	2010	2011	2012	Total
DIM	373.8 (2)	177.8 (1)	177.8 (1)	231.2 (2)	960.5 (6)
FLU			105.2 (1)		105.2 (1)
GLY	1087.2 (3)	2642.4 (3)	933.3 (2)	1999.8 (2)	6662.7 (10)
IPR	213.3 (1)			89.6 (1)	302.9 (2)
TEB	124.2 (3)	54.0 (1)	54.0 (1)		232.2 (5)
TET	40.3 (2)	39.5 (2)	39.5 (2)	39.5 (2)	158.8 (8)
TRI	41.4 (3)	18.0 (1)	18.0 (1)		77.4 (5)

Hydrological and sampling procedures

Rainfall intensity was recorded using a tipping bucket rain gauge. Water discharge was measured at the catchment outlet every 30 s using a bubbler flow module with a 5 mm precision combined with a Venturi channel. As soon as the water level increased above 2 cm, 500 mL of water were sampled every 2 min using an automatic sampler containing 24 polyethylene flasks of 500 mL. Automatic phone calls notified people on duty if the rainfall event exceeded the capacity of the sampler, enabling manual sampling of the rest of the event when necessary. Water samples were then collected and placed on ice for transportation to the laboratory.

Chemical analysis

Samples were filtered to measure the total suspended solid concentration (TSS). Raw and filtered samples were kept at -18 °C in the dark prior to chemical analysis. GLY and AMPA samples were analysed after filtering (0.45 µm). Other compounds were analysed in raw water in order to not underestimate the runoff export via the particulate phase (>0.45 µm). However, for 22 arbitrarily selected samples from the measured runoff events, fungicides were analysed in both filtered and raw forms to investigate the partitioning of those fungicides in the "dissolved phase" (<0.45 µm) and in the particulate phase (>0.45 µm). DIM, FLU, IPR and TET concentrations in raw and filtered water did not differ significantly, indicating that fungicides were predominantly transported in the dissolved phase, which is supported by previous studies (Maillard and Imfeld, 2014). TEB and TRI were not quantifiable in the 22 samples but are hypothesized to behave similarly to TET because they belong to the same triazole family and have a similar log K_{ow} (Table 7.5-133). GLY and AMPA were analysed using HPLC separation with spectrofluorimetric detection after decomplexation of both analytes, followed by a derivatization using 9-fluorenylmethyl chloroformate (FMOC-Cl). The average recovery rates were 100 % and 105 % for glyphosate and AMPA, respectively. The detection and quantification limits were 0.03 µg/L and 0.09 µg/L for glyphosate and 0.04 µg/L and 0.1 µg/L for AMPA, respectively. Other pesticide analyses were performed as follows. After spiking with surrogate standards chlorpyriphos-d_{10} and diuron-d_6, water samples (500 mL) were successively liquid-liquid extracted at 3 pHs (<2, 7 and >12) using a mixture of dichloromethane: ethyl acetate 80:20. The extracts were combined, dehydrated and evaporated under vacuum. The concentrated extract was transferred into a vial and adjusted accurately to 1 mL with ethyl acetate. An aliquot of this extract was solvent exchanged with a mixture of water:methanol (50:50 with 0.1 % acetic acid). Analysis was performed by liquid chromatography/electrospray ionisation tandem mass spectrometry (LC/ESI-MSMS). The remainder of the ethyl acetate extract was analysed by gas chromatography/ion trap tandem mass spectrometry GC/IT-MS-MS. The pesticide quantification limit within the water samples was 0.05 µg/L. Recovery rates ranged between 86 and 96 %.

Data analysis and calculation

Climatic and hydrological data
To compare the amount, intensity and duration of rainfall events, an event index (EVI) was calculated using the following ratio (Baartman *et al*., 2013):

$$EVI = \frac{Imax \times Rtot}{D} \quad (1)$$

where I_{max} is the maximum rainfall intensity [mm/h], R_{tot} is the rainfall amount [mm] and D is the rainfall duration [min]. A high EVI represents a short but intense rainfall event, whereas a low EVI indicates an event with a low intensity but long duration. The catchment response time is defined as the time between the gravity centre of the rain event and the peak outflow.

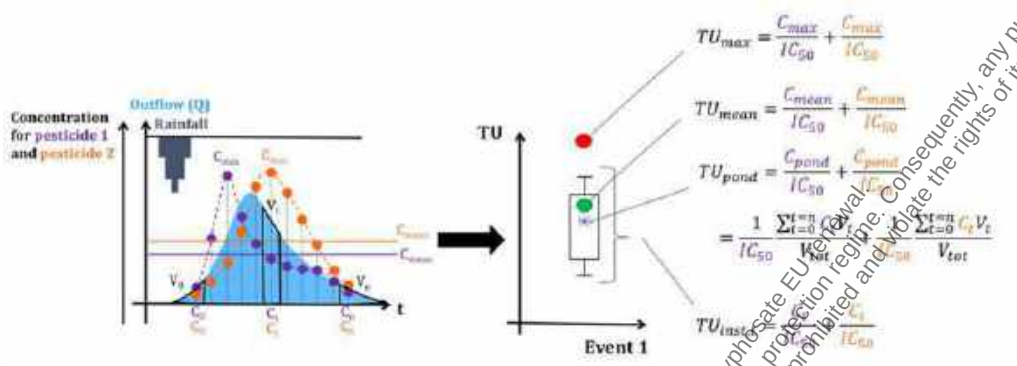
Pesticide export
The maximum pesticide concentration, which was measured, was supposed to be the maximum of the event and was labelled the pesticide peak. To calculate pesticide loads, linearity of the values between two successive concentration data points or flow measurements was assumed. When pesticides were not detected (336 analyses, i.e. 16 %), concentrations were set to zero to calculate the mean concentrations, occurrences and loadings. When pesticide concentrations were detected but lower than the quantification limit (186 analyses, i.e. 9 %), the sample concentration was set to half of the quantification limit. Pesticide export coefficients were estimated as the ratio of the exported loads from a runoff event compared to the cumulative application of the year preceding the studied runoff event. Hydrological characteristics and pesticide concentrations were compared using the paired nonparametric Wilcoxon signed rank test and the Spearman rank correlation test. Statistical tests were performed using the R software.

Ecotoxicological data
The impact of the pesticide mixture toxicity on the aquatic ecosystem was evaluated using the TU approach (Bundschuh *et al*., 2014). TU was estimated as follows for each event:

$$TU = \sum_{i=1}^{n} \frac{C_i}{IC_{50i}} \quad (2)$$

where C_i is the concentration of pesticide i within a mixture of n pesticides [µg/L] and IC_{50i} is the concentration of pesticide i which induces a response halfway between the baseline and maximum after a specified exposure time [µg/L]. In our study, IC_{50} values were taken from the PPDB database (Lewis *et al.,* 2016) and were related to three trophic levels: algal growth inhibition (acute 72 h), invertebrate immobility (acute 48 h) and fish mortality (acute 96 h). Although other species may be more sensitive, for comparison purposes, Daphnia magna and Oncorhynchus mykiss were used to study the effects of all pesticides, except for TET, on invertebrate and fish species, respectively. TET effects on fish species were measured using Lepomis macrochirus. Pseudokirchneriella subcapitata and Scenedemus subspicatus were primarily used to study the effects of the 8 target compounds on green algae. If no data for these green algae species were available (i.e., for GLY, FLU, IPR and TET), IC_{50}-data for any other green algae species were used (Bundschuh *et al*., 2014). Four different methods were used to estimate C_i to test the loss of ecotoxicological information associated with the different sampling methods. The formulas used are illustrated in Figure 7.5-86. Within these formulas, C_i represents: (i) the point concentration within each sample ($C_{inst,t}$ used to estimate $TU_{inst,t}$), (ii) the maximum concentration during the runoff event (C_{max} used to estimate TU_{max}), (iii) the average concentration during runoff event (C_{mean} used to estimate TU_{mean}) and (iv) the discharge-weighted average concentration (C_{pond} used to estimate TU_{pond}). C_{mean} represents the concentration of a pool of samples obtained at regular time intervals, whereas C_{pond} represents the concentration of a pool of samples obtained for a constant outflow volume. $C_{inst,t}$ represents the potential concentration that may occur for a random sample. Estimated TU values were compared to the European Union TU threshold of 0.1 for algae and 0.01 for invertebrates and fish, which are known as the European Uniform Principles (European Commission, 2011). Tus were estimated for all studied runoff events except October, 20 2009 and October, 14 2012. Data for these two events were omitted because not all compounds were analysed.

Figure 7.5-86: Representation of the four different TU_{max}, TU_{mean},TU_{pond}, TU_{inst} calculations for an artificial runoff event with n samplings for two different compounds, called 1 (purple) and 2 (orange)



First flush calculation

A first-flush effect is defined to occur when a disproportionately greater pesticide load is transported by a relatively small proportion of the runoff volume during the beginning of a runoff event. The first flush (FF [%]) is defined as follows:

$$FF_X = \frac{\int_0^{t_x} C(t)Q(t)dt}{\int_0^{T} C(t)Q(t)dt} \times 100 \qquad (3)$$

where X is the defined runoff volume of a sample as a percent of the total runoff [%], here, 10, 25, 50 and 75 %; C(t) [µg/L] and Q(t) [L/s] are the pesticide concentration and the runoff outflow at time t, respectively; T is the duration of the runoff event [min]; and t_x is the time at which X% of runoff has been delivered [min]. A FF_X value significantly larger than X indicates a disproportionate phenomenon. Bertrand-Krajewski *et al*. (1998) assumed that a significant first flush occurred if at least 80 % of the total pollutant mass was transported in the first 30 % of runoff discharged during a rainfall event.

Hysteresis pattern

Runoff events for which at least 2 sample points were quantifiable have been taken along both the rising and falling limb were used in the present study to investigate the hysteresis patterns. To compare the hysteretic loops of different runoff events and solutes, two quantitative indices were used. First, the rotational parameter ΔR, which integrates information on the hysteresis area and direction, was estimated as follows:

$$\Delta R = R \times A_h \qquad (4)$$

where A_h is the normalized hysteresis area, calculated as the polygon area of the convex-hull of the C-Q hysteresis curve after standardizing discharges and concentrations to a unity scale; and R is the hysteresis direction (1 for clockwise, -1 for anticlockwise and 0 for no or an unclear hysteresis pattern). Therefore, ΔR varied between -1 to 1. The magnitude parameter, ΔC, represents the relative change in pesticide concentrations during the runoff event and is measured as follows:

$$\Delta C = \frac{C_{max} - C_{min}}{C_{max}} \qquad (5)$$

where C_{max} and C_{min} are the maximum and minimum pesticide concentrations, respectively.

Results

Characteristics of selected rainfall-runoff events

Twenty rainfall-runoff events occurring between 2009 and 2012 were studied. Each event yielded >1 m^3 total discharge at the outlet of the catchment. These events represented a wide range of rainfall intensities and durations, runoff percentages and volumes (Figure 7.5-87 and Table 7.5-135). Selected rainfall events exhibited return periods ranging from 0 to 10 years (Data from MeteoFrance, Table 7.5-135). The runoff coefficient ranged from 0.3 to 47 %. The catchment response time ranged between 5 and 482 min, with an average of 88.5 min. For events with a high EVI, i.e., intense and short rainfall, discharge occurred rapidly (short response time) ($p < 0.001$) (Table 7.5-135). The concentration of TSS ranged between 11 and 6454 mg/L (Table 7.5-136) and was positively correlated with outflow ($p < 0.001$), suggesting that rill erosion occurred. Maximum pesticide concentrations reached 13, 8, 386.9, 47, 3, 81, 68 and 4.2 µg/L for DIM, FLU, GLY, AMPA, IPR, TEB, TET and TRI, respectively (Table 7.5-136). For each runoff event, maximum pesticide concentrations decreased with increasing time following the last application of the analysed pesticide ($p < 0.016$) (Table 7.5-136). This indicates the occurrence of a dissipation effect, as previously mentioned in literature (Arias-Estévez *et al*., 2008). The exported pesticide and AMPA loads for each of the events are represented in Figure 7.5-87. Maximum exported loads for a single event reached 154, 142, 2229, 660, 39, 185, 255 and 39 mg, for DIM, FLU, GLY, AMPA, IPR, TEB, TET and TRI respectively. This export corresponded to 0.04, 0.13, 0.21, 0.02, 0.23, 1.29 and 0.22 % of the application loads during the preceding year of the runoff event, respectively (Table 7.5-136). Such high export for single event may lead to significant ecotoxicological impact on the surrounding ecosystem.

Figure 7.5-87: Rainfall, outflow, total suspended solids (TSS) and pesticide and degradation product loads (GLY, AMPA, DIM, FLU, IPR, TEB, TET, TRI) for 20 runoff events in a vineyard catchment (Rochefort sur Loire, France)

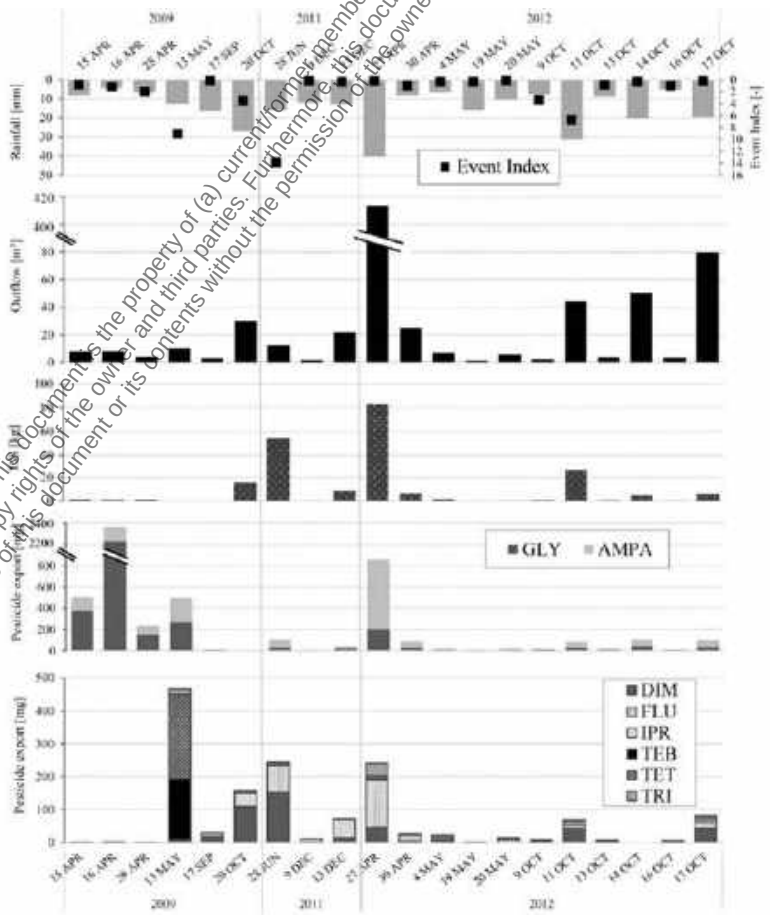


Table 7.5-135: Climatic and hydrology characteristics of the 20 studied runoff events (Rochefort sur Loire, France). Values in bold are extremes. Grey cases represent hysteresis analysis events with at least two measured points along the rising and falling limbs

		2009					2011							2012							
		15 APR	16 APR	28 APR	13 MAY	17 SEP	20 OCT	28 JUN	9 DEC	11 DEC	27 APR	30 APR	4 MAY	19 MAY	20 MAY	9 OCT	11 OCT	13 OCT	14 OCT	16 OCT	17 OCT
Rainfall																					
Rainfall amount	[mm]	8.4	4.4	6.6	12.4	16.4	27.0	15.8	12.0	12.6	**40.0**	8.4	6.6	15.8	10.6	7.6	31.5	8.8	8.1	33.0	19.6
Rainfall duration	[h]	2.6	1.6	1.6	1.6	15.8	4.9	1.5	14.9	7.7	22.1	1.9	1.8	7.5	9.7	1.4	5.0	2.9	3.1	1.8	**24.0**
6 min-peak rainfall intensity	[mm h^{-1}]	14.0	26.0	28.0	68.0	8.0	38.0	**78.0**	14.0	14.0	6.0	14.0	6.0	10.0	8.0	36.0	64.0	16.0	12.0	22.0	14.0
Seven day antecedent rainfall	[mm]	20.0	31.5	6.5	2.6	9.9	**0.4**	2.0	11.7	19.0	38.0	61.6	61.8	3.2	17.6	30.1	0.7	51.1	53.5	64.6	64.0
EVT	[ ]	0.8	1.2	2.0	9.1	0.1	3.5	**13.8**	0.2	0.4	0.2	1.0	0.4	0.4	0.1	3.7	0.1	0.8	0.3	1.0	0.2
Return period	[y]	<2	<2	<2	<2	<2	2-5	2	<2	<2	2-5	<2	<2	<2	<2	2	5-10	<2	<2	<2	<2
Hydrology																					
Total discharge	[m^3]	8.0	8.2	4.0	10.2	2.9	30.1	12.6	1.5	21.9	**413.7**	24.9	0.9	0.9	8.1	0.1	44.0	3.5	30.7	3.3	79.8
Runoff event duration	[h]	3.4	3.5	1.8	7.8	8.6	5.6	1.6	12.2	11.0	**89.7**	24.7	23.1	5.3	12.7	0.1	7.9	4.7	17.9	7.6	30.0
Runoff coefficient	[%]	4.3	8.5	2.8	3.7	0.8	5.1	3.6	0.6	7.9	**47.0**	13.5	4.7	0.3	3.4	0.4	6.4	1.8	11.4	3.0	18.5
Maximum outflow	[L s^{-1}]	3.1	3.9	1.6	5.3	0.5	11.2	11.1	0.3	3.3	10.5	4.1	0.8	0.5	1.2	2.2	**25.2**	1.1	6.0	0.7	3.3
Peak time discharge	[min]	68.0	16.0	53.0	13.5	923.0	141.5	27.5	721.5	428.0	**1095.0**	64.5	119.0	36.5	15.0	58.0	21.5	111.5	301.5	67.0	300.0
Mean outflow	[L s^{-1}]	0.6	0.6	0.6	0.3	0.0	1.4	**2.1**	0.0	0.6	1.3	0.3	0.1	0.0	0.1	0.5	1.2	0.2	0.8	0.1	0.8
Response time	[min]	33	6	33	5	16	27	11	341	116	**482**	24	19	14	58	14	8	19	42	17	286

Table 7.5-136: Number of days after treatment (DAT) [d], export coefficient (EC) [%] and total suspended solids (TSS) [mg/L] and pesticide concentrations [µg/L] (C; provided as min - max and mean ± standard deviation) (DIM, FLU, GLY, AMPA, IPR, TEB, TET, TRI) in the study catchment (Rochefort sur Loire, France). Values in bold signify extremes for each lines. n is the number of samples for each event. Grey cases represent hysteresis analysis events with at least two measured points along the rising and falling limbs. EC are expressed in four classes: "≥0.1" for EC ≥0.1, "≥0.01" for 0.1 N EC ≥0.01, "≥10-3" for 0.01 N EC ≥0.001 and "≥10-6" for 0.001 N EC ≥10-6

*** showed cases when positive exported loads of pesticide occurred while this pesticide was not applied during the previous year.**

2009

	15 APR			16 APR			28 APR		
n	16			6			9		
TSS [mg L⁻¹]	59.2 – 353.6 149.4 ± 40.9			74.5 – 187.3 139.7 ± 42.7			180.2 – 530.6 330.9 ± 105.2		
	C [µg L⁻¹]	DAT [d]	EC [%]	C [µg L⁻¹]	DAT [d]	EC [%]	C [µg L⁻¹]	DAT [d]	EC [%]
DIM	0.1 – 0.3 0.2 ± 0.1	320	$>10^{-6}$	0.3 – 0.3 0.3 ± 0.02	321	$>10^{-6}$	0.3 – 0.4 0.4 ± 0.1	333	$>10^{-6}$
FLU	NA NA	NA	NA	NA NA	NA	NA	NA NA	NA	NA
GLY	17.6 – 59.6 43.2 ± 15.5	9	>0.01	160.7 – **386.9** 285.1 ± 85.2	**0.1**	**>0.1**	24.2 – 51.9 41.3 ± 9.2	12	>0.01
AMPA	7.7 – 20.8 15.3 ± 4.3	–	–	11.9 – 23.2 17.3 ± 4.3	–	–	14.6 – 28 22.5 ± 4.2	–	–
IPR	0 – 0.03 0.01 ± 0.01	232	$>10^{-6}$	0 – 0.03 0.02 ± 0.01	233	$>10^{-6}$	0 – 0.03 0.02 ± 0.01	245	$>10^{-6}$
TEB	0 – 0.03 0.01 ± 0.01	288	$>10^{-6}$	0.03 – 0.03 0.03 ± 0	289	$>10^{-6}$	0 – 0.03 0.01 ± 0.01	301	$>10^{-6}$
TET	0 – 0.03 0.02 ± 0.01	341	$>10^{-6}$	0.03 – 0.03 0.03 ± 0	342	$>10^{-6}$	0.03 – 0.03 0.03 ± 0	354	$>10^{-6}$
TRI	0 – 0.03 0.01 ± 0.01	288	$>10^{-6}$	0 – 0 0 ± 0	289	0	0 – 0 0 ± 0	301	0

	13 MAY			17 SEPT			20 OCT		
n	36			12			34		
TSS [mg L⁻¹]	NA NA			NA NA			13.8 – 1547.5 489.8 ± 385.2		
	C [µg L⁻¹]	DAT [d]	EC [%]	C [µg L⁻¹]	DAT [d]	EC [%]	C [µg L⁻¹]	DAT [d]	EC [%]
DIM	0.2 – 2.3 0.9 ± 0.6	348	$>10^{-6}$	4.7 – 8 6.2 ± 0.9	97	$>10^{-3}$	0.1 – 4.5 3.4 ± 0.9	130	>0.01
FLU	NA NA	NA	NA	NA NA	NA	NA	NA NA	NA	NA
GLY	1 – [illegible] 30.8 ± [illegible]	27	>0.01	0.5 – 1.8 1.0 ± 0.5	154	$>10^{-6}$	NA NA	187	NA
AMPA	2.2 – **37** 16.0 ± 13.[illegible]	–	–	1.5 – 3.6 2.1 ± 0.8	–	–	NA NA	–	–
IPR	0 – [illegible] 0.03 ± 0.03	260	$>10^{-6}$	2 – **3** 2.5 ± 0.3	**60**	$>10^{-3}$	0.06 – 1.7 1.2 ± 0.3	102	**>0.01**
TEB	0 – **81** 16.0 ± 19.6	**0**	**>0.1**	0.08 – 0.19 0.1 ± 0.03	63	$>10^{-6}$	0 – 0.1 0.03 ± 0.02	96	$>10^{-6}$
TET	3.5 – **68** 19.0 ± 16.6	1	**>0.1**	0.17 – 0.86 0.3 ± 0.2	97	$>10^{-3}$	0.07 – 0.4 0.3 ± 0.1	130	>0.01
TRI	0.03 – 4.2 1.6 ± 1.2	**0**	>0.01	0 – 0.14 0.1 ± 0.1	63	$>10^{-6}$	0 – 0.1 0.04 ± 0.04	96	$>10^{-3}$

2011

	28 JUN			9 DEC			13 DEC		
n	21			15			25		
TSS [mg L⁻¹]	29.1 – **6454.5** 2136.9 ± 1720.4			95.2 – 330 128.0 ± 38.1			81.3 – 587.1 260.0 ± 156.0		
	C [µg L⁻¹]	DAT [d]	EC [%]	C [µg L⁻¹]	DAT [d]	EC [%]	C [µg L⁻¹]	DAT [d]	EC [%]
DIM	0.1 – **13** 11.0 ± 3.9	**21**	**>0.01**	0.7 – 1.[illegible] 1.0 ± 0.2	185	$>10^{-6}$	0.4 – 1.3 0.9 ± 0.3	189	$>10^{-3}$
FLU	**0.1 – 8.2** 5.5 ± 2.2	**48**	>0.01	[illegible] – 6 5.3 ± 0.9	212	$>10^{-3}$	1.2 – 5 3.8 ± 1.1	216	>0.01
GLY	0 – 13.7 2.5 ± 2.7	15	[illegible]	0.[illegible] – 0.9 0.4 ± 0.2	240	$>10^{-6}$	0.1 – 4.5 0.8 ± 1.1	233	$>10^{-3}$
AMPA	0 – 7.8 4.2 ± 2.1	–	–	[illegible] – 2.2 1.[illegible] ± 0.3	–	–	0.7 – 2 1.1 ± 0.3	–	–
IPR	0 – 0 0 ± 0	718	0	0 – 0 0 ± 0	882	0	0 – 0 0 ± 0	886	0
TEB	0.03 – 0.07 0.03 ± 0.02	[illegible]	$>10^{-6}$	0 – 0 0 ± 0	217	0	0 – 0 0 ± 0	221	0
TET	0.03 – 1.4 0.9 ± 0.4	[illegible]	>0.01	0.1 – 0.3 0.2 ± 0.05	185	$>10^{-6}$	0.1 – 0.2 0.2 ± 0.04	189	$>10^{-3}$
TRI	0.07 – 0.1 0.08 ± 0.02	53	$>10^{-3}$	0 – 0 0 ± 0	217	0	0 – 0.1 0.02 ± 0.03	221	$>10^{-3}$

2012

	27 APR			30 APR			4 MAY		
n	68			12			16		
TSS [mg L⁻¹]	28.9 – 1071.1 201.9 ± 136.9			96 – 369.6 271.8 ± 90.3			40 – 314.5 208.6 ± 87.1		
	C [µg L⁻¹]	DAT [d]	EC [%]	C [µg L⁻¹]	DAT [d]	EC [%]	C [µg L⁻¹]	DAT [d]	EC [%]
DIM	0 – 0.4 0.2 ± 0.1	325	>0.01	0.1 – 0.3 0.2 ± 0.08	328	$>10^{-3}$	0.1 – 0.2 0.2 ± 0.07	332	$>10^{-6}$
FLU	0.3 – 1.5 0.7 ± 0.4	352	**>0.1**	0.5 – 1.1 0.8 ± 0.3	355	>0.01	0.2 – 0.6 0.4 ± 0.2	359	$>10^{-3}$
GLY	0.1 – 2 0.9 ± 0.5	380	*	0.2 – 3.7 1.1 ± 0.9	392	*	0.1 – 0.5 0.4 ± 0.1	306	*
AMPA	0.1 – 5.7 1.8 ± 0.9	–	–	0.5 – 7.6 3.0 ± 1.9	–	–	0.5 – 3.1 2.3 ± 0.7	–	–
IPR	0 – 0 0 ± 0	1022	0	0 – 0 0 ± 0	1025	0	0 – 0 0 ± 0	1029	0
TEB	0 – 0.03 0.01 ± 0.01	357	$>10^{-3}$	0 – 0 0 ± 0	360	0	0 – 0 0 ± 0	364	0
TET	0.03 – 0.14 0.06 ± 0.04	325	>0.01	0.05 – 0.1 0.08 ± 0.02	328	$>10^{-3}$	0.2 – 3.3 1.8 ± 1.3	**1**	>0.01
TRI	0 – 0.12 0.05 ± 0.05	357	**>0.1**	0 – 0.03 0.01 ± 0.01	360	$>10^{-3}$	0 – 0 0 ± 0	364	0

Table 7.5-136 – continued

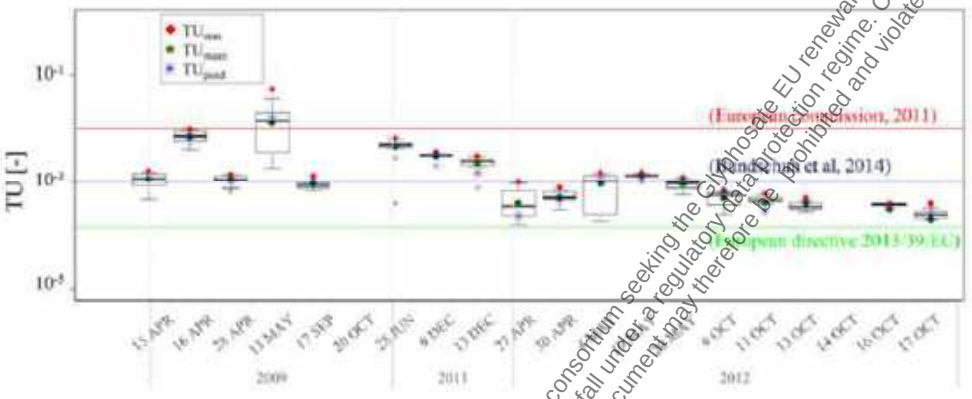
	19 MAY			20 MAY			9 OCT			11 OCT			13 OCT			14 OCT		
n	12			13			12			36			4			5		
TSS [mg L^{-1}]	83.7 –155 125.8 ± 21.1			17.5 –65.9 42.4 ± 12.6			132.5 –702 287.2 ± 153.3			100.6 –1542 355.5 ± 314.9			144.7 –315.2 229.5 ± 72.1			47.1 –130.6 94.1 ± 31.9		
	C [µg L^{-1}]	DAT [d]	EC [%]	C [µg L^{-1}]	DAT [d]	EC [%]	C [µg L^{-1}]	DAT [d]	EC [%]	C [µg L^{-1}]	DAT [d]	EC [%]	C [µg L^{-1}]	DAT [d]	EC [%]	C [µg L^{-1}]	DAT [d]	EC [%]
DIM	0.03 –0.03 0.03 ± 0	347	>10^{-4}	0.03 –0.03 0.03 ± 0	348	>10^{-4}	0.8 –2.3 1.7 ± 0.6	76	>10^{-3}	0 –1.9 1.2 ± 0.5	78	>0.01	0.8 –1.4 1.0 ± 0.3	80	>10^{-3}	NA NA	81	NA
FLU	1.3 –1.7 1.54 ± 0.15	374	*	0.7 –1.4 1.1 ± 0.4	375	*	0.2 –0.4 0.4 ± 0.1	517	*	0 –0.4 0.2 ± 0.1	519	*	0.2 –0.3 0.3 ± 0.1	521	*	NA NA	524	NA
GLY	0.9 –3 1.8 ± 0.7	8	>10^{-4}	0.5 –2.1 1.1 ± 0.6	9	>10^{-4}	1 –3.8 2.9 ± 0.8	112	>10^{-4}	1.4 –3.5 2.4 ± 0.6	114	>10^{-3}	1.4 –2.4 1.9 ± 0.5	116	>10^{-4}	0.5 –2.1 1.0 ± 0.6	117	>10^{-3}
AMPA	2.3 –4.4 3.6 ± 0.5	–	–	1.4 –4.1 2.7 ± 0.9	–	–	1.9 –5.6 4.3 ± 1.0	–	–	2.5 –5.5 3.7 ± 0.9	–	–	2.4 –3.4 3.0 ± 0.5	–	–	0.9 –1.1 1.0 ± 0.1	–	–
IPR	0 –0 0 ± 0	1044	0	0 –0 0 ± 0	1045	0	0.2 –0.4 0.3 ± 0.1	76	>10^{-3}	0 –0.4 0.2 ± 0.1	78	>10^{-3}	0.1 –0.3 0.2 ± 0.1	80	>10^{-3}	NA NA	81	NA
TEB	0 –0 0 ± 0	379	0	0 –0 0 ± 0	380	0	0 –0 0 ± 0	522	0	0 –0 0 ± 0	524	0	0 –0 0 ± 0	526	0	NA NA	527	NA
TET	0.8 –1.5 1.2 ± 0.3	16	>10^{-3}	0.5 –1.1 0.8 ± 0.3	17	>0.01	0.2 –0.4 0.3 ± 0.1	123	>10^{-3}	0 –0.3 0.2 ± 0.1	125	>0.01	0.2 –0.2 0.2 ± 0	127	>10^{-3}	NA NA	128	NA
TRI	0 –0 0 ± 0	379	0	0 –0 0 ± 0	380	0	0 –0 0 ± 0	522	0	0 –0 0 ± 0	524	0	0 –0 0 ± 0	526	0	NA NA	527	NA

	2012							
	16 OCT			17 OCT			Total on the 20 runoff events	
n	3			28			313	
TSS [mg L^{-1}]	65.9 –92 78.07 ± 13.16			11 –145 72.5 ± 27.4			11.0 –6454.6 374.2 ± 720.0	
	C [µg L^{-1}]	DAT [d]	EC [%]	C [µg L^{-1}]	DAT [d]	EC [%]	C [µg L^{-1}]	EC [%]
DIM	0.4 –1.2 0.9 ± 0.4	83	>10^{-3}	0.2 –1.1 0.5 ± 0.2	84	>0.01	0 –13 1.8 ± 2.8	0.06
FLU	0.1 –0.3 0.3 ± 0.1	524	*	0.1 –0.3 0.2 ± 0.04	525	*	0 –8.2 1.6 ± 2.1	0.08
GLY	0.9 –1 0.9 ± 0.05	119	>10^{-4}	0.2 –1.6 0.5 ± 0.3	120	>10^{-3}	0 –386.9 12.1 ± 41.6	0.35
AMPA	0.1 –1.9 1.3 ± 1.0	–	–	0.4 –2 0.8 ± 0.4	–	–	0 –47 5.6 ± 8.1	–
IPR	0.11 –0.27 0.2 ± 0.1	83	>10^{-4}	0 –0.18 0.07 ± 0.06	84	>10^{-3}	0 – 0.4 ± 0.7	0.01
TEB	0 –0 0 ± 0	529	0	0 –0 0 ± 0	530	0	0 – 0 ± 0.7	0.06
TET	0.17 –0.21 0.2 ± 0.02	130	>10^{-3}	0.08 –0.2 0.2 ± 0.03	131	>0.01	0 – 1.1 ± 2	0.19
TRI	0 –0 0 ± 0	529	0	0 –0 0 ± 0	530	0	0 –4.2 0.4 ± 0.7	0.06

Toxicity impact

All runoff water samples contained at least one pesticide with a concentration exceeding 0.1 µg/L (Table 7.5-136). Thus, pesticide levels in the studied catchment continuously exceed mandated acceptable concentrations (European directive 2013/39/EC). Toxic units based on maximum concentrations (TU_{max}) reached 0.29, 0.05 and 0.04 for algae, invertebrate and fish, respectively. The percentage of runoff events that exceeded the European Uniform Principles threshold for these species was 15, 5 and 25 %, respectively. Several researchers questioned the relevance of the TU threshold set by the EU for invertebrates (red line in Figure 7.5-88). Instead, these researchers preferred to use a TU value of 0.001 for invertebrates (blue line in Figure 7.5-88). Based on this threshold, 55 % of events analysed in the present study may represent a risk to the integrity of the aquatic ecosystem. Dilution occurs when these flows reach the Layon River 500 m downstream. However, approximately 182 km^2 of vineyards feed the Layon River, suggesting the potential combination of contaminated flows from >8000 small headwater catchments with features similar to our study site. TU (max and mean) for fish and invertebrates were negatively correlated with seven-day antecedent rainfall ($p < 0.001$), highlighting a dissipation effect with preceding rainfall (Olsson *et al*., 2013). The variations between the different TU estimations are represented for invertebrates in Figure 7.5-88. Surprisingly, very little variation was observed between TU_{mean} and TU_{pond}. There was, on average, 1.6 (and up to 4) times greater TU_{max} than TU_{mean} and 3.4 (up to 30) times greater than TU_{inst}. The method used to estimate TU results in significant differences in the values obtained, which is partly due to the variability of pesticide concentrations patterns throughout the hydrograph.

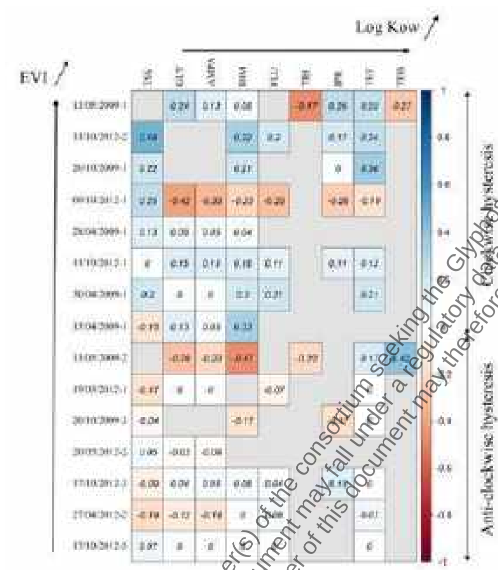
Figure 7.5-88: **Toxic unit for Daphnia magna immobility (acute 48 h) based on observed point concentrations (boxplot), maximum concentrations (red) and mean concentrations (green) for the 20 runoff events (log scale).Horizontal lines represent the toxic unit threshold of the European commission for invertebrates (0.01 in red), based on ecotoxicological studies (Bundschuh *et al.*, 2014) (0.001 in blue) and calculated using mandated acceptable concentrations of 0.1 µg/L for each pesticide (European directive 2013/39/EC) (0.00014 in green)**



First flush and concentration-discharge patterns

The mean and range of the first flushes FF10, FF25, FF50 and FF75 for all chemicals across the 20 runoff events were 9.8 ± 5.2, 25.3 ± 10.2, 50.6 ± 13, 75.5 ± 11.6 %, respectively. It suggests that no disproportionate event occurred. In other words, no "first flush effect" as defined by Bertrand-Krajewski *et al.* (1998) was observed, contrary to expectations. Nevertheless, this phenomenon may occur when pesticides are rapidly mobilized at the beginning of a runoff event if those pesticides are less-sorptive than those in the present study or if their source area is very near the catchment outlet. 15 runoff peaks allowed us to study the differences between the rising and falling limb of the hydrograph (at least two sample points for each rising and falling limb were taken during these events) (Figure 7.5-89). Between 57 and 99 % of the water discharge volume occurred during the falling limb, which may be partly due to natural and artificial drainage features that delayed the flow. TSS and pesticide concentrations did not significantly differ between the rising and falling limb ($p > 0.05$) (except for AMPA and the triazole family: TEB, TET, TRI) and thus require a deeper analysis of concentration patterns. Based on previously determined hysteresis classifications (Bieroza and Heathwaite, 2015), 52 % of the concentration versus discharge graph of quantified suspended solid or pesticide values exhibited clockwise patterns, 27 % exhibited anticlockwise patterns and 21 % had no or an unclear hysteresis pattern (Figure 7.5-89). ΔR ranged from -0.47 to 0.48, with an absolute average of 0.15 ± 0.11. ΔC ranged from 0.21 to 1, with an average of 0.64 ± 0.24. ΔR for TSS and EVI were correlated ($p < 0.01$), indicating that the clockwise hysteresis of TSS occurred for intense rainfall events, whereas an anti-clockwise hysteresis pattern was observed for mild rainfall events. The direction of the hysteresis loops for pesticides were not consistent between substances within an event, nor for one substance across all events. AMPA, DIM, FLU, IPR, TEB and TET presented predominantly clockwise hysteresis patterns (52 %), while TRI exhibited anti-clockwise pattern (67 %) and GLY exhibited unclear pattern (54 %). Figure 7.5-89 shows that for intense events (high EVI), stronger hysteresis patterns (clockwise or anti-clockwise) were predominant, as indicated by a greater loop area. However, this tendency was significant only for GLY, FLU and TET ($p < 0.05$). ΔR for GLY, AMPA, DIM and FLU was significantly and positively correlated with maximum outflow and the runoff coefficient ($p < 0.05$). On the other hand, the number of days since the last application, air temperature and antecedent rainfall did not correlate with ΔR. No hysteresis trends were observed based on the pesticide affinity for water (K_{ow}), as might have been expected. That study found that pesticide molecules with low to moderate solubility resulted in clockwise hysteresis loops while soluble molecules resulted in anticlockwise loops in an 1110 km^2 groundwater-based catchment.

Figure 7.5-89: Hysteresis rotational parameter ΔR (clockwise=positive (blue), anti-clockwise=negative (red) and no or unclear hysteresis pattern=null) for the 15 runoff peaks and TSS, GLY, AMPA, DIM, FLU, TRI, IPR, TET and TEB. Values represent the normalized area of the C-Q hysteresis. Grey cases represent undetected or unavailable data. Runoff peaks are named with the runoff date and a subscript which represents the number of the peak within the runoff event



Discussion

High frequency sampling is costly but reveals important information about the ecotoxicity and underlying hydrological and hydrochemical processes governing pesticide transport in headwater catchments.

Hydrological functioning of catchments

Often, saturation excess runoff is related to low soil depth, good soil structure, high organic matter content, and low erosion potential. On the other hand, Hortonian runoff is associated with steep slopes, the absence of base flow, and crusted soils characterised by low clay and organic matter contents and low structural stability (Descroix *et al*., 2007; Tilahun *et al*., 2016). In this study, indicators of both types of possible runoff scenarios are present such as low soil depth (30-120 cm), 20 % clay content and high structural stability indicating saturation excess runoff but also steep slopes and the absence of a base flow indicating Hortonian runoff. For all runoff events, pesticide concentrations correlated with flow rate (significant except for FLU and TET, $p < 0.05$), indicating a concentration effect and not a dilution effect. The concentrations of the 8 compounds correlated with TSS concentrations ($p < 0.0025$). This suggests that pesticide mobilisation and transport occurred along similar pathways as TSS, which was largely a function of Hortonian runoff associated with intense rainfall. In addition, maximum concentrations are positively correlated with EVIs (only significant for GLY and AMPA, $p < 0.01$). A clockwise hysteresis loop was the most observed pattern within the study site, as was expected for a small catchment (Hudson, 2003; Seeger *et al*., 2004). This suggests the direct and rapid mobilisation of TSS and pesticides via runoff and indicates that drainage had a minimal impact on pesticide and TSS export (Marttila and Kløve, 2010). In the present study, intense rainfall events caused stronger hysteresis patterns (whether clockwise or anti-clockwise) with greater loop areas (Figure 7.5-89). This is partly due to two different, chronological occurrences. For clockwise hysteresis patterns, intense rainfall events rapidly induced surface runoff and a higher runoff coefficient.

Rapid mobilisation of pesticides can thus occur as a flush of available pesticides prior to peak outflow. For anti-clockwise hysteresis patterns, intense rainfall events can activate pesticide sources further from the catchment outlet, less hydrologically connected or dryer (Doppler *et al*., 2014). On the contrary, mild

rainfall events did not possess enough energy or power to rapidly mobilise pesticides nearby or to activate and transport pesticides from further away, resulting in diffuse pollution with a small or non-existent hysteresis area. The shift from a clockwise to anti-clockwise hysteresis pattern for different substances within an event or for one pesticide across runoff events was highly dynamic and dependent on the storm and substance rather than only on the catchment characteristics as previously suggested (Bieroza and Heathwaite, 2015; Thompson *et al.,* 2012) (Figure 7.5-89).

Ecotoxicological impact of runoff events

Maximum TU values were observed for runoff events that occurred very near the application date, for intense rainfall events and after a dry period; in other words, for the first significant rainfall event after application. There are only small differences in the TU_{mean} and TU_{pond} values (Figure 7.5-88) indicating that frequent sampling at short time intervals gives relatively the same value for TU regardless of the method of computation. The potential range of concentrations with random sampling are reflected with the point concentration (or TU_{inst} values). Random sampling, e.g., every month, as often performed by national monitoring programs (Botta *et al*., 2012; Bundschuh *et al*., 2014), may underestimate peak exposure.

This highlights the importance of the sampling method in assessing the ecotoxicological impact of contaminated runoff on nearby ecosystems. Where FLU represented 19 % of the total pesticide load in all runoff in 2011 and 2012, this pesticide accounted for 59, 79 and 96 % of the composite TU value for invertebrates, fish and algae. FLU was extremely persistent and was always detected (>525 days) after a single application of the pesticide on plot B.

Supply limitation vs transport limitation

Pesticides primarily enter agricultural streams during rainfall events *via* runoff; their movement is dependent on the presence of a sufficient amount of the given pesticide and its availability (supply), as well as its ability to be mobilized via runoff (transport). No first flush effect was observed in the present study and the contribution of pesticide exports was similar during almost all runoff events. Pesticide transport rather than pesticide sources appeared thus to be the limiting factor in pesticide exports from the catchment. The sequence of several runoff peaks, with the clockwise followed by anticlockwise runoff peaks was observed on both May 13, 2009 and October 20, 2009 (13/05/2009-1 followed by 13/05/2009-2 and 20/10/2009-1 followed by 20/10/2009-3 in Figure 7.5-89). This sequence supports the hypothesis that an exhaustion effect was present, i.e., the rapid mobilisation of pesticides or suspended solids occurred during the first peak (transport limitation), which limited the source during the second peak (supply limitation) (Bieroza and Heathwaite, 2015; Bowes *et al*., 2009). Degradation, and thus a supply limitation, can be evaluated for GLY in the presence of AMPA. The relationship between AMPA and glyphosate were evaluated by calculating % AMPA as a percentage of the molar load of AMPA compared to the total molar loads of GLY and AMPA (Imfeld *et al*., 2013). A gradual increase in % AMPA from the last application was observed, indicating degradation of glyphosate ($p < 0.05$). % AMPA generally exceeded 60 %, except in April 2009, near the glyphosate application dates, and averaged 67.0 ± 19.3 % across runoff events. AMPA and GLY always followed the same hysteresis patterns; however, % AMPA did not correlate significantly with ΔR or ΔC ($p > 0.05$). TEB and TRI exhibited similar concentration patterns ($p < 0.01$), with first flush calculations that differed significantly compared to the other studied chemicals ($p \leq 0.05$). These pesticides exhibited a predominantly anticlockwise hysteresis pattern. Given that their sorption characteristics fell within the same range as the other pesticides studied (Table 7.5-133), anti-clockwise patterns may be partly due to the application area, which was mainly on the upstream section of plot A. Further location of the application area may delay the pesticide arrival at the outlet of the catchment ([redacted], [redacted] 2012). Hysteresis patterns for the different substances within an event or for an individual substance across events were highly dynamic and shifted between clockwise and anti-clockwise patterns. This may be partly due to (i) the complexity of the studied outflow discharge, which often had multiple peaks and indicated different flow pathways within the catchment (transport limitation) and (ii) the complex interplay between the temporal and spatial evolution of the pesticide stocks related to their application date, amount and mode (foliar or directly on soil), as well as their degradation or their availability via sorption (supply limitation). Few studies address surface dominated catchments or organic pesticides such as in the present study (Pietroń *et al*., 2015; Taghavi *et al.,* 2011), which limited our ability to make direct comparisons.

Conclusion

High frequency sampling is certainly costly but enables the reliable estimation of maximum pesticide concentration and fluxes. Furthermore, it reveals information about the underlying hydrological and hydrochemical processes governing pesticide transport. Altogether, the results highlight that (i) for all runoff events, the pesticide concentrations increased with outflow and significant pesticide export can occur during a single event; (ii) when the TU of the pesticide mixture was analysed, the European Uniform Principles for algae, invertebrates and fish were regularly exceeded (15 %) and FLU was responsible for the majority of the toxicity (59-96 %); (iii) random sampling may result in an up to 30-fold underestimation of the TU for invertebrates obtained using the maximum concentration, highlighting the important role of the sampling methods for assessing peak exposure; (iv) no first flush occurred, and the contribution of the pesticide loads from different section along the hydrograph was mostly homogeneous; and (v) hysteresis patterns were complex and highly dynamic. Individual events can be interpreted in a particular way but not consistently given the complex interactions between hydrology and reactive transport at the study site. The primary limitation of the study was the lack of knowledge about pesticide sources and availability in soils before each rainfall event. This knowledge would help to better interpret hysteresis patterns. Detailed off-site pesticide transport information may support the design and adaptation of mitigation strategies and crop management techniques. For example, here, the absence of an important first flush phenomenon for all of the studied pesticides questions the relevance of mitigation strategies based on the retention of the first part of the runoff volume, such as small storm water wetland. Further field studies that evaluate concentration-discharge patterns for pesticides are needed to better understand the hysteresis behaviour of pesticides and use it as a tool to predict the sources and pathways of pesticides within agricultural catchments. Such an internal signature for a catchment may help researchers to better understand pesticide source availability, mobilisation and transport in runoff water.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article reports the results from a runoff experiment in a French vineyard with different pesticides with a high-frequency setup. Data on glyphosate and AMPA were measured and reported.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/041
Report author	Lerch, R.N. *et al*.
Report year	2017
Report title	Vegetative Buffer Strips for Reducing Herbicide Transport in Runoff: Effects of Buffer Width, Vegetation, and Season
Document No	Journal of the American Water Resources Association (JAWRA) 53(3):667-683.
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

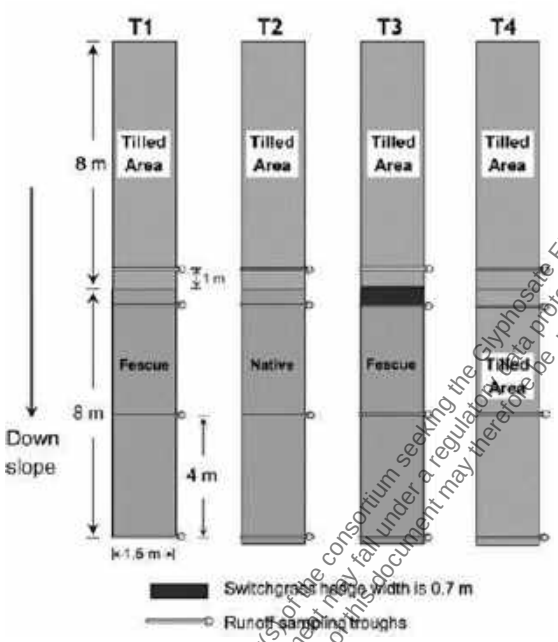
The effectiveness of vegetative buffer strips (VBS) for reducing herbicide transport has not been well documented for runoff prone soils. A multi-year plot-scale study was conducted on an eroded claypan soil with the following objectives: (1) assess the effects of buffer width, vegetation, and season on runoff transport of atrazine (ATR), metolachlor (MET), and glyphosate; (2) develop VBS design criteria for herbicides; and (3) compare differences in soil quality among vegetation treatments. Rainfall simulation was used to create uniform antecedent soil water content and to generate runoff. Vegetation treatment and buffer width impacted herbicide loads much more than season. Grass treatments reduced herbicide loads by 19-28 % and sediment loads by 67 % compared to the control. Grass treatments increased retention of dissolved-phase herbicides by both infiltration and adsorption, but adsorption accounted for the greatest proportion of retained herbicide load. This latter finding indicated VBS can be effective on poorly drained soils or when the source to buffer area ratio is high. Grass treatments modestly improved surface soil quality 8-13 years after establishment, with significant increases in organic C, total N, and ATR and MET sorption compared to continuously tilled control. Herbicide loads as a function of buffer width were well described by first-order decay models, which indicated VBS can provide significant load reductions under anticipated field conditions.

Materials and Methods

Experimental Design

Experiment was established in 2002 at the University of Missouri near Columbia, Missouri. Twelve 1.5 m x 16 m plots with four treatments replicated three times were arranged in a randomized complete block design (Figure 7.5-90). The upper half of each plot (1.5 m x 8 m) was managed under continuous cultivated fallow and served as the source area that received herbicide applications. The lower half of the plots included four vegetation treatments as one set of factors: (1) tall fescue (*F. arundinacea*) (TF); (2) TF with a 0.7-m wide switchgrass (*P. virgatum* L.) hedge at the upslope end of the VBS (Hedge + TF), (3) native warm-season grasses, mainly comprised of Indian grass (*Sorghastrum nutans* L.), eastern gamagrass (*T. dactyloides*), and switchgrass (Native); and (4) continuous cultivated control. Management of the source area under continuous cultivation was used to mimic pre-emergent herbicide application to tilled cropland, which is a common practice in the region. The control treatment represented a non-vegetative treatment for comparison to the grass treatments and tillage was a practical way to maintain consistent conditions. The study was conducted on an eroded Mexico silt loam with an average slope of 5 %.

Figure 7.5-90: **Schematic Diagram Showing One Set of Treatments with Plot Dimensions and Sampler Locations. Treatments were replicated three times. T1, tall fescue (TF); T2, native warm-season grass mixture (Native); T3, switchgrass hedge + tall fescue (Hedge + TF); and T4, Control**



Runoff Event Simulations and Runoff Collection

A rotating-boom rainfall simulator was used to produce uniform antecedent soil water content in the plots before herbicide application and to generate runoff following application. To control antecedent soil water content, simulated rainfall was applied about 24 h before the runoff event until ponding occurred; typically 30-40 min of rainfall was required. Three soil samples were collected from the tilled portion of the plots immediately before the runoff events for determination of water content. Three herbicides, ATR (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine), S-metolachlor (MET) (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide), and glyphosate (GLY) (N-phosphonomethyl-glycine) were applied with a backpack sprayer to the upper 8 m of the plots approximately 16-20 h before simulated runoff was generated. Runoff samples were collected with the initiation of runoff at the 8-m sampler (i.e., runoff was generated over the entire plot area) for a given plot.

Laboratory Analyses

All samples were analyzed for suspended sediment concentration and dissolved and sediment-bound herbicide concentrations. Dissolved-phase herbicide concentrations were determined on filtered samples using magnetic particle enzyme-linked immunosorbent assays

Table 7.5-137: Summary of rainfall simulation and antecedent soil water content data

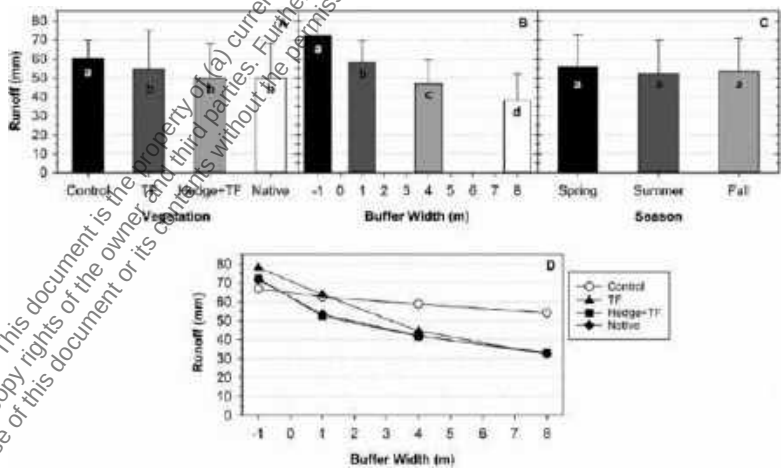
Data Set	Soil Water Content[1] (%)	Runoff Initiation[2] (min)	Rainfall (mm)	Rainfall Rate (mm/h)
Spring 2009	24.8a ± 3.6[3]	17a ± 3	82a ± 3[3]	60a ± 2
Spring 2010	27.4a ± 1.6	15a ± 6	76a ± 7	58a ± 2
Summer 2008	27.1a ± 1.1	17a ± 5	81a ± 8	61a ± 3
Summer 2012	26.6a ± 0.9	14a ± 3	77a ± 4	59a ± 1
Fall 2007	26.6a ± 1.4	15a ± 4	82a ± 6	63a ± 5
Fall 2009	34.5b ± 3.0	17a ± 5	81a ± 6	61a ± 3

[1]Wet weight basis.
[2]At the 8 m sampler.
[3]Mean ± 95% confidence interval. Means in the same column followed by different letters were significantly different at α = 0.05.

Statistical Analyses

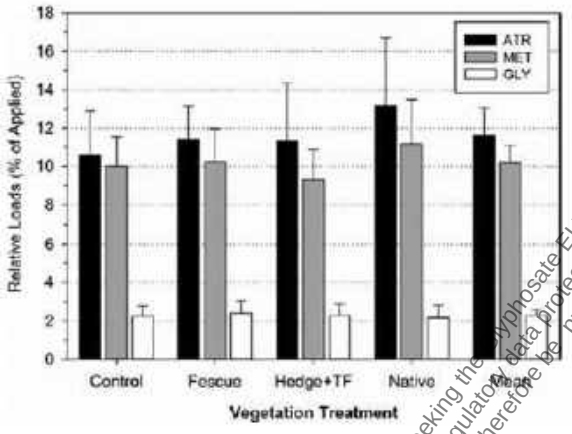
To assess consistency of the rainfall simulator, total applied rainfall, rainfall rate, time to runoff initiation (at the 8 m sampler), and antecedent soil water content were analyzed by one-way analysis of variance (ANOVA) grouped by the individual datasets (i.e., Spring 2009, Spring 2010, Summer 2008, Summer 2012, Fall 2007, Fall 2009) using the Excel add-in, Winstat. If the p value for the ANOVA was ≤0.05, then differences between treatment means were determined by the LSD method at p = 0.05.

Figure 7.5-91: Mean Runoff for the Following Factors: (A) Vegetation; (B) Buffer Width; (C) Season; and (D) Vegetation by Buffer Width Interaction. Error bars are 95 % confidence intervals. Within a main effect, treatments with different letters were significantly different at α = 0.05. Control, unvegetated; TF, tall fescue; Hedge + TF, switchgrass hedge plus tall fescue; and Native, warm-season native grass mixture



All other variables were analyzed as a three-way factorial using the mixed ANOVA procedure (PROC MIXED) in SAS 4.3 with year and plot as random effects. Differences between treatment means were determined by the PDIFF procedure. All main effects, interactions, and mean comparisons were considered significantly different at α = 0.05. Nonlinear regression was used to relate changes in INLs as a function of buffer width using a three-parameter first-order decay model.

Figure 7.5-92: Relative Herbicide Loads, as Percent of Applied, at the -1 m Sampler for Each Vegetation Treatment. Error bars are 95 % confidence intervals. No significant differences between vegetation treatments for any of the herbicides. ATR, atrazine; MET, metolachlor; and GLY, glyphosate



Soil Quality Assessments

General Soil Properties.

Soil samples were collected from 0 to 10 cm depth within the vegetative buffers by compositing at least 20 subsample cores of 1.3 cm diameter. Samples were collected in May 2010 in the Control, TF, and Native treatments and within the switchgrass hedge of the Hedge + TF treatment. For the Hedge + TF treatment, subsamples were collected in proportion to the area covered by switchgrass and TF and composited to achieve representative samples. Samples were stored field moist at 2-4° C until analyses could be completed. Soils were air-dried, mixed, and sieved to 2-mm before conducting basic chemical characterization analyses, including particle size analysis, cation exchange capacity, organic C, total N, and pH using methods reported by Nathan *et al.* (2012). These same treatments were also sampled for bulk density determination using 7.6 cm diameter by 7.6 cm long cores. In May 2011, a set of samples was collected in the same manner as described above for determination of microbial enzyme activities. Methods described by Lin *et al.* (2011b) were used to measure the activities of β-glucosidase (GLU), dehydrogenase (DHG), and fluorescein diacetate (FDA) hydrolysis. To determine saturated hydraulic conductivity (Ksat), two intact soil cores were collected from within the buffers of the four vegetation treatments at 0-10 and 10-20 cm depths in May 2012. The constant head method was used to measure Ksat for most samples while the falling head method was used on some samples with low Ksat values.

Herbicide Sorption

Another set of soil samples was collected from the four vegetation treatments in December 2015 in the same manner as that described previously. These samples were assessed for herbicide sorption using a single concentration batch equilibration method as described by Chu *et al*. (2013). Prior to the sorption experiments, the field moist soils were sieved to 2-mm, root and plant material removed, and moisture content determined. For each herbicide, a stock solution of 1 mg/L in an electrolyte solution of 0.003 M $CaCl_2$ and 0.0015 M NaN_3 (antimicrobial agent) was prepared. Batch equilibration experiments were performed by adding 30 mL of herbicide stock solution to 15.0 g (dry weight) of soil in a 50 mL polypropylene co-polymer centrifuge tube, followed by agitation on an end-to-end shaker at 100 oscillations/min at room temperature (22-25 °C). Preliminary experiments were performed to determine equilibration times for each herbicide: 16 h for ATR and MET; 24 h for GLY. After shaking, the samples were centrifuged for 5 min at 1,850 x g and the herbicide concentration remaining in solution was determined by ELISA using appropriate dilutions for each herbicide. Duplicate subsamples of each plot were analyzed along with soil-free herbicide samples and an electrolyte blank.

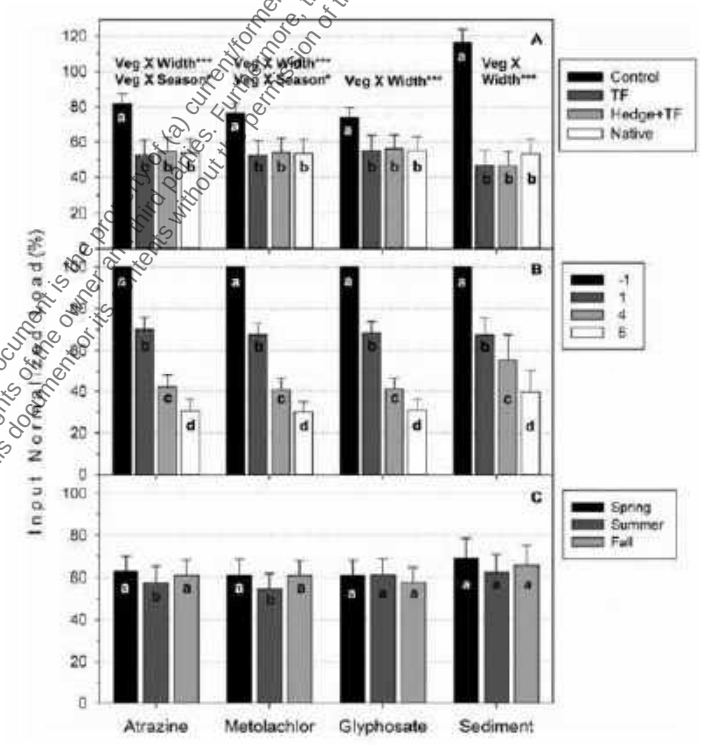
The solid-solution distribution coefficients, Kd (in L/kg), were computed as the ratio of the sorbed to solution concentrations at equilibrium. Statistical analyses to determine vegetation treatment differences in soil quality parameters were determined by either one-way or two-way ANOVA ($\alpha = 0.05$), and mean comparisons were made using the PDIFF procedure with Bonferroni adjustment.

Results and Discussion

Hydrologic Data

The rainfall simulator performed very consistently over the course of the experiment (Table 7.5-137). With the exception of Fall 2009, antecedent soil water content was similar among the datasets. The significantly greater soil water content in Fall 2009 resulted from a series of natural rainfall events within 24 h of all but one of the simulated events. However, the natural rainfall did not significantly affect the time to runoff initiation or the total rainfall applied compared to the other datasets. The average time to runoff initiation varied minimally, ranging from 14 to 17 min. Runoff was significantly affected by both vegetation and buffer width (Figure 7.5-91). The vegetation effect demonstrated that all the grass treatments were comparably effective at reducing runoff relative to the control. The significant vegetation by buffer width interaction occurred due to the greater reductions in runoff depth for the grass treatments as a function of buffer width compared to the control (Figure 7.5-91D). Compared to the runoff input at -1 m, the grass treatments decreased runoff depth by an average of 56 % at 8 m, while the control only decreased runoff by 19 %. TF had greater runoff depth at -1 m than the other vegetation treatments, but all grass treatments were significantly lower than the control at 4 m and 8 m.

Figure 7.5-93: **Mean Input Normalized Loads for atrazine, metolachlor, glyphosate, and sediment by Main Factors of: (A) Vegetation; (B) Buffer Width; and (C) Season. Error bars indicate 95 % confidence intervals. Within a main effect, treatments with different letters were significantly different at a = 0.05. Significance of interactions: *p < 0.05; ***p < 0.001**



Herbicide and Sediment Loads

Relative herbicide loads at the -1 m sampler were consistent over the vegetation treatments (Figure 7.5-92) with no significant differences between treatments for any of the herbicides. Mean relative loads entering the VBS for ATR varied from 10.6 % of applied for the control to 13.2 % for the Native treatment. MET results were similar with relative loads ranging from 9.3 % for the Hedge + TF to 11.2 % for the Native treatments. In contrast, GLY loads were much lower, ranging from 2.2 to 2.4 %, and also much less varied than those observed for ATR and MET. The relative load results indicated that the intense simulated storms represented robust scenarios for testing the ability of VBS to reduce herbicide transport. Dissolved-phase transport as a proportion of total herbicide load at the -1 m sampler was 99 ± 0.3 % (95 % CI) for ATR, 96 ± 0.6 % for MET, and 64 ± 2.0 % for GLY, results that are consistent with previous runoff studies (Wauchope, 1978; Lin *et al*., 2011a). These results demonstrated the much greater soil sorption of GLY compared to ATR and MET and the importance of both dissolved phase and sediment-bound transport to GLY losses in runoff.

The effect of the main factors on herbicide and sediment loss in runoff showed that vegetation treatment and buffer width had the greatest impact on loads while the effect of season was more limited (Figure 7.5-93). Analogous to the runoff results, the vegetation treatment effect showed that all grass treatments were similarly effective at reducing herbicide and sediment loads (Figure 7.5-93A). The three grass treatments significantly reduced herbicide and sediment INLs, and compared to the control, reduced average INLs by 28 % for ATR, 23 % for MET, 19 % for GLY, and 67 % for sediment. For ATR INLs, the grass treatments were less than the control by an average of 31 % at 1 m, 38 % at 4 m, and 43 % at 8 m. Results for MET INLs were similar to those of ATR, but GLY INLs at 1 m showed limited decreases for the grass treatments, with only the Native treatment showing a significant reduction compared to the control. The grass treatments significantly reduced GLY INLs at 4 and 8 m, compared to the control, by an average of 24 and 36 %, respectively. Overall, the results showed that grass treatments mitigated herbicide losses through a combination of reductions in runoff volume and sediment loads, demonstrating the ability of VBS to effectively decrease both dissolved-phase and sediment-bound herbicide transport. Averaged over vegetation treatment and season, INLs decreased with increasing buffer width for all three herbicides and sediment (Figure 7.5-93B), showing the strong influence of width on contaminant load. The effect of buffer width was very similar for all three herbicides. The effect of season was significant only for ATR and MET loads, but sediment INLs showed the same pattern (Figure 7.5-93C).

For both herbicides, summer INLs were significantly less than fall and spring, but the differences were relatively small compared to vegetation and buffer width effects. Compared to fall and spring, the summer INLs were 3-5 % lower for ATR and 7 % lower for MET. The season effect for ATR and MET was largely due to the significant decreases in summer and fall INLs for the Hedge + TF treatment as none of the other vegetation treatments showed any significant seasonal effects. The Hedge + TF treatment decreased ATR and MET summer INLs by 20-23 % compared to spring. Despite no seasonal effect on runoff for the Hedge + TF treatment, these data indicated that increased switchgrass hedge growth and vigor in the summer and fall contributed to reductions in dissolved-phase herbicide loads.

Table 7.5-138: Basic Chemical and Physical Properties of Soil Samples Collected from Four Vegetation Treatments[1]

Vegetation Treatment	Sand (%)	Silt (%)	Clay (%)	Textural Class	Bulk Density (g/cm^3)	CEC[2] (cmol/kg)	Organic C (%)	Total N (%)	pH[3]
Control	22.6a	51.8a	25.6a	SL[4]	1.21a	20.7a	1.4a	0.12a	6.8a
Tall fescue (TF)	22.1a	53.3a	24.6a	SL	1.09a	22.8a	2.2b	0.20b	6.7a
Hedge + TF	16.8a	56.1a	27.1a	SCL	1.13a	23.1a	2.1b	0.18b	6.7a
Native	23.3a	50.6a	26.1a	SL	1.13a	23.5a	2.1b	0.17b	6.6a

Notes: Within a column, means followed by different letters were significantly different at $\alpha = 0.05$.
[1]Soil samples collected from 0 to 10 cm in May 2010.
[2]CEC, cation exchange capacity. Computed as the sum of the ammonium acetate extractable bases and the barium chloride extractable acidity (Nathan *et al.*, 2012).
[3]pH determined in 1:1 soil to water suspension (Nathan *et al.*, 2012).
[4]Textural classes: SL, silt loam; SCL, silty clay loam.

Vegetative Buffer Width and Load Reduction

By measuring herbicide loads at four points along the buffer, the experimental design employed for this study provided the opportunity to relate reductions in herbicide INLs to buffer width and SBAR. Because of the modest seasonal effect on loads, regression equations were developed for each vegetation treatment with data pooled across seasons. The three parameter first-order decay models were significant for all vegetation treatments and herbicides (Figure 7.5-94). This relationship indicated that short VBS widths can be very effective at reducing herbicide loads, even for a high runoff potential claypan soil. Applying the regression equations to a range of buffer widths (from 0.16 to 8 m; SBAR = 50:1-1:1) resulted in predicted load reductions that were within 10 % of each other for the grass treatments, indicating that all three of these VBS types would be similarly effective for reducing herbicide loads in runoff. The highly significant R^2 values (0.700-0.861) for the grass treatment models demonstrated that these models would be useful for predicting expected reductions in herbicide loads.

Table 7.5-139: Herbicide Solid-to-Solution Distribution Coefficients (Kd) and Saturated Hydraulic Conductivity (Ksat) of Soils Collected from Each Vegetation Treatment

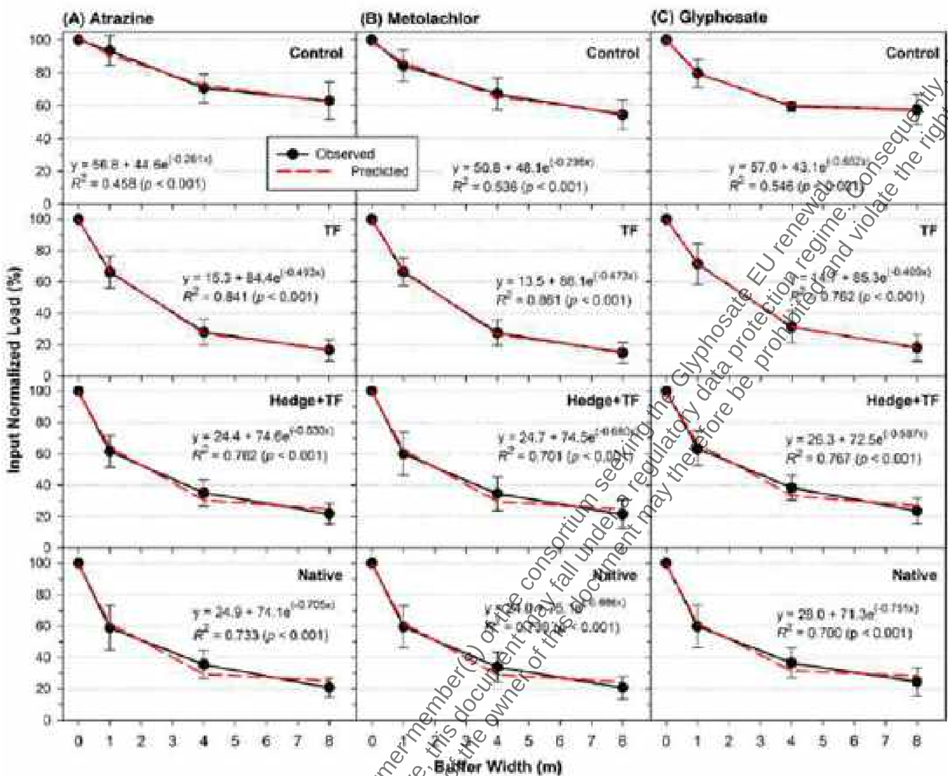
	Sorption Intensity[1]			K_{sat}[2]	
	Atrazine	Metolachlor	Glyphosate	0-10 cm	10-20 cm
Vegetation Treatment	K_d (L/kg)[3]			mm/h	
Control	1.16a ± 0.21[4]	1.92a ± 1.40	156a ± 54.0	120a ± 115	7.5a ± 3.2
Tall fescue (TF)	2.52b ± 0.52	7.11b ± 1.66	137a ± 54.3	190a ± 190	39a ± 8.7
Hedge + TF	2.65b ± 0.47	7.38b ± 1.73	147a ± 20.6	—	—
Native	3.51b ± 0.60	7.36b ± 1.30	171a ± 25.5	180a ± 130	27a ± 24
Mean	2.46 ± 1.07	5.94 ± 3.03	150 ± 40.2	150 ± 120[5]	23 ± 19

[1]Soil samples collected from 0 to 10 cm depth in December 2015.
[2]Soil samples collected in May 2012.
[3]K_d, solid-solution distribution coefficient.
[4]Mean ± 95% confidence interval. Means in the same column followed by different letters were significantly different at $\alpha = 0.05$.
[5]Depth means for K_{sat} were significantly different at $\alpha = 0.05$.

Soil Quality Assessments

Organic C and total N concentrations of the surface soils were significantly increased in the grass treatments, by an average of 53 %, compared to the control (Table 7.5-138). However, basic surface soil (0 - 10 cm) parameters such as texture, bulk density, cation exchange capacity, and pH were not significantly affected by vegetation treatment. The long term inputs and decomposition of plant and root biomass presumably led to the observed accumulation of soil C and N in the grass treatments. Measurement of K_{sat} in surface (0-10 cm) and shallow subsurface (10-20 cm) soils showed no statistical differences among the vegetation treatments, but the surface soils did have significantly greater K_{sat} rates than the subsoil (Table 7.5-139). Surface soils showed variable K_{sat} rates that ranged from 110 to 190 mm/h. A major source of variation was whether or not the claypan horizon was present within the 0-10 cm samples as the depth to the claypan was in the range of 8-12 cm below the surface.

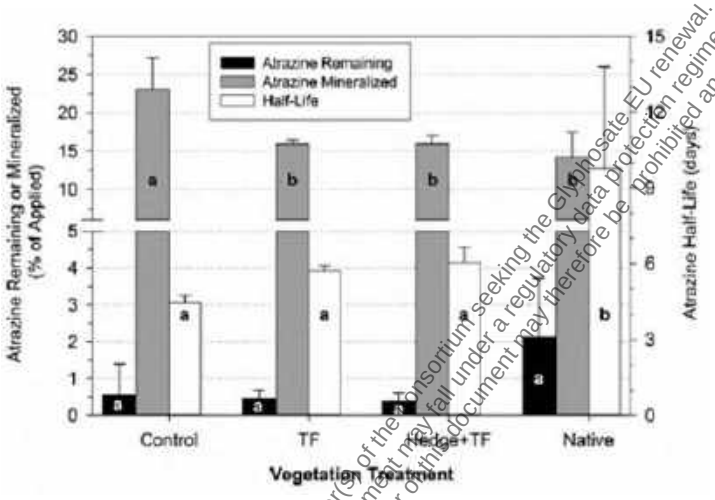
Figure 7.5-94: Regression Equations Correlating Relative Load Reduction (y) as a Function of Buffer Width (x) for: (A) atrazine; (B) metolachlor; and (C) glyphosate. Error bars represent the 95 % confidence interval



Regardless, the profound impact of the claypan on K_{sat} rates could be seen as the subsoil rates were an average of 6.5 times lower than the surface soil. As previously noted, runoff depth was reduced by the grass treatments compared to the control, and the K_{sat} data indicated that the observed reductions were due to slower runoff velocity leading to the increased infiltration and not a function of improved percolation through the soil. Sorption experiments showed that all three grass treatments significantly and similarly increased sorption intensity of ATR and MET (Table 7.5-139). Compared to the control, grass treatments increased K_d values by an average of 2.5 times for ATR and 3.8 times for MET. GLY sorption was not affected by vegetation treatment, with K_d values ranging from 127 to 171 L/kg. The K_d values reported here were similar to those reported for these herbicides in a wide variety of soils. Other possible indicators of improved soil quality, such as herbicide degradation and enzyme activities, showed that VBS had only modest impacts on these biological processes. Results from the ATR degradation study showed that amount of ATR remaining in the soil after 56 days was not significantly different between treatments (Figure 7.5-95). However, the control treatment showed greater ATR mineralization and faster degradation rates than the grass treatments. Microbial enzyme activities were not greatly affected by vegetation treatment as neither DHG nor FDA activities were significantly different among treatments, but GLU activity did show significant increases in the grass treatments compared to the control (Figure 7.5-96). These results were not expected as most studies have reported that VBS enhanced pesticide degradation and increased microbial enzyme activities in soil. The plots used in this study have received ATR application to the source area eight times since 2004, and therefore, microbial adaptation seemed likely given the frequent applications. However, the results suggest greater activity of ATR-degrading genes in the control than the grass treatments. Possible explanations for the findings reported here include: (1) greater labile soil C and N in the grass treatments (Table 7.5-138) resulted in slower and less complete ATR degradation as more energetically favorable substrates were utilized for growth (Figure 7.5-96; greater GLU activity in grass treatments); (2) increased labile soil C and N led to decreased gene copy number

and/or activity of ATR degrading genes in the grass treatments; and (3) greater ATR sorption in surface soil of the grass treatments reduced its bioavailability (Table 7.5-139).

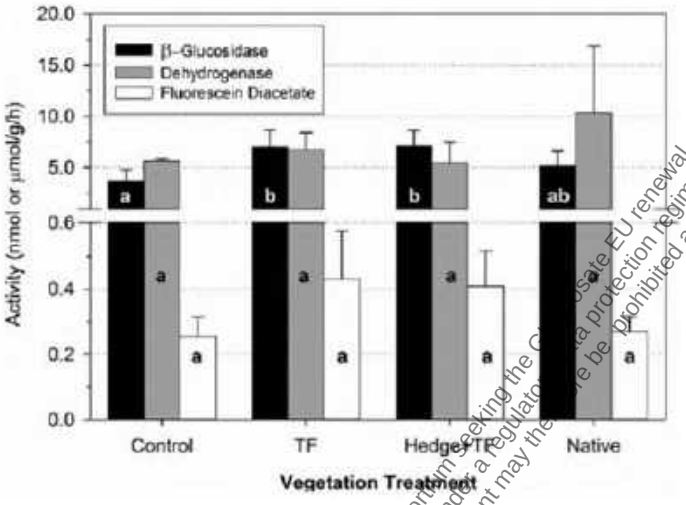
Figure 7.5-95: **Atrazine Degradation in Surface Soil (0-10 cm) Collected from Each Vegetation Treatment. The left y-axis represents atrazine remaining and mineralized after 56 days of incubation. The right y-axis is the atrazine half-life estimated from first order kinetic models. Error bars represent the 95 % confidence interval. Vegetation treatments with different letters were significantly different at α= 0.05**



Conclusions

This study showed that VBS can substantially reduce loads of ATR, MET, and GLY in runoff from a highly eroded claypan soil, a setting known to be the most vulnerable for herbicide losses within the Corn Belt. Thus, VBS were effective for reducing herbicides transported by dissolved-phase and sediment-bound modes. All grass treatments significantly reduced surface runoff via improved infiltration and showed significant reductions in sediment load compared to the unvegetated control. Of the three main factors studied, vegetation treatment and buffer width had much greater effect on herbicide loads than season. Compared to the control, grass treatments reduced herbicide INLs by 19-28 % and sediment INLs by 67 %. These data showed that C3 and C4 grasses used alone or in combination can achieve very similar herbicide and sediment load reductions. Therefore, the choice of VBS grass species appears to be flexible and can be made based on practical considerations such as the site condition, cost and availability of seed, and ease of establishment. Partitioning of dissolved phase herbicide loads retained within the VBS revealed that grasses increased infiltration and adsorption of herbicides compared to bare ground. The results demonstrated that VBS can effectively reduce herbicide loads for soils with limited infiltration or cases in which the SBAR is high (e.g.,>10:1) *via* enhanced herbicide adsorption to soil and vegetation. Grass treatments resulted in modest improvements to surface soil quality 8-13 years after establishment, with significant increases in soil organic C, total N, and ATR and MET sorption. Nonlinear regression analyses showed that herbicide INLs as a function of buffer width were well described by first-order decay models and that VBS can provide significant load reductions when implemented at realistic SBARs. These equations, in combination with existing simulation models that can account for changes in slope, rainfall intensity, and crop management, can be used as the basis for designing VBS that can achieve desired herbicide load reductions while minimizing land taken out of production. This approach provides conservation agencies and landowners a simple and applied tool for effectively implementing VBS to control herbicide losses from cropped fields.

Figure 7.5-96: **Microbial Enzyme Activities in Surface Soil (0-10 cm) Collected from the Vegetation Treatments. Error bars represent 95 % confidence intervals. For each enzyme, treatments with different letters were significantly different at α= 0.05**



3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a runoff experiment to evaluate the effectiveness of vegetative buffer strips in USA. The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/042
Report author	Mottes, C. et al.
Report year	2017
Report title	Relationships between past and present pesticide applications and pollution at a watershed outlet: The case of a horticultural catchment in Martinique, French West Indies
Document No	Chemosphere (2017) 184:762-773
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (Laboratoire Departemental d'Analyses de la Drome)
Acceptability/Reliability:	Reliable

2. Full summary

The understanding of factors affecting pesticide transfers to catchment outlet is still at a very early stage in tropical context, and especially on tropical volcanic context. We performed on-farm pesticide use surveys during 87 weeks and monitored pesticides in water weekly during 67 weeks at the outlet of a small catchment in Martinique. We identified three types of pollution. First, we showed long-term chronic pollution by chlordecone, diuron and metolachlor resulting from horticultural practices applied 5-20 years ago (quantification frequency higher than 80 %). Second, we showed peak pollution. High amounts of propiconazole and fosthiazate applied at low frequencies caused river pollution peaks for weeks following a single application. Low amounts of diquat and diazinon applied at low frequencies also caused pollution peaks. The high amounts of glyphosate applied at high frequency resulted into pollution peaks by glyphosate and aminomethylphosphonic acid (AMPA) in 6 and 20 % of the weeks. Any intensification of their uses will result in higher pollution levels. Third, relatively low amounts of glufosinate-ammonium, difenoconazol, spinosad and metaldehyde were applied at high frequencies. Unexpectedly, such pesticides remained barely detected (<1.5 %) or undetected in water samples. We showed that AMPA, fosthiazate and propiconazole have serious leaching potential. They might result in future chronic pollution of shallow aquifers alimenting surface water.

Methods

Our research analyses farmers' pesticide use practices and water contamination data acquired on an experimental catchment. Our complete dataset rely on different data acquired over different periods: Figure 7.5-97 summarizes data acquired from 2011 to 2013. We started acquiring farming practices before the water sampling campaign to take into account potential pesticide transfer lags. The 67 weeks period lasting from the 11/10/2011 to the 01/02/2013 is an overlapping period of pesticide practices and water quality samples (Figure 7.5-97). For past farming practices, Houdart provided us with the practices of the Ravine catchment farmers for years 2001-2002 (Houdart, 2005).

Study site

The experimental horticultural catchment studied is the Ravine catchment (Mottes *et al*., 2015). It is located on the Northeast side of the Martinique Island, French West Indies (140490200 N, 610701400 W). This catchment is part of the Capot catchment (57 km^2) that provides 20 % of the drinking water in Martinique while being chronically contaminated by pesticides. In Martinique, the climate is tropical humid with a maritime influence. Rainfall pattern is characterized by two seasons: a dry season from January to March and a wet season from June to September. The average annual rainfall on the catchment is 3600 mm. The Ravine catchment covers 131 ha with elevation ranges varying from 312 m to 628 m. The mean slope of the catchment is 14 % with the upper part slopes comprised between 15 and 30 % while the lower part slopes ranges from 0 to 15 %. The land use is agriculture, with more than 200 fields which belong to 20 farms (Figure 7.5-98): 18 % of agricultural lands are chayote (*Sechium edule*), 13 % banana (*Musa* spp.), 6 % pineapple (*Ananas comosus*), 17 % are covered by other horticultural species, 6.5 % by fallow (multiple species), and less than 2 % are covered by roads and tracks roads. Forests, meadows and pastures cover the remaining surface (37.5 %). The soils are andosol (Colmet-Daage and Lagache, 1965; Quantin, 1972), which are young volcanic ash soils with high infiltration rates (Cattan *et al*., 2007; Charlier *et al*., 2008). Drillings showed that subsoil is constituted by a 1-12 m pumice layer and multiple layers of pyroclastic block and ash flow deposits ("nuées ardentes") with different levels of alteration. The total height of block and ash flow deposits exceeds 70 m. Pumices and block and ash flow deposits are porous materials which contain aquifers drained by the volcanic streams (Charlier *et al*., 2008). An in-depth analysis of the hydrological functioning of this catchment is presented by Mottes *et al.* (2015). In particular, they showed that the hydrological functioning of the catchment is dominated by groundwater flows (50-60 % of annual flows) and that aquifers are highly connected to surface water.

Pesticide use survey

Two types of survey among farmers were performed. In a first step, a global survey of the current pesticides used on various cropping systems in 2010 was performed. From this survey, a list of molecules that farmers applied on fields was compiled. This was completed by adding banned pesticides used in the past to the list,

such as chlordecone (banned in 1993), paraquat (banned in 2007), lindane (banned in 1998) or diuron (banned in 2007) and other potential significant pesticides and metabolites that the French water office (ODE) found in water samples at a regional scale. Finally, a final list of 77 molecules (Table 7.5-140) was produced. After this consolidated pesticide list was compiled, Houdart provided a description of the practices of the farmers of the Ravine catchment for years 2001-2002 (Houdart, 2005). Several molecules were found to be applied on the catchment at that time that were not identified in the pesticide list: disulfoton, imidacloprid, methomyl, parathion-methyl, simazine, sulfosate, tebuconazole, terbufos and tridemorph (Table 7.5-140). As a result, these pesticides were not analyzed in water samples (Table 7.5-140). In a second step, all the farmers of the Ravine catchment were surveyed. First, farmers were asked to describe their cropping systems and their strategies to control pests on the different crops they grow. When it was available, the log or notebooks of the farmers were recorded. Second, practice follow up surveys were performed every month from July 2011 to April 2013. During these surveys and for each field, the farmers were asked to detail the field scale practices they performed every week during the previous month. Plantation, harvest, tillage operation, mowing, pruning as well as pesticide applications and other pest management practices were surveyed. The practice application dates were collected, as well as the modalities of application (equipment, localization of practices, dose and commercial product).

Water sampling

The water at the catchment outlet was sampled with an automatic sampler (ISCO 6712, ISCO Incorporation). Throughout each week, that lasted from Tuesday to the next Tuesday unless exception, the sampling frequency of the water in the river was proportional to the stream discharge calculated from the records of a pressure sensor PCDR 1830 (Campbell scientific). Depending on the period, the automatic sampler collected two 100 mL subsamples each time 300-1800 m^3 discharged at the outlet. To avoid pesticides bounding to container, each first subsample was stored in a plastic container while each second subsample was stored in a glass container (Amalric, 2009). During each week, the automatic sampler progressively built the composites samples by adding each new first subsample into the plastic container, and each new second subsample into the glass container. At the end of each week, the two containers containing the composite samples were collected and filled the bottles provided by the laboratory (3 glass bottles: 2 x 1 L + 100 mL and 2 plastic bottles: 150 mL + 100 mL totaling 2.35 L) with aliquots from the composite samples stored in the plastic and glass containers. The composite samples were collected every week from 11/10/2011 to 01/02/2013.

Laboratory analyses

Pesticides concentrations in water samples for the 77 molecules were analyzed by the "Laboratoire Departemental d'Analyses de la Drome" (LDA26). The laboratory has been accredited by Cofrac, the French Accreditation Committee for pesticide analyzes providing guarantees for their technical skills and reliability as well as good management practices. LDA26 complies with ISO 17025 standards for testing and calibration. The methods mobilized for pesticides analysis rely on the EPA-methods 507, 508, 610 and 625. Results are given with a 30 % confidence interval for the analytical error. Depending on pesticides, extraction and analysis methods, limits of quantification for organic molecules ranged from 0.01 to 0.2 µg/L.

Figure 7.5-97: Data acquired from 2011 to 2013 and associated time periods

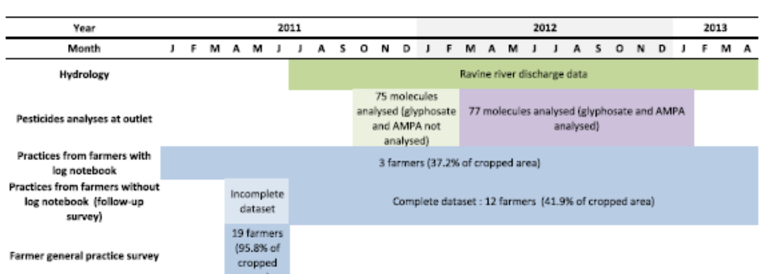
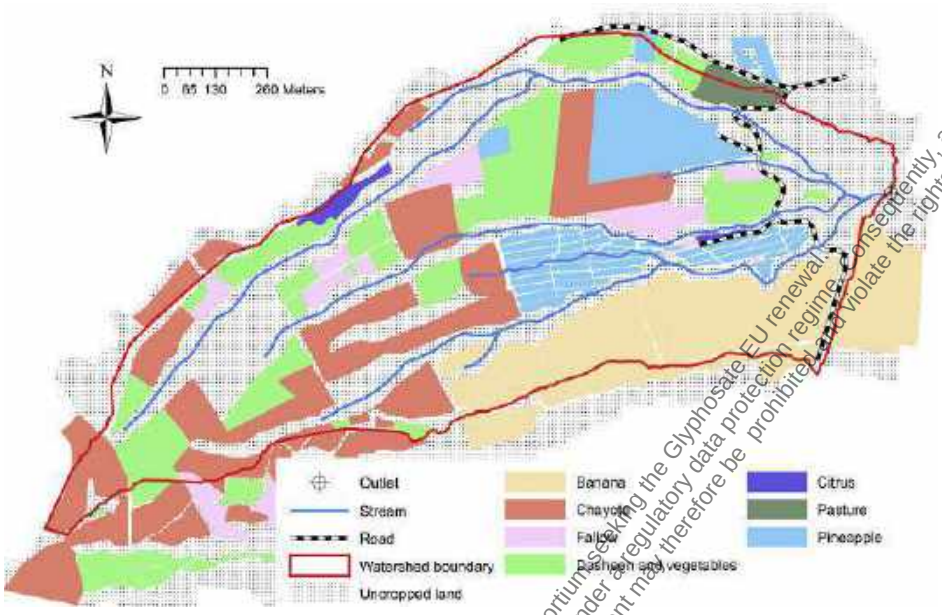


Figure 7.5-98: Land uses of the Ravine catchment



Pesticide application patterns

In order to determine pesticide application patterns, two metrics for each pesticide were calculated: [1] $I_{frapplied}$, a metric of the temporal intensity of the application dynamics. It is defined by the fraction of weeks with applications of the pesticide on the catchment; [2] I_{amount}, a metric of the weekly average amount of pesticide applied on the catchment when it is applied:

$$I_{amount} = \frac{1}{A_{catch}} \times \frac{\sum_{week_{[Qpestapplied_{week}>0]}}^{Nweeks_{[Qpestapplied_{week}>0]}} Qpestapplied_{week} \times e^{-7 \times \left(\frac{\ln(2)}{DT_{50}soil}\right)}}{Nweeks_{[Qpestapplied_{week}>0]}}$$

where $Qpestapplied_{week}$ is the amount of pesticide applied on the catchment during the week "*week*" (g). $e^{-7 \times \left(\frac{\ln(2)}{DT_{50}soil}\right)}$ is a degradation factor derived from a first order degradation kinetics that accounts for potential degradation of the pesticide during 1 week (7 d) with half-life $DT_{50}soil$ (d). A_{catch} is the total area of the catchment (ha). $Nweeks_{[Qpestapplied_{week}>0]}$ is the number of weeks over the considered period with application of the pesticide. I_{amount} is set to 0 for pesticides that were not applied on the catchment in 2011–2013.

We analyzed pesticides application patterns during the practice-monitored period that last from the 1st of June 2011 to the 1st of February 2013 totaling 87 weeks.

Pesticide water pollution

Two metrics for each pesticide to characterize water pollution by pesticide were calculated. First, the frequency of quantification of each pesticide at concentrations higher than 0.1 µg/L in water samples was calculated. Second, an average concentration metric by taking into account weeks with concentrations over 0.1 µg/L was calculated.

$$IConc_{pest} = \frac{\sum_{week_{[Cpest_{week} \geq 0.1\ \mu g\ L^{-1}]}}^{Nweeks_{[Cpest_{week} \geq 0.1\ \mu g\ L^{-1}]}} Cpest_{week}}{Nweeks_{[Cpest_{week} \geq 0.1\ \mu g\ L^{-1}]}}$$

where $Cpest_{week}$ is the concentration of pesticide "*pest*" during the week "*week*" (µg L^{-1}). $Nweeks_{[Cpest_{week} \geq 0.1 \mu g\ L^{-1}]}$ is the number of weeks over the considered period with concentration of the pesticide "*pest*" over 0.1 µg L^{-1}.

Results and Discussion

Table 7.5-140 summarizes pesticides applied on the Ravine catchment in 2001-2002 and in 2011-2013 and pesticides found in water samples in 2011-2013. Farmers applied 27 commercial products corresponding to 17 active ingredients during the 2011-2013 period. Table 7.5-140 indicates that weekly pesticide samples showed contamination of the water at the Ravine catchment outlet. 16 active ingredients at the catchment outlet (Table 7.5-140) were found and provided concentration dynamics for 9 (Figure 7.5-99). Among these, 4 are nowadays prohibited and unreported in the survey (diuron, paraquat, chlordecone and b-HCH), 2 are metabolites or co-products from respectively glyphosate and chlordecone (aminomethylphosphonic acid (AMPA) and chlordecone-5b-hydro) and 10 are still authorized (propiconazol, difenoconazol, dithiocarbamates, copper sulfate, diquat, fosthiazate, diazinon, glyphosate, metolachlor and metaldehyde). Except for banned pesticides, metabolites and metolachlor, farmers of the Ravine catchment declared the use of the measured pesticide in water.

5 pesticide application patterns were found according to the two application metrics calculated from April 2011 to April 2013 (Figure 7.5-100a): [A] high amounts of pesticide applied at high frequency, [B] low amounts of pesticide applied at high frequency, [C] low amounts of pesticide applied at low frequency, [D] high amounts of pesticide applied at low frequency and [E] historical currently unapplied pesticide (removed from Figure 7.5-100a for better readability).

According to Table 7.5-140 and Figure 7.5-99 three types of pesticide concentration dynamics were found: [1] undetected pesticides (all pesticides applied on the catchment but never found in water samples), [2] chronic pollution (pesticides showing pollution periods of several weeks such as chlordecone, diuron, metolachlor and di-thiocarbamates), and [3] peak pollution (pesticide with isolated pollution peaks such as glyphosate, AMPA, propiconazole, difenoconazol, copper sulfate, diquat, paraquat, chlordecone-5b-hydro, fosthiazate, diazinon, b-HCH and metaldehyde). Figure 7.5-100b shows that for the 0.1 µg/L threshold, chlordecone and dithiocarbamates are the two chronic pollutants. Metolachlor concentrations are barely higher than 0.1 µg/L. Figure 7.5-100b also shows that pollutants over the 0.1 µg/L threshold belong to all pesticide application patterns except pattern B (low amounts applied at high frequency).

Table 7.5-140: Characteristics of pesticide used on the catchment. Applications on different crops in 2001 – 2002 and 2011 – 2013, Environmental characteristics (Footprint, 2013): Koc:soil water – organic carbon coefficient, DT50 soil: pesticide half-life in soil, DT50 water: pesticide half-life in water. Detection and quantification ≥0.1 µgL-1 frequencies at the outlet of the Ravine catchment

Active ingredient	Usage	2011–2013					2001–2002					LQ	Koc	DT50 soil	DT50 water	Detection	>0.1 µg L-1
		B	C	P	D	V	B	C	P	D	V	(µg L^{-1})	(mL g^{-1})	(d)	(d)	(%)	(%)
Abamectin	I	–	–	–	–	X	X	–	–	–	X	0.05	–	–	–	0	0
Ametryn (banned)	H	–	–	–	–	–	–	–	X	–	–	0.02	316	37	S	0	0
Azoxystrobine	F	–	–	–	–	X	–	–	–	–	–	0.01	589	78	S	0	0
Bacillus thuringiensis	I	–	–	–	–	–	–	–	–	–	X	–	–	–	–	–	–
Benomyl (banned)	F	–	–	–	–	–	X	–	–	–	X	0.08	1900	67	0.8	0	0
Cadusafos (banned)	N	–	–	–	–	–	X	–	X	–	–	0.02	227 (K_{foc})	38	S	0	0
Copper (copper sulfate)	F	–	–	U	U	X	–	–	–	–	X	20	12000	10000	S	4.5	4.5
Cycloxydim	H	–	–	X	–	X	–	–	X	–	–	0.1	59	0.65	17.4	0	0
Cypermethrin	I	–	–	U	–	X	–	–	–	–	–	0.02	156,250	60	S	0	0
Deltamethrin	I	–	–	–	–	X	–	–	–	–	X	0.02	10,240,000	13	S	0	0
Diazinon	I	–	–	U	–	–	X	–	X	–	–	0.04	609	9.1	138	4.5	1.5
Difenoconazol	F	X	–	–	–	–	X	–	–	–	–	0.05	3760(K_{foc})	134	S	1.5	0
Diquat	H	–	–	–	X	X	X	–	–	–	–	0.05	2,185,000	5342	S	1.5	1.5
Disulfoton (banned)	I	–	–	–	–	–	–	–	X	–	–	–	1345	30	300	–	–
Diuron (banned)	H	–	–	–	–	–	–	–	X	–	–	0.02	813	90	S	81.8	0
Ethoprophos (banned)	N	–	–	–	–	–	–	–	X	–	–	0.04	70	37	S	0	0
Fipronil	I, N	–	–	–	–	–	X	–	–	–	–	0.01	727(K_{foc})	142	S	1.5	1.5
Fluazifop-p-butyl	H	X	–	–	–	–	X	–	–	–	–	0.05	3384	17	78	0	0
Fosetyl-Al	F	–	–	X	–	–	–	–	–	–	–	0.1	–	0.1	S	0	0
Fosthiazate	N	X	–	U	–	–	–	–	–	–	–	0.02	238	13	104	9.1	1.5
Glufosinate-ammonium	H	X	X	–	X	X	–	–	–	–	–	0.1	600	7.4	300	0	0
Glyphosate	H	X	X	X	–	–	X	–	X	–	–	0.1	3424	15	S	6.4	6.4
Imidacloprid (banned)	I	–	–	–	–	–	–	–	–	–	X	–	225(K_{foc})	191	S	–	–
Lambda cyhalothrin	I	–	–	–	–	X	X	–	–	–	–	0.02	493,707	175	S	0	0
Mancozeb (Dithiocarbamates)	F	–	–	–	–	X	–	–	–	–	–	0.1	998	0.1	1.3	22.7	22.7
Metaldehyde	M	–	X	–	–	X	–	–	–	–	–	0.05	240	5.1	S	1.5	0
Oxamyl	N	X	–	–	–	–	–	–	–	–	–	0.1	16.6	7	8	0	0
Methomyl	I	–	–	–	–	–	–	–	–	–	–	–	72	7	S	–	–
Paraffinic oil	F, I	X	–	X	–	–	–	–	–	–	–	–	462000	87	–	–	–
Paraquat	H	–	–	–	–	–	X	–	X	–	–	0.05	1,000,000	3000	S	1.5	1
Parathion-methyl	I	–	–	–	–	–	–	–	–	–	X	–	240	12	21	–	–
Propiconazole	F	X	–	–	–	–	X	–	–	–	–	0.05	1086	71.8	53.5	7.6	3
Pirimicarbe	I	–	–	–	–	X	–	–	–	–	–	–	–	86	S	–	–
Simazine	H	–	–	–	–	–	X	–	–	–	–	–	130	60	96	–	–
Spinosad	I	X	–	–	–	–	–	–	–	–	–	0.02	35,838	17.3	–	0	0
Sulfosate	H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tebuconazole	F	–	–	–	–	–	–	–	–	–	–	–	769 (K_{foc})	63	S	–	–
Terbufos	N	–	–	–	–	–	–	–	–	–	–	–	500	8	6.5	–	–
Tridemorph	F	–	–	–	–	–	–	–	–	–	–	–	6250	24	32	–	–
Chlordecone	I	–	–	–	–	–	–	–	–	–	–	0.01	2500	450	S	100	92.5
Metolachlor	H	–	–	–	–	–	–	–	–	–	–	0.02	120	90	S	87.9	3
β-HCH (lindane)	I	–	–	–	–	–	–	–	–	–	–	0.01	1270	980	732	1.5	0
AMPA	Met	–	–	–	–	–	–	–	–	–	–	0.1	2002	121	–	21.3	21.3
Chlordecone 5b hydro	Met	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	18.2	1.5

B: Banana, C: Chayote, P: Pineapple, V: Dasheen-Other vegetables
I: Insecticide, H: herbicide, F: fungicide, N: nematicide, M: molluscicide, Met: Co-product or metabolite.
X: used, U: unofficial use.
LQ: Limit of quantification.
(K_{foc}): Kfoc (freudlich isotherm) reported.
S: Stable.

Historically applied pesticides

The analysis first showed that water pollution is due to several pesticides, which farmers do not use anymore. Indeed, most of them are now prohibited (e-phy, 2010). This shows that even after 5 to more than 20 years after their ban, they still contaminate water at the catchment outlet. The historical pesticides show 3 types of detection patterns at the catchment outlet. First, chlordecone, diuron and metolachor were detected at a very high frequency throughout the sampling period (Figure 7.5-99, Table 7.5-140); second, Paraquat, b-HCH, chlordecone-5b-hydro are detected only anecdotally (Table 7.5-140), and finally some are not detected anymore such as ametryn, cadusaphos or ethoprophos. Our hypothesis for the first 2 types is that these pesticides are still stocked in soil (DT_{50soil}>75 d) so that they slowly leach into groundwater, soil behaving as pollution source.

Chlordecone, diuron and metolachlor were applied for a long time and on large areas of the catchment. These three pesticides still chronically contaminate water at the outlet. Their detection frequency is higher than 80 % at the catchment outlet and reaches 100 % for chlordecone. Such pollution are characterized by a weekly concentration varying within a narrow range (from 0.05 to 0.77 µg/L for chlordecone; from <0.02

to 0.09 µg/L for diuron and from <0.02 to 0.14 µg/L for metolachlor (pollution peak removed)). We did not observe a strong relationship between water concentrations and rainfall. According to Dores *et al.* (2009), we found metolachlor and diuron to leach in tropical conditions. The three historical pollutants are characterized by long soil half-lives (>75 d). Because persistent and long-term pollution involve the contamination of soils and aquifers, such soil persistence favor permanent pollution of rivers (Cabidoche *et al.,* 2009; Mottes *et al*., 2016). A persistent pollution of the stream by metolachlor was measured with water concentrations under 0.1 µg/L most of the time. The authors expected the ending of a chronic pollution as with diuron. Nevertheless, its use is still authorized on pineapple crop (S-metolachlor compound). The authors suspected an application on the catchment even if no surveyed farmer reported S-metolachlor application. Indeed, a pollution peak (0.39 µg/L) was observed in water samples (Figure 7.5-99e). This pollution peak is consistent with the high transfer rate with runoff found by Dores *et al*. (2009) that could follow applications. This is the reason why this specific use could maintain the long-term pollution of the river. The use of such persistent contaminant of the environment should therefore be stopped in tropical context to avoid any increase of the pollution.

Figure 7.5-99: **Meteorological, hydrological and pollution at outlet time series on the Ravine catchment from 11 October 2011 to (a) daily rainfall; (b) discharge at outlet; (c) chlordecone concentrations, (d) diuron concentrations, (e) metolachlor concentrations, (f) glyphosate concentrations (black), AMPA concentrations (green), (g) fosthiazate concentrations, (h) propoconazole concentrations (black), defenoconazol concentrations (green), (i) dithiocarbamates concentrations.** For detected but unquantified pesticides, we estimated concentrations to quantification limit divided by 3 as suggested by laboratory guidelines

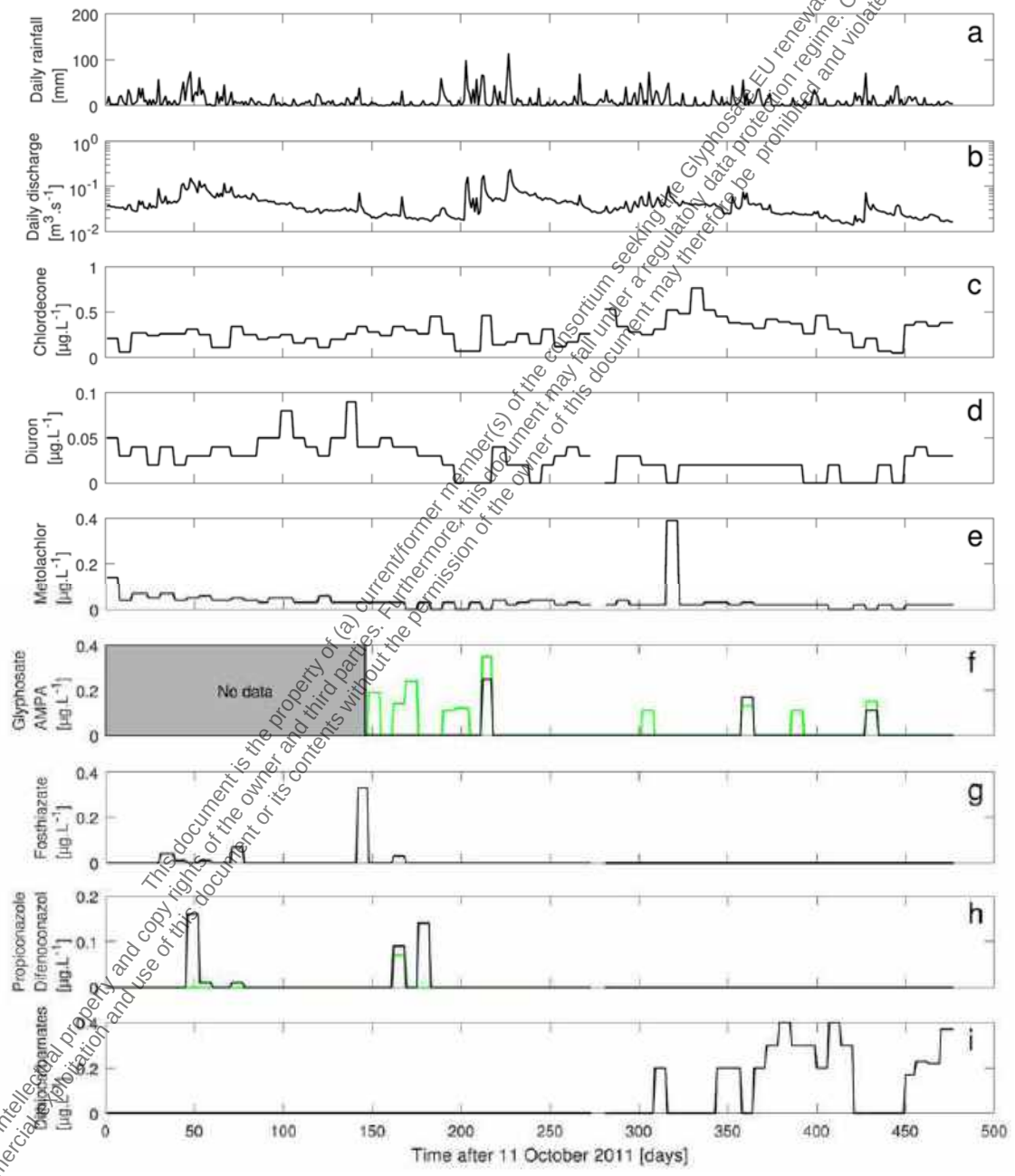
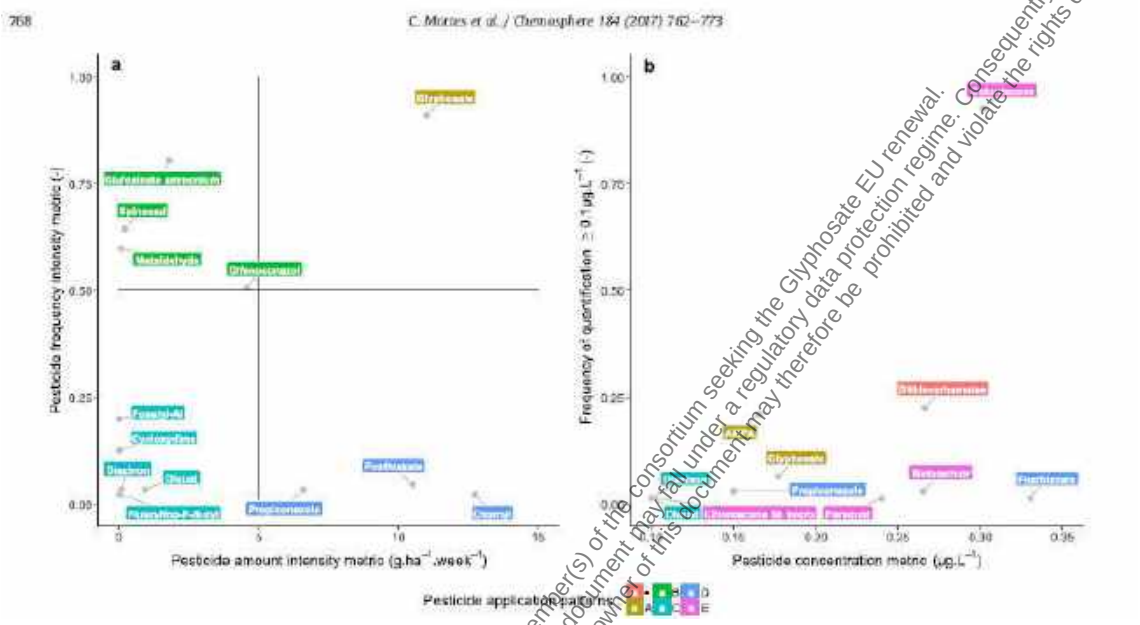


Figure 7.5-100: **Pesticide uses and pollution intensities on the Ravine catchment (a) Pesticide application intensities; (b) Pesticide pollution intensities ≥0.1 µg/L. Pesticide application pattern [-] undefined, [A] high amounts applied at high frequency, [B] low amounts applied at high intensities, [C] low amounts applied at low frequency, [D] high amounts at low frequency, [E] historical currently unapplied pesticides**



Paraquat and b-HCH were used in a less intensive manner or during shorter periods of time than chlordecone, diuron and metolachlor. Chlordecone-5b-hydro is a co-product of chlordecone production that corresponds to a very small fraction of the chlordecone amount applied. Chlordecone-5b-hydro and paraquat were unfrequently quantified at concentrations higher than 0.1 µg/L (Figure 7.5-100b) while b-HCH did not exceed this threshold. The low detection frequencies of these pesticides could be explained by the lower amounts of residues remaining in soil because smaller amounts of these pesticides or co-products were applied on the catchment. It is likely that specific environmental characteristics such as tillage, high water flows, or both led to their remobilization from soil to the catchment outlet. Nevertheless, the small number of detections and the lack of knowledge on the behavior or the spatial and temporal application patterns of these pesticides in the past harms the robustness of this conclusion.

Ametryn, cadusaphos or ethoprophos are pesticides with high dissipation potentials. Charlier *et al.* (2009) clearly demonstrated that cadusaphos quickly contaminated surface water during both high and low flows. Farmers used cadusaphos and ethroprophos as nematicides, they applied both onto the soil. Although these pesticides may have contaminated the environment when they were applied, they were apparently quickly transferred, diluted and/or degraded in the environment leading to no more detection nowadays. At the molecular composition level, we observed that chlordecone, diuron and metolachlor carry at least one chlorine radical, while ametryn, cadusafos and ethoprophos do not. According to our results, we are in the opinion that chlorine radicals could favor the stability and the persistence of molecules in the environment. This is confirmed by Calvet *et al.* (2005) who indicated that chlorine radical decreases the speed of the breaking of aromatic cycles in organic compounds. Henschler (1994) also support this hypothesis by indicating a frequently increased chemical stability of chlorinated organic compounds along with an easier enzymatic conversion. Consequently, the presence of chlorine radical in the molecule could favor the long-term potential pollution of the environment even if the molecule is classified under another organic compound family than organochlorine such as phenylurea, carbamate or triazole.

<u>Pesticides used on the catchment during the sampling period</u>

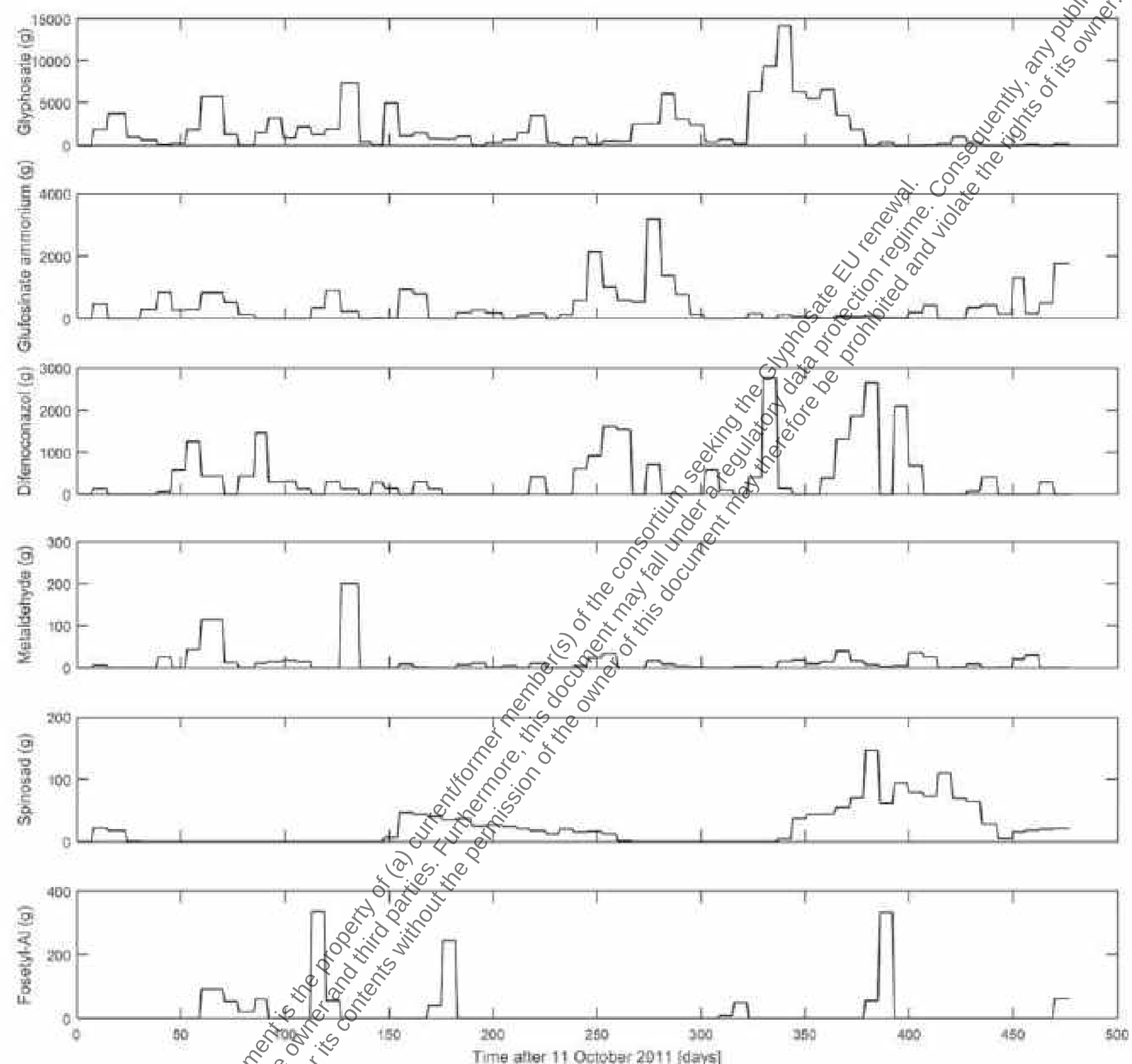
Pesticides regularly applied on the catchment

The survey showed that 5 pesticides were regularly applied on the catchment: glyphosate, glufosinate ammonium, difenoconazol, spinosad and metaldehyde (Figure 7.5-100a). These pesticides were applied on more than 50 % of the weeks during the sampling period. Glyphosate was applied on 90 % of the weeks at very high rates (Figure 7.5-100a and Figure 7.5-101). Glufosinate ammonium was applied 75 % of the weeks at lower rates (Figure 7.5-100a and Figure 7.5-101). Difenoconazol was applied during half of the weeks of the sampling period at intermediate application rates while spinosad and metaldehyde were applied during more than half of the weeks but at low rates (Figure 7.5-100a and Figure 7.5-101). In the water samples, Glyphosate and its metabolite AMPA were quantified over 0.1 µg/L (Figure 7.5-99 and Figure 7.5-100b) which is consistent with its very intensive use at the catchment scale. In spite of their frequent uses, glufosinate ammonium and spinosad were never detected in water samples while difenoconazol and metaldehyde were both quantified only once at concentrations lower than 0.1 µg/L.

Glyphosate is widely used as a general systemic herbicide. Glyphosate and its major metabolite Aminomethylphosphonic acid (AMPA) were frequently quantified at concentrations higher than 0.1 µg/L in our water samples at the catchment outlet. AMPA is a major pollutant detected in 21.3 % samples. Glyphosate was found to have concentrations higher than 0.1 µg/L in 6.4 % samples. For glyphosate pollution peaks, the pollution corresponded to a stormflow event occurring right after the application of glyphosate (Figure 7.5-99f and Figure 7.5-101a). It indicates that glyphosate was quickly degraded or highly adsorbed onto soil particles forming irreversible bounding in agreement with the conclusions drawn by Vereecken (2005) and Borggaard and Gimsing (2008). The surveyed farmers applied glyphosate all year round because weeds are one of the strongest constraints in the humid tropics. Because of this constant application pattern, it is likely that rainfall generating pollution peaks occurred after applications, especially in the tropical climate characterized by heavy and intense rains. AMPA, one of the major glyphosate metabolites, was always present in water samples when we found glyphosate. Nevertheless, AMPA was found with no companion glyphosate during eight weeks over the sampled period. AMPA was found during weeks that are not characterized by significant runoff events. Similarly to chlordecone and diuron, two pesticides which led to permanent contamination at the outlet, AMPA shows a long half-life and a high K_{OC} (Table 7.5-140). In the literature, results from different studies do not agree on the leaching potential of AMPA but some studies showed that AMPA potentially leaches in structured soil conditions (Kjaer *et al*., 2005; Landry *et al.,* 2005; Bergstrom *et al*., 2011). In tropical volcanic catchment conditions, soils are structured with very high infiltration rates (Cattan *et al*., 2007; Charlier *et al*., 2008). Because of the quantification of AMPA outside runoff periods, it is likely that AMPA contaminates at least shallow aquifers on a regular basis. It is likely that glyphosate quickly degrades into AMPA, which is stored in high organic soils, and is leaching to aquifers along with rainfalls. As a result, it was concluded that the widespread and quasi-permanent use of glyphosate on tropical volcanic catchments, such as the Ravine catchment, is likely to result in persistent stream pollution by AMPA within mid-to long-terms.

Glufosinate-ammonium is the second most used herbicide on the catchment. This pesticide was never detected during the weekly analyses, even when runoff events occurred during the same week when farmers applied glufosinate-ammonium. In the literature, glufosinate transfers have been found with that for glyphosate and other herbicides (Screpanti *et al.,* 2005; Shipitalo *et al*., 2008). Anionic retention capacity of andosol (Sansoulet *et al*., 2007) may cause glufosinate ammonium retention in the soils of the catchment. In spite of a high application frequency, the amount of glufosinate-ammonium applied at the catchment scale is lower than glyphosate (Figure 7.5-101) and even lower when considering the degradation rate (Figure 7.5-100a). It might be that pollution is not yet measurable now but could appear in the case of an increase of the amount of glufosinate-ammonium applied at the catchment scale. Glufosinate-ammonium has two identified metabolites that could contaminate the river (3-methyl-phosphinico-propionic acid and 2-methyl-phosphinico-acetic acid) (Footprint, 2013). Unfortunately, their quantifications were outside of the analytic capacity of the laboratory. In the light of this discussion, the authors recommend further investigation on the fate of this pesticide and its metabolites in andosol. They also recommend not to substitute glyphosate by glufosinate-ammonium but rather to find alternatives to exclusive chemical weeding with reduced uses of herbicides.

Figure 7.5-101: Weekly amounts of pesticides applied on the Ravine catchment (g) for glyphosate, glufosinate-ammonium, difenoconazol, metaldehyde, spinosad and fosetyl-al



Difenoconazol has been detected only once in water samples at a concentration below 0.1 µg/L (Figure 7.5-99h). Difenoconazol has an intermediate application pattern at catchment scale in term of frequency and amounts: it is applied on a relatively frequent manner (~50 % of the weeks) at intermediate levels (Figure 7.5-100a). Because of its long soil half-life (85-130 d) reported in the Footprint database (Footprint, 2013) it was expected to detect more frequently difenoconazol in water samples. The only detection occurred on a week characterized by a runoff event the same day that application was performed. That event may have transported the pesticide directly to the outlet during application or right after its application bypassing the soil compartment. This is the reason why the authors are of the opinion that the half-lives of difenoconazol may be lower than the one reported in the Footprint database. This hypothesis is supported by Wang *et al.* (2012) who found short half-life of difenoconazol in water (0.30-2.71 d) and by Mukhopadhyay *et al*. (2011) and Wang *et al*. (2012) who found soil half-life ranging between 4 and 23 d. In the light of this discussion, it is very likely that difenoconazol degraded faster than expected and that such high degradation rates in water explain the single quantification of difenoconazol at the outlet of the Ravine catchment.

Spinosad was frequently used on the banana fields of the catchment. According to Figure 7.5-100a, the amount intensity metric of spinosad is low. The pesticide is applied on banana bunches which are protected by a plastic bag thus limiting washoff and environmental diffusion of that pesticide. The authors are of the opinion that such low application rates under protected conditions limited spinosad transfers to the environment.

Metaldehyde was frequently applied on the catchment but according to Figure 7.5-100a, the amount intensity metric of metaldehyde is very low. Because of such very low amount intensity metric metaldehyde was not expected to be detected in water samples. Nevertheless, it was quantified once below 0.1 µg/L. As for other frequently applied pesticides, the authors are of the opinion that the high application frequency of the pesticide increases the probability of incorrect application conditions on a rainy day that transferred pesticides directly to outlet towards runoff.

The uncertainty surrounding the dithiocarbamates

Dithiocarbamates represent a family of molecules they are mainly used for their fungicide effects. The analytical procedure of the laboratory did not make it possible to identify the specific dithiocarbamate molecules among them. Dithiocarbamates were started to be frequently quantified in the stream from day 309 at concentrations higher than 0.1 µg/L (Figure 7.5-99i). The pollution by dithiocarbamates is the second most intensive after chlordecone (Figure 7.5-100b). Farmers highlighted the intensive use of fungicides on horticultural crops such as tomato, cucumber or pepper but the authors did not have confident enough application dynamics on the catchment to classify the dithiocarbamates application pattern (Figure 7.5-100). Dithiocarbamates were not found any more during high flow periods (Figure 7.5-99). Different hypotheses can be drawn to explain this situation:

(1) The molecules contaminate aquifers but the pollution is diluted below detection limits during high flow periods. However, according to data from the Footprint database (Footprint, 2013), this is unlikely because of the very short reported half-lives of dithiocarbamates (Table 7.5-140). On the contrary, Wilmington (1983), the first manufacturer of mancozeb, the dithiocarbamate used on the catchment, reported soil half-life to range from 4 to 8 weeks. Such values seem to be more realistic and consistent when compared with degradation rates of other pesticides (e.g. Table 7.5-140). (2) The contamination comes from a point source due to inappropriate handling of the unsprayed pesticides fraction. (3) Applications are regularly performed on vegetable crops but no pesticide is sprayed during rainy weeks. (4) Dithiocarbamates were used to produce photodegradable plastic mulches that can be ploughed directly into the soil (Wolfe *et al.*, 1990; Scott, 1997). Degradable plastic mulches are used under pineapple crops but farmers could not attest whether they used photodegradable or biodegradable mulches. In spite of the difficulty to interpret our results, this pollution that appeared at the end of our sampling period is alarming because the stream is polluted in a quasi-persistent manner at high levels. The verification of these different hypotheses would require specific studies on cropping systems using dithiocarbamates and associated transfers to water. In the meantime, improvements of the analysis methodologies are required. Nevertheless, according to the long soil half-life reported by Wilmington (1983) and the K_{OC} of mancozeb (998 mL g^{-1} - Table 7.5-140), we are in the opinion that mancozeb may have contaminated shallow aquifers in our conditions.

Pesticides barely applied on the catchment that generated pollution

Propiconazole and fosthiazate were barely used on the catchment but at high application rates (Figure 7.5-100a). The practice survey showed that both pesticides were applied before the sampling period in response to specific problems such as high sigatoka (*Mycosphaerella* fijiensis, *Mycosphaerella musicola*) pressures or high infestation by nematodes (*Radopholus similis, Pratylenchus coffeae*) on banana fields. Diquat and diazinon were also barely applied but at low rates (Figure 7.5-100a). The four pesticides were detected in water samples at concentrations higher than 0.1 µg/L (Figure 7.5-99 and Figure 7.5-100b) meaning that any intensification of the use of these pesticides will result in pollution at levels higher than the one already observed.

Fosthiazate is an organophosphate nematicide applied onto banana fields. The pesticide was detected during two periods. During the first period (days 30-77), fosthiazate was detected at concentrations lower than 0.1 µg/L (Figure 7.5-99g). During this high flow period the highest concentrations at the peak flow were not observed in spite of a high solubility and a low K_{OC} of the pesticide. This result supports the hypothesis

of a fast transfer toward a shallow aquifer diluted by surface runoff barely occurring in tropical volcanic conditions (Charlier *et al*., 2008; Mottes *et al*., 2015). Later, fosthiazate was detected twice when high rainfall events occurred during a dry period (low average stream discharge). It is likely that the peaks observed during the second period resulted from an unofficial use of the pesticide on pineapple fields before high rainfall events occurred during the dry period (field observations). In the literature, fosthiazate persistence in soil is reported to increase under low pH (Qin *et al*., 2004; Pantelelis *et al*., 2006). Thus, in spite of a short reported soil half-life of 13 d (Footprint, 2013), its persistence in tropical andosols with low pH (Clermont-Dauphin *et al*., 2004) may reach the 47 d values obtained by Pantelelis *et al*. (2006). Its increased stability in tropical volcanic condition can enhance its leaching potential. The contamination of both overland flows and shallow aquifer flows has been observed in similar pedoclimatic conditions by Charlier *et al.* (2009) who studied the transfers of cadusaphos, a nematicide with close molecular characteristics. On the basis of the pollution observed with moderate high flows on the Ravine catchment and results from Charlier *et al*. (2009), there is every likelihood that fosthiazate transfers to catchment outlet toward both overland flows and shallow aquifers.

Propiconazole was detected during a peak flow that took place during the first high rainy event after the beginning of the sampling period (Figure 7.5-99 h). The only reported use for propiconazole occurred 82 d before the beginning of the sampling period. The authors believe that the pollution peaks resulted from that particular pesticide application because a large proportion of the catchment (13 %) was treated on that day by helicopter and because the reported half-life of propiconazole in soil is high 70-200 d (Bromilow *et al*., 1999; Footprint, 2013). Although, propiconazole was reported by several authors to have low leaching potentialities (Bromilow *et al.,* 1999; Kim *et al.,* 2002), Oliver *et al.* (2012) found that propiconazole was transported in a persistent manner from horticultural cropping systems in Australia. Battaglin *et al*. (2011) also observed its presence in United States streams and Toan *et al.* (2013) found that propiconazole significantly contaminated surface water in Vietnam. Propiconazole was frequently found (in 43 % of samples) in a banana oriented catchment in Costa Rica where it was intensively applied (Castillo *et al.,* 2000). Propiconazole pollution dynamics is difficult to interpret because it did not appear systematically during all runoff events; it showed contamination tail during high flow period and a high concentration on weeks without high flow (Figure 7.5-99h). The high soil half-life of the pesticide reminds the ones from historical permanent pollutants (chlordecone, diuron and metolachlor). Propiconazole polluted surface waters in many places but on the Ravine catchment, it did not show clear transfers pathways. The authors suspected however propiconazole to have quickly reached shallow aquifers. Further research on the fate of this pesticide in the specific conditions is warranted, as well as reduction measures to avoid further contaminations of streams. In the French West Indies, application of propiconazole is authorized only once a year. In spite of this restriction, it keeps contaminating water for a long time after being applied. Because this pesticide was found to be a significant water contaminant over the world (Castillo *et al.,* 2000; Battaglin *et al*., 2011; Oliver *et al.,* 2012; Toan *et al.,* 2013) and in the Ravine catchment, we recommend restricting the usage of propiconazole in cases where farmers cannot use alternative techniques, or at least on very small areas of catchments.

Conclusion

The authors have shown that the current and past uses of pesticide in a tropical volcanic catchment resulted in pesticide pollution at catchment outlet and that the approach was relevant to identify potential sources of water pollution at different time scales. Pesticide pollution was not only dependent on the intrinsic characteristics of pesticides but also on the combination of application intensities in terms of frequencies and amounts and on the hydrological functioning of the catchment. Historical pesticides used in horticulture 10-20 years ago resulted in persistent pollution at catchment outlet due to soil and aquifer contaminations. This type of pollution raises the question of the management of the contaminated compartments (such as soils and aquifers) and of the potential implication of such long-term local conditions on larger scale pollution. Pesticides still in use in tropical conditions present serious risk of aquifers contamination. Metolachlor is still authorized while it chronically polluted the catchment outlet. The authors think that the use of glyphosate, fosthiazate and propiconazole could result in mid-to long-term persistent contamination of the stream, as some historical pesticides. In order to avoid the past errors and decrease the risk of long-term pollution of water resources, the only mean to protect them is to reduce or ban the use of these pesticides in horticultural systems. This conclusion raises the question of the design of cropping systems

less dependent on pesticides and their appropriation by farmers. The classification also showed that several pesticides remain undetected in rivers in spite of intensive application patterns. These undetected pollution raise the questions of the underlying processes of the fate of such pesticides. First, the understanding of their fate will make it possible to better anticipate and avoid forthcoming pollution. Second, this will make it possible to assess the potential effect of their increased use in case of farmers shifting of pesticides (cropping system change or regulation evolutions). To assess the three questions raised, the authors recommend further research combining modeling and monitoring to assess the current and future effects of pesticides in tropical horticultural cropping systems on water resources. The combined approach of modeling and monitoring appears to be an interesting approach for co-designing and adjusting cropping systems with farmers.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the monitoring of glyphosate among several pesticides in a horticultural catchment in Martinique, French West India (part of the EU). Methods and results are well described.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/017
Report author	Poiger, T. *et al.*
Report year	2017
Report title	Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS
Document No	Environmental Science and Pollution Research (2017) 24:1588-1596
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Agroscope)
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/043
Report author	Reoyo-Prats, B. *et al*.
Report year	2017
Report title	Multicontamination phenomena occur more often than expected in Mediterranean coastal watercourses: Study case of the Têt River (France)
Document No	Science of the Total Environment 579 (2017) 10–21
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Contaminants found in watercourses are not only the result of anthropogenic activities but also depend on river's seasonal hydrodynamics. This is particularly true in Mediterranean climate regions where long dry periods are interrupted by strong rainfalls. Storm events remobilize particles from soils and sediments and, as a consequence, the load of particulate matter in rivers can be quite considerable, severely affecting water quality. Nevertheless, an absence of fieldwork studies exists concerning the simultaneous dynamics of mixtures of pollutants in river waters, particularly during strong rainfalls and floods. Our study assessed the concentrations of six families of pollutants, including pesticides, at these events, and compared them to those observed at drought sampling periods. We have used as model a typical Mediterranean coastal river from Southeast France, the Têt River, whose hydrodynamics and major elements fluxes have been fairly investigated. As expected, our results show that chemical mixtures due to human activities occur and that they are particularly relevant during storm events. But the results of our study argue that exceptional multi-contamination phenomena actually happen more often than expected because they are linked to recurrent sudden intense rainfall events in the Mediterranean. In particular, combined sewer overflows are responsible for this major issue in urbanized areas, whereas runoff and leaching will be the most important sources of pollutant mixtures occurring at flood flowpeak. After an overview of the sources responsible for chronic multiple stressors events in regions under a Mediterranean climate regime world-wide, we revisit best management measures to reduce risks from the presence of chemical mixtures in the environment.

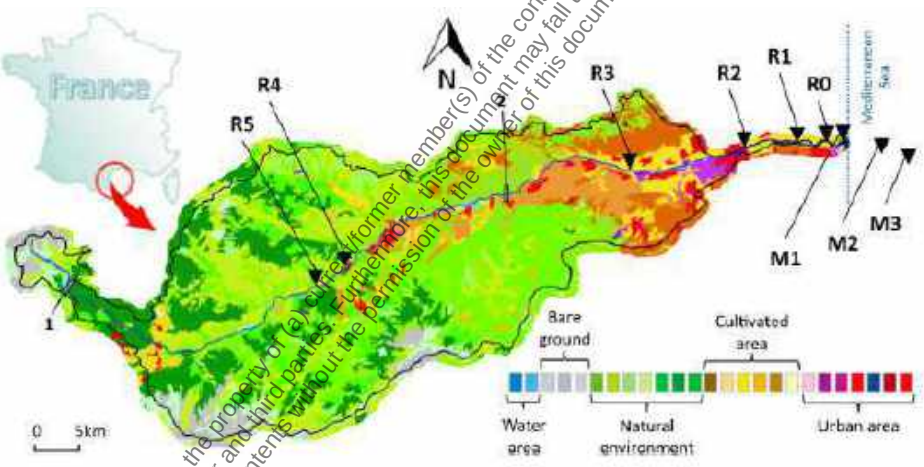
Materials and Methods

Study site and sampling stations

The Têt River is the longest watercourse of the Pyrénées-Orientales department (Southeast France) with a total length of 115 km and a catchment area of 1417 km^2. Two dams partly control the river flow: the Bouillouses dam in the upstream section and the Vinça dam in the plain (see Figure 7.5-102). The Têt River has no major industrial or farm activities along its catchment but is impacted mainly by agriculture and urban activities (see below). It runs through the city of Perpignan, the main city of the department with 120,000 inhabitants. In this study sampling stations were chosen for their contrasted eco-systemic and anthropogenic characteristics along the river course (from R5 to R0 stations) and at the coastal area (from M1 to M3 stations) (Figure 7.5-102). Station R5 is the most upstream station, situated at Serdinya village, 30 km from the source. Upstream there are only little villages and no crop fields. R4 is located at Villefranche village, 4 km downstream R5, and is potentially impacted by fruit tree agriculture developed along the Rotja tributary. R3 is situated at Corneilla-la-Rivière village, 34 km after R4, and is a mildly impacted station with significant vegetable gardening activities and some bigger villages immediately upstream, such as Millas, with a WWTP of 6500 Population Equivalent (PE). R2 is situated 13 km after

R3, in the city of Perpignan but upstream of its WWTP and downstream the Basse tributary, which is highly polluted by agriculture and urban activities. R1 is located 6 km downstream R2 at Villelongue-de-la-Salanque village and is downstream gardening and vineyards cultures as well as the sewage-overflow system and the WWTP of Perpignan city (350,000 PE). R0 is located 3 km downstream R1 station at the closest bridge downstream the WWTP of Perpignan, what allows sampling during flood events. But this station is also downstream of Sainte-Marie-la-Mer and Canet-en-Roussillon vacation resorts WWTPs (24,000 PE and 66,000 PE respectively). M1 is situated at the river mouth, 2 km after R0. M1 is the closest station downstream of vacation resorts WWTPs at drought sampling periods. M2 is approximately 1.5 km from the river mouth. M3 is 3 km offshore besides the CEFREM buoy. Water samples were collected in the summer drought on the 17 September 2013, in the autumn flood on the 17 November 2013 and in the winter drought on the 13 February 2014. M1, M2 and M3 samples from the summer were unfortunately lost for technical reasons. Moreover, to accurately define pollution dynamics during the flood, R0 was sampled a total of 13 times from 16 to 21 November 2013. During this flood, sampling was done from the top of bridges to avoid any danger from sudden water raise and because of the impossibility to access the river during high flow. Marine stations (M1, M2 and M3) were inaccessible as the flood went along with a major sea storm.

Figure 7.5-102: **Land-use map from the Corine Land Cover dataset (European Environmental Agency) for the catchment basin of the Têt River. Sampling stations are indicated as black triangles and dams as grey rectangles (1) Bouillouses and (2) Vinça. See text for details on the main characteristics of sampling stations**



Chemicals analyses

Water sampling, total suspended solids (TSS) and total particulate organic carbon (POC) concentration determination

One (for flood) or two (for drought) 10 L capacity tanks were used for sampling. Tanks had previously been cleansed with 1.5 L of 1 M HCl and rinsed with 2 L of distilled water. Once in the field, tanks were rinsed three times with water from the sample station before being filled. Water samples were maintained refrigerated until processed. For each sample, glass filter columns were washed with distilled water. Sampled water was then filtered until clogging through 0.45 µm porosity GF/F filters using a vacuum pump. Three replicates were carried out per sample, but just two of them were averaged for reporting. The third replicate was kept in case the other two samples gave different results, which did not happen. After filtration, filters were dried at 40 °C for at least 24 h in a clean oven for TSS calculation from dry weight. Dried filters were decarbonized with repeated additions of H_3PO_4 (1 M) and HCl (2 M) and then dried again until no effervescence occurred. Remained sample was filtered in a pre-weighted and a pre-heated GF/F filter (0.7 µm in pore size) followed by repeated additions of HCl at 25 % for inorganic carbon removal. Finally, POC contents were measured using a Leco CN 2000 elemental analyzer.

Pesticides analyses

ISO standards methods (International Organization for Standardization - www.iso.org, 2016) combined with three HPLC methods developed at the Centre d'Analyses Méditerranée Pyrénées (CAMP) were used in this study to detect up to 250 pesticide molecules. A first method started with a liquid-liquid extraction using dichloromethane and 1 L of water sample. Then the sample was evaporated under a dodecane stream (using a Turbovap II) and the residues dissolved in 1 mL of hexane. GC–MS analyses were carried out using a Varian 3800 gas chromatograph with a Saturn 4000 MS detector (ion trap) equipped with DB 5MS capillary column (30 m × 0.25 mm, 0.25 µm; IW Agilent). Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was set at 69 °C for 6 s, programmed to 290 °C at 150 °C/min where it was held for 5 min. The initial oven temperature was 69 °C held for 6 s, heated to 90 °C at 10 °C/min and held for 2 min, followed by 120 °C at 25 °C/min, then 190 °C at 15 °C/min and held for 5 min, followed by 220 °C at 10 °C/min and held for 10 min and finally followed by 320 °C at 5 °C/min and held for 5 min. Molecular ions were monitored for identification via electron ionization (EI) with a full mass range 70–500 *m/z*.

A second method consisted in LC-MS/MS analyses. The derivatization was done by mixing 100 mL water sample with 1.5 mL of FMOC-Cl (1 g/L), 10 mL of sodium tetraborate buffer (19 g/L, pH = 9.2), and 20 mL of acetonitrile (99.9 %). After 24 h of reaction at 4 °C in the dark, reaction was stopped with the addition of 1 mL of orthophosphoric acid (99.9 %). The derivation product was then analyzed by HPLC via an Agilent Bond Elut-PPL 50/PK 9 µm column (100 × 4.6 mm), a volume of 10 mL of methanol was added, the column was then rinsed with 20 mL mQ-water before passing the sample through; all those steps were conducted at a flow of 10 mL/min. The column was then dried for 15 min, before elution was conducted in 10 mL of acetonitrile/methanol (50/50 v/v). Evaporation was then performed with a Turbovap and the extract was obtained via acetonitrile/mQ-water (40/60 v/v) solvent. The LC analysis was conducted at a flow of 200 µL/min using a mixture of two solvents, E (99.9 % acetonitrile/0.1 % formic acid v/v) and F (99.9 % mQ-water/0.1 % formic acid v/v); elution steps were as follows: 0–2 min E at 5 % and F at 95 %, 2–13 min linear gradient from 5 to 100 % for E and 95 to 0 % for F, 13–17 min E holding at 100 % and F at 0 %, finally 17.01 min E and F went back to initial conditions (5 % for E and 95 % for F) until the end at 20 min. The TSQ Quantum ACCESS mass spectrometer used consisted of an HESI source operating with electrospray in the negative-ion mode set at 4 kV, 320 °C, and using collision energy from 16 to 56 eV for glyphosate and from 10 to 15 eV for AMPA. A third and last method started with mixing 100 mL water sample with 100 µL of MCCP D3 standard and 100 µL of formic acid (99 %). Then, the mixture was filtered through a 0.45 µm PTFE membrane and 2 mL were recuperated for the online LC-MS/MS analysis. Sample preconcentration was performed on a Hypersil GOLD C18 5 µm column (2.1 × 50 mm), thanks to a LC pump performing at 0.5 mL/min using a mixture of two solvents, A (99.9 % H_2O/0.1 % formic acid v/v) and B (50 % MeOH/50 % hydrochloric acid CAN v/v); with a step for B solvent going from 5 % to 100 % at 5 min, held until 10 min before going back to 5 %, while A solvent stayed at 95 % except from 5 to 10 min where it was at 0 %. Sample elution was performed on a Hypersil GOLD C18 3 µm (2.1 × 50 mm), via a MS pump at a flow of 300 µL/min using a mixture of two solvents, C (99.9 % acetonitrile/methanol [50/50 v]/0.1 % formic acid v/v) and D (99.9 % mQ-water/0.1 % formic acid v/v); elution steps were as follows: 0–5 min C at 5 % and D at 95 %, 5–15 min linear gradient from 5 to 100 % for C and 95 to 0 % for D, 15–25 min C holding at 10 % and D at 0 %, finally 25.01 min C and D back to initial conditions (5 % for C and 95 % for D) until the end at 28 min. A TSQ Quantum ACCESS mass spectrometer was used in the same conditions as before, except for the collision energy, which was here from 17 to 28 eV.

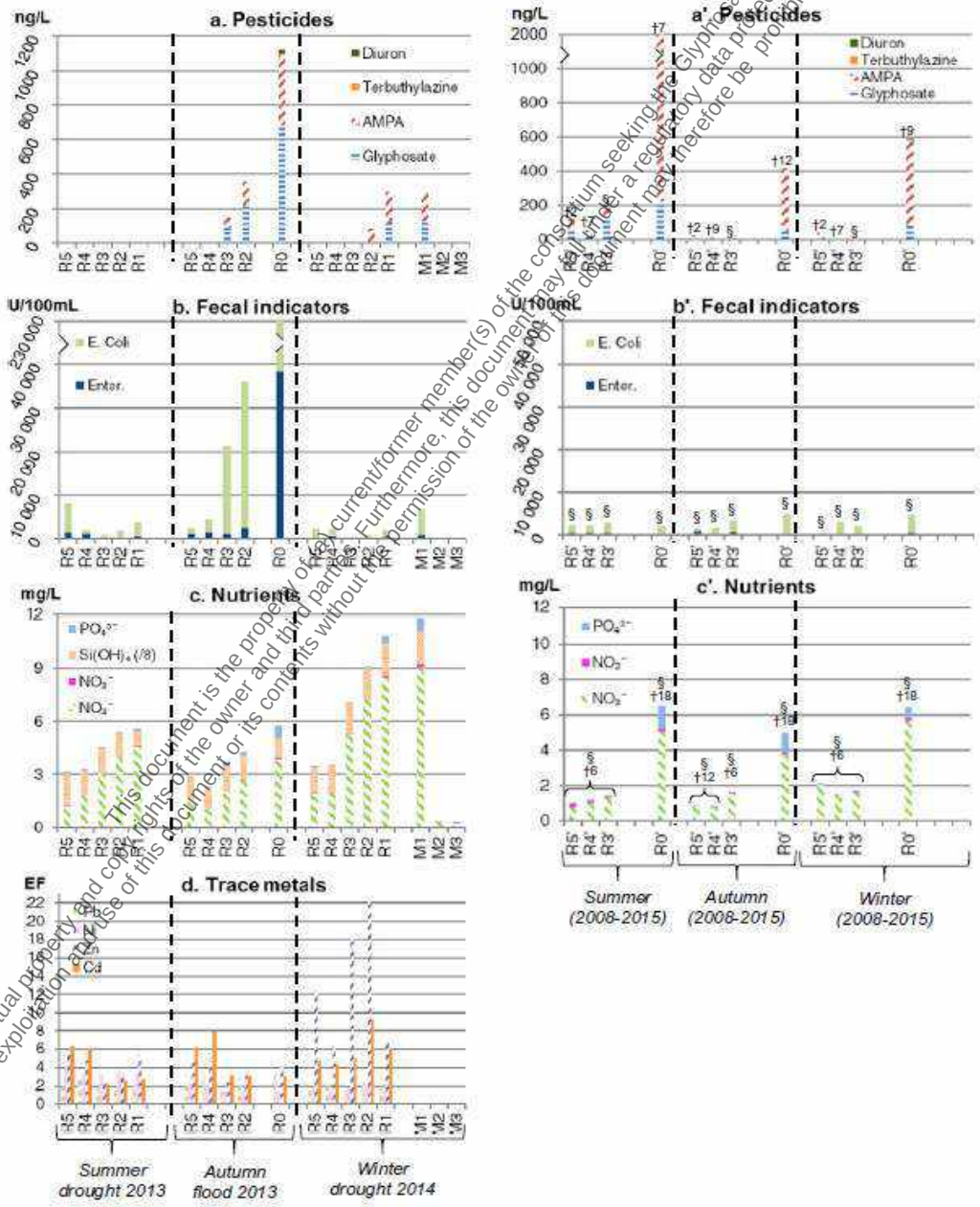
Polycyclic aromatic hydrocarbons (PAHs) and PolyChlorinated biphenyls (PCBs)

PAHs and PCBs were analyzed on 1 L water sample using the ISO 17993 and ISO 6468 standard methods respectively. No PAHs or PCBs were found during the summer drought and the autumn flood, as a consequence they were not further analyzed.

Nutrients

Two replicates samples for nitrate (NO_3^- ± 0.02 µM), nitrite (NO_2^- ± 0.01 µM), phosphate (PO_4^{3-} ± 0.01 µM) and silicate ($Si(OH)_4$ ± 0.05 µM) were collected and stored in 15 mL acid washed polyethylene vials at − 20 °C until used. Samples were analyzed on a Seal-Bran - Luebbe auto-analyzer III according to the colorimetric method of Tréguer and Le Corre (1975) and modified by Aminot and Kérouel (2007).

Figure 7.5-103: Simultaneous variations in contaminants concentrations at the Têt River from our study (a–d) and from government studies averaged per season over years (a'–c'). Variations are reported through space (from upstream to downstream stations) and time (seasonal variations). Notice division correction factor for silicates in c. § stands for CG66 studies of 2008 and 2012, and †x stands for water agency monitoring studies from 2010 to 2015 at x number of samplings



Results

Variation in pesticides concentrations in the Têt River through space and time

The most abundant pesticides found in our study were by far the herbicide N-phosphonomethyl glycine (glyphosate) followed by its microbial degradation product aminomethylphosphonic acid (AMPA), which was present at a much lower concentration. Their highest concentrations were observed in the autumn flood 2013 with a total accumulation of 1119 ng/L at R0 (Figure 7.5-103a). But notice this is not the sample with the highest amount of pesticides in our study (see Figure 7.5-104a and next section for details). Indeed, because we sampled along the whole flood at R0, we chose to represent this station in Figure 7.5-103 by the closest sample in time to that immediately upstream station (R2). Glyphosate and AMPA were also observed at cumulated concentrations of respectively 360 ng/L and 148 ng/L at R2 and R3 stations in autumn, and lower than 300 ng/L at R2, R1 and M1 in the winter drought 2014. However, no pesticides were found at the three most upstream stations (R5, R4 and R3) at any season and neither at the two marine stations (M2 and M3) when measured in winter. Previous government studies (Figure 7.5-103a') found the highest concentrations of pesticides at R0' in summer, with an average total cumulated concentration of 1927 ng/L. In this case, AMPA (1702 ng/L) largely dominated over glyphosate (223 ng/L). Much lower concentrations were found on upstream R3', R4' and R5' during this season. On the contrary, no pesticides were found at any stations in the summer drought 2013 in our study (Figure 7.5-103a). High concentrations of AMPA were also found by government studies at R0' station during autumn (364 ng/L) and winter (496 ng/L) while in the upstream stations comparatively negligible concentrations were found (Figure 7.5-103a'). Regarding the presence of other pesticides, two other herbicides, diuron and terbuthylazine, were also found in our study, but only at the R0 station in autumn, with respective concentrations of 21 and 8 ng/L (but again, see next section). In government monitoring studies, terbuthylazine was only found during autumn at R0' (2 ng/L) while diuron was found at an average concentration of 5 ng/L at R5' and 2 ng/L at R0' during summer, in addition to 4 ng/L at R4' and 9 ng/L at R0' during winter.

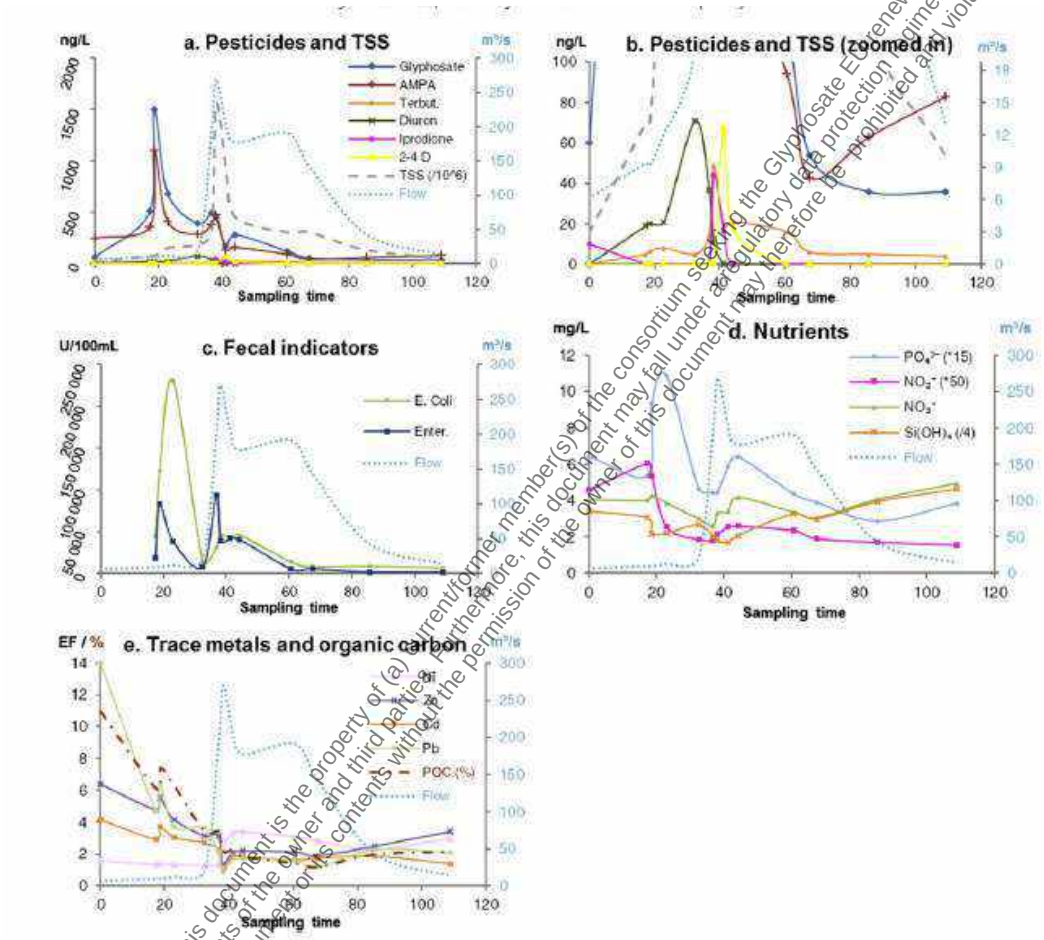
Variations through space and time of other pollutants: Fecal indicators, nutrients and trace metals

Although *E. coli* and *enterococci* were always detected in river waters through the different seasons in all studies (Figure 7.5-103b&b'), much more of these fecal indicators were found in our study during the 2013 autumn flood at the downstream stations. Indeed, concentrations of 20,500, 33,500 and 230,000 U/100 mL of *E. coli* were measured at R3, R2 and R0, respectively, whereas 1670 and 3000 U/100 mL were measured at R5 and R4 during this flood. Thus, at R0, the amount of *E. coli* observed represents > 70 times the values found in summer and winter droughts in all studies. The same highly disproportional values were found during the flood 2013 for *enterococci*, even if they were much less encountered than coliforms (e.g. 38,300 U/100 mL for R1.3 at autumn flood). Exceptional concentrations of fecal pollutants were observed in M1 and R0' in winter (with 5950 U/100 mL and 4380 U/100 mL respectively) and in R5 in summer (6700 U/100 mL). In the two government samplings, however, an average level of only 1755 U/100 mL was found at R5'.

The dynamics of nutrients along the seasons followed a different pattern from fecal bacteria and pesticides. Silicates had the biggest concentrations (Figure 7.5-103c) but, as expected, they did not vary along space and time because they are not directly related to anthropogenic activities, as a consequence they will not be further discussed. Nitrates dominated over nitrites and phosphates and these nutrients progressively enriched along the watercourse (Figure 7.5-103c&c') with no significant differences in their concentrations at the same stations, and this for all seasons with the exception of the winter drought in our study. Indeed, statistically significant higher concentrations of nitrate were observed at this season in our study at downstream stations, from R3 to M1 (Wilcoxon-rank test $P = 0.0312$), with values approximately two times higher than those observed at the two other seasons. For instance, 4.58 mg/L of NO_3^- was measured at R1 in summer and 3.88 mg/L at R0 in autumn, compared to the 8.50 mg/L found at R1 in winter. Only anthropogenic trace metals, i.e. those with EF values higher than 2, are reported in Figure 7.5-103d. Nevertheless, trace metals with EF < 2 values did not show any significant spatial or temporal changes. Zn dominated the other anthropogenic metals during the winter drought with an average EF of 13.2 and a top EF of 22.4 at R2. Contrarily, Zn had an equivalent EF (4.1 in average) in summer and autumn. Furthermore, among all metals, Zn always had the highest EF value on R1 for the three campaigns. In the winter drought, Cd is the second most important anthropogenic metal with an average EF of 5.5 and also a top EF at R2 (9.3). However, Cd had the highest EFs in autumn and in summer, especially at R5 (6.2 and 6.3

respectively) and R4 (7.9 and 6.1 respectively), while the EF of R1 was only respectively 3 and 2.7. Ni and Pb had equivalent rather low EFs in the three seasons, even if a notable difference exists between these two metals, Ni had higher EFs in summer (average of 3.5 vs 2.0) while Pb had higher EFs in autumn (average of 2.7 vs 1.7).

Figure 7.5-104: **Flow variations and dynamics of pollutants during the autumn flood 2013 at the Têt River. Concentrations of pesticides and total suspended solids (TSS) (a and zoom-in b), fecal indicators (c), and nutrients (d), enrichment factors of trace metals and percentage of particulate organic carbon (POC) (e). "Terbut". stands for terbuthylazine. Notice correction factors for nutrients**



Dynamics of pesticides during the flood at the Têt River

As opposed to government monitoring studies, which do not systematically sample during rainy events, in this study we have followed the 2013 autumn flood at R0 for a total of thirteen times (110 h). Figure 7.5-104a represents concentrations of pesticides encountered in the Têt River along this flood. Glyphosate and AMPA dominated in terms of concentrations, with averages of 367 and 300 ng/L, and major peaks of 1500 and 1100 ng/L respectively. These higher concentrations of pesticides occurred approximately at 20 h after the first rains, i.e. at the very beginning of the flood event. Second minor peaks of 490 ng/L of glyphosate and 480 ng/L of AMPA happened 20 h after, coinciding with major combined flow and TSS peaks for this flood event (Figure 7.5-104a). Figure 7.5-104b zooms over the dynamics of other pesticides, in particular the herbicides terbuthylazine, diuron and 2-4D and the fungicide-nematicide iprodione. They were much less important than glyphosate and AMPA, with a maximum average concentration of 13 ng/L and a maximum peak concentration of 71 ng/L for diuron. These pesticides peaked all at the same time, at around 40 h after the first rains, i.e. at the highest flood flow.

Flood dynamic comparison with other contaminants
Regarding fecal contamination, Figure 7.5-104c shows a top peak concentration for *E. coli* of 230,000 U/100 mL at around 20 h and a second lower peak of 45,900 U/100 mL at around 40 h after the first rains. For the *enterococci*, two peaks of approximately 88,000 U/100 mL occurred at the same lapses of time. With respect to nutrients, phosphates had the same concentration dynamics as *E. coli* and pesticides, with a first top peak concentration of 724 µg/L followed by a second smaller peak of 427 µg/L at the same ranges of time (Figure 7.5-104d). Nitrites had a top concentration of 121 µg/L at around 20 h followed by a rapid decrease until 40 h, where it slightly increased to stabilize progressively afterwards. Nitrates and silicates decreased until 40 h, but nitrates behaved as enterococci, they slightly peaked at 20 h and 40 h after the first rains. Phosphates, nitrates and silicates finally tended to increase at the end of the flood event, after 85 h. Anthropogenic trace metals and POC showed different dynamics from the other contaminants (Figure 7.5-104e). They peaked at our first flood sampling point (0 h). Then, they rapidly decreased during the first 17 h of the flood. POC levels decreased from 10.9 to 6.1 while EFs decreased for Pb from 14.0 to 6.5, for Zn from 6.4 to 4.8 and for Cd from 4.1 to 2.9. Ni is the exception here, with stable EFs under 2 during the first 40 h, which then suddenly went up to an average EF of 3, concomitant to the TSS maximum (Figure 7.5-104a), and remained at this value for the rest of the flood. Pb, Zn and Cd had two notable EF peaks, all together, at 20 h and 40 h. After that, their EFs remained stable at around 2 for the rest of the flood.

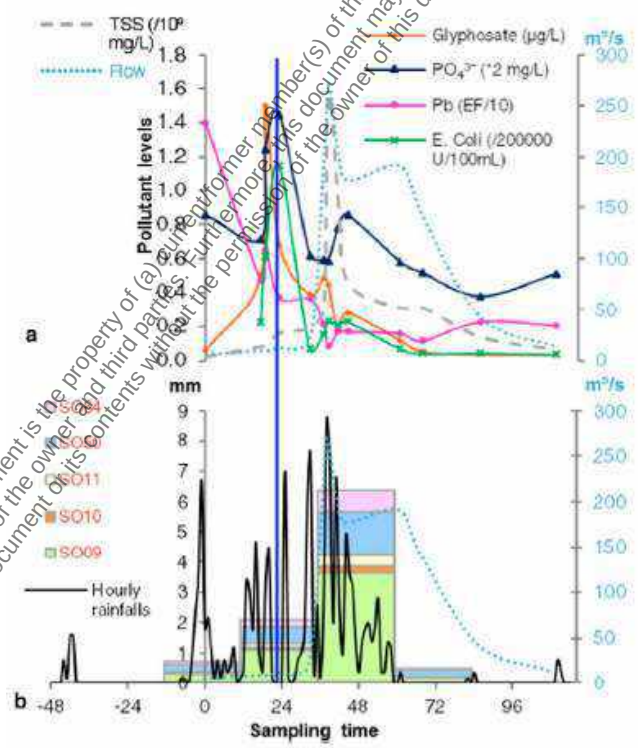
Discussion

Anthropogenic activities drive changes in multiple pollutants concentrations along space and time
At the Têt River, remarkable concentrations of AMPA and some glyphosate were found during summer at very high values at R0’ (Figure 7.5-103a’). The absence of these pollutants at R1, and the comparatively lower presence at R0’ during the other seasons, indicates these molecules are the result of a punctual pollution source, particularly from under-dimensioned activated sludge WWTPs of the summer resorts of Sainte-Marie-la-Mer and/or Canet located immediately upstream R0’. Indeed, this kind of WWTPs cannot be dimensioned for punctual summer-resorts tourism outbreaks but for average annual inputs. In fact, AMPA can be derived from both, detergents and the microbial metabolism of glyphosate, so most likely both wastewater effluents and vegetable or flower gardens leachates arriving to WWTPs are responsible for the presence of this contaminant in the environment. WWTPs are indeed known to be an important source of pesticides to the environment because pesticides are only partially eliminated at WWTPs. The presence not only of pesticides but also of fecal indicators at M1 and R0’ in winter, R0 and R0’ in autumn and the lower levels of fecal pollution in R1 in winter, corroborate this origin. As a matter of fact, the highest amounts of glyphosate and AMPA in our study (Figure 7.5-103a and 7.5-104a) were observed at R0 in the autumn flood 2013, but the impossibility of sampling at R1 during the flood impedes us to rule out the WWTP of Perpignan as responsible of this discharge during storm events (see also next section). The high levels of fecal indicators demonstrate that wastewater is responsible for the presence not only of pesticides but also of the higher concentrations of Pb and phosphates at R0, R2 and R3 during the flood (Figure 7.5-103). Similarly, wastewater inputs to the river can only be responsible for the abnormal fecal indicators levels in our most upstream river station, R5, in summer. Significant levels of other contaminants were not detected in R5, what is not surprising, as this station is surrounded by forests (Figure 7.5-102). Although federal local government studies did not detect this abnormal level of fecal indicators at the same station, poor wastewater treatments at villages upstream R5, which lack WWTPs, must be imputed because our results have been confirmed in successive samplings by our laboratory after 2014 (data not shown).

On the contrary, a diffuse contamination from agricultural seasonal treatments is certainly responsible for the presence of both glyphosate and AMPA in the watercourse in all other cases from all studies during droughts in the Têt River. This pollution is the result of leaching (subsurface flow) and runoff (surface flow) from occasional light rainfalls that occur during summer and winter, as well as, from crops irrigation during these seasons. This is confirmed in our study by the larger presence of nitrates, which are used as fertilizers in crops, and zinc and cadmium, which are known to be naturally present in phosphate minerals used as fertilizers. Furthermore, Zn carbamates are used as fungicides particularly in fruit crops and vineyards. Fruit crops are a major agriculture activity upstream R3 at the Têt River and vineyards are typical upstream R2. High levels of nitrites in this season confirm the origin of these contaminants in winter, at least for R1

and R2, what indicates heavy fertilization nearby these downstream stations. This origin is also confirmed by the absence of abnormal levels of fecal contamination upstream M1. Notice that, in winter, Zn is less important on R1 than on R2 and R3 upstream stations, but its EF is nevertheless still higher than the other three metals. Indeed, at R1, Zn is the metal with the highest EF in the three seasons, which traduces Zn base contamination level due to urban contamination and atmospheric deposition. Nickel does not vary much among stations (Figure 7.5-103d) indicating a non-anthropogenic origin of this metal in the Têt River in spite of an EF N 2. But this element might get concentrated at low water summer levels, explaining higher EFs during this season. High EFs values of Cd and Zn at R4 and R5 in summer and autumn can be attributed to both their higher than expected presence in mountain mother rocks compared to the minimal background values of downstream R1 used to calculate EFs (see Materials and methods section), and also to an atmospheric origin of these trace metals. In fact, as rainfalls are rather frequent in the mountain compare to the plain, atmospheric deposits may be washed more often at up-stream stations.

Figure 7.5-105: **(a) Contaminants dynamics as compared to hydrological variations of different compartments along the 2013 autumn flood of the Têt River. Each pollutant family studied is represented by one contaminant. Notice correction factors used to represent all contaminants together. (b) Rainfalls, volumes discharged by Sewer-Overflows (SO) and river flow along sampling time. Rainfalls are registered every hour while volumes discharged by the sewer-overflows (SO) are registered only every 24 h. Vertical blue line shows the major multicontamination phenomenon imputed to combined sewers overflows**



Pollutants sources in coastal rivers under a Mediterranean climate regime

A thorough study of multiple pollutants dynamics has allowed us to build a complete picture of pollution sources in Med rivers. These sources are largely dependent on two different seasonal periods that characterize Med climate: drought and rainfall. During drought, main sources of pollution are not exceptionally different from other climates, with leaching and comparatively small runoff from crops, farms and urbanized areas (B) contributing to most pollutants found in watercourses. In urbanized areas, leachates are collected into the sewer system (A' and B') and will end up in the WWTP. If not treated, pollutants from leachates and from residences and industrial wastewaters will eventually end up in the watercourse (C). Exceptionally, poorly dimensioned WWTP can contribute to, much greater, punctual pollutions during droughts (C) that can be easily detected via fecal indicators analyses in river water from urbanized areas. During rainy periods, storm events transform leaching into runoff, which brings pollutants to watercourses from all surfaces (a and b). But according to our results, runoff during storm events in Med climate regions produces significant multicontamination phenomena. These phenomena happen for two main reasons: (i) high flow peaks during floods and (ii) Combined Sewer Overflows (CSO) due to sudden and intense rainfalls. Floods will remobilize river sediment-stocked pollutants (d) while CSO will bring both stormwater and wastewater directly into the river (e) without passing through the WWTP, as well as flush sewer pipe sediment-stocked pollutants (d').

Water multicontamination phenomena risk management in Mediterranean climate regions

Water management is recognized as inevitably linked to land. Urbanization, agricultural intensification, afforestation and wetlands removal are reducing the permeability of natural soils along with removing natural catchment areas. As a consequence, drainage intensifies contributing to the increased risk of flooding, at least as much as climate change. Our results show that an exceptionally important multicontamination phenomenon occurs at downstream urbanized areas during intense rainfall events, but still at rather small river water flows compared to typical autumn and spring floods (Figure 7.5-105a). This multiple stressors event was mainly due to CSO, as is the case in other regions when rapid snowmelt or heavy rainfalls occur. Nevertheless, snowmelts and floods are annual or bi-annual events, whereas sudden and intense rainfalls happen many times per year under a Med climate regime, and are actually increasing in frequency due to climate change. Given so, tackling constantly recurring CSOs in Med regions is particularly urgent. One first measure for doing so is to modernize the combined sewer network by transforming it, little by little, into a separate sewer. This way, raw wastewater will be carried into the WWTP even when overflow of the separate stormwater sewer will occur. This solution is, however, very expensive and cannot have much effect on pollutants such as pesticides coming from runoff, which would still be released directly into the river in case of overflow. Nevertheless, as demonstrated upon our results, runoff will contribute to a significant part of the pollution coming into watercourses from CSOs. Therefore, a better solution to cope with this issue is to increase the sewer network capacity by building constructions to temporarily stock combined waters during intense rainfalls before sending them to the WWTP at a smaller pace. In Perpignan city, a storage tank of 13,000 m^3 capacity is operative since October 2015. Comparing chemical mixtures levels before and after these types of constructions will be very interesting as predicted by Llopart-Mascaró *et al*. (2014). Alternative more economic methods consist in tackling the drainage problem at its source, by arranging permeable surfaces and/ or wetlands in urban and peri-urban agricultural areas, which will not only limit runoff from intense rainfalls but also improve the water quality and offer a bigger biodiversity. For instance, in the urbanized areas surrounding coastal rivers, creating public gardens that can be used as storm basins, and settling planted ditches designed to provide hydrological benefits can help to cope with CSO issues. Taking peri-urban crops into account is also essential since natural permeable plots arranged in buffer strips around cultivated crops can limit runoff and take up nutrients and pesticides, thus decreasing storm events consequences from runoff.

Natural permeable plots also provide ecosystem services such as pollinating insects and pest control which would result in a smaller use of pesticides and fertilizers. Indeed, previous solutions cope with the multicontamination peak during heavy rainfalls but we have seen that chemical mixtures can also contribute to the degradation of the water quality along dry periods. They enter the watercourse via leaching and runoff and, in the Têt River, they are due to agriculture only, but industrial and cattle or poultry breeding could also be a source of diffuse pollution in other rivers. In this case, management strategies are going to be dependent on the human activities along the watercourse. The presence of nutrients in our study indicates nearby abuse of fertilizers, so they should be dosed according to agriculture parcels size. This way only the

necessary amounts for plant growing would be added to crops. In any case, solutions to reduce the use and abuse of pesticides would be advising farmers of good practices that take into account environmental toxicity effects of pesticides mixtures through indicators such as the EPRIP 2 (Environmental Potential Risk Indicator for Pesticide 2) or informing them of the benefits of alternative kinds of agriculture. Global management strategies are necessary to assess the environmental risk of chemical mixtures. Monitoring the surface water quality is one of the strategies implemented by federal and local governments for managing watercourses in developed countries. It consists in characterizing water quality not only through the analyses of several contaminants (nutrients, fecal indicators and sometimes also pesticides) as shown in our study, but also by using biomarkers in several river stations. Monitoring can then be complemented with environmental modelling for a better understanding of the managed system. In our case, we have chosen an alternative option, a carefully planned fieldwork study on multiple contaminants along a watercourse. This study can be considered as a step forward for currently undertaken measures for surface waters risk assessment. As a matter of fact, we are now capable of anticipating sources of chronic multiple stressors events in areas under a Med climate regime and, therefore, guiding sustainable management to deal with these sources. Indeed, contrarily to what is currently being implemented (Figure 7.5-103'), government monitoring protocols of Med surface waters should include samplings at pertinent stations during intense rainfalls and floods to better estimate chemical mixtures trends over time and evaluate if undertaken management measures are working. For instance, in order to determine if the storage tank built at Perpignan is useful to minimize CSO, an analysis of multiple contaminants will only make sense when intense sudden rainfalls occur. A next step would be to use environmental fate models in order to better predict long-term impacts of undergoing managed actions.

Conclusion

This study of several families of contaminants concentrations on a relatively well-studied river has allowed a fine understanding of chronic contamination sources, punctual and diffuse, in coastal Mediterranean water courses. We have corroborated that concentrations of pesticides, but also nutrients and fecal bacteria indicators, increased from upstream to down stream stations due to the increase of human activities, and that seasons played a role in these contaminants levels and their sources. Indeed, nutrients and trace metals are found concomitantly with pesticides during winter droughts at all downstream stations, indicating the diffuse origin from agriculture treatments of all these contaminants.

We demonstrated that the high concentrations of pesticides found by monitoring government studies at the most downstream station in summer are due to a punctual source of contamination, i.e. under-dimensioned WWTPs of upstream summer resorts, because immediately further up there are not such high levels of pesticides or even fecal indicators. On the other hand, we found that the highest concentrations of not only pesticides but also all other contaminants studied occur during strong rainfall events and we demonstrate they are mainly due to Combined Sewer Overflows (CSO) in urban areas. Given the current trend of intensification of extreme events, reduction of this chronic multi-contamination phenomenon should be a priority for risk management in Med climate regions worldwide. Solutions include better urban planning and land use as well as monitoring during intense rainfalls to carefully evaluate undertaken management measures. Since we worked on the results here presented, a new storage tank has been built at the city of Perpignan so a study to evaluate if this construction actually helps to improve river's water quality is underway. We also plan to follow the next storm events at high sampling frequency not only at R0 but also at R2 in order to discriminate the contribution of the sewer system upstream this station to CSO. Regarding the second multicontamination phenomenon, which is due to the flood highest flow peak, it would be interesting to model pollutants flow rate along the year. Indeed, during Med floods, these fluxes can represent up to 25 % of the annual total suspended solids. Finally, no studies have yet assessed the ecotoxicology of river water samples impacted by multiple stressors at environmental concentrations. We plan to test the toxicity of water samples from this study that have contrasted levels of chemical mixtures.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:
The article reports the contamination scheme of a Mediterranean river with different pollutant, among others glyphosate and AMPA. The considered approach identified that high concentrations peaks are caused by specific weather conditions, e.g. heavy rainfall after a dry period with consecutive overflow of WWTP, and other sources. The experiment does not focus explicitly on agricultural conditions. Maximum glyphosate and AMPA concentrations measured at 1.500 µg/L and 1.702 µg/L, respectively. The article is considered reliable with restrictions.

<u>Assessment and conclusion by RMS</u>:

1. Information on the study

Data point:	CA 7.5/044
Report author	Desmet, N. *et al.*
Report year	2016
Report title	A hybrid monitoring and modelling approach to assess the contribution of sources of glyphosate and AMPA in large river catchments
Document No	Science of the Total Environment 573 (2016) 1580–1588
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Large river catchments with mixed land use capture pesticides from many sources, and degradable pesticides are converted during downstream transport. Unravelling the contribution of pesticide source and the effect of degradation processes is a challenge in such areas. However, insight and understanding of the sources is important for targeted management, especially when water is abstracted from the river for drinking water production. The river Meuse is such a case. A long-term monitoring data set was applied in a modelling approach for assessing the contribution of waste water treatment plants (WWTPs) and tributaries (sub-basins) to surface water contamination, and to evaluate the effect of decay on the downstream concentrations of glyphosate and AMPA at the point of drinking water abstraction. The results show that WWTPs are important contributors for glyphosate and AMPA in large river catchments with mixed land uses. In the studied area, the river Meuse in the Netherlands, the relative contribution of WWTP effluents is above 29 % for glyphosate and around 12 % for AMPA. Local industries are found to be potentially big contributors of AMPA. Glyphosate entering the river system is gradually converted to AMPA and other degradation productions, which results in downstream loads that are considerably lower than the sum of all influxes. In summer when the travel time is longer due to lower discharge, the first order decay of glyphosate in the river Meuse is estimated to result in about 50 % reduction of the downstream glyphosate.

Materials & Methods

The case study in this paper is the river Meuse which is an important surface water source for drinking water production in the Netherlands. Drinking water standards for glyphosate are frequently exceeded in the river Meuse and high concentrations of the daughter product AMPA are also measured.

Glyphosate and AMPA
Glyphosate (*N*-phosphonomethylglycine) is a broad-spectrum, non-selective herbicide that controls most annual and perennial weeds by inhibiting the amino acid synthesis. Amino- methylphosphonic acid (AMPA) is the primary degradation product of glyphosate but it is also a degradation product of phosphonates which occur in domestic and industrial wastewaters. Phosphonates are used in detergent products and scaling inhibitors in hot water and cooling circuits. Minor applications include bleaching of paper/textile, stabilization of cement and cleaning/polishing of metals.

Study area and available monitoring data
The study area is the downstream part of the Meuse catchment where the river flows through Dutch territory. The Meuse catchment covers 36,000 km^2 and from source to mouth, the main river has a total length of N 900 km. The surface water from the river Meuse is used for drinking water production. The European drinking water standard for individual pesticides is 0.1 µg/L and the Dutch government imposes this limit for surface waters at points of drinking water abstraction. In the river Meuse the threshold of 0.1 µg/L is frequently exceeded for glyphosate and high concentrations of the daughter product AMPA are also measured. The investigated area is a 250 km river stretch between the Belgian- Dutch border (at Eijsden) and the point of drinking water intake "Biesbosch" (at Keizersveer). The land use in the Dutch part of the Meuse catchment is mixed and fragmented with 54–60 % agriculture, 11–34 % urban and 12–28 % nature/forest/water. Glyphosate can originate from both agricultural and urban use. The runoff from agricultural land is mainly a diffuse source of pollution. The runoff in urban areas is generally collected in the sewage system and reaches the river as a point source through a drain, or in the effluent of waste water treatment plants (WWTPs) or due to sewer overflow. The urban areas in the river Meuse basin are mostly clustered around city centers surrounded by densely populated neighborhoods and industrial zones. There are over 50 WWTPs in the Dutch part of the Meuse catchment that discharge their effluent into the surface water. Industrial effluents are the second important point source for pollution in the river Meuse. AMPA can originate from all of these sources, either as a degradation product of glyphosate or as a decay product of phosphonates which occur in domestic and industrial wastewaters.

Monitoring data on glyphosate and AMPA concentrations were obtained from RIWA-Maas (international association of drinking water companies that use the river Meuse as a source). Historical monthly or biweekly monitoring data of glyphosate and AMPA concentrations in the river Meuse were available for the period 1995–2011. The monitoring dataset includes the two bordering locations of the study area: Eijsden (upstream) and Keizersveer (downstream). Furthermore, an extended monitoring dataset was available for 2006, 2008 and 2010. This dataset also includes AMPA and glyphosate concentrations in the main tributaries and in the effluent of WWTPs discharging into the river Meuse along the 250 km stretch considered in this case study. During these extended monitoring campaigns samples were collected on a monthly basis. The tributaries were sampled at downstream locations near the confluence with the river Meuse and the WWTPs were samples at the outflow of the treatment plant (effluent). More details are given in the corresponding RIWA-reports.

Long term series of daily discharge data are available for the river Meuse in Eijsden (since 1950) and in Keizersveer (since 1994). Daily dis- charge data of the main tributaries are available for at least two years be- tween 2006 and 2010. For the WWTPs discharging into the river Meuse, daily effluent discharge data are available in the period 2006–2010, al- though for some WWTPs only one year of data is available. The discharge data were obtained from Rijkswaterstaat (Ministry of infrastructure and the environment of the Netherlands) and RIWA-Maas.

Modelling approach
The River Water Quality Model N°1 (RWQM1) was used to build a model for the downstream Dutch part of the river Meuse, starting at Eijsden (Dutch-Belgian border) and extending to the drinking water intake in Keizersveer. The total length of the modelled river stretch is about 250 km and the required information about geometry and roughness was derived from the hydraulic SOBEK-Maas model which encloses a large database of cross-section characteristics (about 460 sections for the considered stretch of the river Meuse).

The model includes the main course of the river Meuse between Eijsden and Keizersveer, the influx from seven main tributaries (Jeker, Geul, Geleenbeek, Roer, Neerbeek, Niers and Dieze), the influx from two smaller tributaries (Ur and Thornerbeek) and the influx from eight WWTPs discharging effluent into the river Meuse (Heugem, Limmer, Bosscherveld, Stein, Panheel, Roermond, Venlo and Cuijk). Transport of AMPA and glyphosate, as well as the conversion of glyphosate to AMPA and the degradation of AMPA (reaction product not defined and not quantified) are modelled. The conversion of glyphosate to AMPA is considered to be a first-order degradation process. Calculations are performed for a range of kinetic (degradation rate) parameter values. Half-life values (DT_{50}) for glyphosate in water reported in literature range from 1 day to 51 days. The stoichiometric maximum yield is 0.67 g AMPA per g glyphosate, but experimentally measured yields are often lower. Yield values reported for aerobic degradation in water-sediment studies range from 2 % to 16 % (water phase) and up to 27 % (total system) of the total glyphosate applied. The reported yield values are apparent yields calculated as the ratio of AMPA retrieved over glyphosate applied. However, the final amount of AMPA retrieved in the system is not only resulting from glyphosate degradation but is also affected by adsorption, dissipation and further degradation of AMPA it-self. These processes result in a decrease of the amount of AMPA retrieved and therefore the apparent yield generally is lower than the stoichiometric yield of 0.67 g AMPA per g of glyphosate. The stoichiometric yield ratio of 0.67 is applied in our model.

Map of the Meuse catchment on Dutch territory, showing the spatial distribution of land use. Study area of the model is the 250 km river stretch between the Belgian-Dutch border (Eijsden, grey diamond) and the downstream drinking water intake (Keizersveer, grey triangle).

The model only takes into account the water column. As a result, all glyphosate and AMPA that enters the river system is assumed to stay in the water column. Sorption and desorption on sediments is not taken into account. Given the gravelly nature of the sediment, this is considered to be a valid assumption. The river Meuse is characterised by a gravel-bed with bed material diameter D50 of about 16 mm in the upstream part of the study area and a transition towards a more sandy-bed downstream Roermond. Modelled dissipation routes are limited to dilution and degradation. In the model, AMPA is assumed to be the only degradation product of glyphosate. Although AMPA is the major daughter product of glyphosate, other degradation products are known to exist as well, but these are not taken into account here. The model only considers degradation of glyphosate to AMPA and degradation of AMPA without further specification of degradation products.

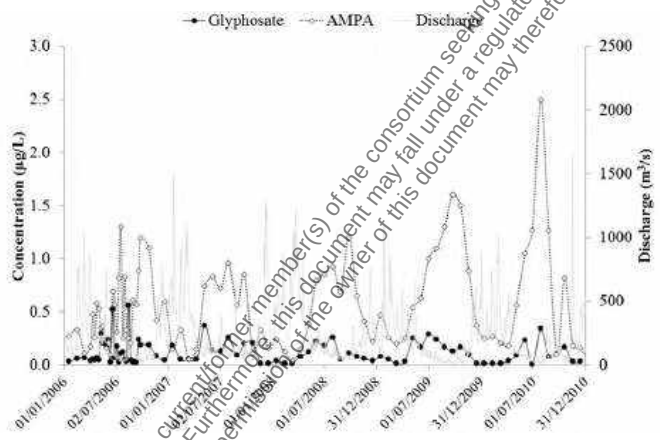
Model input data, boundary conditions and model output
The model requires discharge and concentration data at the boundaries, i.e. the upstream boundary at Eijsden, the tributary boundaries, and WWTPs effluents. These boundary conditions are defined based on available discharge (daily) and concentration data (biweekly or monthly) for the river, its main tributaries and WWTPs discharging into the river. The data were obtained from RIWA-Maas and Rijkswaterstaat.

Starting from 2006, daily discharge data and monthly or biweekly concentration data are available for the upstream boundary and for the main tributaries. The daily discharge data are directly used as input for the model. The concentration monitoring data, on the other hand, are used to generate, at each boundary, a single set of representative concentration levels (glyphosate and AMPA) based on the monthly mean concentration obtained for the available dataset with measured concentrations in the period 1995–2011. This dataset contained 15–22 observations per calendar month at the upstream boundary and 18– 29 observations per calendar month at the downstream drinking water intake. There are less observations in winter months than in spring and summer months due to lower frequency of monitoring and focus on the application period in the monitoring before 2002 (i.e. lower frequency monitoring in winter). The monthly mean value (based on the available monthly and biweekly data) is assigned to the 15th day of the month

and in-between the concentration level is interpolated to generate a set of daily concentration values. This represents the average pattern of concentration time series throughout a year.

For some tributaries and WWTPs the available dataset lacks measurements in one or two months (in winter). The missing monthly concentration level in tributaries is estimated taking into account the relative seasonal variation of the concentrations in the river. While for WWTPs the missing effluent concentration of a missing month is assumed to be equal to the concentration level in the previous month because WWTPs with concentrations measurements for each month do not indicate any clear seasonal or other pattern in the effluent concentrations. There were no more than two successive months with missing data in the WWTP effluent data series. Simulated concentrations are compared with measured values at the drinking water intake. At the downstream end of the modelled river stretch (drinking water intake at Keizersveer), the monitoring data are resampled and represented in the same way as described above for the boundary input data. Model results are compared with this generated set of representative concentration levels based on the average concentration measured in a particular month over several years.

Figure 7.5-106: Glyphosate and AMPA concentrations and discharge measured in the river Meuse near the upstream boundary at Eijsden



Results & Discussion

Measured concentrations in the river Meuse

Figure 7.5-106 and Figure 7.5-107 show the measured time series of river water AMPA and glyphosate concentrations for the period 2006–2010 at the upstream boundary and at the drinking water intake, respectively. In that period, maximum glyphosate concentrations were 0.7 µg/L at the upstream border and 0.3 µg/L at the downstream drinking water abstraction. Maximum AMPA concentrations were 2.5 µg/L at the upstream border and 3 µg/L at the downstream drinking water abstraction. In general, higher concentrations of both glyphosate and AMPA were measured at low discharge of the river (summer) and lower concentrations were measured at high discharge (winter). Plotting concentrations versus discharge showed an inverse relation between AMPA concentration and river discharge, although correlation coefficients were rather small ($R^2 = 0.64$ at the upstream boundary and $R^2 = 0.46$ at the down- stream water intake). For glyphosate, however, the relation with dis- charge was less pronounced with considerably lower correlation coefficients ($R^2 = 0.19$ at the upstream boundary and $R^2 = 0.11$ at the downstream water intake). The better correlation of AMPA concentrations with discharge points to a more constant load of AMPA and thus more constant source influxes. The differences between glyphosate and AMPA in concentration pattern and correlation with discharge, indicates that different sources are involved. The temporal variation of glyphosate and AMPA shows a similar pattern at first glance that seems to be related to seasonal dynamics. There is large seasonal variation of the discharge in the river Meuse. If the load is constant, the concentrations will be inversely related to the river discharge when dilution is the main cause of temporal variation. The correlations analysis be- tween concentration and discharge, shows that for AMPA dilution is a likely possible reason for the observed temporal variation with high concentrations in summer (at low

discharge) and low concentrations in winter (at high discharge). For glyphosate, however, the seasonal variation of the concentrations has poor correlation with the discharge. The likely possible reason for the observed temporal dynamics in glyphosate concentrations is the seasonal pattern of the use of glyphosate as pesticide. The application period ranges from March till October. The load increases when more glyphosate is applied and therefore also the concentrations increase.

At the upstream boundary the median glyphosate concentration was 0.08 µg/L and the 90th percentile value reached 0.27 µg/L (monitoring dataset 1995–2011). The median AMPA concentration was 0.50 µg/L (monitoring dataset 1995–2011). About 45 % of the glyphosate concentrations exceeded the drinking water standard of 0.1 µg/L and 21 % of the concentrations exceeded the standard by at least a factor of 2. About 20 % of the AMPA concentrations exceeded the threshold of 1 µg/L. At the drinking water intake, the median glyphosate concentration was 0.07 µg/L and the 90th percentile value was 0.15 µg/L. The median AMPA concentration was 1.10 µg/L. About 32 % of the glyphosate concentrations exceeded the drinking water standard of 0.1 µg/L, but only 2 % of the concentrations doubled the standard. About 52 % of the AMPA concentrations exceeded the threshold of 1 µg/L and 5 % exceeded the level of 2 µg/L. From the concentration ranges and the time series (Figure 7.5-106 and Figure 7.5-107) it is clear that glyphosate concentrations in the river decreased along the 250 km river stretch between the upstream boundary and the drinking water intake. This is contrary to the AMPA concentrations which show considerable increase along the trajectory. Due to the increase of AMPA concentrations and the decrease of glyphosate concentrations along the river stretch the ratio of AMPA to glyphosate increases accordingly. At the upstream boundary, the median ratio is 5.9 while at the drinking water intake the median ratio is 12.7.

Measured concentrations in tributaries and WWTP effluents
In several tributaries concentrations were higher than in the river Meuse. Highest concentrations of glyphosate were found in the tributary Jeker and in WWTP effluents (highest concentrations measured at WWTPs Panheel and Roermond). The highest concentrations of AMPA were found in the tributaries Ur and Geleenbeek and in the WWTP effluents (with the highest concentrations measured at WWTPs Gennep and Panheel). Extremely high AMPA concentrations were found in the river Ur (average 28 µg/L, maximum 130 µg/L), while glyphosate concentrations were only moderate (average 0.7 µg/L, maximum 3.8 µg/L). This small tributary is mainly discharging effluent from an industrial waste water treatment plant. In 2005 and 2006, RIZA (Rijkswaterstaat, department of water) measured very high AMPA concentrations (up to 69 µg/L) in this effluent and concluded that this originated from the application of zinc phosphonates in the industrial cooling circuit. In 2008 and 2010, RIWA included the tributary Ur in its monitoring campaign for the river Meuse. In 2010 very high concentrations of AMPA (up to 130 µg/L) were found in the Ur but in 2008 the highest concentration measured in the Ur was only 4.1 µg/L.

Figure 7.5-107: Glyphosate and AMPA concentrations and discharge measured in the river Meuse near the drinking water intake at Keizersveer

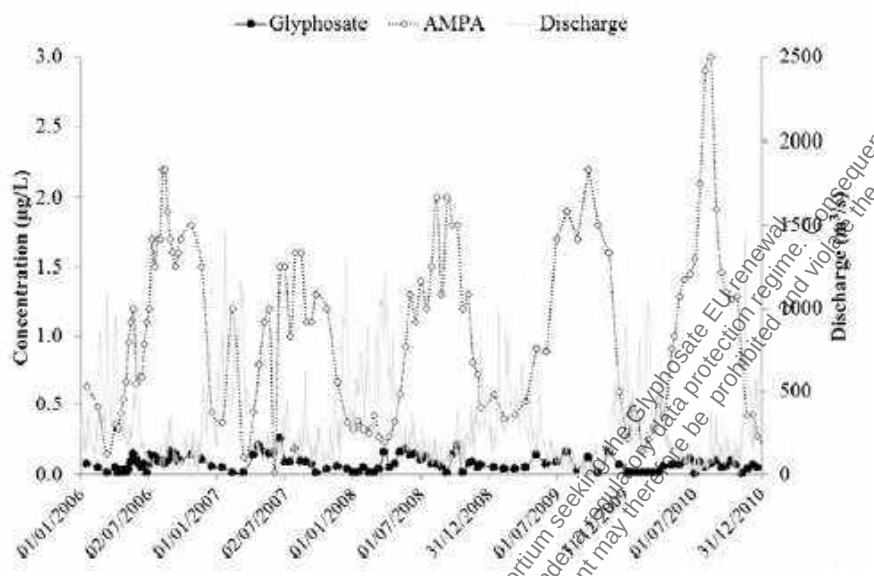


Table 7.5-141: Percentile values for the AMPA/Glyphosate ratio at the upstream boundary (Eijsden) and at the downstream drinking water intake (Keizersveer), based on measurements in the period 2006–2010

	AMPA/glyphosate ratio				
	P10	P25	P50	P75	P90
Upstream	1.67	3.52	5.93	9.33	16.38
Intake (downstream)	0.57	9.00	12.73	20.77	34.18

Table 7.5-142: Range of glyphosate and AMPA concentrations measured in the period 2006–2010 in the river Meuse (upstream at Eijsden and downstream at Keizersveer), in the main tributaries and in the effluent of WWTPs discharging into the river Meuse

	Glyphosate (µg/L)			AMPA (µg/L)		
	Mean	Min.	Max.	Mean	Min.	Max.
Meuse - upstream	0.13	<0.02	0.66	0.62	0.04	2.50
Meuse - downstream	0.08	<0.02	0.26	1.06	0.02	3.00
Jeker	2.18	0.26	12.00	1.62	0.05	4.50
Geul	0.41	<0.05	3.20	0.98	0.05	3.10
Ur	0.67	<0.05	3.80	27.79	0.20	130.00
Geleenbeek	1.33	0.05	4.60	3.14	0.05	10.00
Thornerbeek	0.46	<0.05	2.23	2.38	0.70	6.20
Roer	0.10	<0.05	0.80	0.56	0.05	1.50
Neerbeek	0.06	<0.05	0.20	0.37	0.05	0.98
Niers	0.25	<0.05	4.80	1.61	0.20	4.80
Dieze	0.23	<0.03	0.71	1.45	0.23	4.30
WWTPs	1.70	<0.05	6.63	3.06	0.25	7.06

Figure 7.5-108: Measured and simulated discharge in the river Meuse at the downstream drinking water intake

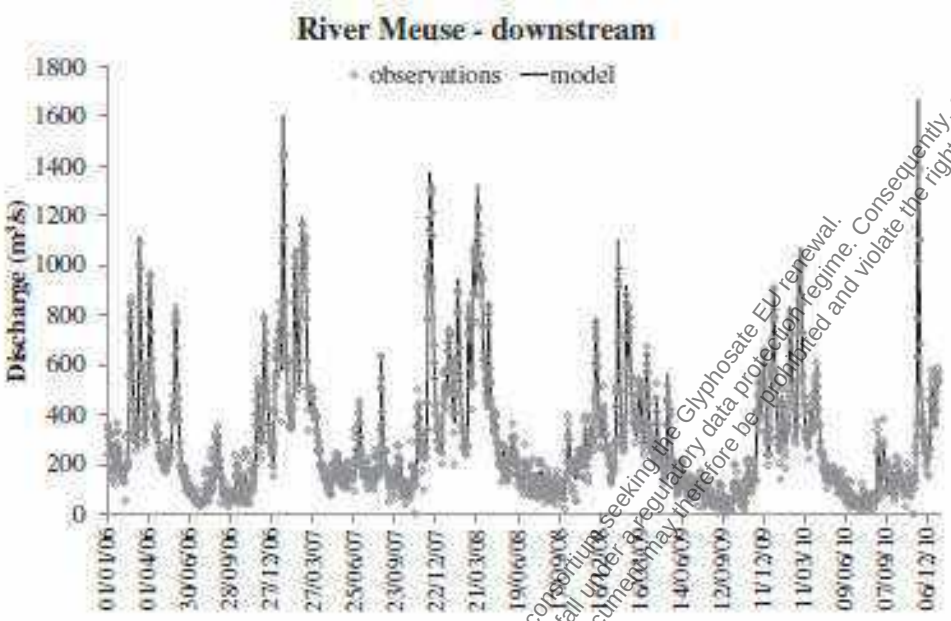
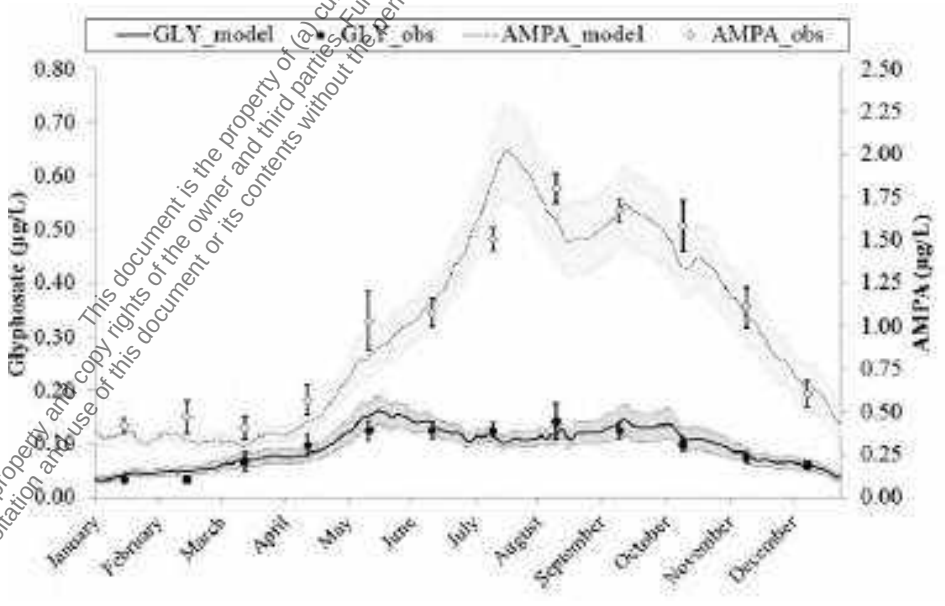


Figure 7.5-109: Simulated and measured monthly mean concentrations of AMPA and glyphosate at the drinking water intake for an average year based on data from 1995 to 2011. Best fit obtained using the degradation parameter settings: glyphosate DT_{50} = 3.6 d, AMPA DT_{50} = 52.5 d. The shaded area represents the model uncertainty



Hydraulics

Observed and simulated daily-averaged discharges in the river at the downstream point of the modelled river stretch are presented in Figure 7.5-108. The hydraulic routing of the Meuse river is very well reproduced by the model (R^2 = 0.94, NRMSE = 4 %, CV(RMSE) = 21 %). Up to discharges of 1000 m^3/s the river hydraulics are very well simulated. At peak discharges, the model sometimes overestimates and sometimes underestimates the maximum level, but it is not a systematic deviation. The increase in deviation between observed and simulated values at higher discharges is partially related to the temporal resolution of the flow input data. More accurate simulation of peak discharges requires sub daily input data. However, daily averaged values are sufficient for this study because the focus of the analysis is not on peak flow events.

Degradation of glyphosate to AMPA

The key parameter for the degradation process is the substance half- life value (DT_{50}). In order to estimate glyphosate half-life time and AMPA half-life time in the modelled part of the river Meuse, DT_{50} parameter values were varied within a given range (Table 7.5-144) and the model results were compared with the glyphosate and AMPA concentrations measured at the drinking water intake (Figure 7.5-109). The root mean squared error (RMSE) and sum of squared errors (SSE) were used as calibration statistics. Best fit results were obtained with glyphosate half- life time of 3.6 days and AMPA half-life time of 52.5 days (Figure 7.5-109, R^2 = 0.86). With these parameter settings both AMPA and glyphosate concentrations in the Meuse at the drinking water intake were well simulated by the model. The resulting correlation between observed and simulated values was R^2 = 0.92 for AMPA and R^2 = 0.86 for glyphosate. Since only the water phase is considered in the current modelling approach, the simulated degradation may in reality partly reflect dissipation to the sediment (eventually followed by degradation in the sediment). Water-sediment studies show that dissipation to the sediment can be an important pathway for glyphosate and AMPA losses from the water column. However, water to sediment ratios applied in these studies are quite low and merely representative for ditches and small water courses. In our case water to sediment ratios are generally much higher and therefore it can be assumed that dissipation to the sediment will be more limited compared to the aforementioned water-sediment studies.

In the current model AMPA was considered to be the only degradation product of glyphosate and the stoichiometric yield of 0.67 g AMPA per g glyphosate was applied. However, the apparent yield was b 0.67 g/g, because degradation of AMPA was also considered in the model. The apparent yield is the actual amount of AMPA originating from glyphosate measured or simulated divided by the amount of glyphosate de- graded. The apparent yield is less than the stoichiometric yield when the daughter product is further degraded. Due to further degradation of AMPA, the apparent yield of AMPA over glyphosate varies with the residence time in the river system. The smaller the flow velocity in the river, the larger the residence time, and the more degradation of AMPA occurs. So, the apparent yield of AMPA from glyphosate degradation was lower at low discharges in the river. At low discharges (in summer), the calculated hydraulic residence time between the upstream boundary and the drinking water intake is about 10 days. Using the best fit degradation parameter estimates (glyphosate DT_{50} = 3.6 d, AMPA DT_{50} = 52.5 d) the corresponding apparent yield of AMPA was about 0.58 g per g glyphosate.

The simulated apparent yield of AMPA in the river at the drinking water intake was higher than the apparent yield reported in several water-sediment studies, ranging between 0.05 and 0.27. However, the duration of these water-sediment studies is about 100 days or more. Applying the degradation parameters of the best fit model (glyphosate DT_{50} = 3.6 d, AMPA DT_{50} = 52.5 d) for a residence time of 100 days resulted in an apparent yield of about 0.18 g AMPA per g glyphosate, which is comparable to the yield observed in the water-sediment studies.

Contribution of sources

In order to distinguish the contribution of glyphosate degradation to the AMPA concentrations in the river, the calibrated model results (glyphosate DT_{50} = 3.6 d, AMPA DT_{50} = 52.5 d) were evaluated against the results of a reference run without glyphosate and AMPA degradation. The percentage of AMPA originating from glyphosate degradation is calculated as the relative difference between simulated AMPA concentrations with (best fit run) and without degradation (reference run). The effect of glyphosate decay on the downstream glyphosate and AMPA concentrations in the river varies with discharge because discharge affects the residence time of the water and thus the time available for degradation to occur. The

conversion of glyphosate to AMPA and other degradation products during transport, results in a reduction of the glyphosate concentrations. The half-life for glyphosate in the river Meuse is estimated at 3.6 days. Since the travel time over the 250 km river stretch can reach up to 10 days, the effect of decay on glyphosate concentrations can be considerable. In summer when the travel time is longer due to lower discharge, the first order decay of glyphosate in the river Meuse is estimated to result in about 50 % reduction of the downstream glyphosate concentrations (Figure 7.5-109 versus Figure 7.5-110).

According to our model results, the contribution of glyphosate decay to the observed AMPA concentrations at the drinking water intake ranged between 2 % and 10 %, and was highest in summer, at low discharge (Figure 7.5-111). In absolute concentration levels glyphosate degradation resulted in an average increase of the AMPA concentrations by 0.06 µg/L. At high discharge and limited residence time, the concentration increase due to glyphosate decay was b 0.01 µg/L. At low discharge, however, the contribution of glyphosate to AMPA concentration levels increased up to 0.15 µg/L. The percentage of AMPA originating from glyphosate is maximal in spring and does not coincide with the highest concentration levels in the river which are observed in summer.

In order to estimate the contribution of AMPA and glyphosate inflow from tributaries and WWTPs, scenario runs were performed with the calibrated model including and excluding each of the tributaries and the WWTPs. The scenario results are used to calculate the contribution of the tributaries and the WWTPs to the load of AMPA and glyphosate at the drinking water intake. In our approach only WWTPs discharging directly into the river Meuse were regarded as contributions from WWTP effluents because WWTPs discharging into a tributary of the Meuse were accounted for in the contribution of the tributary. As described before, note that the tributary Ur is mainly discharging effluent from an industrial waste water treatment plant (Volz, 2009). The relative contribution of upstream influx, tributaries and WWTP effluents to the concentrations in the river at the drinking water intake is shown in Figure 7.5-112 for glyphosate and in Figure 7.5-113 for AMPA, and is summarized in Table 7.5-143. The model scenario analysis shows that influx at the upstream border had a contribution of 56 % in the load of both AMPA and glyphosate. The WWTPs accounted for 12.6 % of the glyphosate load and 5.3 % of the AMPA load. Considering only the influxes on Dutch territory (so excluding the upstream influx at the upstream border) the relative contribution of WWTP effluents was 29 % for glyphosate and 12 % for AMPA. This includes only the WWTPs that discharged directly into the river. The tributary influxes of glyphosate and AMPA also originated partly from WWTP effluents that are discharged upstream on the tributaries. So the total contribution of WWTPs is expected to be larger. Several studies already pointed out the importance of WWTPs as a source of glyphosate and AMPA inputs to surface water. However, assessments quantifying the contribution of the WWTP effluent loads are rarely made. For a small catchment (25 km^2) with mixed land use in Switzerland, the contribution of glyphosate originating from urban areas to the load during selected rain events was estimated at N 50 % based on targeted monitoring of stream surface water, urban drainage water and WWTP effluents. Blanchoud *et al.* (2007) estimated the urban contribution to pesticides (among which glyphosate) in the Marne catchment (12,762 km^2) at about 50 %.

Based on the average concentration levels measured in the Ur (which are used as input for the model), this tributary accounted for 12 % of the AMPA load. But, one should note that the concentrations measured in the river Ur vary over a large range (see Table 7.5-142). So the contribution of the Ur to the AMPA load is probably quite variable in time and depends on the concentrations in the industrial effluent. The influxes from the tributary Dieze accounted for 10 % of both AMPA and glyphosate loads at the drinking water intake. The influxes from the tributary Jeker contributed considerably more to the glyphosate load (7 %) than to the AMPA load (2 %). The monthly variation in the relative contribution of upstream influx, WWTP effluents, tributary Ur (discharging mainly industrial effluent) and other tributaries to the concentrations in the river at the drinking water intake is shown in Figure 7.5-112 for glyphosate and in Figure 7.5-113 for AMPA. The model scenario results also indicate that the relative contribution of different sources is quite variable throughout the year. The seasonal variation is larger for glyphosate than for AMPA. The contribution of AMPA influx at the upstream border ranged from 38 % to 68 %, while the contribution of glyphosate upstream influx ranged from 25 % to 75 %. The WWTP effluents accounted for 3 % to 9 % of the AMPA load and 13 % to 21 % of the glyphosate load. For both glyphosate and AMPA, the highest contributions from WWTPs occurred in summer. Main WWTP contributions for glyphosate occur from May until October. Main WWTP contributions for AMPA occur from June until September.

Figure 7.5-110: **Simulated and measured monthly mean concentrations of AMPA and glyphosate in the river Meuse at the drinking water intake for an average year based on data from 1995 to 2011. Reference run: without any degradation of glyphosate and AMPA. The shaded area represents the model uncertainty**

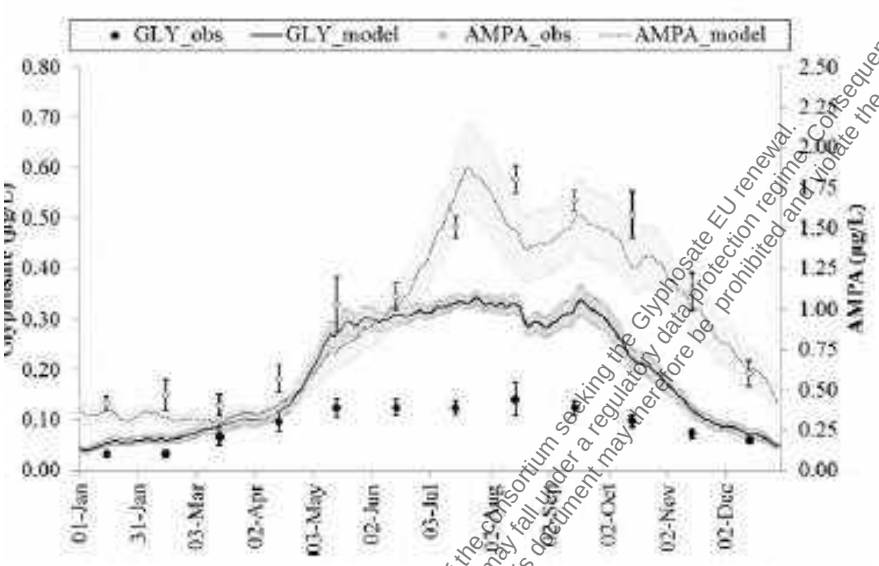


Figure 7.5-111: **Simulated relative contribution of glyphosate degradation to AMPA concentrations in the river at the drinking water intake. AMPA concentrations and river discharges**

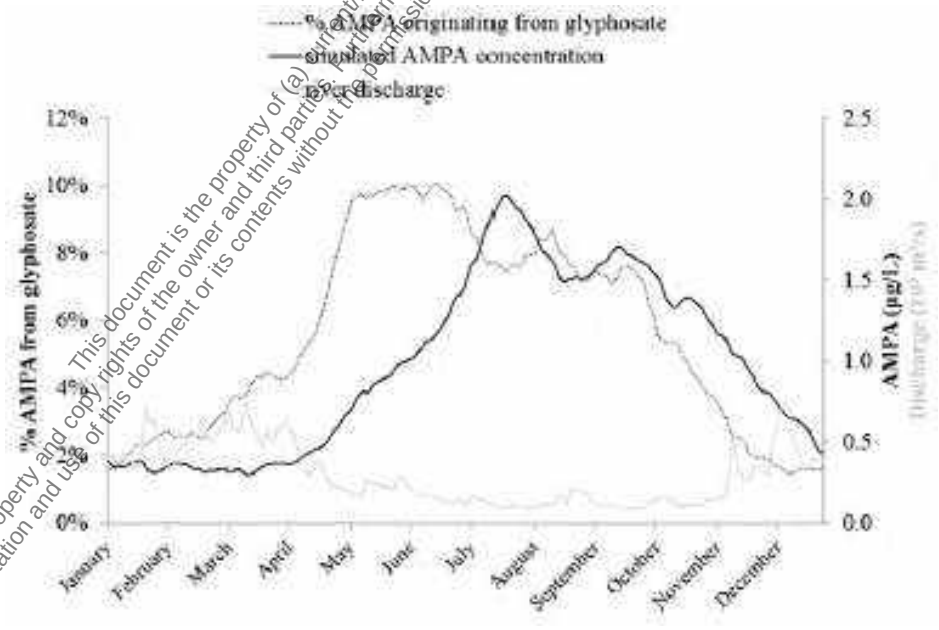


Figure 7.5-112: Monthly variation in the relative contribution of upstream influx, WWTP effluents, tributary Ur (discharging mainly industrial effluent) and other tributaries to the glyphosate concentrations in the river Meuse at the drinking water intake

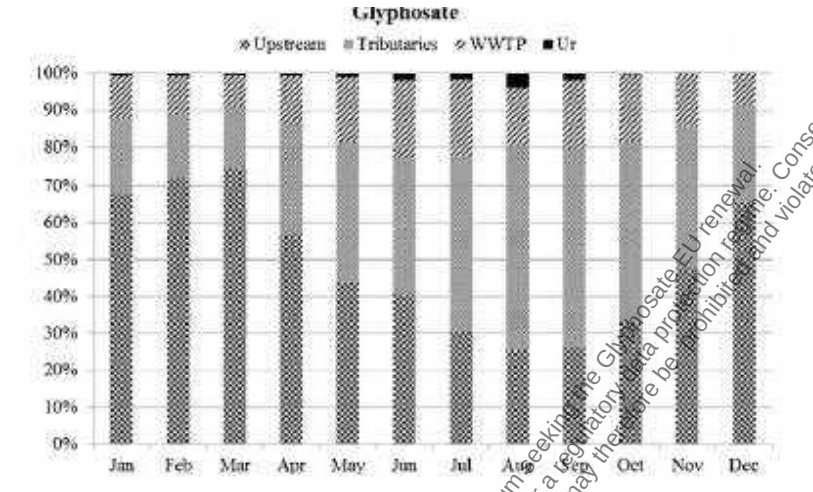


Figure 7.5-113: Monthly variation in the relative contribution of upstream influx, WWTP effluents, tributary Ur (discharging mainly industrial effluent) and other tributaries to the AMPA concentrations in the river Meuse at the drinking water intake

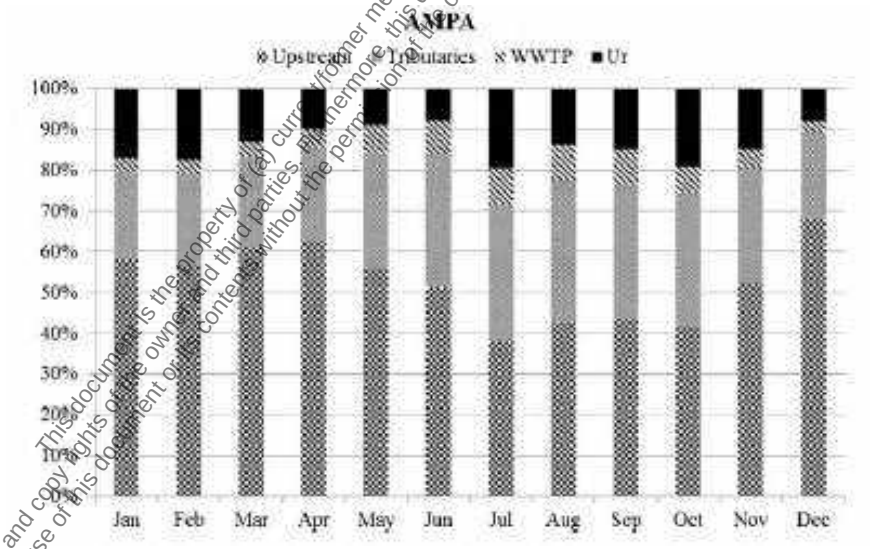


Table 7.5-143: Statistics on the contribution of upstream influx, tributaries and WWTP effluents to the discharge of the river at the drinking water intake

		River at upstream boundary	Sum of tributaries	Sum of WWTPs
Average		78.6%	20.0%	0.8%
Percentiles	P10	52.1%	41.8%	2.0%
	P50	71.8%	24.2%	0.9%
	P90	85.1%	15.7%	0.6%

Table 7.5-144: Range of DT50 values (min-max) used for calibration of the glyphosate and AMPA degradation rate and obtained best fit values

	Half-life time, DT_{50} (d)			Degradation rate, k (1/d)		
	Min	Max	Best fit	Min	Max	Best fit
Glyphosate	1	105	3.6	0.0066	0.6931	0.1925
AMPA	7	105	52.5	0.0066	0.0990	0.0132

Conclusion

Our results show that the application of a river model facilitates the assessment of pesticide loads and source contributions in dynamic downstream areas of a river catchment based on low-frequency (monthly) concentration data and high-frequency (daily) hydraulic data. The variability of pesticide concentrations and discharge in the study area impedes such assessments based on monitoring data soley. Our study illustrates how to overcome the limitations of low-frequency pesticide concentration data by means of modelling. The results further indicate that the effect of local measures to reduce the exposure concentration at the point of drinking water abstraction, is limited by dominant transboundary loads. In order to apply the model for decision making on pesticide use at specified locations, a dynamic coupling to detailed landscape information (urban areas, agriculture, land use, soil type, etc.) is needed. The application of a model- ling approach as proposed in this study in river management and decision making requires modelling expertise and sufficient information of the river system to develop an adequate water quality model. The results obtained for the modelling approach can be used as such in management to target measures sources with the largest load contribution. In the future the modelling approach can be re-used to assess the effect of taken measures on the loads and concentrations based on additional simulations updated with the recent monitoring data.

In large river basins, insight in the spatial distribution of pesticide in- fluxes along the main river course is important for policy makers in prioritizing certain areas for specific management actions. Local reduction programmes clearly affecting local concentrations might fail to show the expected impact on the larger scale due to fluxes coming from other (transnational) sub basins, hydrological variations, limited spatial and temporal resolution of monitoring data, and other larger scale is- sues. Recommendations to improve river basin management are targeted monitoring in sub basins and at the outlets of waste water treatment plants (WWTPs) and modelling the whole catchment to distinguish between sources and to derive cost-effective programme of measures. The model scenario results also indicated that the relative contribution of different sources varies throughout the year. The seasonal variation is larger for glyphosate than for AMPA.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports a hybrid monitoring and modelling approach to evaluate different sources of glyphosate and AMPA in the Meuse River in the Netherlands and their decay in the waterbody. Waste-water treatment plants and tributaries were considered as entry routes of the substances. The experiment does not consider or model explicitly the contribution of agricultural application of the substances. The measured maximum concentrations of glyphosate in the river Meuse was 0.7 µg/L and in its tributaries was 12 µg/L. Also, the measured maximum concentrations of AMPA in the river Meuse was 3 µg/L and in its tributaries was 130 µg/L.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/045
Report author	Larsbo, M. *et al*.
Report year	2016
Report title	Surface Runoff of Pesticides from a Clay Loam Field in Sweden
Document No	Journal of Environmental Quality 45:1367-1374
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Pesticides stored at or close to the soil surface after field application can be mobilized and transported off the field when surface runoff occurs. The objective of our study was to quantify the potential pesticide losses in surface runoff from a conventionally managed agricultural field in a Swedish climate. This was achieved by measuring surface runoff volumes and concentrations in runoff of six spring-applied pesticides and autumn-applied glyphosate and its metabolite aminomethylphosphonic acid (AMPA). Measurements were performed for 3 yr both during the growing seasons and during intervening winter snowmelt periods on a clay loam field close to Uppsala. During growing seasons, surface runoff was generated on only five occasions during one 25-d period in 2012 when the infiltration capacity of the soil may have been reduced by structural degradation due to large cumulative rainfall amounts after harrowing. Concentrations in surface runoff exceeded Swedish water quality standards in all samples during this growing season for diflufenican and pirimicarb. Surface runoff was generated during three snowmelt periods during the winter of 2012-2013. All of the applied pesticides were found in snowmelt samples despite incorporation of residues by autumn plowing, degradation, and leaching into the soil profile during the period between spraying and sampling. Concentrations of glyphosate ranged from 0.12 to 7.4 µg/L, and concentrations of AMPA ranged from 0 to 2.7 µg/L. Our results indicate that temporal changes in hydraulic properties during the growing season and when the soil freezes during winter affect pesticide losses through surface runoff.

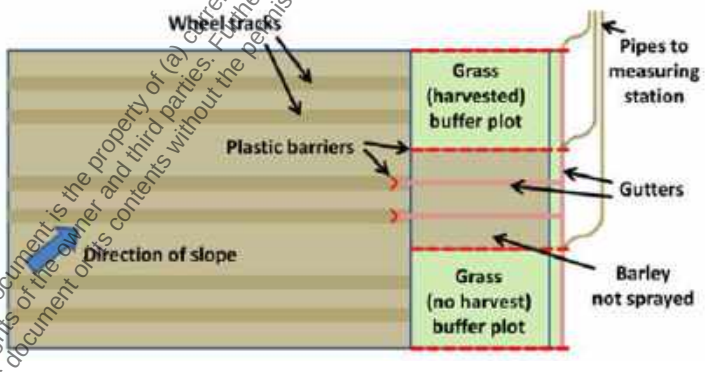
Materials and Methods

Site Description and Experimental Set-up

The field site is located close to Alsike church about 15 km south of Uppsala in eastern Sweden. The experimental field is about 0.42 ha (72 × 50 m), with a slope in the north-south direction of about 1 %. The soil is a clay loam (32.3 % clay, 33.1 % silt, 34. % sand) and has an organic carbon content of 13 g/kg. The field was conventionally managed (i.e., autumn plowed to a depth of about 20 cm, harrowed to a depth of about 6 cm before sowing) and sown with spring barley during the years when measurements were made (2012-2014). Before the start of the project during the years 2007 to 2010, the field was under ley, and no pesticides were applied. In autumn 2010, glyphosate (1440 g/ha) was applied to the field, and the field was plowed. Oat was sown and treated with 2-methyl-4-chloro-phenoxyacetic acid (MCPA) (500 g/ha) and tribenuron methyl (5.6 g/ha) in spring 2011. In 2010, a 6-m-wide grassed buffer strip was established along the south side of the field. The buffer strip was divided into four blocks, each containing three plots (6 by 6 m). The plots within a block were randomly assigned one of three treatments: permanent grass with no harvest, permanent grass harvested once a year, or no buffer zone (i.e., the plot was sown with the same crop as the rest of the field). Each buffer zone plot is drained with a central 6-m long drain pipe at 1 m depth. The rest of the field is not drained.

Surface runoff was collected in an open permanent gutter at the bottom edge of each plot. During the growing season, surface runoff was only monitored from the plots sown with barley (i.e., with no grassed buffer zone) because preliminary experiments with a rainfall simulator showed that the infiltration capacity of the grassed plots was so large that it would be highly unlikely that any surface runoff would pass across the plots without infiltrating. In the plots sown with barley, temporary collection gutters were installed after spraying, which led the water directly from wheel tracks that were created during pesticide spraying to the permanent gutters (Figure 7.5-114). Surface runoff was monitored from all 12 plots during winter and spring snowmelt periods because the infiltration capacity at such times is limited by frozen soil.

Figure 7.5-114: Schematic illustration of one block with gutters collecting water from the wheel tracks during the growing season



Gutters were open and, hence, could collect rain falling directly on them. To calculate surface runoff collected from the wheel tracks in summer, this volume was subtracted from the total collected volume. The volume that fell directly on the gutters was estimated by the average volume collected from plots without temporary collection gutters. We assumed that surface runoff during winter and spring was dominated by snowmelt, so no corrections were made for precipitation falling directly on the gutters.

The surface runoff water was led to an automated measuring station where water volumes were measured using a tipping bucket system. Flow proportional subsamples were taken every 2 L (0.006 mm) of surface runoff. Pesticide concentrations (see below) were measured in bulk samples collected on an ad hoc basis after periods with surface runoff. Relative losses of pesticides from each of the four plots monitored during growing seasons and 12 plots during snowmelt were estimated by dividing the pesticide mass in surface runoff collected from a plot by the mass applied to one twelfth of the field area (0.035 ha). Pesticide losses

from the field through surface runoff not captured by the collection gutters may have occurred because the direction of the slope of the field was not perpendicular to the gutters (Figure 7.5-114). During the growing season, wheel tracks directed surface runoff toward the collection gutters.

Precipitation was measured automatically at an hourly resolution at the site using a professional rain gauge (MJK automation AB). The rain gauge was not heated, which means that the amount of precipitation falling as snow was uncertain because it was only registered on melting. Therefore, for winter seasons we used daily precipitation data from Ultuna climate station located about 7 km north of the field site. Measurements of air temperature were also taken from Ultuna. The whole field except the buffer zone was sprayed each year in spring during 2012-2014 with the herbicides MCPA, clopyralid, fluroxypyr, and diflufenican; the fungicide prothioconazole; and the insecticide pirimicarb. In the autumns of 2012 and 2013, the field was sprayed with the herbicide glyphosate. All applications were performed at doses commonly used in Sweden using commercial products. Spring applications of pesticides were done perpendicular to the buffer zone starting outside each plot (Figure 7.5-114), whereas glyphosate applications were done parallel to the buffer zone. The permanent gutters were cleaned after spraying to remove any pesticide contamination caused by spray drift. Because degradation of prothioconazole is very fast (<1 d), the major metabolite formed in soil, prothioconazoledesthio (maximum formation in soil 49.4 %) (PPDB, 2016), was analyzed instead of the parent compound. The major metabolite of glyphosate, aminomethylphosphonic acid (AMPA) (maximum formation in soil, 29.0 %) (PPDB, 2016), which is more persistent in soil than the parent compound, was also analyzed. In addition to the applied pesticides and two metabolites, the fungicide carbendazim, which was not applied at the site during the experiment, was also detected in most surface runoff samples.

Analytical Methods

Spring-applied pesticides were analyzed using an automated on-line, solid-phase, extraction-liquid chromatography- tandem mass spectrometry procedure as described by Jansson and Kreuger (2010). About 95 pesticides are simultaneously measured with this method, which is why pesticides that were not applied during the course of the experiment, such as carbendazim, could be detected. Before analysis the samples were spiked with internal standard followed by filtration through a 0.2-μm regenerated cellulose filter. Limits of detection were in the 0.001 to 0.010 μg/L range, and limits of quantification were in the 0.002 to 0.050 μg/L range. Glyphosate and AMPA were analyzed in aqueous phase and bound to particles because both forms are known to contribute to leaching. The method used to separate the two phases is described in detail in Ulén *et al*. (2012). Limits of detection and limits of quantification for the aqueous phase were 0.010 and 0.025 μg/L, respectively, for glyphosate and 0.020 and 0.050 μg/L, respectively, for AMPA. Limits of detection and limits of quantification for the particle-bound fraction were and 0.035 and 0.050 μg/L, respectively, for glyphosate and 0.050 and 0.10 μg/L, respectively, for AMPA.

Infiltrometer Measurements

Four to five replicate tension infiltrometer measurements were performed on one, four, and three occasions during the growing seasons of 2012, 2013, and 2014, respectively. Measurements were done on uncompacted soil and, when present, in wheel tracks. We used two identical tension infiltrometers with 20-cm-diameter infiltration discs. A layer of fine sand was first placed on the soil surface to ensure good contact between the soil and the porous disc. Measurements were performed in a sequence from low to high pressure potentials at -6, -4.5, -2, and -1 cm in 2012 and at -6, -3, and -1 cm in 2013 and 2014. Near-saturated hydraulic conductivities were calculated from steady-state infiltration rates using the approach outlined in Ankeny *et al*. (1991). The hydraulic conductivities at -1 cm pressure potential give a good estimate of the saturated hydraulic conductivity providing there are no vertically oriented continuous pores with a diameter larger than 3 mm.

Statistics

Effects of the different buffer zone treatments on surface runoff volumes, pesticide concentrations, and losses during spring snowmelt periods when all 12 plots were used were analyzed accounting for the randomized block structure of the experimental field with the ANOVA tool implemented in CoStat. Differences between mean pesticide concentrations and losses between sampling events and between substances as well as differences in near-saturated hydraulic conductivities between measurement dates and between uncompacted areas and wheel tracks were analyzed using *t* tests assuming equal variances. Differences were considered significant for *p* values <0.05. Statistical significance should be interpreted

with caution because the limited number of replicate samples did not allow us to test the underlying assumptions of normality and equal variances. Hydraulic conductivities were log-transformed before statistical analysis.

Results and Discussion

Tension Infiltrometer Measurements

The results from the tension infiltrometer measurements are presented in Figure 7.5-115 and Supplemental Figure 7.5-116. Generally, the variation in hydraulic conductivities between replicate measurements was large both for undisturbed soil and wheel tracks at all supply pressure potentials (average coefficient of variation was 62 %). The hydraulic conductivities in August 2012 were significantly higher in the uncompacted soil than in the wheel tracks at supply pressure potentials of -1 and -2 cm, which is in line with the results presented by Ankeny *et al*. (1995). In the growing season of 2013, the hydraulic conductivity for the uncompacted soil was significantly higher in May than in the subsequent measurements at the -1 and -6 cm supply pressure potentials (Figure 7.5-115).

Figure 7.5-115: Temporal development in hydraulic conductivity rates at the supply pressure potentials of -1 cm (left) and -6 cm (right) during the growing seasons 2013 (top) and 2014 (bottom)

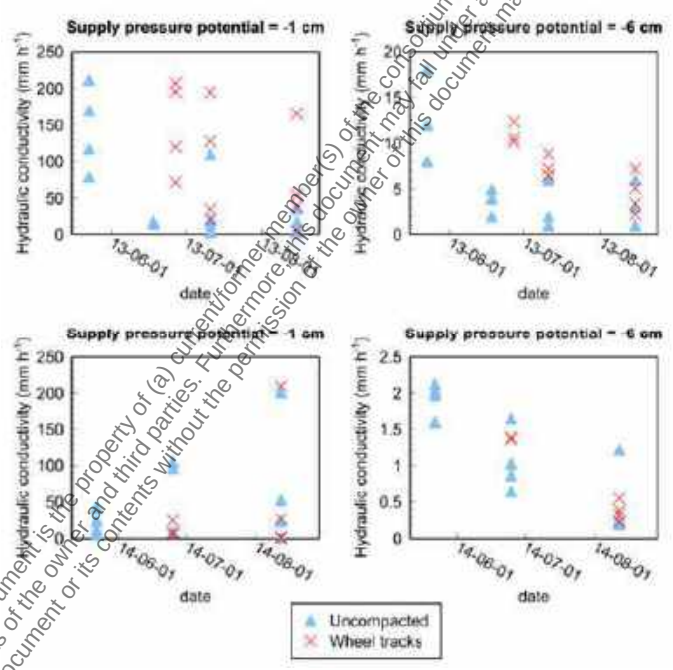
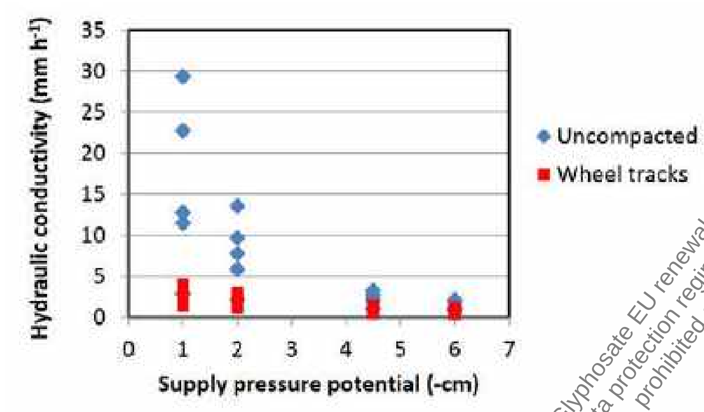


Figure 7.5-116: Hydraulic conductivities measured in August 2012 at supply pressure potentials between -1 cm and -6 cm



A significant decrease in the hydraulic conductivity was also apparent in the wheel tracks at -6 cm supply pressure potential. Hydraulic conductivities were significantly higher in the wheel tracks than in the uncompacted soil at -6 cm supply pressure potential in June and July 2013 and at -1 cm supply pressure potential in June 2013. These unexpected results can be explained by the formation of a surface crust before the pesticide application in 2013. This crust was destroyed at pesticide application in the tracked areas by the pressure exerted by the tractor tires, which recreated a fine aggregated structure at the soil surface. These results show that under certain conditions a surface crust may have a stronger influence on near-saturated hydraulic conductivity than traffic-induced compaction. In 2014 the hydraulic conductivities at a supply pressure potential of -6 cm were significantly lower in August than in the preceding measurements for the uncompacted soil and significantly lower in August than in June for the wheel tracks. There were no significant changes with time at -1 cm supply pressure potential. There were no significant differences in hydraulic conductivity between uncompacted soil and wheel tracks in 2014.

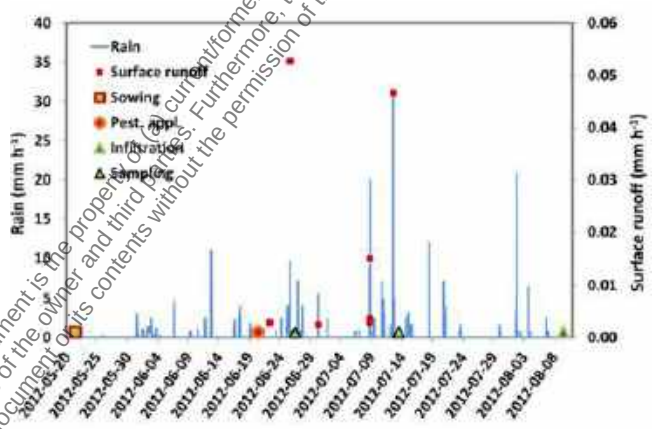
Our results show a complex behavior, but some general trends are apparent. The hydraulic conductivity during the growing season decreases with time from harrowing. The decrease in hydraulic conductivity is most apparent at the -6-cm supply pressure potential. A possible explanation is that the natural processes acting to regenerate structure during the growing season (e.g., shrinkage crack formation and burrowing animals) create pores that are too large to conduct water at -6 cm pressure potential. There were large differences in the measured hydraulic conductivities at -6 cm pressure potential between the years 2013 and 2014 (note the different scales in Figure 7.5-115 [top right vs. bottom right panels]). The first measurements in 2013 were made directly after sowing before any rain had affected the structure in the harrowed layer. The hydraulic conductivity on this occasion (average, 14 mm/h) was more than 7-fold higher than on the first measurement occasion in 2014 (average, 1.9 mm/h), which was made almost a month after sowing, by which time 50.2 mm rain had fallen on the soil. The hydraulic conductivity at -6 cm supply pressure potential in 2013 remained significantly higher than in 2014 throughout the growing season. These results indicate that the amount of rain that falls early in the growing season when the soil surface is unprotected by crops has a strong effect on hydraulic conductivities.

Runoff Events during the Growing Season

During the three monitored growing seasons, surface runoff was only generated on five occasions during a 25-d period in 2012. On these occasions cumulative runoff volumes from the plots were between 23 and 64 L (0.065-0.18 mm). This corresponds to an average runoff coefficient for the five events of 0.17 %, which is small compared with those measured in comparable studies (Riise *et al*., 2004; Siimes *et al*., 2006). The limited number of times when runoff was generated during these three growing seasons suggests that this soil has a small potential for surface runoff under conventional management in this climate. The near-saturated hydraulic conductivity in wheel tracks was smallest (between 1.4 and 4.0 mm/h) in August 2012 (significantly smaller than all other occasions except August 2013 and August 2014). Unfortunately, we did not measure near-saturated hydraulic conductivity at the time when surface runoff was generated.

However, rainfall intensities were much larger than the hydraulic conductivity at -1 cm pressure potential measured in August 2012 on a number of occasions during the growing season (Figure 7.5-117). Near-saturated hydraulic conductivities were generally larger than rainfall intensities during the growing seasons of 2013 and 2014 when no surface runoff was generated. A likely explanation for the smaller near-saturated hydraulic conductivity and the generation of surface runoff during the growing season of 2012 is the formation of a well-developed surface seal due to the larger cumulative rainfall amounts in the period after soil tillage when the soil was unprotected by crops (Fiener *et al*., 2011; Le Bissonnais *et al*., 2005). The cumulative rainfall amounts during the 30-d period after sowing were 85.6, 35.2, and 50.2 mm for the years 2012, 2013, and 2014, respectively. The differences in near-saturated hydraulic conductivities may also have been influenced by differences in soil water contents at pesticide spraying when the wheel tracks were created, which affects susceptibility to compaction (Batey, 2009; Strudley *et al*., 2008). The cumulative rainfall amounts during the week preceding pesticide application were 26.8, 14.2, and 6 mm for 2012, 2013, and 2014, respectively, which suggest wetter soil conditions during 2012. The infiltration capacity of the soil is not only dependent on the saturated hydraulic conductivity but also, among other things, on the antecedent soil water content, which determines the hydraulic gradient driving infiltration. Because both properties vary with time and space, it is difficult to relate runoff events to specific measurements of the near-saturated hydraulic conductivity only. Physically based models that account for the complex interactions between rainfall, infiltration, and the near-surface hydraulic properties are powerful tools for increasing process understanding (Assouline, 2004). Models of surface seal development and water flow through sealed soils have been shown to reproduce measured data on an event basis, but their implementation has been hampered by a lack of data for long-term model evaluations under field conditions (Assouline, 2004).

Figure 7.5-117: Hourly rainfall measured at the field site and average surface runoff during the growing season of 2012. Field operations and dates when bulk samples for pesticide analysis were taken and tension infiltrometer measurements were performed are indicated

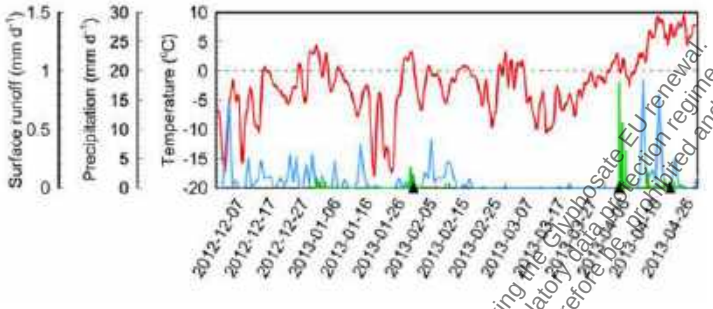


Runoff Events during the Winter Seasons

Surface runoff was generated during three snowmelt periods in 2013, once in January and twice in April. Data on runoff volumes were unfortunately lost for one of these periods. The total runoff volumes for the remaining two periods were between 130 and 2100 L (0.38-6.0 mm). The runoff coefficient for the period December 2012 to March 2013 was 2.6 %. Daily air temperatures and precipitation during the periods December 2012 to March 2013 and December 2013 to March 2014 are presented in Figure 7.5-24. The total precipitation in these two periods was 124 and 185 mm, respectively. Corresponding average temperatures were -3.9 and 1.4°C. The long periods with temperatures below 0°C during the winter of 2012-2013 resulted in snow accumulation on the field and soil freezing. These conditions produced surface runoff during three snowmelt periods. This indicates that the soil remained frozen, which reduced the infiltration capacity. In contrast, the limited amount of precipitation during cold periods in the winter of 2013-2014 did not result in significant snow accumulation on the field. It also seems likely that the

infiltration capacity was less affected by freezing due to the higher temperatures during the winter of 2013-2014. There were no significant effects of buffer zone treatment or block on runoff volumes.

Figure 7.5-118: Daily precipitation (blue line) and air temperature (red line) data from Ultuna climate station and average surface runoff (green line) measured at the field site during winter 2012-2013. Surface runoff sampling times are indicated by triangles.



Pesticide Concentrations

Spring-Applied Pesticides

All applied compounds were detected in all samples. Average concentrations were higher (0.83-7.3 µg/L) during the first runoff event compared with the second event (0.55-4.1 µg/L) for all compounds, although differences were significant only for MCPA. Riise *et al*. (2004) and Siimes *et al*. (2006) also reported the highest concentrations in surface runoff in the first events after pesticide application. Swedish water quality standards below which no effects on surface water ecosystems are assumed have been estimated by the Swedish Chemicals Agency for about 100 pesticides and degradation products (Swedish Chemicals Agency, 2016). Concentrations in surface runoff exceeded water quality standards in all samples during the growing season for diflufenican and pirimicarb. Concentrations of MCPA exceeded water quality standards in all samples taken on 26 June. However, these are in-field concentrations, and whether pesticides in runoff reach surface waters depends on the connectivity to the stream. In addition, dilution would significantly reduce concentrations in receiving surface waters, considering the small surface runoff volumes. All of the applied pesticides were also found in surface runoff collected during snowmelt in the winter of 2012-2013. There were no significant effects of buffer zone treatment or block on pesticide concentrations. Concentrations were about two orders of magnitude lower than in the preceding summer. During the intervening period, residues of the spring-applied pesticides were incorporated by autumn plowing and also degraded and leached into the soil profile. All these processes acted to reduce concentrations in surface runoff. Average concentrations of diflufenican exceeded Swedish water quality standards values for all three sampling occasions during snowmelt.

The fungicide carbendazim, which was not applied to the field, was detected in all samples at concentrations in the same range as some of the recently applied pesticides. Carbendazim has not been included in any products approved for use in Sweden since 1999. This result suggests that degradation of carbendazim is either much slower under Nordic conditions than would be indicated or that there was another source of this compound. Carbendazim is a metabolite of the fungicide thiophanate-methyl, which has been registered for use in Sweden since the mid-1970s, with one product currently approved. However, the degradation in soil of thiophanate-methyl to carbendazim is very fast (PPDB, 2016), and we have no record of any recent use of thiophanate-methyl at the site.

Glyphosate and Aminomethylphosphonic Acid

Concentrations of glyphosate in aqueous phase and bound to particles ranged from 0.12 to 7.4 µg/L and from 0.12 to 2.7 µg/L, respectively; the corresponding AMPA concentrations ranged from 0 to 2.7 µg/L and from 0 to 0.85 µg/L. It is possible that some of the glyphosate and AMPA found in surface runoff originated from the glyphosate application in 2010. There were no significant effects of buffer zone

treatment or block on glyphosate or AMPA concentrations. Average concentrations of both substances in the aqueous phase decreased (although not significantly) from the first sampling occasion to the last. Concentrations of glyphosate and AMPA in the aqueous phase were on average 2.2- and 5.1-fold higher, respectively, than in the particle-bound fraction. However, the only statistically significant differences were found between concentrations of AMPA in solution and bound to particles for the sampling on 4 and 21 April. There were no significant correlations between runoff volumes and concentrations for glyphosate and AMPA. The only comparable study that we are aware of (Siimes *et al.,* 2006) reported glyphosate concentrations in the aqueous phase in runoff during snowmelt of between 0.08 and 0.94 µg/L. However, in their study glyphosate was sprayed on bare soil (silt loam) in July at half the dose used in our study.

Pesticide Losses

It was not possible to calculate pesticide losses from the samples taken on 5 Apr. 2013 because data on runoff volumes were not available. The number of plots that generated surface runoff was 10 (31 January), 7 (5 April), and 4 (21 April) for the three events during snowmelt in 2013. This suggests that the losses on 5 April were of the same order of magnitude as the losses during the other two runoff events. Tile drainage has been shown to reduce pesticide losses through surface runoff (Burgoa and Wauchope, 1995; Kladivko *et al*., 2001). It is therefore likely that pesticide losses during snowmelt would have been larger if the buffer zones had not been drained. Quantification of the effects of the tile drainage on the losses through surface runoff was beyond the scope of this study. The total relative losses of the spring-applied pesticides varied between 0.0012 % for MCPA and 0.0091 % for diflufenican (Table 7.5-145). These losses in surface runoff were small compared with those reported by Riise *et al.* (2004) and Siimes *et a*l. (2006) because the fraction of rainfall routed to surface runoff was smaller. Although runoff concentrations were much higher during the growing season than in snowmelt, winter losses of the spring-applied pesticides were of the same order of magnitude due to the much larger runoff volumes. The coefficients of variation in total losses for the spring-applied pesticides were between 70 and 100 % and between 220 and 350 % for the growing season and snowmelt periods, respectively. One of the plots dominated (66-100 %) the losses of most of the spring-applied compounds during snowmelt. There were no significant effects of buffer zone treatment or block on pesticide losses. The total losses of glyphosate and AMPA in both phases were 0.021 % of the applied amount of glyphosate. Due to the small runoff volumes, losses were small compared with the 0.13 % losses reported by Siimes *et al*. (2006). We did not find any clear relationships between compound properties and the relative losses in surface runoff (Table 7.5-145), but the timing of runoff losses was significantly affected.

Table 7.5-145: Losses of pesticides in surface runoff

Date	Clopyralid	MCPA†	Fluroxypyr	Diflufenican	Prothio-conazole	Pirimicarb	Aqueous phase		Particle bound	
							Glyphosate	AMPA‡	Glyphosate	AMPA‡
	% of applied amount									
26 June 2012	0.0016	0.0012	0.0037	0.0013	0.0011	0.0027	NA§	NA	NA	NA
13 July 2012	0.0014	0.00092	0.0018	0.0015	0.00086	0.0021	NA	NA	NA	NA
Sum	0.0030	0.0012	0.0055	0.0028	0.0020	0.0048	NA	NA	NA	NA
31 Jan. 2013	0	0	0.00032	0.0018	0.00028	0.000077	0.0054	0.0074	0.0015	0.00031
5 Apr. 2013¶	–	–	–	–	–	–	–	–	–	–
21 Apr. 2013	0	0	0.0014	0.0045	0.0047	0.0029	0.0030	0.0014	0.0019	0.00045
Sum	0	0	0.0017	0.0062	0.0050	0.0030	0.0084	0.0088	0.0034	0.00076
Total sum	0.0030	0.0012	0.0072	0.0091	0.0070	0.0078	0.0084	0.0088	0.0034	0.00076

† MCPA, 4-chloro-2-methylphenoxyacetic acid.
‡ AMPA, aminomethylphosphonic acid. Losses of AMPA are given as percentages of the applied amount of glyphosate.
§ NA, not available.
¶ Pesticide losses could not be estimated because data on runoff volumes were lost.

Conclusions
Our results show that the temporal variation in near-saturated hydraulic conductivity during the growing season may be large and that this variation influences the potential risk for pesticide losses in surface runoff. This study also shows that the weather conditions during winter that determine snow accumulation and soil freezing affect pesticide losses in runoff during snowmelt periods. Both spring-applied pesticides and glyphosate, which was applied in the autumn, were found in snowmelt surface runoff samples when runoff occurred. Modeling approaches for pesticide losses through surface runoff should account for the temporal variability in soil hydraulic properties due to seedbed consolidation and surface sealing and, for cold climate, should include the effects of freezing and thawing on the infiltration capacity of the soil. The modeling approaches currently used in risk assessment for pesticides in the European Union do not explicitly account for these processes (FOCUS, 2001, 2014).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a runoff experiment on a field site in Sweden with realistic cultivation conditions.
The runoff of glyphosate and AMPA was measured over a period of 3 years.
The article is considered reliable

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/005
Report author	Napoli, M. *et al*.
Report year	2016
Report title	Transport of Glyphosate and Aminomethylphosphonic Acid under Two Soil Management Practices in an Italian Vineyard
Document No	Journal of Environmental Quality 45:1713-1721 (2016)
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the soil monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/046
Report author	Schreiner, V. *et al.*
Report year	2016
Report title	Pesticide mixtures in streams of several European countries and the USA
Document No	Science of the Total Environment 573 (2016) 680-689
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Given the multitude of pesticides used in agriculture, adjacent streams are typically exposed to pesticide mixtures. Previous studies analysed the ecological risks of a few pesticide mixtures or were limited to an individual region or crop, whereas a large scale analysis of pesticide mixtures is missing. Routine monitoring data from Germany, France, the Netherlands and the USA comprising a total of 4532 sites and 56,084 sampling occasions was analysed with the aim of identify the most frequently detected pesticides, their metabolites and mixtures. The most frequently detected compounds were dominated by herbicides and their metabolites. Mixtures mostly comprised of two up to five compounds, whereas mixtures in the USA and France had clearly less compounds than those of Germany and the Netherlands. The number of detected pesticides and thereby the size of mixtures is positively correlated to the number of measured pesticides ($r = 0.57$). In contrast, a low relationship was found to the ratio of agricultural areas within the catchment ($r = 0.17$), and no relationship was found to the size of the catchment ($r = 0.06$). Overall, our study provides priority mixtures for different countries that may be used for future ecotoxicological studies to improve risk assessment for stream ecosystems.

Materials and Methods

We compiled pesticide monitoring data of lotic surface waters from databases from Germany, France, the Netherlands, and the USA (Table 7.5-146). We retrieved the data from France from EIONET (Reporting Obligations Database (ROD); River quality (EWN-1) - Eionet, 2014), the data from the Netherlands from www.bestrijdingsmiddelenatlas.nl and the data from Germany were provided by the regional water quality authorities. The US dataset was generated by harmonizing and combining datasets from the National Water-Quality Assessment Program (NAWQA Data Export, 2014) and the Water Quality Data Portal (WQP, 2014). Sites within a 10 m distance from both datasets were considered as identical and entries from them were merged. The data from France, the Netherlands and the USA covered the country-level, whereas the German data were restricted to four German states (Rhineland-Palatinate, North Rhine-Westphalia, Saxony and Baden-Württemberg). Nevertheless, we refer to this data as Germany to enhance readability. The used chemical concentrations originated exclusively from grab water samples. Data pre-processing consisted of the following steps: (I) To obtain a spatially-balanced monitoring data set for each region and country, and thus to enhance comparability, we used the Generalized Random Tessellation Stratified method (GRTS; R package: spsurvey) and randomly sampled subsets with maximised spatial balance. The subset size was chosen as the maximum number of sites that showed no spatial clustering (as measured by the $\chi 2$ statistic). This method reduced the used number of sites per country (Table 7.5-146). (II) Non-detects and duplicate entries were removed after assigning a Chemical Abstract Service (CAS) registry number to each chemical. (III) We limited the data to the years of 2008-2012 (only for the German states of Baden-Württemberg and Rhineland-Palatinate the years of 2006-2010 and for North Rhine-Westphalia the years of 2005-2009 were used), because these data had an increased number of sampling occasions compared to preceding years. These steps resulted in a total of 4532 sites with 56,084 sampling occasions. On average, 12 sampling occasions were performed per site, ranging from 6 in the USA to 27 in France. Up to 779 different pesticides

and their metabolites were included in the analysis, with the data set from Netherlands contributing most with 637 different pesticides and their metabolites (Table 7.5-146; Figure 7.5-119). Differences in the analysed pesticides and their metabolites between the different countries were illustrated using multidimensional scaling based on the binary Jaccard distance.

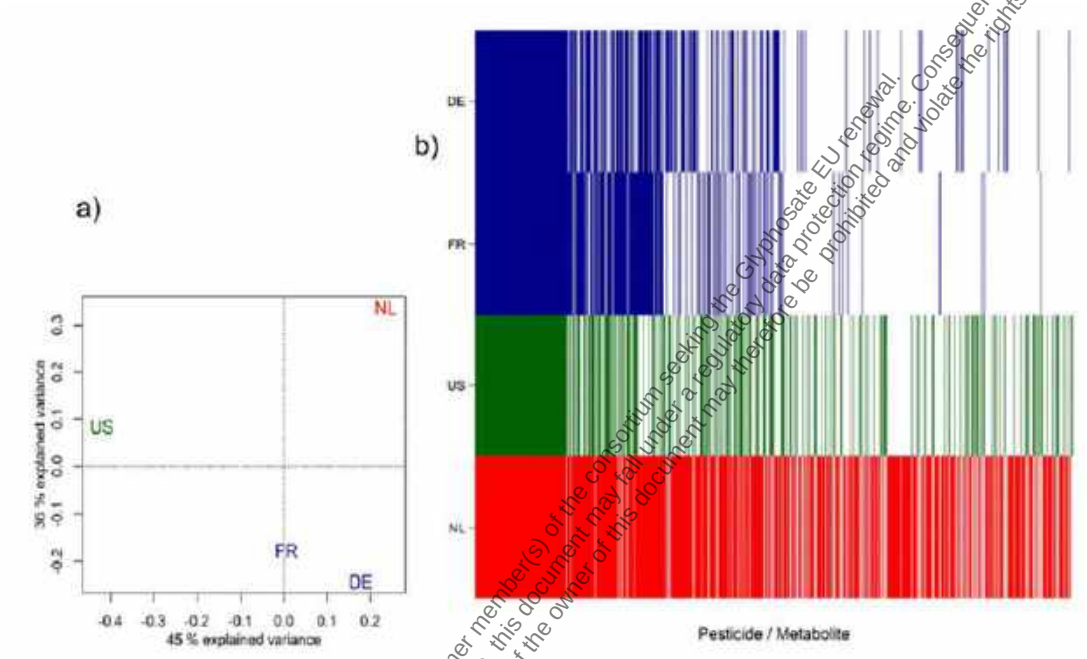
Table 7.5-146: Overview of data sets analysed with information of detection rates and numbers of compounds and mixtures within the different countries

	DE	FR	NL	US
Sites remaining after GRTS [%]	72	63	70	62
Sites after GRTS	1037	950	320	2225
Sampling occasions after GRTS	12,177	25,586	5112	13,209
Median sampling occasions per site	8	26	8.5	3
Analysed compounds	297	292	637	321
Mean No. compounds analysed per sampling occasion	85.1	27.6	83.4	36.7
Detected compounds	205	115	267	127
No. most frequent compounds	132	25	68	14
Mean size mixtures all compounds, ±SD	7.0 ± 4.8	3.0 ± 1.6	4.8 ± 3.4	2.2 ± 1.2
Sites with compounds, all compounds [%]	85.1	78.1	90.3	23.5
Sites with mixtures, all compounds [%]	49.4	13.7	62.3	16.2
Sampling occasion with pesticide exposure, all compounds [%]	69.3	32.7	82.1	26.1
Detected core compounds	40	40	38	29
No. most frequent core compounds	33	16	19	9
Mean size mixtures core compounds, ±SD	4.1 ± 2.3	2.5 ± 1.0	3.6 ± 1.8	2.5 ± 0.7
Max size mixture, core compounds	20	9	14	6
Sites with compounds, core compounds [%]	80.3	73.1	85.3	21.4
Sites with mixtures, core compounds [%]	36.2	7.6	36.1	15.0
Sampling occasion with pesticide exposure, core compounds [%]	59.9	24.8	60.2	24.6

DE: Germany; FR: France, NL: Netherlands, US: United States of America. "No. most frequent (core) compounds": number of compounds after establishing level of most frequent compounds (c.f. Fig. S2). Compounds = pesticides + metabolites, GRTS = Generalized Random Tessellation Stratified, SD = standard deviation. For the same table with differentiation between the German states see Table S2.

Figure 7.5-119: **a) Multidimensional scaling of the analysed pesticides and their metabolites in the different countries. b) Comparison of the analysed pesticides and metabolites from the different countries**

Each line represents one compound. France and Germany were coded with the same colours in both graphs to highlight concordance of the analysed compounds (see a). For number of analysed pesticides andmetabolites in each country, see Table 7.5-146. DE: Germany, FR: France, NL: Netherlands, US: United States of America.



Identifying most frequently detected pesticides and mixtures

We calculated the relative occurrence (p) of each pesticide and metabolite (compound) (i) for sampling occasions as well as at sites as: $p_i = \Sigma y_i / n$ where n is the number of sampling occasions or sites and y is 1 if compound was found in a site or on a sampling occasion, otherwise 0. Additionally, we calculated the percentage of sites and sampling occasions were at least one compound was detected (percentage of sites and sampling occasions where $\Sigma p_i > 0$). We identified most frequent mixtures composed of different types of pesticides (herbicides, insecticides and fungicides). Compounds that occurred at <5 % of sites were omitted from further analysis as they lead to an inflation of the number and occurrence frequency of mixtures. For example, consider the case of two compounds A and B occurring on 100 sampling occasions and the compounds X, Y, and Z each occurring on 4 sampling occasions. This could result in multiple ternary (ABX, ABY, ABZ) or quaternary (ABXY, ABXZ, ABYZ) mixtures with low relative occurrence frequency. Subsequently, for each mixture the absolute number of compounds (size), the number of the different pesticide types and the occurrence frequency at sites as well as sampling occasions was calculated. For the German data set, the analysis was firstly conducted separately for the four German states and subsequently the results were aggregated weighted by the number of analysed sites or sampling occasions.

Calculation of size and relative land cover of catchment areas in Germany

For each site analysed in Germany, we quantified land cover types in its catchment by following a four step procedure: (i) Extraction of the stream network from a digital elevation model that shows the highest concordance with a mapped stream network of the German state, using the open-source software algorithm ATRIC, (ii) snapping the sites to the nearest segment of the extracted stream network, (iii) automatically delineating the upstream catchment polygon for each fitted site from the DEM using ATRIC and (iv) overlaying the catchment polygons with the CORINE land cover datasets and subsequently calculating the percentage of six land cover types (arable land, permanent crop, forest, meadows, water bodies and other). The analysis was limited to Germany because only for Germany mapped stream networks were readily

available. Besides, in the case of the Netherlands, geomorphology does not allow for derivation of stream networks from a DEM.

Associations with monitoring characteristics
We scrutinised whether characteristics of the monitoring programs influence the detection of pesticides and its mixtures using the following response variables: size of mixtures and number of detected compounds. We correlated (Pearson's correlation) these response variables with the number of analysed pesticides and metabolites per sampling occasion and the size of catchment areas of sampling sites. For Germany, we also correlated the response variables with the areal proportion of agriculture, of arable land and of permanent crop land within the upstream catchment. This was done using a cubic regression spline with a Poisson distribution.

Direct comparison of mixtures from different countries - core compounds
Given that the compound spectrum varied between countries (Figure 7.5-119), we analysed the data for 44 core compounds that were measured in all countries and German states. Most of these (29) were herbicides and metabolites with a herbicide as parent compound. Additionally eleven insecticides and four fungicides were part of the core compounds. These core compounds enabled a direct comparison of mixtures from different countries. We tested for differences in the size of mixtures between the countries as well as for differences in mixtures composition using analysis of variance (ANOVA) followed by a Tukey-HSD (Honestly Significant Difference) test for pairwise comparison. Pre-processing of data, statistical analysis and visualisations were performed using R, version 3.1.1.

Results

Most frequently detected pesticides and metabolites
The spectrum of analysed pesticides and metabolites varied strongly between countries (Figure 7.5-119a and b). The monitoring data of France and Germany showed a high concordance in the total number of analysed compounds (Germany: 297, France: 292, Table 7.5-146) and identity of analysed compounds in comparison to the Netherlands and the USA (shown with different colours in Figure 7.5-119). The different spectrum of analysed pesticides and metabolites resulted, in several compounds among the most frequent pesticides and metabolites that were country-specific, particularly for the Netherlands, such as Bitertanol, Flonicamid and Flutolanil (Table 7.5-147).

Table 7.5-147: List of the most frequently detected pesticides and metabolites with their relative occurrence at sites of the different countries

The compounds are ordered alphabetically. Each listed compound occurred in at least one country at a minimum of 10 % of the sites

Compound	CAS	Pesticide type	DE	FR	US	NL
1,2,3,4,5,6-Hexachlorocyclohexane	58899	IN	8.7	11.2	0.7*	10
2,4-D	94757	HB	9.1*	4.9*	3.0*	18.4*
2,6-Dichlorobenzamide	2008584	HB	0.2	0.1	0	28.4
AMPA	1066519	M	12.2	13.2	2.3	37.8
Atrazine	1912249	HB	24.3	42.0	19.2*	11.3
Azoxystrobin	1318860338	FU	18.4*	0.7*	0*	27.8*
Bentazon	25057890	HB	23.2*	7.7*	0	37.5*
Bitertanol	55179312	FU	0	0	0	15.9
Boscalid	188425856	FU	38.6*	0*	0*	12.8*
Carbendazim	10605217	FU	16.3*	1.5	0	55.3
Chloridazon	1698608	HB	13.7*	0.8*	0	29.4*
Chlorpropham	101213	HB	0*	0.2*	0*	31.9*
Chlorpyrifos	2921882	IN	19.2	6.6*	1.7*	3.8*
Chlortoluron	15545489	HB	13.0*	4.6*	0	8.4
Clomazone	81777891	HB	19.8*	0.1*	0*	2.8*
Desethylatrazine	6190654	M	12.2	5.5	3.0	2.2
Desethylterbuthylazine	30125634	M	34.3	10	0	4.4
Dichlobenil	1194656	HB	0	0.4	0*	11.9
Diflufenican	83164334	HB	33.4*	0.3*	0	0.9*
Dimethachlor	50563365	HB	19.8*	0.1*	0	0
Dimethenamid	87674688	HB	20.9	7.5	1.2*	15.3
Dimethoate	60515	IN	7.6*	0.2*	0.6*	20*
Dimethomorph	110488705	FU	1.6*	1.1*	0*	18.4*
Diuron	330541	HB	54.1*	55.3	1.3*	38.1
Epoxiconazole	133855988	FU	10.1*	1.5*	0	6.9*
Ethofumesate	26225796	HB	3.6*	1.3*	0*	32.5*
Flonicamid	158062670	IN	0*	0*	0*	11.9*
Flufenacet	142459583	HB	23.1*	0*	0*	1.3*
Fluroxypyr	69377817	HB	0	0.1*	0*	14.7*
Flurtamone	96525234	HB	23.1*	0.1*	0	0.3
Flutolanil	66332965	FU	0*	0*	0*	19.1*
Glyphosate	1071836	HB	9.7*	12.1*	0*	30*
Hexachlorobenzene	118741	FU	15.5	4.0	0.4	2.5
Igarol 1051	28159980	FU	26.4	0	0*	0
Isoproturon	34123596	HB	62.0*	53.6*	0	37.5*
Linuron	330552	HB	2.0	10*	0*	28.4*
MCPA	94746	HB	22.5*	43.2*	0.4*	44.4*
Mecoprop	93652	HB	24.9*	5.4*	0	38.4
Metalaxyl	57837191	FU	7.9	0.5	0.1*	21.3
Metamitron	41394052	HB	10.5*	0.7*	0	12.8*
Metazachlor	67129082	HB	45.5*	2.3*	0	19.1*
Metolachlor	51218452	HB	31.1*	7.9	11.6*	36.6
Metribuzin	21087649	HB	3.2*	0.1*	3.6*	11.6*
Napropamide	15299997	HB	18.3*	0.4*	0*	0*
p,p'-DDD	72548	IN	10.5	5.9	0.5	0.9
p,p'-DDT	50293	IN	20.9	7.5	0.9*	3.1
Pencycuron	66063056	FU	0.8*	0*	0*	14.4*
Pendimethalin	40487421	HB	12.7*	0.6*	2.6*	5.0*
Pirimicarb	23103982	IN	7.9*	0.1*	0*	24.1*
Pronamide	23950585	HB	14.3*	3.1*	0.5*	13.4*
Propamocarb	24579735	FU	0.6*	0*	0*	11.6*
Propiconazole	60207901	FU	17.4*	0.1*	0.2*	3.8*
Prosulfocarb	52888809	HB	2.2*	0.4*	0	15.9*
Quinmerac	90717036	HB	15.6*	0*	0	0.3*
Simazine	122349	HB	29.0	19.9	1.6*	15.3
Tebuconazole	107534963	FU	15.6	2.6*	0*	17.5*
Terbothylazine	5915413	HB	55.1*	0.1	0	33.1*
Terbutryn	886500	HB	37.4	2.9	0	5.6
Terbuthylazine, 2-hydroxy	66753079	M	10.9	0	0	0

DE: Germany; FR: France; NL: Netherlands; US: United States of America. IN: insecticide, HB: herbicide, FU: fungicide, M: metabolite. * indicates that the respective pesticide was approved during the time frame of the data used for this study (EC, 2015; EPA, 2016 personal communication). See Table S4 for differentiation between German states.

In addition, pesticide detections varied strongly between the countries across sampling occasions (26 % for USA to 82 % Netherlands) and sites (24 % for USA to 90 % for the Netherlands (Table 7.5-146). The most frequently detected compounds, occurring at least at 10 % of sites, were mainly herbicides and their metabolites belonging to the chemical classes of phenylurea (Diuron (DCMU), Isoproturon), chlorotriazine (Terbuthylazine, Atrazine) and organophosphorus herbicides (Glyphosate) (Table 7.5-147). In some countries, fungicides (Propiconazole, Germany; Boscalid, Germany; Carbendazim, the Netherlands) and insecticides (Lindane (γ-HCH), France; Fipronil, USA; Imidacloprid, the Netherlands) were among the most frequently detected pesticides. Although 34 % and 19 % of the analysed compounds were insecticides and fungicides, both pesticide types were less frequently detected in comparison to herbicides.

Table 7.5-148: List of the most frequent mixtures from the different countries with the ratio of occurrence at sites and sampling occasions as well as the number of compounds (size). Order of compounds based on CAS numbers

[%] Occurrence site	[%] Occurrence sampling occasions	No. compounds	Compounds
GERMANY			
7.6	0.1	2	Diuron (HB), Isoproturon (HB)
3.0	0.1	2	Atrazine (HB), Desethylatrazine (M)
2.4	0.2	2	Boscalid (FU), Isoproturon (HB)
2.0	0.2	2	Isoproturon (HB), Metazachlor (HB)
1.9	0.2	2	Boscalid (FU), Terbuthylazine (HB)
1.9	0.1	2	Isoproturon (HB), Terbuthylazine (HB)
1.5	0.1	2	Isoproturon (HB), Terbutryn (HB)
1.5	0.1	2	Irgarol 1051 (FU), Isoproturon (HB)
1.5	0.1	2	Simazine (HB), Terbuthylazine (HB)
1.4	0.1	2	Isoproturon (HB), Diflufenican (HB)
FRANCE			
18.0	1.5	2	Diuron (HB), Isoproturon (HB)
13.6	1.1	2	Diuron (HB), MCPA (HB)
10.1	0.6	2	Atrazine (HB), Diuron (HB)
9.2	0.7	2	Atrazine (HB), Isoproturon (HB)
8.5	0.5	2	Isoproturon (HB), MCPA (HB)
7.2	0.4	2	Atrazine (HB), MCPA (HB)
6.4	0.4	3	Diuron (HB), Isoproturon (HB), MCPA (HB)
6.0	0.3	3	Atrazine (HB), Diuron (HB), Isoproturon (HB)
5.2	0.3	3	Atrazine (HB), Diuron (HB), MCPA (HB)
4.1	0.2	2	Simazine (HB), Diuron (HB)
NETHERLANDS			
7.2	1.5	2	AMPA (M), Glyphosate (HB)
4.7	0.3	2	Carbendazim (FU), Imidacloprid (IN)
3.8	0.6	2	Diuron (HB), Isoproturon (HB)
3.4	0.4	2	Bentazon (HB), Isoproturon (HB)
3.4	0.2	2	Carbendazim (FU), Isoproturon (HB)
3.1	0.2	2	Carbendazim (FU), Diuron (HB)
3.1	0.3	2	Bentazon (HB), Mecoprop (HB)
3.1	0.3	2	Mecoprop (HB), MCPA (HB)
2.8	0.2	3	Bentazon (HB), Mecoprop (HB), MCPA (HB)
2.5	0.3	3	Carbendazim (FU), Imidacloprid (IN), Floaicamid (IN)
USA			
6.3	2.5	2	Atrazine (HB), Metolachlor (HB)
3.8	3.2	3	Atrazine (HB), Acetochlor (HB), Metolachlor (HB)
1.6	0.7	2	Atrazine (HB), Desethylarrazine (M)
1.3	0.3	4	Atrazine (HB), Acetochlor (HB), Metolachlor (HB), Desethylatrazine (M)
1.2	0.8	4	Alachlor (HB), Atrazine (HB), Acetochlor (HB), Metolachlor (HB)
1.0	0.3	2	Atrazine (HB), Acetochlor (HB)
1.0	0.3	4	Atrazine (HB), Metribuzin (HB), Acetochlor (HB), Metolachlor (HB)
0.9	0.5	5	Alachlor (HB), Atrazine (HB), Metribuzin (HB), Acetochlor (HB), Metolachlor (HB)
0.8	0.2	3	Atrazine (HB), Metolachlor (HB), Desethylatrazin (M)
0.7	0.4	4	AMPA (M), Atrazine (HB), Acetochlor (HB), Metolachlor (HB)

HB: herbicide, IN: insecticide, FU: fungicide, M: metabolite, No.: number. See Table S5 for differentiation between German states.

Most frequently detected mixtures

The 10 most frequently detected mixtures were mostly binary or ternary and composed of herbicides and consisted of compounds that represented the most frequent individual compounds in the countries.

The number of compounds constituting the 10 most frequent mixtures ranged from 5 in France to 12 in Germany (Table 7.5-148).

Associations with monitoring characteristics

The number of detected compounds as well as mixture size (Table 7.5-146) correlated moderately positive with the total number of analysed compounds per sampling occasion (Figure 7.5-120). Both correlated negligibly with catchment size for all countries, and only weakly with the fraction of arable land or of total agricultural area within the catchment areas of Germany (Table 7.5-149). However, the mean number of detected pesticides increased from 3 to 7 compounds when the fraction of total agricultural area within the catchment area increased from 20 % to 40 %.

Figure 7.5-120: Relationship between number of detected and of analysed compounds (on a logscale)

Solid line indicates a 1:1 ratio of detected: analysed compounds, dashed lines indicate 1:5, 1:10, 1:20 and 1:50 ratios. Colours indicate the number of individual sampling occasions with this respective relationship.

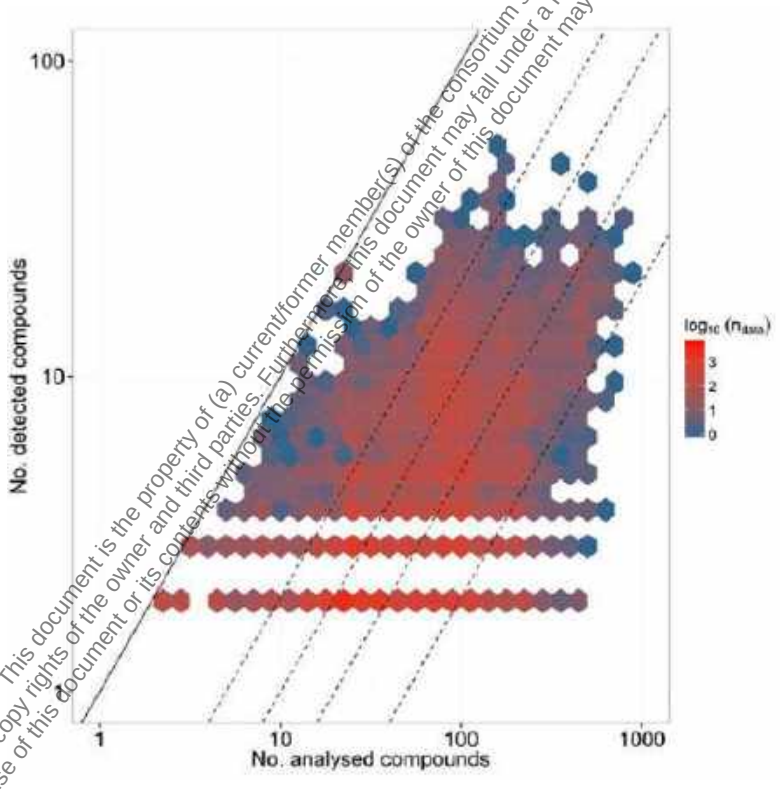


Table 7.5-149: Correlation coefficients and corresponding confidence intervals (CI) concerning associations with monitoring characteristics

	r	95% CI	n
No. analysed compounds ~ n detected compounds	0.57	0.56–0.57	56,084
No. analysed compounds ~ size mixture	0.54	0.54–0.55	56,084
No. catchment size ~ n detected compounds	0.06	0.05–0.07	56,084
No. catchment size ~ size mixture	0.06	0.05–0.07	56,084
% arable land in catchment area ~ n detected compounds	0.17	0.15–0.19	12,177
% arable land in catchment area ~ size mixture	0.18	0.16–0.20	12,177
% agricultural area in catchment area ~ n detected compounds	0.19	0.17–0.21	12,177
% agricultural area in catchment area ~ size mixture	0.20	0.18–0.22	12,177

no.: Number, all correlations with arable land and agricultural area in catchment area only refer to data from Germany.

Core compounds - composition and size of detected mixtures

The pesticide mixtures for the core compounds that were analysed in all countries consisted mainly of herbicides (Figure 7.5-121), where Atrazine, Simazine and the metabolites AMPA with a herbicide as parent compound were dominating. For France, herbicide mixtures accounted for 94 % of mixtures, whereas for Germany, only 48 % of mixtures were solely comprised of herbicides, due to frequent mixtures with fungicides (e.g. Metalaxyl, Propiconazole) and insecticides (Chlorpyrifos). For all countries, insecticides contributed negligibly to mixtures, although one quarter of the analysed core compounds were insecticides. Considering that only four of the 44 analysed core compounds were fungicides, they were comparatively overrepresented in the mixtures of Germany and Netherlands with 41 % and 18 % of all mixtures containing fungicides (Figure 7.5-121). Generally, the relative occurrence of mixtures decreased with an increase of mixture size (Figure 7.5-122). Binary and tertiary mixtures dominated in surface waters as detected in all countries. Only for the German data, larger mixtures occurred also frequently, which was mainly based on mixtures from the German state Baden-Württemberg. Baden-Württemberg also had significantly larger mixture sizes compared to the other countries and German states (all $p < 0.001$, all 95 % confidence intervals exclude 0).

Figure 7.5-121: Relative amount of mixtures from core compounds for the main pesticide types

DE: Germany; FR: France, NL: Netherlands, US: Unites States of America. Dark green: mixtures of only herbicides, light green: herbicides in mixture, blue: fungicide in mixtures, red: insecticides in the mixtures. Metabolites were assigned the pesticide type of their parent compound.

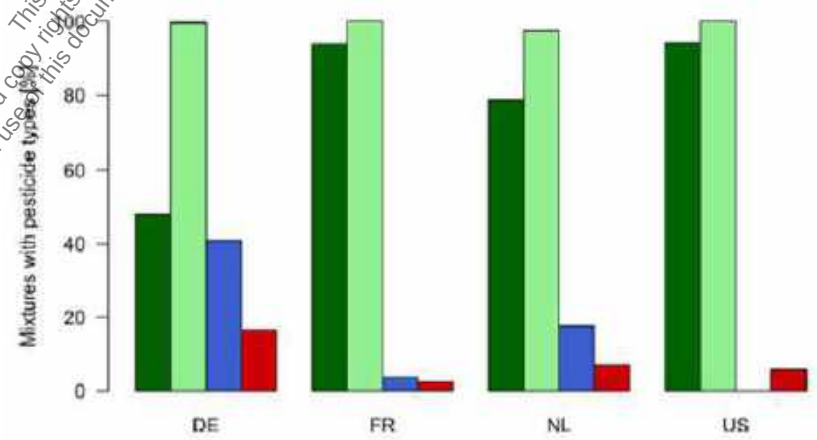
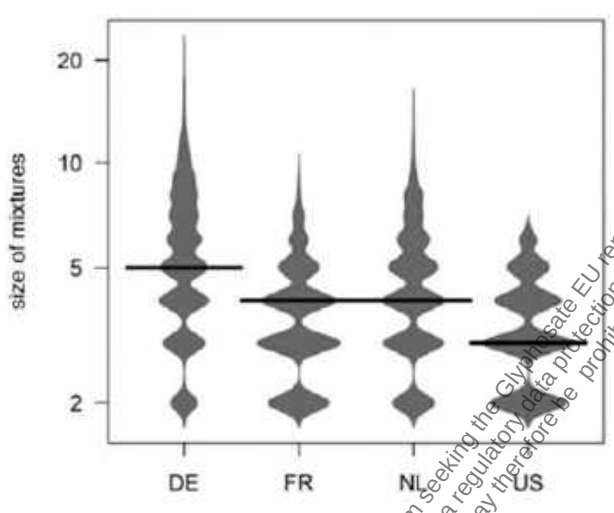


Figure 7.5-122: Distribution of mixture size for the different countries for the core compounds
The black solid line gives the median. Y-axis on logarithmic scale. DE: Germany, FR: France, NL: Netherlands, US: United States of America.



Discussion

Most frequently detected pesticides and mixtures
Herbicides and metabolites with herbicides as parent compounds were the most frequently detected pesticide group in our study, of which Isoproturon, MCPA and Atrazine were the most frequent herbicides. This result is in accordance with several other studies that identified herbicides as the most frequently detected compound group. With approximately 83,000 t, the combined herbicide use in France, Germany and the Netherlands was a factor of 12 higher than insecticide and 50 % higher than fungicide use. Based on these application quantities, herbicides enter streams usually in relatively high concentrations, which together with their typical high water solubility and persistence simplifies detection in chemical analysis, especially in comparison to insecticides. Despite herbicides in the USA being applied 2.5 times more frequently than insecticides, presumably due to different climate conditions than in Europe, the ratio of herbicide to insecticide detections was similarly low as for the European countries. In our study, Glyphosate was not considered in the analysis for the USA, although it is frequently applied, due to a lack of data from the regular monitoring. Other monitoring programs included Glyphosate and detected it frequently. The exclusion of the Glyphosate and its metabolites in the regular monitoring can be attributed to its difficult analysis, where the high polarity complicates detection using liquid chromatography, and high costs using alternative methods. Fungicides were in our study detected in all countries except for the USA, in contrast to other studies which detected fungicides in the USA. This lack of detection in the USA may be explained by the fact that fungicides were rarely part of large scale monitoring programs used in our analysis. Additionally, the usual application pattern of fungicides leads to relatively low but continuous concentrations of these compounds in streams.

The limits of quantification (LOQ) for the USA for fungicides in our study were in average 12-fold higher as those of other countries, which might contribute to the low detection frequency. The streams in the German state Baden-Württemberg showed a high percentage of mixtures with fungicides (93 %) in comparison to other countries and German regions (0-24 %). This is mainly due to the most frequently detected fungicides Metalaxyl and Propiconazole, which occurred at 58 % and 90 % of the sites respectively. In Baden-Württemberg, the compounds were analysed in almost all sites (98 % for both) and all sampling occasions (94 % and 92 % for Metalaxyl and Propiconazole). In the other regions and countries, except for the German state Saxony where the monitoring was similar to that of Baden-Württemberg, they were analysed in <66 % and 36 % of sites and sampling occasions. In the other countries the rather high detection rate of Metalaxyl and Propiconazole can also be attributed to the comparatively low LOQ of 1 ng/L for both compounds that was only reached for Baden-Württemberg and was for example 15-fold higher in Saxony.

The LOQ from these compounds in the other German states and countries ranges from 5-fold higher in Rhineland-Palatinate up to 80-folds higher in France. Finally, differences in agricultural land use and consequently in pesticide use may partially explain differences in detection patterns. A study in Switzerland showed that by decreasing the LOQ in pesticide analysis, the number of detected compounds could be increased up to 67 % corresponding to 30 to 50 individual compounds in this study. This decrease of LOQs can be necessary to appropriately evaluate potential ecological risks from pesticides. For our dataset, the ratio of LOQ and LC_{50} of the most sensitive taxa differed strongly from 0.0003 (10^{th} percentile) to 4.1 (90^{th} percentile). Decreasing the LOQs is still required for many compounds for a comprehensive ecological assessment. Insecticides were the least frequently detected compound group. The most frequently detected insecticides were DDT, Pirimicarb and Chlorpyrifos. The most frequently detected mixtures from the different countries consisted of two or three compounds with mainly herbicides and metabolites with a herbicide as parent compound. This small size of frequently detected mixtures is partly also due to the limitation to compounds detected at >5 % of sites. Without this limitation the average size of the mixtures would be higher. The single compounds of the most frequent mixtures reflect the most frequent single compounds from all analysed surface waters. Frequently detected mixtures in corn and soybean growing areas showed comparable number of compounds to our study (two to four compounds and were exclusively composed of herbicides (Belden *et al*., 2007). Mixtures with Acetochlor, Metolachlor and Atrazine dominated the most frequently detected mixtures in this study from the USA as well as in our results from the US monitoring data. Mixtures with these compounds were absent in other countries, which can be explained by to the fact that the herbicide Acetochlor is not authorized in the EU. Compounds such as Diuron, Atrazine, Simazine and Isoproturon that were often contained in frequently detected mixtures were also detected in a different climate zone.

Associations of detected compounds and mixtures with monitoring characteristics

Our results show that the number of detected pesticides and size of mixtures were correlated to the number of analysed compounds. On average, to detect one pesticide, between 5 and 20 pesticides had to be analysed (Figure 7.5-120). Due to analysis of a high number of randomly detected compounds might not be feasible during routine monitoring, a selection of compounds motivated by current use of pesticides, sales or crop-related use recommendations should be included to analysis.

The number of detected compounds and size of mixtures were not associated with the size of the upstream catchment (r = 0.06). We expected that a larger catchment size would result in a higher number of detected pesticides due to (i) higher amount of pesticide use in a larger catchment, and (ii) a typically larger variety of crops in larger catchments, associated with a higher diversity of applied pesticides. The lack of such a relationship with catchment size may be a result of dilution, i.e. that water body size also increases with catchment size and dilutes pesticide concentrations. Increasing catchment size is related to longer stream distances and consequently transport times of compounds, and increasing transport time may lead to different degradation and transformation processes, as well as partitioning into the sediment phase, which in turn decreases concentrations, and consequently detection frequency. Flow velocity (not considered in analysis due to lack of data) might be a factor in determining, in addition to the duration a compound occurs in a stream and the related dilution factor and degradation, the amount and grain size of sediments, which might influence adsorption from compounds and subsequently the detection rate of pesticides in grab samples.

In contrast to the size of the catchment upstream of the sampling site, the fraction of agricultural area was weakly correlated with the number of detected pesticides and size of mixtures in Germany (r = 0.17). Nevertheless, the number of detected pesticides increased from 3 to 7 when the agricultural area in the catchment area exceeded 20 % based on the larger area with pesticide use. Other studies in different countries found a clear footprint of agriculture in terms of effects in stream ecosystems for a higher ratio of agriculture within the catchment of 40 % in Germany and France and the USA.

Differences in pesticide detections between countries

The size of mixtures in countries differed between Germany and the Netherlands on the one hand (mean size of mixtures of 7.0 and 4.8, respectively) and USA and France on the other hand (mean mixture size of 3.2 and 3.0, respectively). These groups also differed in the number of analysed compounds per sampling

occasion. Whereas in Germany and the Netherlands over 80 compounds were analysed, in the USA and France only 30 compounds were analysed (Table 7.5-146). This stresses again, as already shown above and other studies, that a high number of analysed compounds is crucial for a representative picture of the pesticide load of streams. Even when restricting the analysis to the core group of pesticides measured in all countries, these differences prevailed, though to a lower degree. France and the USA had a mean size of mixtures of 2.5 core compounds, whereas average mixtures in Germany and the Netherlands contained 4.7 and 3.6 compounds. These differences in the size of mixtures of core compounds may be caused by differences in the LOQ between the different countries. For 52 % of all compounds, the LOQs were lowest in Germany, potentially increasing the detection frequency. The USA had the lowest LOQ for only 5 % of compounds and, presumably partly related to this, the lowest detection frequencies. The low number of core compounds detected in the USA and France compared to Germany and the Netherlands could be caused by: (i) soil properties, (ii) the slope and (iii) the distance of agricultural areas, but also by (iv) crop type.

For instance, in the USA and France legumes are grown on relatively large area (36 % and 12 %) in comparison to Germany and the Netherlands (0.5 % and 6 %) and legumes were shown to reduce runoff during rainfall events and the related pesticide input in streams by up to 95 % for full gown plants. Finally, agricultural areas in the USA are often dominated by large fields and crop monocultures (average farms of 95 ha) and compared to the other countries (average farms: France 54 ha, Germany 56 ha, the Netherlands 26 ha) a lower farm density. Based on the assumption of a lower farm density and of a homogeneous selection of pesticides within a farm, the number of different pesticides in streams could be lower due to the lower number of pesticides applied. This study provides priority pesticides and pesticide mixtures from streams of Germany, France, the Netherlands and the USA. Using these priority mixtures in ecotoxicological risk assessment could help to improve the estimation of mixture effects in aquatic ecosystems. Additionally, this study suggests that through improved routine pesticide monitoring, by increasing the number of analysed pesticides, improving analytical performance in terms of lowering LOQs and the use of alternative sampling methods to grab sampling, monitoring would provide a more realistic picture of the exposure situation and the number of detected pesticides would likely increase.

Conclusions
Pesticides in streams typically occur in mixtures of two to five compounds, in which herbicides are clearly dominating. The size of detected mixtures is influenced by the number of analysed compounds, the LOQs, but also the proportion of agriculture in the upstream catchment and the sampling method. We identify frequently detected pesticides which may inform the ecological risk assessment for stream ecosystems. Nevertheless, a comprehensive assessment of exposure to pesticide mixtures, would require a decrease of the LOQ for many compounds and widening the spectrum of compounds considered in monitoring programs.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article summarizes monitoring results of pesticides in some EU Member States and the USA. Glyphosate measurements were derived from databases of national or regional government agencies in Germany, France, the Netherlands and the USA and were reported and evaluated.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/047
Report author	Stenrød, M.
Report year	2015
Report title	Long-term trends of pesticides in Norwegian agricultural streams and potential future challenges in northern climate
Document No	Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 2015 Vol. 65, No. Supplement 2, 199–216
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities (Bioforsk)
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The objective of the present study was to identify environmental challenges of pesticide use in the northern climate by evaluating long-term pesticide monitoring data compiled by the Norwegian Agricultural Environmental Monitoring Programme. Pesticide use data and pesticide concentrations measured in stream water from six small agricultural catchments in Norway were analysed. Observed trends in pesticide detection frequencies, measured concentrations and cumulative risk from the six monitoring sites were compared. The results demonstrated the need for continued focus on the herbicides metribuzin and aclonifen, and potential concerns regarding use of the fungicide prothioconazole and the insecticide imidacloprid. The six monitoring sites represented the diversity of intensively cropped areas in Norway and differed with respect to estimated cumulative risk. Vegetable and potato cropping areas showed not only the highest level of total environmental risk, but also a statistically significant decreasing trend over the monitoring period. Cereal cropping areas exhibited no statistically significant time-dependent trends in the studied parameters but did show an increase in fungicide use that requires continued attention. The need for risk assessment of mixture toxicity effects and improved monitoring strategy is also discussed. In conclusion, the present results imply that the current global focus on multiple stressors and mixture toxicity of pesticides in stream water is equally relevant in cold climatic conditions.

Materials and Methods

Monitoring sites
Monitoring data representative of pesticide use in Norwegian agriculture are obtained through annual farmer surveys of pesticide applications in the Skuterud, Mørdre, Heia, Vasshaglona and time catchments, which cover areas dominated by production of cereals, potatoes and vegetables, as well as meadows and pastureland. Here, pesticide application data are given as total area (ha) sprayed with herbicides, fungicides and/or insecticides (not reflecting the number of applications per season) and total amount (kg) of the different groups of pesticides applied.

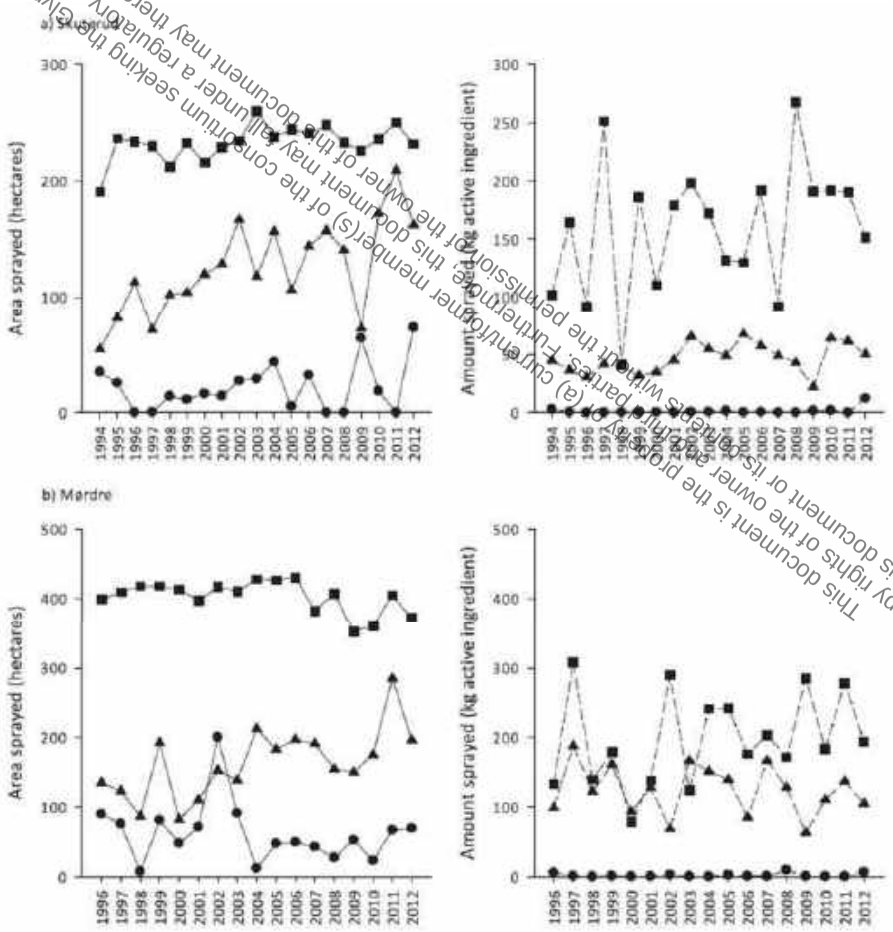
Water sampling and pesticide analyses
The water sampling is mainly by flow proportional composite sampling on average over a period of 14 days. A small water samples is taken each time a predetermined volume of water passes the monitoring station and all sub-samples are collected and stored in a glass container kept in a refrigerator. During the growing season (April–October), the water samples are analysed for pesticides.

Results

Pesticide use

The data collected in the JOVA catchments indicate considerable variation in the use of different pesticides over time (Figures 7.5-123 to 7.5-125). Herbicides have dominated in cereal production (Figure 7.5-123), but the changes in use over the monitoring period differed between the catchments. Considering the area sprayed with herbicides, there has been an increasing trend for Skuterud ($r = 0.5$, $p = 0.035$) but a decreasing trend in Mørdre ($r = -0.5$, $p = 0.037$). Also, the area sprayed with fungicides increased markedly in both Skuterud ($r = 0.7$, $p = 0.001$) and Mørdre ($r = 0.6$, $p = 0.005$). The amounts applied varied substantially between years, but no significant trend over time was detected. No statistically significant trends in insecticide use were found for the monitoring period, and application of such chemicals was generally low, although larger areas were sprayed in some years. For the catchments with agricultural production dominated by a combination of potatoes and cereals (Heia) or vegetables and potatoes (Vasshaglona; Figure 7.5-35) the area sprayed with pesticides was quite stable throughout the monitoring period, and no statistically significant time-dependent trends could be discerned.

Figure 7.5-123: Area (left panel) and amounts (right panel) of herbicides (■), fungicides (▲) and insecticides (●) applied in the JOVA catchments Skuterud (a) and Mørdre (b) throughout the monitoring period 1995–2012

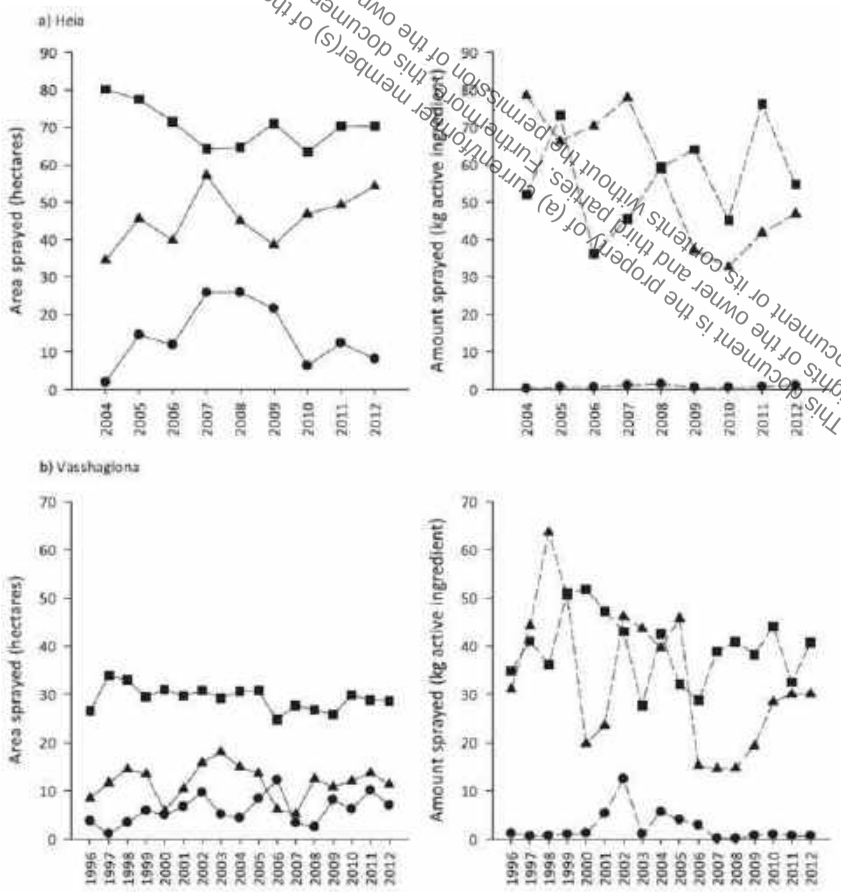


However, a statistically significant decreasing trend in amount of fungicide applied was noted for both the Heia ($r = -0.8$, $p = 0.007$) and the Vasshaglona ($r = -0.5$, $p = 0.042$) catchment. Analysis of data from the Time catchment, an area dominated by meadows and pasture, showed less use of pesticides (Figure 7.5-125) and no statistically significant time-dependent trends.

Pesticide detections

The JOVA programme has detected 61 different pesticides (including both active ingredients and metabolites) in stream water in the monitored catchments; 24 herbicides (Table 7.5-150), 25 fungicides (Table 7.5-151) and 12 insecticides (Table 7.5-152). The results indicated that although herbicides constituted 77 % of all pesticide detections, only about 9 % were at concentrations exceeding the corresponding MF values. Fungicides represented 20 % of all detections, and 6 % of those were at levels above the MF value. Relatively few insecticides were detected (only 3 % of the detections), but up to 50 % of these exceeded the MF value. In all over the 18-year monitoring period, pesticides were detected at concentrations exceeding the MF value on 408 occasions (excluding double sampling in 2004 and 2007 in the Heia catchment; Table 7.5-153). These detections gave a MEC/MF ratio ≥ 1 and are here assumed to indicate risk to aquatic organisms. Throughout the entire monitoring period and for all six catchments, a mean of two pesticides were detected in each sample analysed, and the corresponding figure for 2011 and 2012 was three pesticides per sample (data not shown). Calculation of the cumulative risk, that is, the measured concentrations of all pesticides in a sample in relation to the respective MF value, resulted in 367 samples with Σ(MEC/MF) ≥1 (Table 7.5-153). In these 367 samples, 57 different pesticides were detected, which included those with MEC higher than the MF values (Tables 7.5-149 to 7.5-151); two exceptions to this were DDT and terbuthylazine, which were at levels higher than the MF values in samples not reaching a cumulative risk score of >1.

Figure 7.5-124: **Area (left panel) and amount (right panel) of herbicides (■), fungicides (▲) and insecticides (●) applied in the JOVA catchments Heia (a) and Vasshaglona (b) throughout the monitoring period 1995–2012. The boundaries for the Heia catchment were altered in 2004, and hence, only data for the years 2004–2012 are shown**

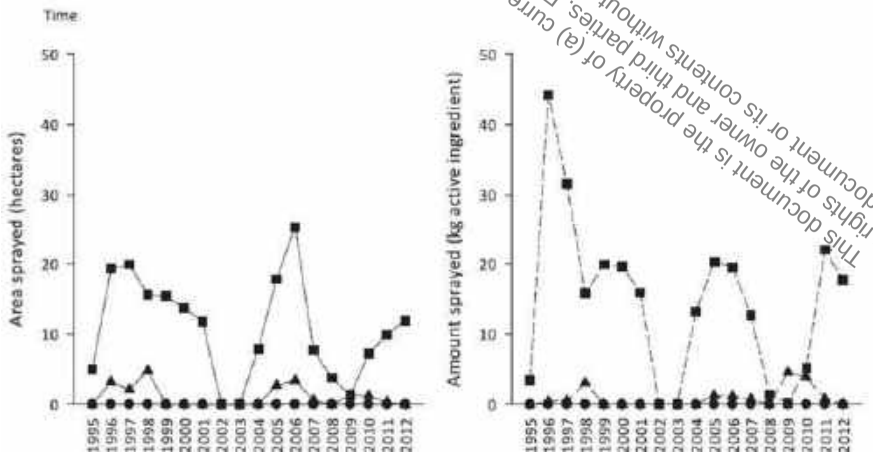


Trends in pesticide detections
Statistically significant differences ($p < 0.001$) between the monitoring sites were found for the median cumulative risk values (Table 7.5-154, Figure 7.5-126). The results indicated statistically significant differences between the Heia catchment dominated by potatoes/vegetables/cereals and those with mainly grain/fodder crops. Furthermore, there was a tendency (i.e. $p \leq 0.1$) towards differences between Heia (after 2004) and Vasshaglona catchments ($p = 0.091$) and Vasshaglona and Time catchments ($p = 0.066$; Table 7.5-154). However, no such statistically significant differences could be found for the 75th percentile values. The multiple comparisons method used assures low risk of false rejection of a H_0 hypothesis assuming equality between groups but also makes it difficult to assert statistically significant differences in the data due to the large variability and large proportion of zero values within each group.
Trend analysis on the individual monitoring sites, showed statistically significant time-dependent trends towards reduction in the Heia, Vasshaglona and Time catchments during the period 1996–2012 (Table 7.5-155). The sampling site and area monitored in the Heia catchment were changed in 2004, but the positive development seen as reduced detection frequency, measured concentrations and cumulative risk could be shown for the sampling points and areas used during both of the monitoring periods in this catchment (i.e. 1996–2004 and 2004–2012). No statistically significant time-dependent trends were evident for the Skuterud, Mørdre and Hotran catchments.

Discussion

Pesticide use
The large year-to-year variation in pesticide use observed in the JOVA catchments (Figures 7.5-123 to 7.5-125) indicates the need for long-term time series as a reference for evaluating single-year results. The trend in herbicide use increased in one of the grain crop catchments (Skuterud) but decreased in the other catchment with such crops (Mørdre), which might be explained by differences in tillage practices (ploughless tillage vs spring ploughing).

Figure 7.5-125: Area (left panel) and amount (right panel) of herbicides (■), fungicides (▲) and insecticides (●) applied in the JOVA catchment time throughout the monitoring period 1995–2012



The illustrated data clearly demonstrate the substantial variability caused by management practices and weather conditions, which in turn affects the necessity and possibility of plant protection, and the changes in use caused by approval conditions (e.g. bans, reduced recommended doses and new approvals), and pricing and taxation of plant protection products. However, the influence of these factors is not given further consideration here.

Detected pesticides and potential concerns

The substantial number and levels of pesticide detections shown by the JOVA monitoring data for the period 1995–2012 document the potential environmental concerns connected with the present practices in pesticide use in Norway. Furthermore, climate change projections indicate a forthcoming increase in use of these chemicals in the northern countries. Metribuzin, propachlor, linuron and aclonifen are systemic herbicides, which affect the photosynthesis in selected weeds in potato and vegetable production, and were among the pesticides most frequently found to exceed the MF value over the monitoring period (Table 7.5-151). Two of these compounds, metribuzin and aclonifen, are still in use.

Table 7.5-150: Detections of herbicides in rivers and streams of the JOVA catchments during the monitoring period 1995–2012, categorised according to frequency of detections exceeding the MF level (MF values for 31 December 2013)

		Detections					
Herbicide	No. of samples analysed	No.	% of samples analysed	No. of detections exceeding MF value	MF value[1] (µg/L)	Mean conc. (µg/L)	Max. conc. (µg/L)
Metribuzin	2259	462	21	249	0.058^{d}	0.17	12
Propachlor	2259	82	4	20	0.29^{c}	1.61	68
Linuron	2259	139	6	20	0.56^{c}	0.29	2.9
Aclonifen	2139	35	2	15	$0.12^{c,5}$	0.22	1.5
Isoproturon[2]	1016	21	2	3	$0.3^{c,5}$	0.11	0.45
Metamitrone	2259	122	5	3	10^{c}	1.03	42
Terbutylazine	2259	1	<1	1	0.2^{d}	–	0.09
Phenmedipham	432	9	2	1	1^{c}	0.47	2.2
Glyphosate[3]	83	74	89	0	28^{c}	0.15^{4}	4.0^{4}
Bentazone	2259	638	28	0	80^{c}	0.24	6.9
MCPA	2259	624	28	0	13^{d}	0.32	9.7
Dichlorprop	2259	299	13	0	15^{d}	0.22	10.5
Mecoprop	2259	295	13	0	44^{d}	0.12	1.8
2,6-dichlorobenzamide (BAM) (metabolite)	1550	145	9	0	$21^{b,c}$	0.04	0.6
Fluroxypyr	1982	103	5	0	$10^{b,d}$	0.18	1.5
2,4 – D	2259	78	4	0	2.2^{d}	0.10	1.1
Simazine	2259	73	3	0	$1.0^{5,c}$	0.07	0.57
Clopyralid	1546	44	3	0	71^{c}	0.26	2.4
Dicamba	1715	18	1	0	$4.5^{b,d}$	0.08	0.25
Pinoxaden	96	1	1	0	$0.91^{b,c}$	–	0.029
Pyridate metabolite	96	1	1	0	$4.93^{b,c}$	–	0.11
Chlorprofam	1546	9	<1	0	5^{c}	0.29	1.4
Flamprop	1546	1	<1	0	19^{a}	–	0.16
Atrazin	2259	2	<1	0	$0.6^{5,c}$	–	0.03
Sum herbicides		3276		309			

[1] MF value based on chronic NOEC for most sensitive test species ([a] fish, [b] invertebrate, [c] algae, [d] aquatic plant) and assessment factor according to European guidelines (EC 2011).
[2] Special analysis (fewer samples) until 2004.
[3] Special analysis, not analysed after 2001.
[4] Highest detected concentration during study of runoff event, mean for ordinary samples.
[5] MF value set equal to EQS (Directive 2013/39/EU).
[6] MF value based on acute toxicity data due to deficiencies in the available data.

These compounds currently represent the herbicides most often detected above MF levels and hence, they require continued attention. Swedish national pesticide monitoring has provided comparable results regarding these substances with concentrations measured in stream water higher than MF values in 49 % and 22 % of the detections, respectively. An environmental quality standard for aclonifen was included in the list of priority substances of the WFD in 2013 (Directive 2013/39/EU), confirming the broader relevance of apprehension regarding this herbicide. Fenpropimorph, propiconazol, prochloraz and the metabolite prothioconazole-desthio were the top four fungicides in the JOVA data with respect to detections exceeding the MF value (Table 7.5-152), and all of these compounds are currently in use. Prothioconazole-desthio is the major metabolite of a fungicide that was recently (in 2008) approved in Norway for control of Fusarium spp. in grain crops. Due to rapid degradation of the parent compound prothioconazole in the environment this metabolite which is moderately persistent in field soil, is most often encountered in stream water samples. Prothioconazole-desthio is also more toxic to aquatic organisms (especially fish) than the parent compound, which implies potential future concern in Norway.

The insecticides found at concentrations exceeding the MF value (Table 7.5-153) have mainly been used in production of vegetables, potatoes and berries. In general, insecticides are highly toxic to aquatic organisms (mainly invertebrates (Daphnia spp.) and fish) and, consequently, have very low MF values. The present results call for increased attention on measuring environmental concentrations of the fungicide

metabolite prothioconazole-desthio and the insecticide imidacloprid (included in the analysis since 2011), which were detected in a large proportion of the analysed samples and frequently at concentrations above MF (in 64 % and 44 % of the detections, respectively). By comparison, the national pesticide monitoring in Sweden detected quantifiable amounts of prothioconazole-desthio and imidacloprid in nearly 20 % of the samples that were assessed and the measured concentrations were above MF in 27 % and 8 % of the samples, respectively. These results regarding detections as percentage of samples analysed are comparable to the JOVA data, whereas the percentage above MF is considerably lower. Mesocosm studies with the invertebrate test species Chironimus riparius (EFSA 2008b) have demonstrated the potential toxicity of imidacloprid (a neonicotinoid) in the aquatic environment.

Trends in pesticide detections

Taking into account the high input of pesticides (due to production of potatoes and vegetables, which require frequent use of pesticides) Heia and Vasshaglona had the highest cumulative risk compared to the other JOVA catchments. The Time catchment, which has very little use of pesticides and a low cumulative risk, also showed a reduction in environmental load over the period, possibly chiefly due to some high concentrations of insecticides measured early in the monitoring. Notwithstanding, considering the large increase in the number of substances analysed during the monitoring period as well as a substantial lowering of the quantification limits in the analyses, an increase in environmental load could have been expected instead, especially in the catchments dominated by grain crops with increased use of pesticides. The reduction in load that was noted might have been partly due to the coverage of the analyses still being incomplete in comparison with the vast variety of plant protection products used in the JOVA catchments. It has been reported that the more comprehensive a pesticide screening is, the more reliable are the results of water quality assessments. The herbicide diquat dibromide, which is a desiccant that has been used in potatoes and other crops for several decades, is not assessed in the JOVA catchments. The environmental load caused by this long-term use should be studied to ensure that leaching and negative effects in soil are low, despite the strong sorption of diquat dibromide to soil that can lead to increased persistence and potential accumulation. The catchments dominated by grain crops (Skuterud, Mørdre and Hotran) showed no statistically significant time-dependent trends. However, this might not provide the complete picture, because several currently used fungicides were only recently (2011) included in the analyses, and the widely used glyphosate and sulfonylurea herbicides were not assessed at all.

Need for risk assessment of mixture toxicity effects

The present results on pesticide concentrations and potential cumulative risk in agricultural streams imply that although pesticide use is lower in northern European countries compared to the EU countries with more intensive agricultural practices and pesticide-demanding crops (e.g. France, Spain, Italy), there are concerns regarding residues in stream water and potential negative effects on aquatic organisms. Such effects assumedly include impacts of herbicides on growth of aquatic plants and algae, of fungicides on invertebrates (i.e. Daphnia spp.), fish and algae, and of insecticides on invertebrates (water dwelling growth stages for insects) and fish, with reference to the most sensitive test species indicated above (Tables 7.5-149 to 7.5-151). Furthermore, considering that samples from the main spraying season often contain more than 10 different pesticides, it seems that mixture toxicity should be included in the interpretation and follow-up of monitoring results.

Table 7.5-151: Detections of fungicides in rivers and streams of the JOVA catchments during the monitoring period 1995–2012, categorised according to frequency of detections exceeding the MF level (MF values for 31 December 2013)

Fungicide	No. of samples analysed	Detections		No. of detections exceeding MF value	MF value[1] (µg/L)	Mean conc. (µg/L)	Max. conc. (µg/L)
		No.	% of samples analysed				
Fenpropimorph	1982	21	1	20	0.016[a]	0.81	12
Propiconazole	2259	97	4	9	0.13[c]	0.14	7.2
Prochloraz	2139	14	<1	9	0.05[b]	0.11	0.25
Prothioconazol-desthio[2]	96	14	15	9	0.033[a]	0.11	0.53
Fluazinam	1646	23	1	2	1.2[a]	0.32	2.5
Azoxystrobin	1050	106	10	1	0.95[b]	0.11	0.5
Cyprodinil	1375	45	3	1	0.18[b]	0.07	0.29
Fenamidon[2]	96	4	4	1	0.25[b]	0.21	0.68
Carbendazim[2]	96	1	1	1	0.03[b]	–	0.039
ETU[3] (metabolite)	59	14	24	0	20[b]	0.26	3.0
Pencycuron[2]	96	12	13	0	4.96[b]	0.18	0.42
Trifloxystrobin-metabolite (CGA321113)	432	53	12	0	32[b]	0.11	0.46
Metalaxyl-m	2259	258	11	0	7.5[a]	0.12	1.62
Boscalid[2,3]	98	8	7	0	12.5[a]	0.12	0.33
Kresoxim (metabolite)	1216	69	6	0	10[a]	0.26	1.5
Iprodion	1982	67	3	0	12[a]	0.29	5.3
Mandipropamid[2]	96	3	3	0	0.76[b]	0.13	0.24
Fenhexamid	432	9	2	0	10.1[a]	0.29	1.4
Picoxystrobin	432	5	1	0	0.36[a]	0.02	0.03
Cyazofamid[2]	96	1	1	0	0.25[c]	–	0.03
Tiabendazol	2139	3	<1	0	1.2[a]	0.13	0.22
Penconazol	1715	6	<1	0	6.9[b]	0.08	0.28
Pyraclostrobin	432	2	<1	0	0.4[b]	–	0.1
Imazalil	797	2	<1	0	0.86[a]	–	0.64
Trifloxystrobin	797	2	<1	0	0.19[c]	–	0.03
Pyrimethanil	1594	4	<1	0	16[c]	0.05	0.11
Kresoxim-methyl	432	1	<1	0	0.7[c]	–	0.01
Sum fungicides		844		53			

[1]MF value based on chronic NOEC for most sensitive test species ([a]fish, [b]invertebrate, [c]algae, [d]aquatic plant, [e]mesocosm study) and assessment factor according to European guidelines (EC 2011).
[2]Detected due to expansion of chemical analysis from 2011.
[3]Two samples from Heiabekken analysed in 2010.
[4]MF value based on acute toxicity data due to deficiencies in the available data.

Table 7.5-152: Detections of insecticides in rivers and streams of the JOVA catchments during the monitoring period 1995–2012, categorised according to frequency of detections exceeding the MF level (MF values for 31 December 2013)

Insecticide	No. of samples analysed	Detections		No. of detections exceeding MF value	[1]MF value (µg/L)	Mean conc. (µg/L)	Max. conc. (µg/L)
		No.	% of samples analysed				
Chlorfenvinphos	2259	26	1	26	0.00025[2,b]	0.08	0.37
Azinphosmethyl	2139	11	<1	11	0.0034[a]	0.24	0.64
Diazinon	2259	12	<1	12	0.017[b]	0.14	0.49
Lindan	2259	33	2	5	0.08[b]	0.06	0.16
Imidacloprid[3]	98	9	9	4	0.2[b,e]	0.42	1.5
Pirimicarb	2259	21	1	4	0.09[b]	0.07	0.47
Alphacypermethrin	2139	2	<1	2	0.0001[b]	–	0.01
Dieldrin	1050	1	<1	1	0.01[5,a]	–	0.16
Esfenvalerat	1715	1	<1	1	0.0001[a]	–	0.06
DDT and metabolites	2259	1	<1	1	0.025[5,a]	–	0.06
Permethrin	2259	1	<1	1	0.0006[b]	–	0.02
Dimethoate	2259	18	<1	0	4[b]	0.17	0.75
Sum insecticides		136		68			

[1]MF value based on chronic NOEC for most sensitive test species ([a]fish, [b]invertebrate, [c]algae, [d]aquatic plant, [e]mesocosm study) and assessment factor according to European guidelines (EC 2011).
[2]MF value based on acute toxicity data due to deficiencies in the available data.
[3]Detected due to expansion of chemical analysis from 2011.
[4]Two samples from the stream Heiabekken analysed in 2010.
[5]MF value set equal to EQS (Directive 2013/39/EU).

A mixture toxicity risk evaluation of the JOVA pesticide monitoring data from 2012 suggested that single substances or simple mixtures tend to predominate in the calculated cumulative risk quotients based on the sum of MEC/PNEC ratios and the sum of toxic units for each standard test species group.

Table 7.5-153: Detections of pesticides exceeding the MF value (values for 31 December 2013) and number of stream water samples with cumulative risk ≥ 1 in Norwegian agricultural catchments monitored in the JOVA programme during the period 1995–2012

		Exceeding MF value		Cumulative risk ≥ 1	
Year	No. of samples	no. of pesticides	% of samples	no. of samples	% of samples
1995	120	28	23	22	18
1996	157	34	22	31	20
1997	208	41	20	38	18
1998	185	48	26	43	23
1999	189	33	18	33	18
2000	106	21	20	16	15
2001	123	10	8	10	8
2002	130	28	22	23	18
2003	123	24	20	22	18
2004[1]	126	30	24	29	23
2005	125	21	17	21	17
2006	120	23	19	21	18
2007[1]	120	18	15	17	14
2008	111	9	8	9	8
2009	112	13	12	13	12
2010	113	2	2	2	2
2011	42	8	19	7	17
2012	54	17	31	11	20
Sum	2264	408	–	367	–
Mean		23	18	20	16

[1]Parallel grab and composite sampling was performed in the Heia catchment; only results from composite sampling are included here.

Need for improved monitoring approaches

A continuous challenge is to ensure that the analytical methods employed are updated in relation to the plant protection products that are in use, while at the same time keeping the costs of monitoring at a minimum. Several of the most widely used pesticides are not included in the evaluations performed within the JOVA programme due to analytical and economic limitations, and this incomplete coverage affects the risk assessments based on the monitoring results. The most evident deficiencies in the JOVA analyses concerns the sulfonylurea herbicides and herbicides with glyphosate as the active ingredient. Another important challenge in monitoring of pesticide residues in surface water is being able to measure the (peak) pesticide concentrations that actually occur. The main sampling method in the JOVA catchments (i.e. flow-proportional composite sampling) involves a period of storage before analysis, and other technical aspects connected with sample pre-processing and analysis might lead to an underestimation of the pesticides present in a water sample from a given time period. For some pesticides the quantification limits of the analyses are too high to allow determination at environmentally relevant concentrations, and thus, these substances might occur at potentially harmful levels even though they are not detected through the monitoring. However, lower concentrations can be detected by using passive sampling devices rather than composite water sampling. Also, the sampling of stream water in the JOVA catchments is restricted to the spraying season (May–September), which might yield insufficient monitoring results under such cold climatic conditions. There are indications that degradation of pesticides is delayed in cold climates, which entails an elevated risk of transport during autumn, winter and spring flow events. Furthermore, research has suggested that the mobility of pesticides is increased by soil freezing and by large losses during snowmelt.

Conclusion

The main objective of the present study was to identify environmental challenges associated with use of pesticides in the northern climate by examining trends in detection frequencies, measured concentrations and cumulative risk observed in the long-term pesticide monitoring data collected in the JOVA programme. These data indicate that the environmental load of pesticides used in Norwegian agriculture has decreased in the JOVA catchments from 1995 to 2012. During this monitoring period both the frequency of detections

and pesticide concentrations in streams were reduced in areas predominantly growing heavily sprayed potato and vegetable crops, and possibly also in areas dominated by meadows and pasture and thus with lower levels of pesticide use.

Table 7.5-154: Median and 75th percentiles for the summed monthly relative detection frequency, measured concentration, and cumulative risk, for Norwegian agricultural catchments monitored in the JOVA programme

		Detection frequency*		Measured concentration**		Cumulative risk***	
Location	Time period	Median	75th percentile	Median	75th percentile	Median	75th percentile
Skuterud (N = 135)	1996–2012	0.96	1.44	0.54	1.49	0.08[ab]	0.84
Mørdre (N = 113)	1996–2012	0.92	1.43	0.51	1.19	0.04[ab]	1.19
Heia (N = 82)	1996–2004	1.02	1.42	0.52	1.24	0.30[ac]	1.03
Heia (N = 60)	2004–2012	1.03	1.28	0.60	1.39	0.75[c]	1.23
Vasshaglona (N = 135)	1996–2012	0.86	1.45	0.64	1.20	0.21[abc]	0.84
Hotran (N = 116)	1996–2012	0.89	1.48	0.32	1.13	0.09[ab]	0.65
Time (N = 102)	1996–2012	0.90	1.36	0.68	1.43	0.01[b]	0.08

Note: Test for pair wise comparisons with Scheffe's adjustment for multiplicity: not sharing the same letter indicates statistically significant difference in the quantile between test groups (i.e. location) at a 5% test level.
N, number of months with analyses of pesticides in stream water.
Quantile regression (QUANTREG procedure, SAS statistical software) for testing of equality between quantiles (i.e. Rank_Normal test performed for median and 75th percentile): *p = 0.724; **p = 0.082; ***p = 0.0001

The JOVA catchments chiefly characterised by cereal production plausibly face future challenges related to increased use of fungicides, and they showed no significant reduction in the environmental load of pesticides over the monitoring period. In general, the presence of pesticides in stream water can be explained mainly by the use of pesticides on nearby land areas and the prevailing weather conditions. Most of the pesticides detected in stream water in the JOVA catchments are currently used in Norwegian agriculture. The present results indicate that continued attention should be focused on the herbicides metribuzin and aclonifen, which were monitored throughout the period 1995–2012. Concerns are also emerging with regard to the fungicide prothioconazole (i.e. the metabolite prothioconazole-desthio) and the insecticide imidacloprid, which was more recently included in the JOVA programme, and thus these substances should be scrutinised in the coming years. In many cases, detection frequencies and concentrations of the mentioned pesticides are comparable to those noted in areas with more intensive agriculture than that performed in Norway and the Nordic countries. Pesticide use is probably lower in colder climates compared to more temperate zones, but the current results do not indicate that the environmental challenges of pesticides are at a lower level in the colder areas. It is not possible to draw broader conclusions from this study due to the following limitations: incomplete coverage of pesticides and metabolites, insufficient sampling techniques that did not consider short-term peak concentrations, and inadequate data on yearly variations in pesticide occurrence. The detection frequencies, measured concentrations and estimates of cumulative risk observed in this study imply that the current global focus on multiple stressors and mixture toxicity of pesticides in stream water is equally relevant in cold climatic conditions. This suggests that risk assessment of monitoring results and MEC should be based on a more holistic approach that includes pesticide monitoring, ecotoxicity studies of pesticide mixtures occurring in the field, and modelling strategies.

Figure 7.5-126: Summed monthly relative cumulative risk over the monitoring period shown for the six JOVA catchments

x denotes grab samples from the first sampling site [1996–2003]; + indicates samples from the 2nd [current] sampling site [2004–2012]). Month number refers to January 1994 as month number 1.

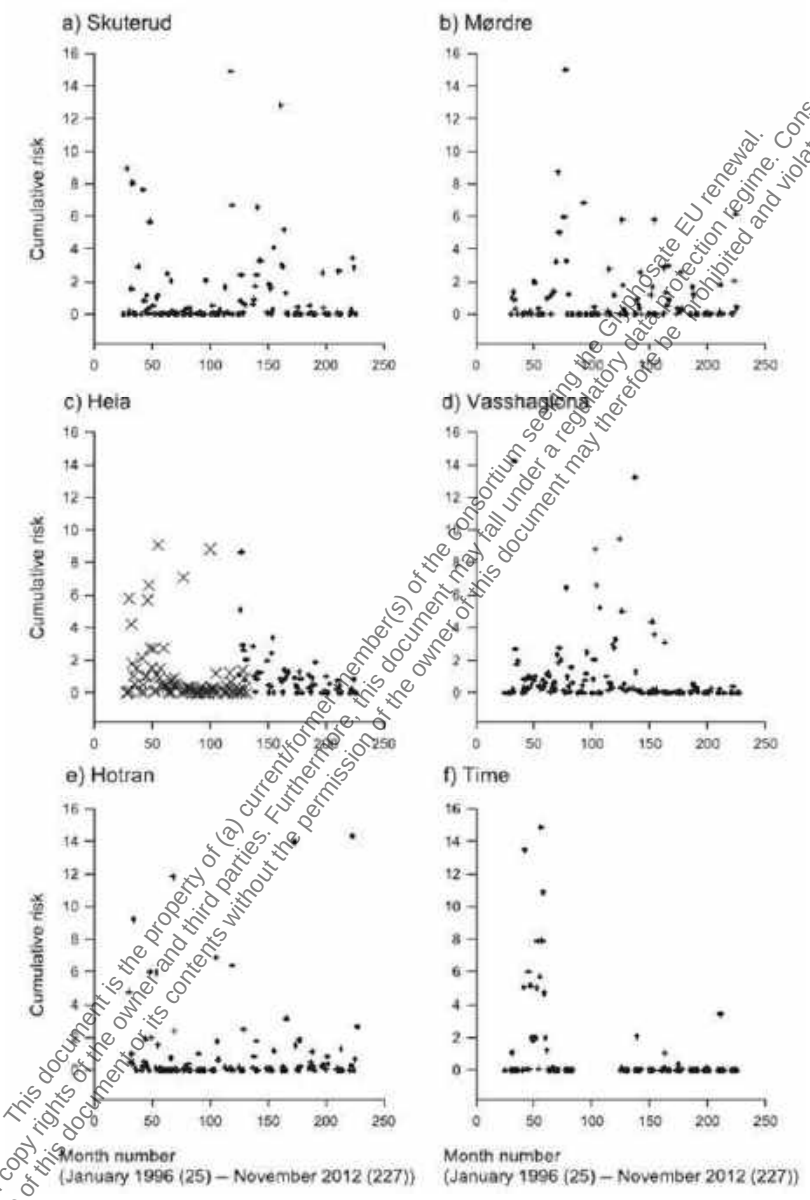


Table 7.5-155: Summary of the results of non-parametric trend analysis (Kendall's Tau) for the summed monthly relative detection frequency, concentration, and cumulative risk, for Norwegian agricultural catchments monitored in the JOVA programme

		Detection frequency		Measured concentration		Cumulative risk	
Location	Time period	Kendall's Tau	p	Kendall's Tau	p	Kendall's Tau	p
Skuterud (N = 135)	1996–2012	−0.04	0.4719	−0.05	0.3788	−0.01	0.8935
Mørdre (N = 113)	1996–2012	0.07	0.2554	0.01	0.8732	0.03	0.7420
Heia (N = 96)	1996–2004	−0.34	<0.0001	−0.35	< 0.0001	−0.29	<0.0001
Heia (N = 60)	2004–2012	−0.20	0.0273	−0.32	0.0003	−0.44	<0.0001
Vasshaglona (N = 135)	1996–2012	−0.21	0.0004	−0.11	0.0520	−0.25	<0.0001
Hotran (N = 116)	1996–2012	0.01	0.9152	0.08	0.2317	0.02	0.7770
Time (N = 102)	1996–2012	−0.19	0.0059	−0.19	0.0045	−0.29	<0.0001

N, number of months with pesticide analyses of stream water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article evaluates data from Norwegian monitoring programs for pesticides to identify trends and future challenges for the Norwegian agriculture. For glyphosate, deficiencies in the monitoring methods were reported and only few information on the active ingredient is reported. Maximum glyphosate concentration of 4 µg/L.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/048
Report author	Székács, A., *et al*.
Report year	2015
Report title	Monitoring Pesticide Residues in Surface and Ground Water in Hungary: Surveys in 1990–2015
Document No	Journal of Chemistry, Vol. 2015, 717948, 01.01.2015
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities (Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre)
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Over 2000 surface, ground and raw drinking water samples have been analyzed in the frame of different monitoring projects in Hungary and watercourses in neighboring countries between 1990 and 2015. Effects

of pesticide contamination on ecological farming and drinking water supply have been assessed. Main water pollutant ingredients of agricultural origin in Hungary are herbicides related to maize production. After EU pesticide re-registration, diazinon, atrazine, and trifluralin gradually disappeared as contaminants. High levels of water soluble pollutants (e.g., acetochlor) in surface water result in temporarily enhanced levels in raw drinking water as well. Extreme levels observed for herbicide residues were of agrochemical industrial origin.

Materials & Methods

In this work, a total of 49 pesticide residues and degradation products, belonging to different chemical classes, were monitored in Hungary. Water samples have been collected in the frame of seven monitoring projects in over twenty sampling campaigns between 1990 and 2015. Each sampling campaign had defined objectives and corresponding sampling regimes. In certain sampling campaigns, soils on cultivation fields were also sampled. Selection of target pesticides was done on the basis of their use and persistency. Determination of the selected analytes was performed using solid phase extraction (SPE) of water samples (1000x concentration factor) followed by GC-MS with or without derivatization, while determination of neonicotinoid insecticides was carried out by HPLC and glyphosate was measured by ELISA.

GC Analysis. Analytical sample preparation and GC/MS procedure was a multiresidue pesticide analysis method applied by survey authorities in Hungary and modified and validated in our laboratory. Acidic ingredients, for example, chlorophenoxy acid type herbicides, were eluted from graphitized carbon black SPE cartridges in a second fraction and were then subjected to derivatization to silyl esters using t-butyldimethylsilyl N, N-dimethylcarbamate as silylating agent and trifluoroacetic acid catalyst. GC-MS analysis was performed on a Varian Saturn 2000 workstation equipped with a Varian CP 8200 autosampler (Varian Inc., Walnut Creek, CA, USA). Quantification of the selected pesticides was performed using matrix-matched calibration. The estimated values of the limits of detection (LODs) were in the range 0.4–5.5 ng/L.

HPLC Analysis. Determinations of neonicotinoid type pesticide active ingredients were performed on Younglin YL9100 HPLC system equipped with YL9150 autosampler (Younglin Co., Anyang Korea). Compounds were separated on a C18 column (Agilent Extend-C18, 150 mm × 4.6 mm i.d., 5 μm) equipped with an Agilent Guard column (12.5 mm × 4.6 mm i.d., 5 μm) at 40 degrees. UV detector signals were recorded at λ = 252 nm and λ = 269 nm. Eluent flow rate was 1.0 mL/min during the isocratic elution until 8 minutes (70 : 30 = A : B eluents, A = 90 % water: 10 % MeOH, B = MeOH). External calibrations based on the results for standard solutions (Pestanal) were used for quantification. If low concentration ranges required, HPLC-MS/MS measurements were carried out on a Bruker AmaZon SL ion trap instrument (Bruker AXS GmbH, Karlsruhe, Germany) operated in the positive electrospray ionization mode, upon SPE preparation of samples. Retention times were 2.42 min for thiamethoxam and 3.38 min for its decomposition product, clothianidin. LOD determined with standard solutions and with UV detector lied at 10 μg/L. External calibration based on the results obtained for 12 standard solutions in the range of concentrations between 10 μg/L and 150 mg/L. Determinations obtained upon SPE (Sep-Pak C18) with standard solutions and with MS/MS detector allowed LODs of 4 ng/L for thiamethoxam and 17 ng/L for clothianidin. Calibration solutions were prepared from a stock solution by dilution with water.

ELISA. As desirable low LODs for glyphosate and AMPA were not achieved even after their labor-intensive extraction followed by derivatization prior to GC-MS analysis, for determination of glyphosate in ground and surface water, an immunoanalytical method, the commercially available ELISA method (PN 500086) by Abraxis LLC (Warminster, PA, USA), was applied. Measurements were carried out in 96-well microtiter plates according to manufacturer instructions. Comparative results with LC-MS or LC-MS/MS demonstrated the reliability of this competitive ELISA method; therefore, we have used it in our monitoring studies. The main drawback of the method is that it does not detect AMPA; therefore, due to the fast decomposition of glyphosate its environmental occurrence can be underestimated. On the other hand a comparative study has established that immunoassay overestimated glyphosate and detected a trace level in a sample deemed uncontaminated by LC-MS/MS.

Results

Nationwide Survey of Pesticide Residues in Surface Water in Hungary. A national survey (Project OMFB 02193/1999; Monitoring of pesticide residues in surface and ground water, 1999–2002) was launched together with the National Service for Plant and Soil Protection (NSPSP) to assess chemical contamination levels in water bases in Hungary, to explore the points of vulnerability, and to identify pesticide residues in surface and ground water throughout the country. An additional aim was to inspect whether chemical loads on the environment decreased due to the introduction and implementation of integrated pest management (IPM) practices and the spread of ecological (organic) agriculture and to indicate whether pesticide contamination occur as point source or diffuse contaminants. Thus, 332 surface and raw drinking water samples were collected at 90 sites in Hungary. The overall numbers of water samples collected and analyzed were 118, 119, and 95 in 2000, 2001, and 2002, respectively. Among these samples 24, 16, and 11 were tap-water samples provided by Wedeco Waterworks Hungary or collected in the region of Vác in 2000–2002, respectively. In the first year of the survey (2000) 32 % of water samples were found to be contaminated mainly by acetochlor and atrazine up to the level of 10000 ng/L and prometryn have also been found at lower concentrations (1–10 ng/L). Two point contamination sources of industrial origin were identified in the region of Balatonfüzfö and the Northern Hungarian Chemical Works (Sajoécseg). In 2001, 58 % of samples contained pesticide residues above the LODs. Earlier mentioned ingredients showed similar pattern; 36 % of samples were polluted by atrazine and among them 3 % are at concentrations above 1000 ng/L, whereas the same ratios for acetochlor were 16 % and 6 %. Thus, acetochlor occurred less frequently, but higher concentrations have been determined. Prometryn was found in 7 % of the samples at levels of 100–10000 ng/L. Among other pollutants trifluralin (10–10000 ng/L), metribuzin (100– 1000 ng/L), and terbutryn (10–1000 ng/L) were detected in 1– 3 % of samples. Although diazinon was often (36 %) found, its levels were usually low (10–100 ng/L). Regarding seasonal variation of residues it is worthy of note that one-third of samples polluted by atrazine and/or diazinon were collected prior to pesticide application, indicating persistency of these active ingredients under appropriate circumstances. The last year of the project (2002) focused on contaminated areas; therefore, 91 % of collected samples contained one or more pesticide active compound. Maximum levels for atrazine and acetochlor remained high (over 15000 ng/L and 46000 ng/L) and contamination rates for these ingredients were 44 % and 31 %, respectively. Prometryn was detected in 18 % of samples up to 1270 ng/L. Frequently found diazinon (65 %) at levels 10–100 ng/L and in 3 % of samples terbutryn (467–1671 ng/L) were determined. Regarding raw drinking water samples there was only a single case when acetochlor has been detected during the first two years. However, in the autumn of 2002, acetochlor contamination in raw drinking water was observed in the region of Vác near river Danube. Its concentration in raw drinking water occurred to be near 100 ng/L, sometimes exceeding the MRL for drinking water in the EU. To our surprise simultaneously collected surface water samples from river Danube contained similar concentration of this ingredient (80 ng/L). Acetochlor contamination of raw drinking water was also detected in Veröce (34–64 ng/L), but here the levels remained under MRL. As the contamination levels in the river were not extremely high, results indicated the pesticide content passed through bank filtration and water treatment (e.g., chlorination) and occurred at unmodified levels in tap water.

Assessment of Point Source Pesticide Contamination in Hungary.
On the basis of results obtained in the nationwide survey, regions of identified point source contamination sites were monitored (Project KvVM-KAC; Revision of pesticide active ingredients regarding environmental assessment and monitoring results, 2003). Sampling was carried out mainly near Lake Velence and in two regions of Lake Balaton (Balatonfüzfö and Tihany). This project, supported by the Hungarian Ministry of Environment and Water, was also connected to the revision of pesticides considering environmental aspects and pesticide residue monitoring data. In the region of Balatonfüzfö extensive sampling was performed (62 samples) in order to assess the extent and severity of earlier detected point source contamination of industrial origin. Additional 21 sites at Lake Balaton, 14 sites at Lake Velence, and 11 sites in Budapest and other regions were sampled. Surface and raw drinking water samples were collected at 80 sites in May and at 28 additional sites in June and August, 2003. Sampling was repeated at polluted sites in June and/or August. Thus, overall 135 surface and raw drinking water samples were analyzed during the project.

The contamination rate was found to be as high as 61 %, and in accordance with earlier results, surface water samples collected in the region of Balatonfüzfö contained high or extremely high levels of atrazine and acetochlor. Maximum concentration of atrazine was 8240 ng/L and 7540 ng/L in surface water and ground water, respectively. The corresponding values for acetochlor were found to be 13950 ng/L and 10070 ng/L, respectively. In addition, acetochlor could be measured in 56 % of the tap water samples reaching the level 1075 ng/L. Lower levels of prometryn (up to 1025 ng/L) and terbutryn (up to 605 ng/L) have also been found. The quality of effluent waters originated from the industrial site of Nitrokémia Chemicals Works was of high concern, as contaminated water bodies flow through basins and ponds into stream Séd and then reach Lake Balaton. Concentrations of atrazine and/or acetochlor in these water courses were in the range of 2000–6000 ng/L, and sometimes exceeded the level of 10000 ng/L. Additional 18 sites in the neighborhood showed higher levels for acetochlor probably due to its leaching from contaminated soil around the area of Nitrokémia Works.

Atrazine was not detected and diazinon occurred in a single case at a level of 538 ng/L. In the region of Tihany, the highest concentration was found to be 424 ng/L in surface water, 359 ng/L in Lake Balaton, and unfortunately appeared in a drinking water sample at a level of 249 ng/L (Csopak). South from the point contamination source half of samples from Lake Balaton were contaminated by acetochlor reaching the maximum concentration of 1547 ng/L, whereas 332 ng/L was measured in Channel Sió. A similar pattern was observed at Lake Velence: 316 ng/L was determined in a surface water sample, whereas high contamination rates (88 %) were observed in the lake itself with levels up to 702 ng/L and 2970 ng/L as a peak concentration. Comparing the concentrations determined in water samples collected at a certain polluted site in May, July, and September, the levels of acetochlor, terbutryn, and prometryn ingredients decreased and similar tendency have been usually observed for levels of atrazine. High levels for atrazine and acetochlor have been detected due to improper technology applied for washing pesticide containers (Papkeszi). More than half (56 %) of the raw drinking water samples collected in this polluted region near to Nitrokémia Works or above LOD. Contamination levels were in the range of 116 to 1075 ng/L.

Transnational Survey of Seasonal Pesticide Contamination in Rivers in the Carpathian Basin.
To assess the extent of pesticide contamination carried by rivers, in given cases through national frontiers (Project HUSK/0901/2.1.2/0076; Agrowater, 2011–2013), samples collected from Danube, Tisza and Vág rivers, streams, Lake Balaton, and other surface waters and some of drinking water samples were analyzed. Samples were collected in February 2011 before pesticide application along the Danube River, and the same sites from Hainburg (Austria) through Bratislava-Komarno (Slovakia) to numerous sampling points in Hungary, Mohács being the most Southern point, were revisited for repeated sampling after pesticide application during a one-month period after the middle of May. Other sites in the catchment area (Tisza, Balaton, and Vág) and tap water have also been sampled. Monitoring was conducted at eleven sampling sites along the river in the winter and at 31 sampling sites in the summer. Monitoring continued in 2012 and 2013, but sampling has been restricted to Danube River (Budapest). Sixteen surface water samples from Danube and 12 tap water samples were taken twice a week in May and June and four additional samples from Lake Velence in the middle of June in 2012. Similar sample collection from Danube has been performed in 2013, but sometimes it had to be cancelled due to flood in the middle of June. Therefore only twelve samples were analyzed in that year. All surface water samples contained traces of some pesticide residues (trifluralin, alachlor, and chlorophenoxy acids) in February indicating their slow degradation and dissipation rate. Withdrawn ingredient, alachlor, could be detected only in the winter sampling regime at low levels (0.7–10.3 ng/L). In the summer sampling regime (May-June) the ratio of surface water samples that exceeded the maximum concentration of 100 ng/L for individual pesticides was 41 %, and 18 % of samples contained total pesticide residue above 500 ng/L. Regarding the ingredients and the typical levels results were in accordance with those obtained for samples in Békés county earlier. Acetochlor was the most frequently found pollutant. It was present in all but one surface water samples collected in May and June and typically higher concentrations (75–711 ng/L) have been observed in May than in June (23– 162 ng/L). Metolachlor the second most frequently detected ingredient polluted 65 % of samples collected and levels in Danube were 31–241 ng/L. No special pattern for pollutants' concentrations could be observed along the river. Earlier often detected and banned persistent water pollutants also appeared in samples collected in May and June. Similarly to results found in 2011–2013, atrazine was detected in 13 % of samples at levels 17–40 ng/L, in addition trifluralin (25 %, 4–31 ng/L) and ethofumesate (19 %, 12–27

ng/L) also often occurred. Less frequently diazinon (16 %, 6–10 ng/L) and prometryn (10 % 7–40 ng/L) were observed.

Results in 2014 and 2015 (Project AD006; Assessment of (bio)chemical, biological main and side-effects of organic microcontaminants of agricultural origin, monitoring, and determination in environmental and biological samples, 2014–2016) showed a similar pattern seen in 2011, but acetochlor the earlier most frequently found pollutant has not been observed, in contrast to metolachlor that was present in 75 % surface water samples collected in May and June (45–365 ng/L). No special temporal variation in time for metolachlor concentrations could be observed. Atrazine could be detected in 13 % of samples at levels 17–40 ng/L, often occurred trifluralin (25 %, 4–31 ng/L) and ethofumesate (19 %, 12–27 ng/L). Less frequently were observed diazinon (13 %, 6– 10 ng/L) and prometryn (6 % 7–40 ng/L). The vast majority of surface water samples (92 %) contained neonicotinoids below LOD, while the highest concentrations (10–41 μg/L) were measured from temporary shallow water bodies after rain events in early summer. Only thiamethoxam and its decomposition product clothianidin were detected among neonicotinoids. These levels are in agreement with recent findings reported for neonicotinoids as surface water polluting contaminants.

Ecotoxicological Analysis.
Given surface water contaminants were subjected to targeted ecotoxicological analysis. Thus, special emphasis was given the combined toxicity and ecotoxicity of glyphosate and its formulating adjuvants, as well as to distribution and ecotoxic effects of neonicotinoid active ingredients. Although glyphosate presents lower acute toxicity than other herbicides, its widespread use and difficulties in detection prompts cautious assessment for combination effects as well. It has been evidenced to cause toxicity and genotoxicity in aquatic organisms and amphibians and teratogenicity in amphibians and birds and has been shown to induce endocrine disrupting effects as well, the latter effect being highly synergized by polyethoxylated tallowamine (POEA) and other commonly used formulating agents in glyphosate-based herbicide preparations. As an immediate consequence of the above toxicological and ecotoxicological concerns and as these substances have proven to be persistent under typical application conditions, glyphosate and its metabolite AMPA are required to be regularly monitored in surface and ground waters. Combinational ecotoxicological effects were proven in our hands as well, on various aquatic organisms. Moreover, adjuvant enhanced cytotoxicity has been evidenced on cell lines of animal and human origin. Our preliminary results indicate that a newly emerging pesticide class of neonicotinoids can be found in environmental water samples as well. Sporadically clothianidin was found in ponds near to maize and sunflower crops emerged from treated seeds. These compounds are used mainly as seed dressings, and the portions not uptaken by target crops contaminate the environment. They accumulate in soil and due to their good water solubility they appear in water resources. As neonicotinoids exert systemic action, the active compounds are translocated and distributed throughout the entire plant; therefore, consumption of different parts of plants (pollen, nectar) could be harmful to insects. Novel ways of intoxication for bees have also been explored, that is, water collection from guttation liquid. They appeared in potatoes and high contamination rates were reported for fruits and vegetables, as well as honey samples. Serious bee poisoning events and risk assessment of EFSA in January 2013 led the European Commission to the conclusion that a high risk for bees cannot be excluded except by imposing further restrictions for two years involving withdrawal of authorization of neonicotinoids and ban of treated seeds for different crops. The restriction applies to the use of 3 neonicotinoid active ingredients (clothianidin, imidacloprid, and thiamethoxam) for seed treatment, soil application (granules), and foliar treatment on crops attractive to bees, including certain cereals. Our findings prompted us to expand our investigations to these target compounds as well as to other polar pollutants amendable only by LC-MS analysis.

Discussion
Pesticides residues in surface waters have routinely been detected in nationwide studies. The rate of contaminated (detectable) samples ranged between 2 and 51 %. In the period of 1994–2000, the most common contaminants were atrazine (6 %), acetochlor (4 %), propisochlor (1.5 %), metolachlor (1.5 %), diazinon (1 %), and 2,4-D (1 %). Key contaminants were atrazine and to some extent isoproturon, being found in several cases at above 100000 ng/L. Results of the national survey between 1999 and 2002 and other studies on problem areas also indicated diffuse contamination of surface and ground water in Hungary. Surprisingly high contamination rate, 32–61 %, was found in monitoring projects. Two point contamination sources of industrial origin were identified in the region of Balatonfüzfö (Nitrokkfonf

Chemicals Works) and Sajóecseg (Northern Hungarian Chemical Works) connected to former pesticide producers. Atrazine and acetochlor were found in soils in Balatonfüzfö (Nitrokémia Ipartelepek) at alarmingly high concentrations reaching 10–400 ng/g; therefore, the levels of these ingredients in surface waters in surroundings, for example, in the Séd stream, exceeded the level of 10000 ng/mL. Extremely high levels were measured around Sajóecseg not only for acetochlor, but occasionally concentrations for atrazine, prometryn, and terbutryn were above 1000 ng/L in the same sample. Sometimes concentrations in soil were as high as ingredient content in formulated pesticides. At these sites due to exceedingly high residue levels phytoremediation is impossible. Point contamination source due to illegal pesticide deposit has also been explored in Gyomaendröd. Apart from these extremities typically more than half of surface and ground water samples contained one or more pesticide active ingredient. Temporal alterations of residue concentrations have been characterized by bimodal pattern. Whereas pesticide contamination in soil samples appeared to be more uniform in time, contamination rates and levels in water are time dependent. As amounts of precipitation strongly influence leaching of pesticides, levels determined depend not only on pesticide application, but also on meteorological conditions. As expected, the highest levels of pesticide pollution appeared in water samples collected in late spring and autumn campaigns but rarely occurred in waters sampled in August. Although high contamination rates have been found, but due to the improvements of analytical methods, low LODs can be achieved for most target compounds and trace levels of contaminants are detected. One of the minimum performance criteria for analytical methods applied for monitoring chemical pollutants corresponds to the limit of quantification (LOQ). According to the WFD, LOQs should be equal or less than 30 % of the relevant Environmental Quality Standards (EQSs). Legally only concentrations measured above the MRL are significant and samples containing pollution below the MRL are regarded as pesticide-free by authorities. Independently from toxicological considerations for individual ingredients, MRLs for pesticide residues in drinking water and ground water in the EU have been set to a common standard value (100 ng/L). Directive 2013/39/EU proposed maximum allowable concentrations (MAC) and annual average (AA) for levels of priority compound sand certain other pollutants in inland surface and other surface waters as EQSs. Values were set for a number of pesticides including alachlor, atrazine, simazine, diuron, isoproturon, chlorfenvinphos, chlorpyrifos, endosulfan, trifluralin, hex-achlorocyclohexanes (HCHs), DDT, aldrin, dieldrin, endrin, and isodrin. MAC values for some of detected water pollutants in Hungary are 700 ng/L and 2000 ng/L for alachlor and atrazine, respectively, but for trifluralin no MAC value is applicable. In our surveys, these levels have rarely been exceeded, only in the cases of point contaminations, where higher concentrations were determined for atrazine. In contrast to the above mentioned limits, pesticide-free means zero level of residues for the public, and it is often a source of confusion or contradiction between the authorities and civil society.

The results confirmed that ecological fields could be contaminated *via* irrigation water; therefore, it should also be monitored especially in corn cultivation regions. Although withdrawal of some water pollutants (atrazine in 2007, diazinon in 2008 and trifluralin in 2009) probably improved water quality, the use of certain water resources as irrigation water in ecological farming should/has to be restricted. As it was observed later in project MONTABIO, withdrawal of the above mentioned ingredients resulted in their gradual disappearance. Atrazine could be detected only in samples collected at Gyomaendröd in 2010, while earlier it had been detected in samples from Békéscsaba and Orosháza. Trifluralin often detected as a soil pollutant has, due to its limited water solubility, quite long dissipation time. Therefore, it could be detected in water samples in all years between 2008 and 2010. Diazinon was often found in water samples collected in 2008, not detected in 2009, but in 2010 eight ground water samples contained this insecticide. They appeared even in 2011; thus their dissipation is slow. Frequent occurrence and temporarily high levels of acetochlor, as well as metolachlor, might be related to their use instead of atrazine in Hungary. Detections of acetochlor in surface water probably contributed to its withdrawal in EU in 2012. The temporal pollution "plaques" of herbicide residues in rivers upon broad field application of herbicides pollute potential irrigation water sources and pose risk to the drinking water supply. Concentrations of acetochlor and metolachlor reported in this study are comparable to those found in the Danube River basin in Serbia (80 and 150 ng/L). In contrast to this Serbian study terbuthylazine was not detected in the surveys.

Atrazine was used predominantly as herbicide in maize monocultures in Hungary. In contrast to DDT, which was banned first in the world in Hungary in 1968, atrazine was being used up to the last date possible by derogation measures upon its ban in EU in 2007. It was often detected in the US, for example, in ground water together with other pesticide active ingredients (simazine, metolachlor, etc.). Diazinon insecticide

was also banned in 2007. Trifluralin active ingredient is banned in Hungary since 2009; acetochlor used mainly as a herbicide in maize crops was banned in 2012. Some of these compounds are on the list of the 45 priority substances. Atrazine was present at higher levels only in samples belonging to extreme point source contamination. Concentrations at these sites sometimes exceeded the values of 2000 ng/L established by the legislation as the MAC for atrazine in inland surface waters. Its levels in other water samples were far below the MRL, and upon withdrawal, its levels and occurrence frequency seem to decrease. Trifluralin, which is often detected as a water pollutant in our studies at low concentrations due to its poor water solubility, is also listed as priority substance, although with no applicable MAC value. Compared to our findings (19–70 ng/L) lower levels were reported for atrazine (<5 ng/L) from all parts of Danube in August, 2011, but its metabolite desethylatrazine could be detected at levels 5–20 ng/L with maximum levels around Budapest. Regarding chlorophenoxy acid type herbicides 2,4-D is one of the most widely used herbicides in the world and mixtures of mecoprop, dichlorprop, and MCPA are often applied. As our results indicate these compounds often occur in surface water and amounts of 2,4-D can be usually quantified (56–186 ng/L in 2011). Similar results have been reported in a study conducted in August and September, 2011, with limited number of target compounds belonging to pesticides. The highest concentrations for 2,4-D were found in the area around Budapest (~50 ng/L), whereas in the Austrian-Slovakian part of the Danube and in the downstream part lower concentrations (~20 ng/L, ~10 ng/L) were measured. Despite of the fact that glyphosate is the most frequently used herbicide in Hungary, as well as worldwide, there is little known information about its levels in the environment. Due to its fast decomposition and low detectability it is rarely measured. Regarding contamination rates and levels of glyphosate, the great contrast between sampling regimes is explained by differing agricultural locations, and, to a greater extent, catchment area characteristics, resulting in varying leaching or runoff of glyphosate to surface water. Contamination rates and levels found are strongly influenced by amounts of natural precipitation. Glyphosate contamination reported in large scale environmental water contamination studies was similar to our results. Byer *et al*. analyzed over 700 samples in Canada using an ELISA method. Concentrations were above LOD (100 ng/L) in 33 % of the samples collected in 2007, with peak values (up to 12000 ng/L) in late spring/early summer and fall. A monitoring study in Norway found frequent occurrence of glyphosate and AMPA in surface water (54 % of 540 surface water samples in 1995–1999). Monitoring in Catalonia, Spain, between 2007 and 2010, reported a 41 % contamination rate in the ground water samples analyzed. Similar findings were reported in the United States, as well as in Canada in 2004-2005 (21 % of 502 samples contained glyphosate or AMPA at very high maximum concentrations of 41 and 30 ng/mL, respectively).

Conclusion

During this period detectable pesticide residues at low concentrations occurred in alarming proportions of the surface water samples analyzed over decades. Hardly were found samples with pesticide residues below the analytical LOD, even in natural protection or recreational areas. Among monitored pesticides, the most frequently found ingredients are mainly used in maize production. High and periodic herbicide residue levels mostly reflect current herbicide usage, while low to moderate levels of certain pesticides (e.g., trifluralin) indicate a general diffuse contamination countrywide. However, high concentrations observed at point sources were not due to agricultural pesticide application but were related to the pesticide production industry. Contamination levels in ecological fields were substantially lower than that of intensively cultivated fields. However, residues are present in organic cultivation and cause exposure due to persistent organic pollutants (POPs) in soil and due to contamination of irrigation water. Occurrence of banned ingredients may indicate illegal pesticide use or slow decomposition in the given environmental matrix. Among often detected water pollutants some ingredients (atrazine, diazinon, and trifluralin) have been withdrawn in the meantime that can improve water quality. However, as the obtained results show, these compounds and their residues can still be detected in environmental matrices due to their slow degradation rate. Observed pesticide residue levels in surface waters correlate with current pesticide applications and rates. The ongoing process of pesticide reevaluation in the EU resulted in reregistration of only 27 % of the authorized pesticide active ingredients between 1995 and 2009. In turn, the range of available pesticides registered for crop and horticultural plant protection has substantially changed in Hungary after 2004 as the country became a full member of the EU. Among insecticides and acaricides, as well as fungicides and antimicrobials, numerous active ingredients have been withdrawn and replaced by new types (novel pyrethroid, neonicotinoid insecticides, triazole, and strobilurin fungicides). The most radical changes occurred among herbicides that represent over half of the pesticide market. In addition to

several thiocarbamates (EPTC, butylate), major triazines (atrazine, cyanazine, terbutryn, and prometryn) and chloroacetamides (propachlor, alachlor, propisochlor, and acetochlor) have been gradually banned. Moreover, the shrinkage in herbicide active ingredients led to the predominance of glyphosate on the herbicide market with over 30 various currently registered glyphosate-based formulations. However, on the basis of the resulting increase in environmental occurrence and exposure routes of glyphosate, as well as its recent classification in Group 2A (probably carcinogenic to humans) by the International Agency for Research on Cancer glyphosate is likely to face restrictions on its use in the near future, which will, in turn, affect its levels in environmental matrices. Certain replacement (and only later banned) compounds (e.g., acetochlor) occurred as surface water contaminants. Thus, main surface water contaminants were triazines (atrazine, propisochlor), chloroacetamides (acetochlor, metolachlor), and phenoxycarboxylic acids (2,4-D, MCPA) during the late nineties, followed by triazines (atrazine, prometryn, and diazinon) and chloroacetamides (acetochlor) after the turn of the millennium, while glyphosate and neonicotinoids are more frequently detected advancement of analytical techniques.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports monitoring results for pesticide residues in surface and groundwater in Hungary. For Glyphosate a specific analytical method was used as with the methods used for other substances, no reliable LOD's were achieved. Only limited information on the results for glyphosate were reported. A maximum glyphosate concentration of 1 µg/L was reported.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/049
Report author	Tang, T. *et al.*
Report year	2015
Report title	Quantification and characterization of glyphosate use and loss in a residential area
Document No	Science of the Total Environment 517 (2015) 207–214
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Urban runoff can be a significant source of pesticides in urban streams. However, quantification of this source has been difficult because pesticide use by urban residents (e.g., on pavements or in gardens) is often unknown, particularly at the scale of a residential catchment. Proper quantification and characterization of pesticide loss via urban runoff requires sound information on the use and occurrence of pesticides at hydrologically-relevant spatial scales, involving various hydrological conditions. A monitoring study in a residential area (9.5 ha, Flanders, Belgium) was conducted to investigate the use and loss of a widely-used herbicide (glyphosate) and its major degradation product (aminomethylphosphonic acid, AMPA). The

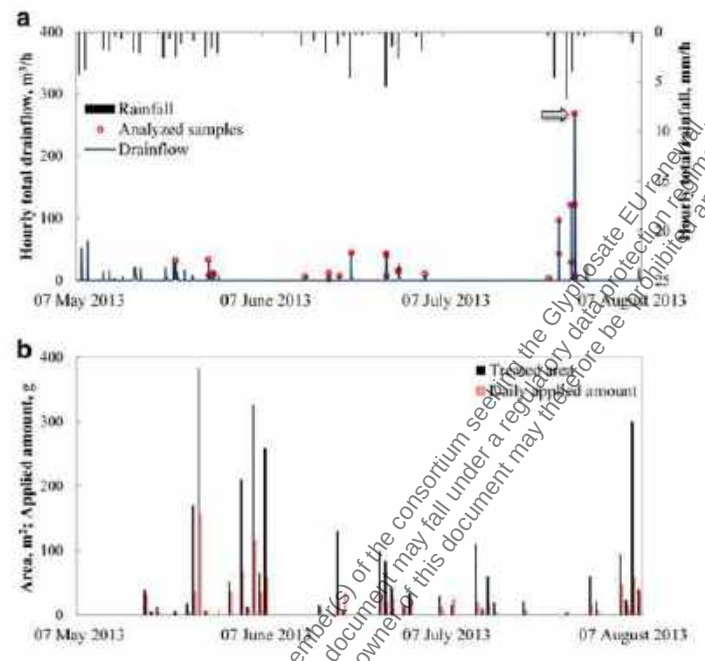
study covered 13 rainfall events over 67 days. Overall, less than 0.5 % of glyphosate applied was recovered from the storm drain outflow in the catchment. Maximum detected concentrations were 6.1 µg/L and 5.8 µg/L for glyphosate and AMPA, respectively, both of which were below the predicted no-effect concentration for surface water proposed by the Flemish environmental agency (10 µg/L), but were above the EU drinking water standard (0.1 µg/L). The measured concentrations and percentage loss rates could be attributed partially to the strong sorption capacity of glyphosate and low runoff potential in the study area. However, glyphosate loss varied considerably among rainfall events and event load of glyphosate mass was mainly controlled by rainfall amount, according to further statistical analyses. To obtain urban pesticide management insights, robust tools are required to investigate the loss and occurrence of pesticides influenced by various factors, particularly the hydrological and spatial factors.

Materials and Methods

Site description
The residential area was situated in the municipality of Meerhout, northern Belgium (Flemish region). The area was deliberately selected such that it had a separate storm drain system, had no glyphosate inputs from agriculture, industry or government authorities, and represented a typical Belgian residential coverage by non-hard and hard surfaces. Government authorities confirmed that herbicides were not applied by the municipality before and during the study period in the area.

Survey on glyphosate use
The survey was conducted by visits to all the households and questionnaires, between mid-May and early August. 100 households (89 %) were interviewed about their general pesticide usage behaviors and asked to participate in the study on 15–18 May 2013. Households, who agreed to participate, were given a questionnaire to record their glyphosate use. The recorded information included date and hour of application; type of surface, surface material and weed level; treated area and treatment methods (spot or areal treatment). Meanwhile, upon their requests, participants were supplied with commercial glyphosate products of either 1 L (Roundup Spray, 8069G/B) or 5 L (Roundup Spray Pump'N Go, 10100G/B). Bottles of the products were weighed before being supplied to and after collection from the participants to determine the total amount used by each household during the study.

Figure 7.5-127: **(a) Rainfall, drainflow and analyzed samples during the study, with indication of the peak discharge (grey arrow, hourly total: 286 m^3/h; minute measurement: 9.1 m^3/min) of the period, and (b) daily treated area (m^2) and daily applied amount (g) of glyphosate, indicating maximum daily treated area and maximum applied amount (gray bar, 382.5 m^2 with 157.4 g)**



Water sampling and chemical analysis

Rainfall was recorded by an ISCO 674 rain gauge that was installed near the outlet of the storm drain. Discharge was measured by an ISCO 750 area velocity module at the outlet. Both rainfall and discharge were recorded at 1-minute intervals, between 7 May and 7 August 2013. Samples were analyzed using liquid chromatography and tandem mass spectrometry (LC–MS/MS), after the standard pretreatment procedure, which include filtration, derivatization and extraction. The limits of quantification (LOQ) were 0.1 µg/L for both glyphosate and AMPA. Stability tests were carried out by spiking water samples from the drain outflow with two glyphosate additions (1 µg/L and 36 µg/L). For feasibility reasons, a selected number of samples were analyzed (Figure 7.5-127a), including 23 event samples from13 rainfall events, 1 background sample and 1 dry-weather sample.

Table 7.5-156: Influencing factors that were statistically analyzed against the concentration and load of glyphosate

Type of factors	Factors	Definition/method of calculation
Factors related to application	Antecedent applied amount	The total amount applied by all residents before a given rainfall event and after the previous event
	Cumulative applied amount	The total applied amount since the start of load calculation at a given time
	Weighted residence time	Average duration between the start of rainfall and the time of all applications before a given rainfall event, weighted by the amount of each application
	Application rate	Antecedent applied amount divided by the total treated area before a given rainfall event
Hydrological factors	Rainfall amount	Depth of rainfall in a given rainfall event
	Antecedent dry period	The duration between the given and its previous rainfall event
	Rainfall intensity	Rainfall amount divided by the rainfall duration in a given event

Data analysis

The mass of glyphosate recovered from the outflow during a given event, referred as event load, was calculated from the measured discharge and concentrations in a time-weighted manner. The event loss rate of glyphosate was subsequently calculated as event load divided by the applied amount of glyphosate between the given and its antecedent rainfall event. For the whole period, the overall loss rate was calculated as total load divided by the total applied amount. The load calculations were done for a period of 67 days between the first (23 May) and last (28 July) event with sample analysis. Glyphosate concentration and event load were analyzed statistically to identify their relationships with a set of predefined controlling factors. The predefined factors mainly included hydrological factors and the pattern of glyphosate use (Table 7.5-156). The dependent variables included glyphosate event load, concentrations of glyphosate and AMPA for the first two analyzed samples from the 13 rainfall events, and the event mean concentration (EMC, event load/event discharge). Via Microsoft Excel 2010, two statistical approaches were applied, bivariate analysis and multivariate analysis, using the correlation analysis and regression analysis, respectively. Linear relationships were assumed during initial bivariate analyses.

Table 7.5-157: Treated area by surface material, as defined in the questionnaire

Type of surface materials	Applied amount		Treated area	
	g	%	m^2	%
Asphalt or tile with cemented jointing	60	5.8%	112	4.0%
Concrete (or clay) slab with sand jointing	654	63.1%	1892	67.6%
Cobblestone with sand jointing	96	9.3%	241	8.6%
Gravel, stone	173	16.7%	425	15.2%
Path on bare soil	9	0.8%	13	0.5%
Vegetated soil	34	3.3%	75	2.7%
Not classified	11	1.0%	40	1.4%
Total	1036	100%	2798	100%

Results and discussion

Hydrology

Field evidence shows no direct runoff flowed to the receiving stream, other than discharge from the storm drain system. The 3-month total discharge corresponds to 12.7 % of the total rainfall volume from the study

area, with event transfer rates (= event discharge/event rainfall volume) of 6.7 %–23.6 %. According to the bivariate analyses, the event transfer rate was most significantly affected by rainfall amount, followed by rainfall intensity (Table 7.5-158).

Glyphosate use, detection and loss

Glyphosate use

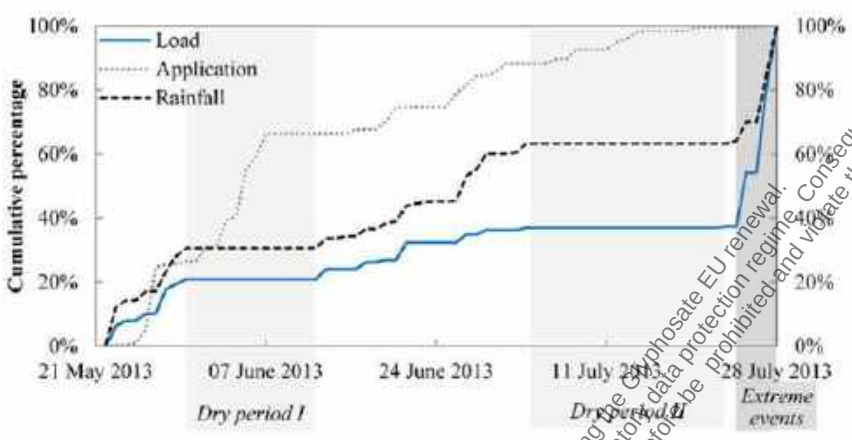
The use by local residents was likely the only source of glyphosate in the study area during the study, based on our surveys. 57 households (51 %) participated and 50 households (45 %) used the products during the study, though interviews during the first survey showed that only 36 households (32 %) used herbicides and 21 households (19 %) used glyphosate-containing herbicides on an annual basis. It was therefore assumed that the remaining 49 % of households did not use glyphosate during the study. The 45 % of households who applied the products was somewhat high, compared with typical residential outdoor use of weed killers. The high percentage in the study was ascribed to the participation of some residents (22 households, 20 %) who do not commonly use weed killers due to the provision of free products, and the high number of retired residents (53 %) in the study area, which was not the case in generic surveys cited above. Consequently, the 45 % herein represents a worst-case scenario for Belgian residential use, though no national data is available for further comparison. As expected, glyphosate was mainly applied on hard surfaces, such as driveways and paths in gardens, and two-thirds of the treated surfaces were concrete slabs (67.6 %, Table 7.5-157). During the study, total treated area amounted to approximately 2798 m^2 (Figure 7.5-127b), with a total amount of 1.04 kg. The maximum daily amount (157.4 g, Figure 7.5-127b) was contributed by 8 households on 27 May. 74 % of this amount was applied by 3 households, two of which were located close to the drain outlet. Application on this day resulted in the highest glyphosate concentration during the study on the next day (28 May).

Detection of glyphosate and AMPA

The background sample had concentrations of 0.3 µg/L for both glyphosate and AMPA, indicating that glyphosate had likely been applied in 2013 before the study started. However, the glyphosate residues, due to applications before the study started, had limited influence on the detection of glyphosate and AMPA, and the estimation of load and loss rate. According to previous studies, the majority of glyphosate loss takes place within the first 5 mm of rainfall after application and repeated rainfall events further reduce the available residues. While within the 10 days before the survey started, the study area had 4 rainfall events with rainfall amount > 5 mm and another 4 events with rainfall amount between 1 mm and 5 mm (Figure 7.5-127a). These rainfall events can wash off most of the available residues either on landscapes or in the storm drain system. Both glyphosate and AMPA were detected in all analyzed samples, with maximum concentrations of 6.1 µg/L and 5.8 µg/L, respectively. The maximum glyphosate concentration resulted from the large quantity applied (157.4 g) on the previous day and the short distance of the major application sites to the drain outlet, as mentioned above.

The concentrations described here agree with those found in more urbanized catchments, though glyphosate concentration varies by orders of magnitude among different catchments. All measured concentrations in this study were below the predicted no-effect concentration (PNEC, 10 µg/L) for surface water proposed by the Flemish environmental agency, but higher than the EU drinking water standard for individual pesticides (0.1 µg/L). Glyphosate from this residential area is hence likely to have low ecotoxicological significance. An additional sample was automatically taken at the end of the first dry period (Figure 7.5-128), probably related to runoff from irrigation in gardens or from car wash. The sample had glyphosate and AMPA concentration of 3 µg/L and 16 µg/L, respectively. The high concentration of AMPA can be ascribed to the long residence time (19 days), compared with event samples. Because AMPA degrades reportedly slower than glyphosate, it accumulated on hard surfaces or in drainage standing water and resulted in high AMPA levels. Meanwhile, AMPA may have originated from other sources, such as car wash detergents. Car wash detergents may contain phosphonate-containing compounds as chelating agents and AMPA is a key metabolite of such compounds.

Figure 7.5-128: The evolution of cumulative load primarily follows that of the cumulative rainfall, and extreme events after dry period II (dark gray) significantly contributes to the total load of glyphosate



Loads and loss rates

The total glyphosate load from the 14 events (including load from dry-weather discharge) amounted to approximately 3.7 g, 0.45 % of the applied amount of glyphosate during the 67 days. Nevertheless, the 3 unanalyzed events also carried glyphosate load and should be considered. Assuming glyphosate concentration therein equaled to the mean measured concentration of all samples (2.32 µg/L), total glyphosate load was 3.9 g, 0.48 % of the applied amount. Furthermore, accounting for the loads of both glyphosate and AMPA, loss rates were 0.79 % and 0.84 %, without and with the inclusion of the unanalyzed events, respectively. Therefore, the overall loss of glyphosate alone is expected to be lower than 0.5 %, while including loads from both compounds resulted in a loss rate of less than 1 %. Nevertheless, including both compounds can overestimate the total loss, due to other sources of AMPA.

Glyphosate loads and temporal dynamics

As shown in Figure 7.5-128, the evolution pattern of the cumulative load (as fractions, including load from unanalyzed events) resembled that of cumulative rainfall, but was disproportionate to that of cumulative applied amount, reflecting the dominant influence of rainfall on glyphosate fluxes. Moreover, the cumulative percentage of load was constantly lower than that of rainfall and applied amount, resulting from the substantial contribution of loads by relatively heavy rainfall events after dry period II (dark grey, Figure 7.5-128). The 4 days had one-third of the total rainfall of the 67 days, including the two heaviest rainfall events. This rainfall resulted in two-thirds of the glyphosate load of the period, despite that the antecedent applied amount was very limited. The result implies that a high proportion of retained glyphosate can be washed off from the applied sites during heavy rainfall events. Considering that more than 90 % of the treated areas were hard surfaces (Table 7.5-157), this highlights the importance of heavy rainfall events in glyphosate loss from hard surfaces.

Table 7.5-158: Levels of significance by bivariate correlation analysis of the factors influencing the concentration, event load and loss rate of glyphosate

Dependent variables \ Factors		Source-dependence: factors related to application				Driving-force-dependence: hydrological factors			
		Weighted residence time	Application rate	Antecedent applied amount	Cumulative applied amount	Rainfall amount	Antecedent dry period	Rainfall intensity	Water transfer rate[a]
Water transfer rate						**		*	
Concentration	EMC_Glyph[b]	*		*			×		
	EMC_AMPA[c]	*		×	×	×		*	
	Glyph_1[d]	*		**			*		
	AMPA_1[e]	*			*		×	*	
	Glyph_2								
	AMPA_2							×	
Load	Event load				×	**		*	***
	Cumulative load		×		**	**		×	***
Event loss rate			×			***			*

***: p ≤ 0.001 (strong significance).
**: 0.001 < p ≤ 0.01 (moderate significance).
*: 0.01 < p ≤ 0.05 (weak significance).
×: 0.05 < p ≤ 0.1 (very weak significance).
Gray cells: p > 0.1 (no significance).
a: Water transfer rate is not considered in multivariate analyses, because it is not an independent factor.
b: EMC_Glyph: Event mean concentrations of glyphosate.
c: EMC_AMPA: Event mean concentrations of AMPA.
d: Glyph_n: glyphosate concentration in the n[th] analyzed sample in a given event.
e: AMPA_n: AMPA concentration in the n[th] analyzed sample in a given event.

Loss rates

Since we did not consider the glyphosate residues before the antecedent rainfall event, the event loss rates calculated in our study are expectedly higher than the actual rates and considered worst-case scenarios. Event loss rates ranged from 0.04 % to 23.36 %, though the overall loss rate was below 0.5 %. The widely-varying rates confirm the need to cover a wide range of rainfall events to better estimate and characterize glyphosate loss. Notably, the percentage loss referred herein resulted from glyphosate attenuation and retention not only on land surfaces, but also in the storm drain system. The overall loss rate (<0.5 %) agrees with previous glyphosate studies in urban catchments, but is considerably lower than direct loss from hard surfaces or roadsides. Many reasons can explain the low loss rate in this study and other field studies. In this study, major reasons included the relatively high percentage of permeable surfaces, high fraction of concrete blocks as application sites, and strong adsorption of glyphosate onto concrete and deposits. In this study, more than 90 % of treated areas were hard surfaces, such as sidewalks and driveways (Table 7.5-157). First, some hard surfaces are connected to permeable surfaces, such as front gardens. Glyphosate runoff can be routed to permeable surfaces, retained and infiltrated thereon, as confirmed by the low water transfer rate. Second, the majority of treated surfaces (63 %) are made of small concrete slabs with sand jointing.

Concentration of glyphosate and AMPA

Based on the bivariate analyses, glyphosate concentrations, particularly those of the 1[st] analyzed samples and EMCs, were mainly influenced by antecedent applied amount, weighted residence time and antecedent dry period (with decreasing level of significance), while AMPA concentrations were mainly influenced by rainfall intensity, weighted residence time and cumulative applied amount. The discrepancy reflects the wash-off behavior of glyphosate is probably different from that of AMPA. The former depends mainly on the glyphosate availability, determined by the applied amount and site dissipation as a function of residence time and field conditions. Whereas for AMPA, the strong influence of rainfall intensity implies that AMPA wash-off is associated with wash-off of particulates. Overall, significance levels of the correlations range from weak to moderate. Multivariate analyses of the factors gave unsatisfactory results with model significance $p > 0.01$ and variable significance $p > 0.1$ in all tests ($N = 10$, 7 tests in total, details not shown). Therefore, no dominating factors can be attributed to the concentration variation of glyphosate and AMPA from our dataset. The unsatisfactory predictions indicate that these factors cannot sufficiently explain the

concentration dynamics. One important unaccounted factor is the spatial properties of the catchment and application sites, which influenced the hydrological behaviors and the complex interactions among glyphosate, surface runoff and treated surfaces. Therefore, more spatially-explicit considerations are needed for the catchment properties, particularly materials of the treated surfaces and their connectivity to the storm drain inlets or streams.

Event load and loss rate

According to the bivariate analyses, event glyphosate load is mainly influenced by the rainfall amount, intensity and is weakly influenced by cumulative applied amount (Table 7.5-158). The three factors can explain 70 % of the variations in the event load ($p = 0.003$, N=13), according to the multivariate analyses. The resulting regression model confirms the significant influence of rainfall amount ($p = 0.002$), but rejects that of rainfall intensity ($p = 0.11$) and cumulative applied amount ($p = 0.42$). There have been no reported studies in which factors contributing to herbicide loss were directly investigated under field conditions. Notably, the regression model is site-specific and the statistical significance should be carefully considered due to the uncertainties originated from load estimation, quantification of the factors and the limited number of events in the statistical analysis. Additionally, a strong correlation ($p < 0.005$) between loss rate and rainfall amount was observed, underlining again the hydrological dominance on glyphosate loss.

Conclusions

This study investigated the use and loss of glyphosate from a typical Belgian residential area, aiming to realistically quantify glyphosate loss via surface runoff related to use by local residents and to investigate the controlling factors. The study covered 13 rainfall events of various amounts and intensities, and provided a representative quantification of glyphosate loss. It is among the few studies that have quantified pesticide loss from residential catchments over a relatively long period (67 days) and addressed the influencing factors under field conditions. Despite that a high number of households used glyphosate during the study, glyphosate was found at concentrations below the surface water PNEC proposed by VMM. The overall loss rate of glyphosate (<0.5 %) was substantially lower than loss from hard surfaces at laboratory and plot scales. However, glyphosate load and loss rate varied considerably among rainfall events. The overall low loss is related partially to the high fraction of permeable surfaces and concrete slabs being the main treated surfaces in the study area, whereas variations in event load and loss rate are predominantly determined by rainfall (amount and intensity). Additionally, multivariate analyses suggested that rainfall and application cannot adequately explain the concentration variations. This promotes the need to account for other important factors, such as the surface material and connectivity to the drain inlets of the application sites. For robust analyses of different factors or to obtain management insights, a spatially-distributed hydrological model is beneficial to account for the spatial properties and urban hydrology. To develop such tools, in-depth understanding of pesticide behaviors in urban environments is needed, including how pesticides interact with different surface materials (e.g., asphalt and concrete), and to what extent adsorption and desorption take place and allows for residue wash-off.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes a modelling exercise to quantify and characterize the loss of glyphosate in a residential area to surface waters in Belgium. Overall, less than 0.5 % of applied glyphosate was recovered from the storm drain outflow. Maximum detected concentrations were 6.1 µg/L and 5.8 µg/L for glyphosate and AMPA, respectively. The authors concluded that measured concentrations and percentage loss rates could be attributed partially to the strong sorption capacity of glyphosate and low runoff potential in the study area.

The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/050
Report author	Gasperi, J. *et al.*
Report year	2014
Report title	Micropollutants in urban stormwater: occurrence, concentrations, and atmospheric contributions for a wide range of contaminants in three French sites
Document No	Environmental Science and Pollution Research (2014) 21:5267-5281
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

This study is aimed at: (a) providing information on the occurrence and concentration ranges in urban stormwater for a wide array of pollutants (n = 77); (b) assessing whether despite the differences between various catchment areas (land use, climatic conditions, etc.), the trends in terms of contamination level are similar; and (e) analyzing the contribution of total atmospheric fallout (TAF) with respect to sources endogenous to this contamination. The studied contaminants include conventional stormwater contaminants (polycyclic aromatic hydrocarbons (PAHs), Zn, Cu, Pb, etc.), in addition to poorly or undocumented pollutants such as nonylphenol and oetylphenol ethoxylates (NPnEO and OPnEO), bisphenol A (BPA), polybrominated diphenyl ethers (PBDEs), a wide variety of pesticides, and various metals of relevance (As, Ti, Sr, V). Sampling and analysis were performed using homogeneous methods on three urban sites with different land use patterns located in three distinct French towns. For many of these pollutants, the results do not allow highlighting a significant difference in stornmwater quality at the scale of the three urban sites considered. Significant differences were, however observed for several metals (As, Cr, Cu, Ni, Sr and Zn), PAHs, and PBDEs, though this assessment would need to be confirmed by further experiments. The pollutant distributions between dissolved and particulate phases were found to be similar across the three experimental sites, thus suggesting no site dependence. Lastly, the contributions of TAF to stormwater contamination for micropollutants were quite low. This finding held true not only for PAHs, as previously demonstrated in the literature, but also for a broader range of molecules such as BPA, NPnEO, OPnEO and PBDEs, whose high local production is correlated with the leaching of urban surfaces, buildings, and vehicles.

Materials and Methods

Site description and sampling procedure

Three urban catchment areas, one on each observatory, were considered in this study, i.e., Sucy in Paris, Pin See in Nantes, and Chassieu near Lyon. These areas are all drained by conventional separate storm sewers (Table 7.5-159). They range from 30 to 228 ha, and their impervious surface coefficients vary between 27 % (Sucy) and 75 % (Chassieu). Heavy traffic loads are reported in Sucy. On each site, total atmospheric fallout (TAF) and stormwater at the catchment outlet were simultaneously collected. Depending on the site, between seven and 24 events were sampled (Table 7.5-160).

Table 7.5-159: Urban catchment characteristics and description

Site	Location	Area (ha)	ISC (%)	Land use	Rain events	
					Total[a]	Compound[b]
Sucy	Southeastern Paris	228	21	Residential	24	4–14
Pin Sec	Northeastern Nantes	30	49	Single- and multi-family dwellings	18	9–14
Chassieu	Eastern Lyon	185	75	Industrial	7	2–5

ISC impervious surface coefficient (in percent)
[a] Number of rain events collected on the site
[b] Number of rain events for a group of compounds

Sampling was conducted over a 23- month period (from July 2011 to May 2013). Due to the large volumes required for these analyses (more than 20 1 for all pollutants in order to obtain suitable TSS masses for the particulate phase), it was not possible to analyze all contaminants during each single rain event. Two sampling configurations were thus deployed on each site: one for APnEO, polybrominated diphenyl ether (PBDE), and pesticide monitoring and another configuration for PAH, glyphosate, AMPA, and metal monitoring. Hence, between two and 14 events were sampled for a given family of compounds on a given catchment (Table 7.5-159). The number of rain events considered for each family and each catchment is listed in the individual result tables. The main characteristics of these events, including precipitation depth (H, in millimeters), mean intensity over the rain event and maximum 5-min intensity (I_{mean} and I_{max}, in millimeters per hour), and preceding dry weather period (PDWP; in days), are shown in Table 7.5-160. These rain events feature relatively low rainfall intensities, with no extreme rainfall amounts collected. Precipitation depth (from 1.2 to 50 mm) and duration (00:35 to 60:35) both cover wide ranges. To avoid contamination or sorption, TAF was collected in a 1-m² stainless steel collector for organic pollutants and two 0.5-m² plastic collectors for metals and glyphosate. TAF values were measured for the period spanning the studied rain event and the preceding dry weather period. Atmospheric collectors were set up on rooftops at two sites and/or away from potential local sources, such as heavy road traffic, on all three sites. At the catchment outlet, stormwater was sampled using automatic samplers equipped with Teflon® pipes and plastic or glass bottles; samples were then controlled through a flowmeter in order to derive flow proportional EMC.

Table 7.5-160: Rain event characteristics on the three study sites (min-max and median values)

	H[a] (mm)	Duration (hh:mm)	I_{mean} (mm h^{-1})	I_{max}[b] (mm h^{-1})	PDWP[c] (day)
Sucy (n=24)	1.2–50.0	00:35–26:20	0.43–3.77	2.4–24	0.17–9.18
	5.45	06:52	1.48	7.92	2.1
Pin Sec (n=18)	1.2–39.9	02:40–60:35	Not estimated	2.4–28.8	0.19–22.29
	15.1	19:14	–	11.4	2.60
Chassieu (n=7)	2.4–50.0	03:07–31:38	0.8–1.7	4.7–22.7	0.2–9.8
	18.8	14:31	1.2	12.2	2.8

Min–max values, as well as median values
[a] Depth of precipitation
[b] I_{max} evaluated over 5-min intervals
[c] *PDWP* preceding dry weather period

Conventional water quality parameters and pollutants analyzed

Conventional water quality parameters, such as total suspended solids (TSS) and total dissolved and particulate organic carbon (TOC, DOC, and POC), were analyzed for each rain event collected in terms ofTAF and stormwater. A total of 77 pollutants were monitored, including 14 metals, 30 pesticides, 16 PARs, nine PBDEs, bisphenol A (BPA), and seven alkylphenols (APnEO, including nonylphenol (NP) and

nonylphenol mono- (NP1EO) and diethoxylates (NP2EO), octylphenol (OP) and octylphenol mono- (OP1EO) and diethoxylates (OP2EO), and nonylphenol acetic acid (NP1EC)). Table 7.5-161 provides the full list of targeted molecules, the analytical methods employed, and the usual abbreviations. All compounds were analyzed over both the dissolved and particulate phases in order to evaluate their potential for transfer and further treatment processes. For all organic compounds, the dissolved and particulate phases were analyzed separately and not deduced from the total and dissolved phases because separate extraction of the two phases was found to be essential for an accurate quantification of contaminant levels.

Table 7.5-161: Pollutants analyzed and analytical methods

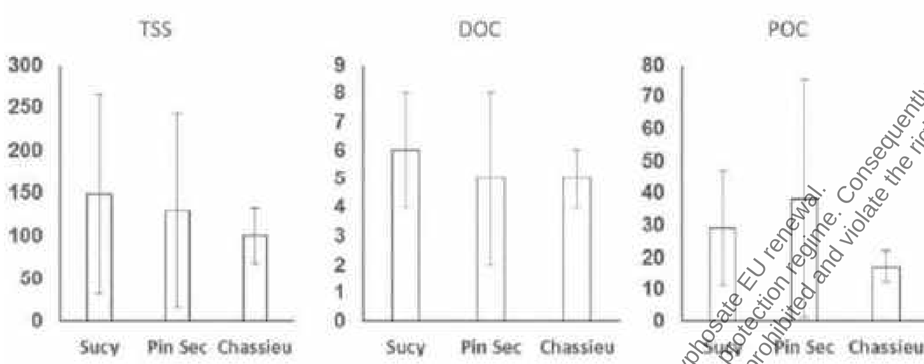
Groups (n=77)	Methods[a]	Substances and abbreviations
Metals (n=14)	ICP–MS ICP–AES	Arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), platinum (Pt), vanadium (V), cobalt (Co), molybdenum (Mo), strontium (Sr), barium (Ba), titan (Ti)
PAHs (n=16)	GC–Tof	Naphthalene (N), acenaphthylene (Acyl), acenaphthene (Acen), fluorene (F), phenanthrene (P), anthracene (A), fluoranthene (Fluo), pyrene (Pyr), benzo(*a*)anthracene (BaA), chrysene (Chry), benzo(*b*)fluoranthene (BbF), benzo(*k*)fluoranthene (BkF), benzo(*a*)pyrene (BaP), indeno(*cd*)pyrene (IP), dibenzo(*ah*)anthracene (DahA), benzo(*ghi*)perylene (BPer)
Pesticides (n=30)	GC–MS LC–MSMS LC–Fluo	Metaldehyde, glyphosate, amino methyl phosphonic acid (AMPA), glufosinate, chlorfenviphos, diuron, endosulfan A, folpel, isoproturon, aldrin, dieldrin, isodrin, mecoprop, 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methyl phenoxy acetic acid (2,4-MCPA), triclopyr, carbendazim, isothiazolinone, irgarol 1051, terbutryn, acetochlor, metolachlor, pendimethalin, epoxiconazole, tebuconazole, fenpropidine, chlorothalonil, metazachlor, diflufenicanil, deltamethrine
PBDEs (n=9)	GC–MS	BDE-28 [tri], BDE-47 [tetra], BDE-99 [penta], BDE-100 [penta], BDE-153 [hexa], BDE-154 [hexa], BDE-183 [hepta], BDE-205 [octa], BDE-209 [deca]
Bisphenol A and APnEOs (n=1+7)	LC–MSMS	Bisphenol A (BPA), nonylphenol (NP), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), nonylphenol monocarboxylate (NP1EC), 4-tert-octylphenol (OP), octylphenol monoethoxylate (OP1EO), octylphenol diethoxylate (OP2EO)

ICP–MS inductively coupled plasma with a mass spectrometer, *ICP–AES* inductively coupled plasma with atomic emission spectroscopy, *GC–MS* gas chromatography with a mass spectrometer, *GC–Tof* gas chromatography with a time-of-flight mass spectrometer, *LC–Fluo* liquid chromatography with a fluorescent detector, *LC–MSMS* liquid chromatography with a tandem mass spectrometer

[a] Analytical methods

As regards the analytical methods employed, metals were analyzed by inductively coupled plasma (ICP)-mass spectrometry for the most part or by inductively coupled plasma with atomic emission spectroscopy for Zn. All organic pollutants were analyzed by either gas or liquid chromatography with a fluorescence detector or with a simple, tandem, or time-of-flight mass spectrometer for both the dissolved and particulate tractions. All pollutants were quantified using internal standards. To avoid analytical bias, all analyses for a given class of contaminant were conducted by the same reference laboratory. Field blank results indicate no particular contamination from sampling devices and/or sample pre-treatment procedure for most pollutants monitored (n = 77). A low contamination by nonylphenol could, however, be observed (<5 ng/L), but this value was far less than levels found in TAF or stormwater.

Figure 7.5-129: Concentrations (mean ± SD, in milligrams per liter) of conventional water quality parameters for stormwater on the Suey (*n* =24), Pin Sec *(n* = 18), and Chassieu *n* = 7) catchments



Result interpretation methodology

Concentrations will be compared first across study sites and then to data from the literature, i.e., NURP database for the USA and QASTOR database in France. To compare sites, the statistical distribution of stormwater EMC data for each site will be assessed. In this study, log-normal distributions have been tested at 5 % significance levels hence both the mean and standard deviation (SD) of EMCs (estimated distribution) have therefore been calculated first in log space and then transformed into arithmetic space. Based on similar methodology, the statistical distributions of each pollutant EMC will be evaluated and the differences in pollutant EMCs across sites assessed using the Kruskal-Wallis test at 5 %. For pollutants showing site-to-site differences, individual site concentrations will be presented. When no difference has been identified, data from all three sites will be pooled and global statistical parameters provided. The last parts will present the distribution of pollutants between the dissolved and particulate phases, as well as the contributions of total atmospheric fallout to stormwater contamination.

Results and discussion

Conventional water quality parameters

Conventional water quality parameters (TSS in milligrams per liter, DOC and POC in milligrams of C per liter) are provided in Figure 7.5-129. On each site, EMCs for TSS, DOC, and POC are log-normally distributed (Shapiro-Wilk test, α = 0.05, *W*=0.93 for Sucy and Pin Sec, *W*=0.79 for Chassieu), and no significant differences appear across the three sites (Kruskal-Wallis test, α =0.05, *p* value=0.478, 0.167, and 0.102 for TSS, DOC, and POC, respectively).

Table 7.5-162: Occurrence (in percent) of pollutants in TAF and stormwater

Substances	Sucy		Pin Sec		Chassieu	
	TAF	Outlet	TAF	Outlet	TAF	Outlet
Metals	8 events	8 events	15 events	15 events	5 events	5 events
>80 %	As, Cd, Cu, Ni, Pb, Sr, Ti, V, Zn					
Co	57 %	57 %	0 %	86 %	40 %	80 %
Mo	25 %	25 %	31 %	31 %	20 %	20 %
Pt	63 %	63 %	54 %	50 %	20 %	80 %
PAHs	8 events	8 events	7 events	7 events	4 events	4 events
>80 %	N, Acen, F, P, Fluo, Pyr					
Acyl	75 %	75 %	50 %	71 %	75 %	75 %
A	25 %	75 %	25 %	43 %	25 %	100 %
B(*a*)A	63 %	88 %	25 %	100 %	50 %	100 %
Chry	88 %	100 %	50 %	100 %	75 %	100 %
B(*b*)F	75 %	100 %	50 %	100 %	100 %	100 %
B(*k*)F	71 %	100 %	25 %	100 %	50 %	100 %
B(*a*)P	50 %	75 %	0 %	100 %	50 %	75 %
IP	25 %	75 %	0 %	86 %	0 %	75 %
D(*ah*)A	0 %	63 %	0 %	14 %	0 %	0 %
B(*ghi*)P	50 %	75 %	25 %	100 %	25 %	100 %
Pesticides	7 events	7 events	8 events	8 events	4 events	4 events
<20 %	Metaldehyde, chlorfenviphos, endosulfan A, folpel, aldrin, dieldrin, linuron, 2,4-D, trichlopyr, isothiazolinone, irgarol 1051, terbutryn, acetochlor, *S*-metolachlor, pendimethalin, epoxiconazole,					
	Tebuconazole, fenpropidine, chlorothalonil, metazachlor, diflufenicanil					
Isoproturon	100 %	100 %	33 %	29 %	75 %	100 %
Diuron	67 %	100 %	67 %	71 %	75 %	100 %
Carbendazim	67 %	100 %	0 %	71 %	75 %	100 %
2,4-MCPA	50 %	33 %	0 %	29 %	75 %	75 %
Mecoprop	50 %	50 %	0 %	0 %	75 %	25 %
Glyphosate	50 %	40 %	67 %	70 %	75 %	75 %
AMPA	50 %	40 %	67 %	50 %	75 %	75 %
Glufosinate	50 %	40 %	67 %	70 %	50 %	75 %
Diflufenicanil	17 %	40 %	0 %	29 %	0 %	0 %
PBDEs	12 events	12 events	7 events	7 events	2 events	2 events
>80 %	BDE-47, BDE-209					
BDE-28	88 %	100 %	80 %	75 %	100 %	100 %
BDE-99	100 %	100 %	80 %	50 %	100 %	50 %
BDE-100	100 %	100 %	80 %	75 %	100 %	100 %
BDE-153	63 %	75 %	40 %	50 %	50 %	50 %
BDE-154	38 %	38 %	0 %	25 %	0 %	0 %
BDE-183	50 %	50 %	0 %	50 %	0 %	100 %
BPA/APnEO	12 events	12 events	7 events	7 events	2 events	2 events
>80 %	BPA, OP, OP2EO, NP, NP1EO, NP2EO, NP1EC					
OP1EO	50 %	63 %	75 %	50 %	50 %	100 %
<20 %		20–50 %		50–80 %		>80 %

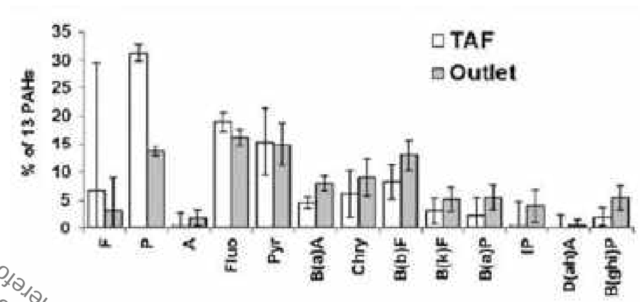
The measured TSS concentrations are in good agreement with those reported on the same sites in previous studies. On the Chassieu catchment, based on on-line turbidity measurements from 2004 to 2011, the average TSS concentration during storm events was estimated at around 75 mg/L. The concentrations found on these sites (mean values of 148, 129, and 100 mg/L) are much lower, however, than those previously reported in France by Saget (1994): a TSS of between 170 and 550 mg/L (with a median of roughly 420 mg/L) on Paris sites (QASTOR database). High concentrations of TSS found by Saget (1994) might reflect poor quality local sewer connections leading to the discharge of wastewater into the separate sewer. Since 1994, considerable EMCs display similar statistical parameters to those reported in the NURP database, i.e., a mean and median TSS concentration of approximately 174 and 113 mg/L, respectively.

Micropollutants

Occurrence of micropollutants on each catchment

The occurrences (in percent) for each pollutant monitored, as well as the number of rain events considered, are reported in Table 7.5-162 for TAF and stormwater. Out of 77 pollutants monitored, between 42 and 48 substances (including metals, PAHs, PBDEs, APnEOs, and BPA) were systematically detected, while 20 to 25 substances exhibited occurrence rates of less than 25 %.

Figure 7.5-130: PAH fingerprints (in percent, mean ± SD) in TAF and stormwater at the catchment outlet



Overall, the occurrence profiles were quite homogenous across the three sites, except for some pesticides or low-level compounds. Out of 14 metals monitored, almost all were systematically detected in TAF and stormwater at each catchment outlet, except for Co, Mo. and Pt. As regards their occurrence rates, no clear difference appeared from atmosphere to catchment outlet. It would therefore appear that the 14 trace metals analyzed within the scope of this survey were ubiquitous in both atmospheric deposition and stormwater, with no significant differences across the three sites. For Co, Mo, and Pt, the levels in TAF and runoff were below their detection limits. For PAHs, six compounds (N, Acen, F, P, Fluo, and Pyr) were systematically observed in TAF and stormwater regardless of the site considered. Regardless of the site under investigation, the PAH fingerprints were quite homogenous from one rain event to the next and from one site to the next. Yet, as illustrated in Figure 7.5-130, TAF and stormwater present different PAH fingerprints. PAH patterns for TAF: are characterized by the predominance of low molecular weight PAHs (LMW; two to four aromatic rings) compared to heavy molecular weight PAHs (HMW; i.e., five to six aromatic rings) as attested by a mean LMW/HMW ratio of approximately 12. This difference traduces direct deposition on urban surfaces of HMW PAHs emitted by either combustion (vehicle exhaust) or petroleum sources (rubber tires, oil leakage, asphalt materials) whereas the LMW PAHs can be transported over large distance via the atmosphere, as, in urban context, the PAH distributions in stormwater reflect a mixture of pyrolytic and petrogenic contamination.

Table 7.5-163: Pollutant concentrations (mean ± SD) in stormwater Substances Sucy displaying site-to-site differences

	Substances	Sucy		Pin Sec		Chassieu		References
		Mean	SD	Mean	SD	Mean	SD	
Metals[a]	Events	8		15		5		
	As	1.18	0.80	4.04	2.70	0.88	0.66	–
	Cr	3.55	2.54	1.95	1.46	6.20	5.00	2.1–14[d] 3.0–19[e] 2.1–20[f]
	Cu	38.00	28.41	14.87	11.33	34.62	29.19	10.0–197[e] 5.8–37[f] 6.6–54.8[g]
	Ni	2.88	1.97	3.14	2.28	6.64	[illegible]	2.1–8.5[f] 2.2–32[d]
	Sr	112.83	79.62	28.98	29.46	51.40	34.17	–
	Zn	212.35	145.08	126.34	87.06	238.78	196.77	64–536[d] 52–502[e] 32–320[f] 176–140[g]
PAHs[b]	Events	8		7		4		
	Fluo	217	193	105	72	97	65	169[h]
	Pyr	176	156	104	72	88	59	170[h]
	$\sum_{13}$ PAHs	1,237	1,127	723	491	644	406	
	$\sum_{16}$ PAHs	1,362	1,227	892	604	1,135	770	3,300[h]
PBDEs[b]	Events	12		[illegible]		2		
	BDE-209	25	23	90	111	86–98[c]		
	$\sum_{8}$ PBDEs	23	23	101	113	237–98[c]		

[a] Metal concentrations in micrograms per liter, [b] Concentrations in nanograms per liter for organic pollutants, [C] Only two events collected, [d] Lamprea and Ruban 2011a, [e] Rossi (1998), [f] Sabin *et al.* (2005), [g] NURP database, mean and median values, [h] Zgheib *et al*. (2011a, b), median values

Of the 30 pesticides evaluated, 19 compounds broken down into five herbicides (metazachlor, terbutryn, pendimethalin, trichlopyr, and acetochlor), five fungicides (folpel, epoxiconazole, fenpropidine, chlorothalonil, and tebuconazole), six insecticides (chlortenviphos, endosultan, aldrin, dieldrin, isodrin, and deltamethrine), and three algaecides/imolluscicides (isothiazolinone, irgarol 1051, and metaldehyde)-were never detected in stormwater or with an occurrence rate of below 20 %, regardless of the catchment considered. The detection limits of most of these compounds lie in the range of 2 - 7 ng/L. Of these compounds, some (such as aldrin and dieldrin) are now banned: The non-detcction may be explained by having been phased out from use in France. In spite of reports surrounding the leaching of additives from recent construction materials, terbutryn, irgarol 1051, and isothiazolinone were also not detected. As a matter of fact seven herbicides (glyphosate. glufosinate and its degradation product AMPA, diuron, isoproturon, mecoprop, and 2,4-MCPA) and one fungicide (carbendazim) were frequently observed in stormwater, and this finding remained independent of the site tested. In general, these compounds exhibited occurrences varying between 20 and 100 % in runoff, e.g., mecoprop 0-50 %, isoproturon 29- 100 %, and 2,4-MCPA 29- 75 %. More details on their occurrence rates are provided in Table 7.5-163. A similar trend was observed in TAF. As regards occurrence, slightly higher rates of these herbicides were noted at the scale of larger basins (i.e., Sucy and Chassieu), compared to Pin Sec. Given that the pesticide presence in stormwater is highly dependent on site and peripheral activities, this could suggest that the pesticide use could tend to be more limited and specific on smaller sites. This finding may also reflect the results of the new policy being implemented in the Nantes Metropolitan Area targeting a drastic reduction in pesticide use on public spaces. The Pin Sec catchment is in fact affected by the same kind of this policy. Chassieu, which has, the less restrictive policy in terms of pesticide reduction, shows the higher level of occurrence in TAF and Stormwater for most of the pesticides analyzed.

Table 7.5-164: Pollutant concentrations (mcan ± SD, Q20 and Q80) in stormwater displaying no site-to-site differences

		Mean	SD	Q20	Q80	Reference
Metals[a] (n=28)	Cd	0.32	0.31	0.11	0.39	0.5–2.2[d]
	Co	3.45	3.13	1.00	3.68	–
	Mo	7.68	13.09	1.10	12.05	–
	Pb	21.52	20.73	6.79	33.22	175–131[e]
	Ti	27.80	28.60	9.70	37.50	
	V	4.86	2.84	2.55	6.79	
Pesticides[b,c] (n=19)	Glyphosate	337	806	95	198	
	Glufosinate	756	10,121	6	389	
	AMPA	824	7,077	16	469	
	Diuron	1,213	10,784	25	795	
	Isoproturon	88	929	3	53	
	Carbendazim	213	1,355	7	[illegible]	
	Mecoprop	3	7	1	[illegible]	
APnEO and BPA[c] (n=21)	BPA	552	510	207	[illegible]	<LOD–107,000[f]
	OP	61	37	[illegible]	[illegible]	
	OP1EO	23	25	[illegible]	22	
	OP2EO	10	11	[illegible]	14	
	NP	359	228	[illegible]	509	<LOQ–7,300[f] 160–920[g]
	NP1EO	347	[illegible]	69	428	
	NP2EO	164	[illegible]	52	141	
	NP1EC	466	[illegible]	160	374	

[a] Metal concentrations in micrograms per liter, [b] For pesticides, the site-to-site differences were not tested, [c]oncentrations in nanograms per liter for organic pollutants, [d] Rossi (1998), [e] NURP database, mean and median values, [f] Kalmykova *et al.* (2013), [g] Bressy *et al.* (2012), d10-d90 values

Diuron and glyphosate are used as total herbicides, and their presence in stormwater may be explained by its application on different types of urban surfaces. At the scale of the Paris conurbation and prior to 2008, diuron accounted for about 31 % of urban pesticide use. At present, in spite of its recent ban in France (December, 2008) from phytophannaceutical products, diuron is being increasingly added to building facade paints and renders in order to provide antialga1 and antifungal protection. Glyphosate is widely used by municipalities and home gardeners; this tendency has been verified in a recent survey conducted at Pin Sec, which showed that in spite of information delivered by local authorities, herbicides (and especially glyphosate) are still being used.

Table 7.5-165: Percentage of metals and organic pollutants in the particulate phase of stormwater (mean ± SD)

	<20 %	<50 %	50–80	>80 %
Metals	Sr (13±10)	As (48±18)	Cd (63±30) Mo (63±40) Ni (54±18) V (62±18) Zn (60±23) Cu (73±13)	Co (80±34) Cr (85±11) Pb (94±4) Ti (91±9)
PAHs		N (44±28)	A (60±44) F (70±31)	Acyl (88±12) Acen (88±28) [illegible] (94±12) Fluo (98±5) [illegible] (97±3±5) BaA (100±22) Chry (97±4) B(*b*)F (99±2) B(*k*)F (99±2) B(*a*)P (100±1) IP, d(*ah*)A (100[a]) B(*ghi*)P (99±1)
Pesticides	Diuron (6±41) Glyphosate (14±43)	Glufosinate (43±46) Isoproturon (42±43)		
PBDEs			BDE-28 (71±33), BDE-100 (55±34)	BDE-47 (95±33) BDE-99 (86±42) BDE-209 (99±7)
APnEO and BPA	BPA (18±11) OPIEO (14±23)	OP (45±32) OP2EO (32±16) NP (48±22) NPIEO (39±20) NP2EO (29±17) NPIEC (38±30)		

[a] Detected only in the particulate phase

Based on experimental batch tests conducted on surfaces of varying imperviousness, Blanchoud *et al.* (2007) estimated the transfer coefficients (i.e., the ratio between quantity of pollutants at the catchment outlet and quantity of pollutants input on this catchment) to equal roughly 60 % for diuron and 25 % for glyphosate. Carbendazim were also reported to be leached from new antifouling paints and renders. Mecoprop and 2,4-MCPA are mainly applied for yards, parks, and railway maintenance.

Out of the nine PBDEs monitored, high occurrence rates were observed for five compounds (BDE-28, 47, 99, 100, and 209) while other congeners (BDE- 153, 154, 183, and 205) were less frequently detected. Due to growing environmental and human health concerns, penta- and octa-BDE and, more recently, deca-BDE have been banned in Europe though they are still being detected. To date, however, no study has focused on their occurrence in runoff. Their presence in runoff was nevertheless anticipated since PBDEs are found in TAF and have commonly been added to building materials, automotive parts, plastics, and electronic equipment. Lastly, BPA and APnEO (NP, NPIEO, NP2EO, NPIEC, OP, OPI EO, and OP2EO) were systematically observed in runoff and TAF. Nonylphenol ethoxylate (NPnEO; 80 %) and octylphenol ethoxylate (OPnEO; 20 %) are widely used in industrial and domestic applications, such as lubrication, oil additives, detergents, and antistatic agents.

The presence of NP and OP in stormwater had been expected since both compounds are used in paints, concrete, building materials, asphalt, and certain vehicle parts. Nonylphenol acetic acid (NPI EC), which is a degradation product of NPnEO, is also frequently identified in both matrices. BPA is primarily used as a monomer in the manufacturing of polycarbonate plastics, renowned for its high resistance to shocks and

temperature (e.g., plastic windows, car bumpers), as well as in epoxy resins. BPA is also an admixture introduced during the production of PYC, varnishes, and paints and in the fonnulation of some car products (brake fluid, tires).

Concentration ranges of pollutants in Stormwater
The statistical parameters of EMC distributions are indicated in Table 7.5-163 for pollutants that display site-to-site differences and Table 7.5-164 for the other pollutants.

Metals - From an overall standpoint, metal EMC ranges varied by one or more orders of magnitude from one sample to another. It should be highlighted that the INOGEV project has contributed new information on the elements As, Co, Mo, Pt, Sr, Ti, and V, which had seldom been reported in the literature previously. For Mo (1 - 12 µg/L, Q20 and Q80), CO (1 - 3.5 µg/L), Pb (7- 35 µg/L), V (3- 7 µg/L), Ti (10-37 µg/L), and Cd (0.12- 0.42 µg/L), our results do not indicate any site-to-site differences at the scale of the three urban sites studied. Statistical parameters of the EMC distribution are reported in Table 7.5-164. For As, Cu, Cr, Ni, Zn, and Sr, differences between sites appeared and concentrations on each site are given in Table 7.5-163. Higher Cr and Ni concentrations were found at Chassieu, most likely as a result of local industrial activities. The highest Cu. Zn. Sr. and Ti concentrations were reported at Sucy. Interestingly, these metals are known to originate from vehicle brake linings and tires, thus suggesting that differences could be highly correlated with traffic density. Initial estimations actually revealed significantly different traffic densities on each site. The Zn contamination might also be attributed to leaching from roofs, gutters, street fumiture, etc. The higher Ni and Cr concentrations measured at Chassieu could be explained by the presence of industries on this catchment, but these concentrations did remain low.

PAHs -The PAH results are discussed on the basis of total concentrations, Whereas no significant difference was found for TAF across the three sites, statistical analyses revealed significant site-to-site differences for total PAH concentrations in stormwater. Moreover, Chassieu (644 ng/L for Σ_{13} PAHs, Table 7.5-164) and Pin Sec (723 ng/L) presented lower concentrations than Sucy (1,237 ng/L). Another interesting point is that even though TSS concentrations vary within the same range on all three sites, the differences observed are primarily tied to the PAH contents of the particles collected. The median PAR content found in Sucy (approximately 19,000 ng/g) far surpasses that reported for Chassieu (6,000 ng/g) or Pin Sec (7,000 ng/g). On the whole, the stormwater concentrations are much higher than those observed in TAF, thus indicating a local production source. No correlation was found between PAHs, TSS, and dissolved and particulate organic carbon levels (Speam1an test, R^2 <0.3). In addition and based on the limited number of rain events, no seasonal correlation was identified. As previously mentioned for vehicle-derived metals, the contamination in stormwater likely reflects a difference in road traffic density and type from one catchment to another. In accordance with the extensive literature, PAHs are indeed emitted by vehicle traffic via gas exhaust, tire wear, and spilled oil. The highest concentrations were consistently found for Sucy, which is subjected to much higher traffic density. The industrial catchment of Chassieu generated the lowest PAH concentrations, except for the extremely high concentrations of naphthalene measured on some samples. These low PAH concentrations were unexpected, due to the numerous industrial and logistics activities in Chassieu as well as the proximity to Lyon's dense highway corridor, yet they remain consistent with the low traffic density inside the catchment.

Pesticides - Among the most widely detected pesticides, glyphosate (95- 198 ng/L, Q20 and Q80), AMPA (16-469 ng/L), diuron (25- 795 ng/L), and glufosinate (6-389 ng/L) are all non-selective herbicides and were predominantly in stormwater. Isoproturon (3- 53 ng/L) and carbendazim (7- 195 ng/L) were detected at lower concentration levels, while the remaining pesticides (mecoprop, 2,4-D, 2,4-MCPA) did not generally exhibit concentrations reaching 5 ng/L. Given the limited number of rain events for these compounds (from four to eight events, depending on the site), the difference in herbicide concentrations between sites was not statistically tested and instead the data were pooled (Table 7.5-164).

High glyphosate concentrations were measured on Pin Sec, where municipal use of this pesticide is limited. At the scale of our three study sites, it can reasonably be assumed that glyphosate is being used by private gardeners. Diuron and carbendazim were reported to be leached at high concentrations from new antifouling paints and renders. This source would be consistent with the much lower concentrations measured on Chassieu (with industrial –type buildings), compared to Sucy and Pin Sec, though it remains

limited to relatively new or recently renovated facades. Despite the ban, dated supplies of diuron-based pesticides might still be in use by private gardeners or it has accumulated in the soils. High herbicide concentrations were occasionally observed (1,500-3,000 ng/L) independent of the site or period under consideration. These high concentrations depend on various factors affecting the quantity of pesticides remobilized, such as the time interval between applications and rainfall, the level of imperviousness of the treated surface, or the characteristics of the rain events.

PBDEs - Of the eight PBDEs detected in runoff, deca-BDE (BDE-209) displayed the highest concentration, in ranging from 23 to 251 ng/L (Q20 and Q80 on the full dataset) and with a median relative contribution to Σ_8 PBDEs of around 90 %. The other congeners varied overall within the 0.5- 3.0- ng/L range. For tri- to hepta-BDEs, BDE-47 and BDE-99 were the most abundant congeners, with mean relative abundances of 5 and 3 %, respectively. While the PBDE contamination of the atmospheric compartment is known, no experimental data on PBDE levels in stormwater were available. Although no geographical difference was noticed for TAF contamination, significant site-to-site differences were observed for stormwater contamination (Σ_9 PBDE, Kruskal-Wallis test, α = 0.05, p value=0.017, Table 7.5-163). This finding suggests that land use and/or building materials applicable to these sites might affect runoff differently. Lower PBDE concentrations were actually found in Sucy, as compared to the other sites. To date, any more comprehensive explanation has not been provided. For all sites under consideration, BDE-209 concentrations at the catchment outlet were significantly higher than those either measured in TAF during this study (0.4-8.6 ng/L) or reported for Sweden in urban areas (2.5- 14.4 ng/L for Σ_8, PBDEs).

Bisphenol A and APnEO - For BPA and APnEO, no site differences were observed (Kruskal-Wallis test, α =0.05, p value = 0.035 for BPA and p value = 0.111 for APnEOs). The statistical parameters associated with their distributions are listed in Table 7.5-164. The mean EMCs of BPA and NP were estimated at 552 and 359 ng/L. For both compounds, these concentrations were much higher than those reported for rainwater in Paris and in the same overall range as results for runoff and landfill leachate in Sweden. On the French national scale, NP levels were also comparable to those reported by Bressy *et al*. (2012), NP and nonylphenol ethoxylates (NP1EO and NP2EO) were predominant, in comparison with OP and octylphenol ethoxylates (OP1EO and OP2EO). In our study, NP tends to exhibit higher concentrations than NP1EO and NP2EO; these findings contrast with the Swedish results. Regardless of the site and rain event considered, the alkylphenol distributions remained fairly homogenous, as characterized by the following order: NP (42±25 %>NP1EO (25±11 %) ~NP1EC (21±9 %>NP2EO (12±4 %). For the first time, the presence of NP1EC has becn reported in runoff, with concentrations significantly greater than those measured in TAF (<3 ng/L).

Table 7.5-166: Contributions (in percent) of TAF to stormwater pollution (mean ± SD)

	$C_{[TAF]}/C_{[Outlet]}$<10 %	$C_{[TAF]}/C_{[Outlet]}$<20 %	$C_{[TAF]}/C_{[Outlet]}$>20 %
Water parameters	TSS (8±11) POC (9±13)		DOC (25±15)
Metals	As (7±4) Sr (9±7)	Ti (13±24) Pb (11±12) Cu (20±17)	Cu (20±17) Cr (32±53) Ni (32±31) Cd (33±29)
	Sr_{Sucy} (3±1) $Sr_{Chassieu}$ (5±3) Zn_{Sucy} (9±5) Cr_{Sucy} (10±5) $Cr_{Chassieu}$ (8±6)	$Sr_{Pin\ Sec}$ (14±6) $Zn_{Chassieu}$ (15±9)	$Zn_{Pin\ Sec}$ (86±127) $Cr_{Pin\ Sec}$ (55±68)
PAHs	B(*a*)P (4±11) I(*cd*)P (1±4), B(*ghi*)P (4±8)	A (13±34) Fluo (17±19) Pyr (14±14) Chry (13±20) B(*b*)F (14±21) B(*k*)F (11±20) B(*a*)A (11±20)	[illegible] (68±[illegible]) Acy (34±25) Flene (40±45) T (28±20) P (26±22)
PBDEs	BDE-47 (6±4) BDE-99 (9±15) BDE-154 (5±6)	BDE 28 (8±[illegible]) BDE-100 (16±[illegible]) BDE-153 (12±16) BDE-209 (7±19)	
APnEO and BPA	BPA (4±3) NP1EC (4±10) OP (8±5) BPA (4±4)	NP (19±46) NP1EO (18±21) OP1EO (16±21) OP2EO (17±13)	NP2EO (32±21)

$C_{[TAF]}/C_{[outlet]}$ concentrations found for total atmospheric fallout/concentrations measured in stormwater at the catchment outlet

Distribution of pollutants between dissolved and particulate phases

The distributions of all pollutants between dissolved and particulate phases are shown in Table 7.5-165. For all pollutants examined, no significant differences across the three sites were remarked, thus suggesting that this distribution is not site-dependent but rather correlated with the physical and chemical properties of the compound under consideration. This assessment could prove useful in the choice of stormwater treatment device. Most metals were mainly bound to the particulate phase (>50 %), except for Sr. This tendency was more pronounced for Co, Cr, Pb, and Ti and to a lesser extent for Cu. The remaining metals (As, Cd, Ni, Y, Mo, and Zn) yielded an intermediate behavior since the mean particulate phase ranged from 48± 18 % (As) to 63±40 % (Mo). In accordance with typical stormwater findings, most organic pollutants studied herein are preferentially associated with particles. Despite the fact that log K_{ow} does not accurately describe the behavior of all pesticides, this coefficient can still be used as an indicator to predict the pollutant distribution between dissolved and particulate fractions. Other parameters however, might also affect the partitioning, e.g., molecular structures and charges.

Contribution of atmospheric deposition to storm water pollution

For each pollutant, the contributions of total atmospheric fallout to stormwater pollution have been calculated. At the scale of the rain event, the ratio between TAF and stormwater concentrations was evaluated; the mean +SD values of this ratio are given in Table 7.5-166. Except for several individual substances, the contributions of TAF were on the whole rather weak and median values generally did not exceed 30 %. For metals and as a result of low concentrations found on all three sites for TAF total atmospheric fallout accounted for less than 20 % of the stormwater pollution for six metals (As, Pb, Sr, Ti, V, and Cu) though in some cases (Cd, Cr, Ni) did exceed 30 %. For As, Sr, and V, this contribution did not exceed 10 %. Overall, the ratios between TAF and stormwater were quite similar at the scale of these three sites, except for Cr, Sr, and Zn. Differences were readily observed for Cr (55 % at Pin Sec vs. 8 % and 10 % at Chassieu and Sucy), Sr (14 % at Pin Sec vs. 5 % and 3 % at Chassieu and Sucy), and Zn (86 % at Pin

Sec. vs. 9 % and 15 % at Chassicu and Suey). A very high atmospheric Zn contribution was observed on Pin Sec (86± 127 %), which was mainly due to the first three campaigns (December 2011 through March 20 12), during which unusually high atmospheric concentrations were measured (122- 537 µg/L). These increased concentrations may be attributed to specific works involving zinc sheets in the vicinity of the sampling device; however, this hypothesis could not be verified. Long-range transportation is rejected as an explanation since TSS did not increase during this period.

For PAHs, PBDEs, APnEO, and BPA, atmospheric contributions remained low, thus confirming a strong local production for all compounds. Except for PAHs and NP, this production has not been highlighted in the literature for such a broad panel of substances. For other families, such as APnEOs and PBDEs, local production from road, urban surfaces, and vehicle leaching would be expected since these compounds are used in building materials and automobile parts. As mentioned for PAHs, the sources of these compounds now need to be investigated more thoroughly. Consequently, samples from street runoff will soon be analyzed as a follow-up to this work.

Conclusion

This paper has been developed as part of the INOGEV project being carried out by the three French Observatories in Urban Hydrology (OPUR, OTHU, and ONEVU) focusing on stormwater quality and intended to deliver the initial conclusions drawn from a new more extensive French dataset. This study has provided, for a wide array of pollutants and three distinct sites featuring distinct land use patterns and contexts, a knowledge and comparison of their occurrence rates and concentration ranges in stormwater with the same experimental procedures for each site. Relevant data have been derived for newly targeted metals (As, Ti, Sr and V) and heretofore poorly documented organic pollutants, such as nonylphenol and octylphenol ethoxylates, PBDEs, certain pesticides, and BPA. Such a database could be used to develop a relevant decision-making aid for urban Stormwater practitioners and watershed managers in evaluating the stormwater contribution to the pollution of receiving waters. For many pollutants, the results obtained during this monitoring program do not highlight any significant difference in stormwater quality across the three urban sites studied, with variability from one site to another being of the same order of magnitude or less than variability from one event to another.

This study has not only confirmed the initial conclusions drawn at the scale of three Paris sites (Zgheib *et al*. 20 II a) but has reinforced them since the urban sites considered in the INOGEV project are more highly contrasted than those initially examined. This study, however, also underscores significant site-to-site differences for several metals (As, Cr, Cu, Ni, Sr, and Zn), as well as for PAHs and PBDEs.

Like for stormwater quality, this study reveals no significant differences in the distribution between dissolved and particulate phases across all sites, which suggests that this distribution is not site-dependent but instead correlated with the physical and chemical properties of the compound being examined. In accordance with typical stormwater observations, most metals were primarily bound to the particulate phase: (a) >50 % for As, Cd, Mo, Ni, V, Cu, and Zn and (b) >80 % for Co, Cr, Pb, and Ti. for organic pollutants, their distributions between dissolved and particulate phases depend heavily on their chemical and physical properties; moreover, it appears that the octanol-water coefficient (log K_{ow}) of these substances may be used to roughly predict their behavior. Log K_{ow} serves as an empirical predictive approach for easily determining the distributions between dissolved and particulate phases of pollutants, yet the relation between K_{ow} (or another coefficient, like K_{oc} or K_d) and substance distribution in stormwater still requires further investigation.

In conclusion, this study has highlighted that the contributions of TAF were either rather low or very low for quality parameters and micropollutants, with median values not exceeding 30 % except for certain individual substances. This extremely relevant finding underscores local production not only for PAHs, as previously demonstrated in the literature, but also for a broader range of substances such as BPA, APnEOs, and PBDEs. This local production is correlated with leaching from urban surfaces, buildings, and vehicles, although their actual sources must now be more thoroughly investigated. In pursuing this work and in addition to the initial conclusions delivered, a deeper analysis between groups of pollutants (correlation trends) will be carried out in order to select representative substances to be studied. Atmospheric and stormwater fluxes at various temporal scales will also soon be evaluated and compared in order to assess

the relative contribution of atmospheric inputs. Stormwater quality relative to rain event characteristics will also be studied. Subsequent investigations will rely on developing a methodology and tools for estimating annual stormwater pollutant fluxes at the scale of urban sites based on on-line turbidity measurements.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports the results from a monitoring exercise for micorpollutants in total atmospheric fallout (TAF) and stormwater of three French urban catchment areas. Occurrence of glyphosate and AMPA (in percent) were reported in TAF and stormwater. Among other pollutants, the concentrations of glyphosate and AMPA, expressed as mean ± SD, Q20 and Q80 were also reported. The results provide a comprehensive overview on the occurrence of glyphosate and AMPA in the stormwater of urban areas. However, the focus is not on agricultural areas.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/051
Report author	Maillard E., Imfeld G.
Report year	2014
Report title	Pesticide Mass Budget in a Stormwater Wetland
Document No	Environmental Science & Technology 2014, 48, 8603−8611
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Wetlands are reactive landscape zones that provide ecosystem services, including the improvement of water quality. Field studies distinguishing pesticide degradation from retention to evaluate the sink and source functions of wetlands are scarce. This study evaluated based on a complete mass budget the partitioning, retention, and degradation of 12 pesticides in water, suspended solids, sediments, and organisms in a wetland receiving contaminated runoff. The mass budget showed the following: (i) dissolved pesticides accounted for 95 % of the total load entering the wetland and the pesticide partitioning between the dissolved phase and the suspended solids varied according to the molecules, (ii) pesticides accumulated primarily in the <250 µm bed sediments during spring and late summer, and (iii) the hydrological regime or the incoming pesticide loads did not influence the pesticide dissipation, which varied according to the molecules and the wetland biogeochemical conditions. The vegetation enhanced the pesticide degradation during the vegetative phase and the pesticides were released during plant senescence. The dithiocarbamates were degraded under oxic conditions in spring, whereas glyphosate and aminomethylphosphonic acid (AMPA) degradation occurred under reducing conditions during the summer. The complete pesticide mass budget indicates the versatility of the pesticide sink and source functions of wetland systems.

Materials and Methods

Description of the Stormwater Wetland

The studied stormwater wetland is located at the outlet of a 42.7 ha vineyard catchment in Rouffach (Alsace, France). The daily rainfall and evapotranspiration were measured at a weather station located on the catchment (Meteo France, station no. 68287003).

Sampling of Water, Organisms, and Sediments in the Wetland

The runoff discharges and volumes were continuously monitored from March 23 to September 28, 2011 (i.e.,over 189 days) using bubbler flow modules combined with a Venturi channel at the inlet and a V-notch weir at the outlet of the stormwater wetland. Water samples (300 mL) were collected at the wetland inlet and outlet every 3 m^3 using automatic samplers. Integrative water samples (150 mL) were also collected at the center of the wetland forebay every 2 h to be representative of the forebay water. The detailed hydrological budget is provided in Table 7.5-167. Discrete flow- and time-proportional water samples obtained over a week were combined in single composite samples prior to analysis. Additional sampling campaigns were conducted monthly in the wetland from 23 March (day 0) to 07 September (day 168), 2011 on days 0, 28, 56, 84, 111, 140, and 168 to quantify the pesticides in the wetland compartments, that is, the aqueous phase, TSS, bed sediments, vegetation, algae, and invertebrates. A grid-cell sampling was conducted by dividing the forebay area into four equal rectangular cells (9 × 6 m). The subsamples were collected at the center of each cell, and the four subsamples were pooled to obtain a single composite sample for each wetland compartment. For each sample type and sampling campaign, a portion of the fresh collected composite samples was weighted, dried at 80 °C for 1 week, and weighted again to estimate the (bio) mass of the wetland compartment, and another portion was maintained at -20 °C for chemical analyses.

Table 7.5-167: Hydrological and Pesticide Mass Budget in the Stormwater Wetland (Rouffach, Alsace, France)

			spring (March 23–May 10)		summer (May 10–August 10)		late summer (August 10–September 07)		season (March 23–September 07)	
parameter		unit	inlet	outlet	inlet	outlet	inlet	outlet	inlet	outlet
Wetland Parameters	rainfall intensity	[mm h^{-1}]	5.0 ± 3.3		30.4 ± 17.9		17.0 ± 10.8		21.1 ± 17.2	
	quiescent period[a]	[days]	13.5 ± 9.6		6.4 ± 2.3		6.5 ± 2.2		8.8 ± 6.6	
	hydraulic Residence Time[a]	[days]	6.7 ± 3.0		14.7 ± 8.4		26.9 ± 8.1		14.1 ± 9.6	
	vegetation cover	[%]	80		80		100		85	
	vegetation density	[stems m^{-2}]	35–150		175		200		140	
	weekly TSS load	[kg]	12.8 ± 12.6	1.6 ± 1.5	186.9 ± 297.5	1.9 ± 1.6	15.9 ± 15.8	0.6 ± 0.8	123.4 ± 248.1	1.5 ± 1.5
	weekly DOC load	[kg]	1.2 ± 0.1	0.4 ± 0.1	2.4 ± 1.2	0.9 ± 0.5	1.0 ± 0.5	0.5 ± 0.4	1.2 ± 1.2	0.6 ± 0.4
Pesticide Loads[b]	glyphosate	[mg]	1063/10	nd/nd	68099/1672	664/28	1712/26	39/0.30	48784/1708	703/34
	AMPA	[mg]	408/0.23	15/0.78	4328/224	784/14	455/7.16	55/3.67	3391/231	854/18
	dithiocarbamates	[mg]	nd/.94	nd/nd	nd/922	nd/1.93	nd/nd	nd/nd	nd/1016	nd/1.93
	kresoxim-methyl	[mg]	nd/nd	nd/nd	72/nd	24/nd	nd/nd	nd/nd	72/nd	24/nd
	pyrimethanil	[mg]	nd/nd	nd/nd	367/nd	196/0.07	6.54/0.12	12/0.02	375/0.12	207/0.09
	metalaxyl	[mg]	283/nd	nd/nd	962/nd	218/nd	11/nd	3.21/nd	1256/nd	221/nd
	tetraconazole	[mg]	nd/nd	nd/nd	292/2.69	17/nd	17/0.13	4.63/0.03	309/2.73	21/0.03
	difenoconazole	[mg]	nd/nd	nd/nd	nd/nd	nd/nd	nd/0.04	nd/nd	nd/0.04	nd/nd
	fludioxonil	[mg]	nd/nd	nd/nd	nd/nd	nd/nd	3.37/1.83	46/0.17	3.37/1.83	46/0.17
	spiroxamine	[mg]	nd/2.28	nd/0.43	251/47	30/0.04	11/1.94	nd/0.14	262/52	30/1.41
	cyprodinil	[mg]	nd/nd	nd/nd	nd/nd	nd/nd	nd/1.45	nd/0.04	nd/1.45	nd/0.04
	cyazofamid	[mg]	nd/nd	nd/nd	57/nd	nd/nd	nd/nd	nd/nd	57/nd	nd/nd
Dissipation	pesticide mass	[mg day^{-1}]	31.32/1.90	0.26/0.02	610.90/33.34	22.48/0.52	83.30/1.49	6.15/0.40	336.37/17.94	12.54/0.33
	total dissipated fluxes of pesticides[c]	[mg day^{-1} m^{-2}]	0.10/0.01		1.84/0.10		0.25/0.003		1.02/0.06	
	pesticide storage compartment	[–]	bed sediments and plants		water and TSS		bed sediments		bed sediments	
	pesticide dissipation process	[–]	plant uptake		biodegradation		sorption on bed sediment		biodegradation	

[a]Mean ±2s. [b]Values are reported as "dissolved"/"solid-bound" loads, respectively at the inlet and the outlet of the wetland, for the three phases of the investigation period. [c]The total dissipated fluxes of pesticides were calculated as follow: (total inlet pesticide load - total outlet pesticide load)/number of days of the phase/wetland area (319 m^2).

Chemical Analyses

The dissolved oxygen, pH, conductivity, redox potential and temperature were directly measured in the field using WTW multi 350i portable sensors at the center of the four cells of the forebay and in the 6 piezometers of the gravel filter. The hydrochemical characteristics (TIC, DIC, NPOC, DOC, TKN, $PO4^{3-}$, P_{tot}, NO_3^-, NO_2^-, NH_4^+, Mn^{2+}, Fe^{2+}, Fe_{tot}, SO_4^{2-}, Mg^{2+}, Na^+, Cl^-, and K^+) were determined in the water samples using FR EN ISO standards and laboratory procedures. Ten fungicides that is, cyazofamid, cyprodinil, difenoconazole, dithiocarbamates (metiram-zinc and mancozeb), fludioxonil, kresoxim methyl, metalaxyl, pyrimethanil, spiroxamine, tetraconazole, 1 herbicide, glyphosate, and its degradation product

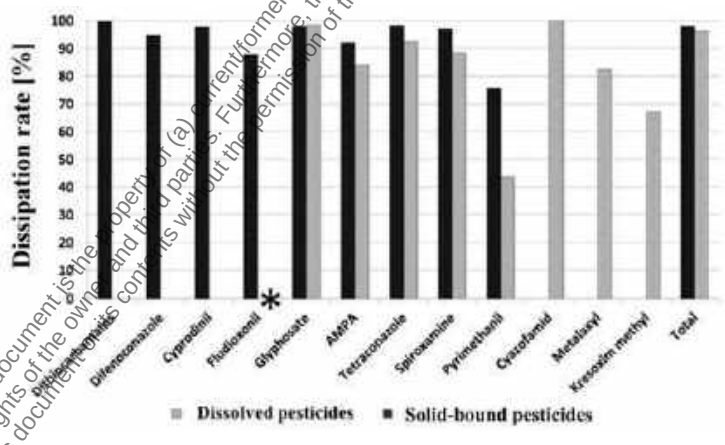
AMPA were analyzed because of their widespread use and high frequency of application on the catchment. The fungicides and herbicides were quantified by LC−MS/MS following SPE extraction according to the NF XPT 90-210 standards and procedures. The dithiocarbamates (metiram-Zn + mancozeb) were quantified by GC−MS/MS via the headspace quantification of CS2 following the acid-catalyzed hydrolysis of dithiocarbamate in a $SnCl_2$+HCl solution. Glyphosate and AMPA were quantified following derivatization with fluorenemethoxycarbonyle (FMOC).

Results and Discussion

Pesticide Dissipation by the Wetland

The dissipation rate of the total pesticide loads by the wetland was 96.3 %. The total dissolved pesticide load that entered the wetland during the investigation period (23 March to 28 September) was 56.6 ± 13.2 g (<0.7 µm) and 58.9 ± 13.9 g (<0.22 µm) and accounted for 95 % of the total inflowing load, whereas the load of solid-bound pesticides was only 3.0 ± 1.0 g. The dissolved pesticides loads in the fractions <0.7 and <0.22 µm did not significantly differ at the inlet and at the outlet of the wetland. This highlights that pesticides were predominantly transported in the dissolved phase, in agreement with previous study. During the investigation period, 2.1 g of dissolved pesticides and 0.06 g of solid-bound pesticides were released by the wetland (the average daily flux of total pesticides was 11.6 mg/day) (Table 7.5-167). The average Kd and Koc values calculated for the pesticide significantly differed between the wetland inlet and outlet, and the forebay. Field Kd and Koc values should be cautiously considered as limits of pesticide quantification in TSS (10 µg/kg) were 2 orders of magnitude higher than limits in water (0.1 µg/L) due to the analytical difficulty to extract solid bound-pesticides.

Figure 7.5-131: Dissipation rates of dissolved (<0.7 µm) and solid-bound pesticides (>0.7 µm) in the stormwater wetland (Rouffach, Alsace, France) from 23 March to 7 September 2011. ∗fludioxonil dissipation in the dissolved phase was negative (−1267 %)



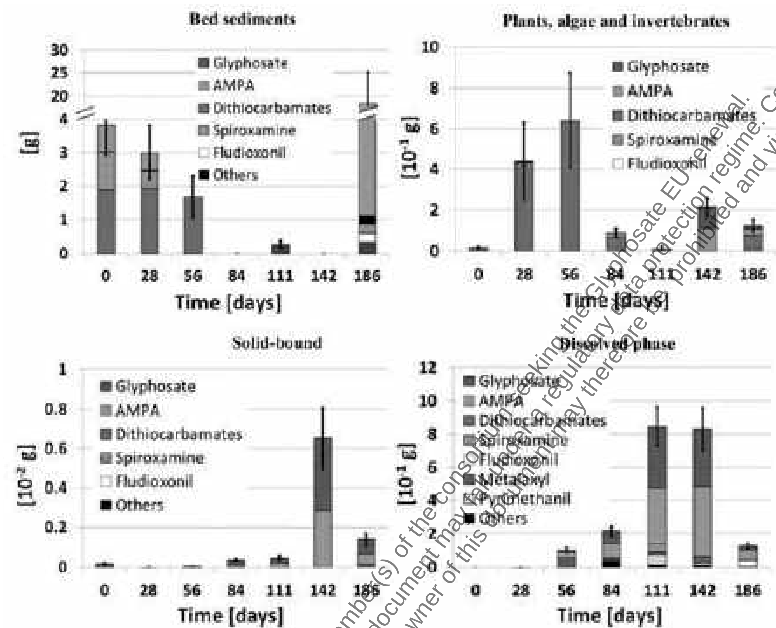
The weekly dissipation rates of the dissolved pesticides averaged 96.2 ± 8.2 %, but ranged from negative values for fludioxonil (−1266 %) to 100 % for cyazofamid (Figure 7.5-131). Fludioxonil entered the wetland during the late summer (after day 147) and larger fludioxonil loads were released by the wetland (59.3 mg) compared with those entering (4.3 mg), which indicates the persistence of fludioxonil since the previous agricultural season. The dissipation rate of the total solid-bound pesticides (>0.7 µm) was 98 % and ranged from 75.5 % for pyrimethanil to 99.8 % for the dithiocarbamates (Figure 7.5-131), underscoring the high capacity of the wetland to trap solidbound pesticides through settling processes. The hydrological conditions did not influence the dissipation of the dissolved and the solid-bound pesticides because no correlation was found on a weekly basis between the dissipation of total pesticide loads and the average quiescent period (the time between two runoff events) or the hydraulic retention time (HRT) in the wetland. Glyphosate (48.8 g; 86.3 %), AMPA (5.4 g; 9.5 %), metalaxyl (1.3 g; 2.2 %), pyrimethanil (0.4 g; 0.7 %) and tetraconazole (0.3 g; 0.5 %) primarily contributed to the inflowing load of dissolved pesticide (<0.7

µm), whereas glyphosate (1.7 g; 56.7 %), the dithiocarbamates (1.0 g; 33.7 %), AMPA (0.2 g; 7.7 %), spiroxamine (0.05 g; 1.7 %) and tetraconazole (0.003 g; 0.1 %) contributed to the solid-bound load. The mean AMPA fraction (%AMPA, calculated on a weekly basis as a percentage of the total molar loads of glyphosate and AMPA) was 27.6 ± 20.4 % at the wetland inlet and 68.5 ± 33.0 % at the outlet, which indicates that glyphosate was degraded into AMPA in the wetland as described previously. The overall dissipation rate of glyphosate was 98.5 %, whereas that of AMPA was 84.3 %, which highlights that AMPA was more persistent in the wetland compared to glyphosate.

Due to the dense wetland vegetation cover and the relatively high photodegradation half-life times (DT_{50} photolysis > 10 days), significant pesticide photodegradation is not expected for the studied pesticides, with the exception of cyazofamid (DT_{50} photolysis = 0.1 days), which contributed to only 0.1 % of the total inflowing pesticide load. Hydrolysis is expected to be negligible in the wetland conditions, except for the dithiocarbamates (with a DT_{50} of 1.3 days, pH 7, and 20 °C). It is noteworthy that the dithiocarbamates were only found in association with the suspended solids, which supports the idea that solid-bound dithiocarbamates were more stable than dissolved dithiocarbamates. Pesticide loss by volatilization can be neglected in the mass budget for pesticides with a lowvapor pressure (<0.18 mPa), and estimates of the total mass loss by volatilization for pyrimethanil, metalaxyl and spiroxamine (vapor pressure <3.5 mPa, nondimensional Henry constant $<10^{-7}$) are in the range of the analytical error (<1 % of the total mass budget). Consequently, in our study, a negative pesticide mass budget that cannot be attributed to storage in any of the wetland compartments can be attributed to biodegradation, except in the case of cyazofamid. The pesticide mass budget made it possible to distinguish three seasonal phases during the investigation period with respect to pesticide inputs, distribution, degradation and retention, as follows: spring (23 March (or day 0) to 18 May), summer (19 May to 10 August) and late summer (11 August to 07 September (or day 168)) (Table 7.5-167, Figures 7.5-132 and 7.5-133).

Seasonal Change in the Pesticide Distribution and the Wetland Source/Sink Functions.
On day 0 (March 23), the amount of pesticides stored in the wetland was 3.8 g, 91.6 % of which was found in the fine bed sediments (50−250 µm), 8 % in the medium bed sediments (250−1000 µm), 0.2 % in the vegetation and <0.3 % in the other compartments. The dithiocarbamates (1.9 g), spiroxamine (1.1 g) and AMPA (0.8 g) primarily contributed to the total pesticide load. The amount of pesticides stored on day 0 corresponds to the pesticides used in the previous winegrowing period because no pesticides were used in the catchment before day 0.

Figure 7.5-132: Monthly pesticide mass budget (g) in the bed sediments, plants and invertebrates, suspended solids and dissolved phase of the stormwater wetland (Rouffach, Alsace, France). The error bars correspond to the analytical uncertainty. The errors for the pesticide loads were calculated via error propagation based on the uncertainty of the individual pesticide concentration measurements and the mass estimate for each wetland compartment



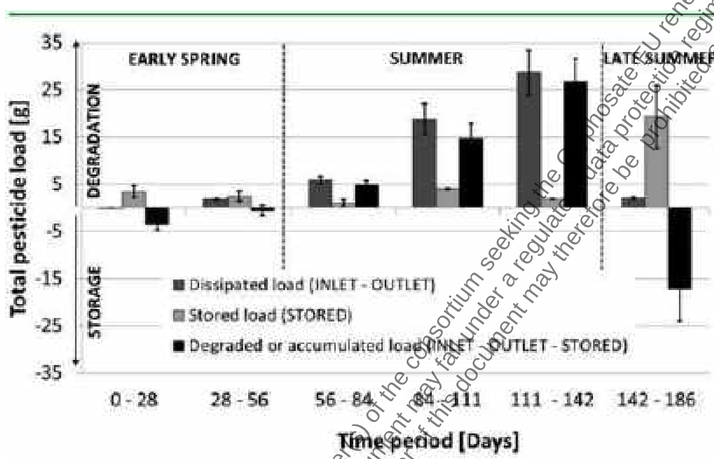
Spring

During the spring (day 0 to day 56), the wetland received 3.1 % of the total incoming load during the investigation period and acted as a pesticide sink. The dissolved pesticides accounted for 94.3 % of the total incoming load. Metalaxyl and spiroxamine were only found in the dissolved phase, whereas the dithiocarbamates were exclusively associated with the TSS and accounted for 88 % of the total solid-bound load. 16.3 mg of pesticides were released by the wetland, corresponding to 0. 9 % of the inflowing load. The pesticides accumulated in the wetland sediments (1.7 g on day 56; 69.3 % of the total load stored) and the vegetation (0.6 g; 26.4 %) because the amount of pesticides found in the wetland on day 56 (2.4 g) exceeded that entering the wetland (1.8 g) (Figure 7.5-133, Table 7.5-167). However, the pesticide amount stored in the wetland decreased from 3.8 to 2.4 g. The primary contributors to the total pesticide load in the wetland compartments were as follows: the dithiocarbamates >spiroxamine > AMPA > glyphosate. Biodegradation of spiroxamine, glyphosate and AMPA occurred in the wetland as indicated by (i) decreasing load of spiroxamine and AMPA in the bed sediments (from 1.1 g to 0 for spiroxamine and from 0.8 to 0.03 g for AMPA) without any increase in the other compartments, and (ii) the release of only 15.9 mg of AMPA (the maximum AMPA concentration was 0.2 µg/L), 0.4 mg of spiroxamine and no glyphosate, although 1.1 g of glyphosate and 0.4 g of AMPA entered the wetland during the spring. The degradation of spiroxamine and AMPA occurred under aerobic conditions prevailing in the wetland forebay during the spring, as indicated by the average oxygen concentration (3.9 ± 4.1 mg/L), the redox potential (50 mV ± 160 mV), as well as release of nitrate and sulfate by the wetland. Nitrate release may either occur by nitrification or result from vegetation decay, and the release of sulfate by the wetland supports sulfite and sulfide oxidation. Pesticides were partly translocated from the bed sediments to the vegetation during the spring. The biomass of the aerial plant parts increased from 0.5 to 3.7 kg/m^2 and that of the roots from 1.6 to 8.2 kg/m^2 during the vegetative phase. The dithiocarbamates were taken up by the plants, as indicated by a decrease of the dithiocarbamates load in the fine bed sediments (from 1.6 to 1.3 g between days 0 and 56) and an increase in the vegetation (from 7.1 mg to 508.7 mg in the roots and from 9.1 mg to 128.0 mg in the aerial parts), whereas 2.3 mg of the dithiocarbamates were found in the algae on day 56 (i.e., <0.1 %

of the total pesticide load stored in the wetland). Dithiocarbamates did not accumulate during the summer in the wetland, although the degradation of dithiocarbamates decreased over time (Figure 7.5-132).

Figure 7.5-133: Pesticide mass budget highlighting the pesticide storage vs degradation in the stormwater wetland (Rouffach, Alsace, France)

The dissipated pesticide load (INLETload − OUTLETload) refer to the load stored (STOREDload) or degraded (INLETload − OUTLETload − STOREDload >0). The accumulated load (INLETload − OUTLETload − STOREDload <0) is the load accumulated in the wetland from one period to another. The errors for the pesticide loads were calculated via error propagation based on the uncertainty of the individual pesticide concentration measurements and the mass estimate for each wetland compartment.



Summer

During the summer (day 56 to day 142), the wetland acted as a pesticide sink and degradation was the primary dissipation process. This resulted in low pesticide accumulation in the wetland despite large pesticide inputs (Figures 7.5-132 and 7.5-133, and Table 7.5-167). During the investigation period, 52.5 g of dissolved and 2.9 g of solid-bound pesticides entered into the wetland, which represented 93 % of the total input load. The total pesticide amount released by the wetland during the summer was 1.9 g, corresponding to 3.4 % of the total inflowing load. Anoxic conditions prevailed, as indicated by the dissolved oxygen concentrations (0.3 ± 0.3 mg/L) and the redox potential values (−20 to −120 mV), whereas the nitrate and sulfate mass budgets indicate nitrate (−69 ± 42 %) and sulfate reduction (−51 ± 25 %) in the wetland from the end of June (day 91). Anoxic conditions in the wetland were compatible with pesticide degradation, as shown for glyphosate and AMPA, whereas dithiocarbamates degradation appeared to be less efficient.

The total pesticide amounts stored in the wetland were 0.3, 1.2, and 1.1 g between days 56 and 84, day 84 and 111, and day 111 and 140, respectively, which accounted for less than 6 % of the total load entering the wetland. This result indicates that pesticide degradation was the prevailing process during the summer (Figures 7.5-132 and 7.5-133). The stored pesticide loads were 1 order of magnitude lower than that found during the spring, even though the pesticide input in the wetland was larger during the summer (Table 7.5-167). The largest pesticide loads were found in the dissolved phase of the wetland forebay (203.0 mg on day 84 and 765.4 mg on day 142), which indicates limited pesticide storage in the sediments and vegetation due to the regular mixing of the forebay water during runoff events (the average quiescent period was 6.4 ± 2.3 days, indicating more frequent runoff events in the summer than in the spring). Other relevant storage compartments in the wetland were the fine bed sediments (270.7 mg on day 111) and the plant roots (from 55.8 mg on day 84 to 177.5 mg on day 142). The average vegetation density was 175 stems/m^2, that is, 4 times higher than in early spring (Table 7.5-167). The plant roots accumulated glyphosate (101.0 mg or 0.3 mg/m^2 wetland) and AMPA (76.5 mg or 0.2 mg/m^2 wetland) (Figure 7.5-132), indicating sorption onto the roots and/or plant uptake. The pesticide loads in the algae and invertebrates accounted for 10.4 % (31.3 mg) and 0.13 % (0.4 mg), respectively, of the total stored pesticide load on day 84. Algae were not

observed in the wetland after day 84 and during the late summer. It is noteworthy that pesticides taken up by organisms may be quickly and irreversibly conjugated in less-extractable forms, leading to an underestimation of the pesticide amounts stored in plants, algae, and invertebrates.

Late summer

During the late summer, the wetland mostly acted as a pesticide sink with moderate pesticide degradation and primary storage in the fine bed sediments. Pesticides were not used in the vineyard catchment after day 132 (02 August). Anaerobic conditions prevailed, as indicated by the mass depletion of nitrate (37 %) and sulfate (28 ± 53 %) by the wetland. 2.2 g of dissolved pesticides and 38.8 mg of solid bound pesticides entered wetland, corresponding to only 3.8 % of the total inflowing pesticide load (Table 7.5-167). The total pesticide amount released by the wetland during the late summer was 0.2 g, corresponding to 7.3 % of the inflowing load. The pesticides were stored as follows: in the fine bed sediments (18.4 g of pesticides) > plant roots (122.6 mg) >dissolved phase (105.4 mg) > coarse bed sediments (46.1 mg) > the TSS of the forebay (12.1 mg). The total pesticide load stored in the wetland consisted of AMPA (17.4 g), glyphosate (321.2 mg), fludioxonil (306.5 mg), and spiroxamine (284.1 mg) and was greater than during the summer, except in the dissolved phase, the TSS and the vegetal biomass, which primarily stored pesticides during the summer (Figure 7.5-132). Although the vegetation cover was denser during the late summer (200 stems/m^2), the plant root biomass was lower than in the summer (−32 %) and the evapotranspiration decreased, indicating vegetation senescence during the late summer. Hence, plant decay may also have contributed to the pesticide accumulation in the bed sediments by increasing both the content of organic matter and the diversity of the carbonaceous sorbent materials. The accumulation of AMPA in the fine bed sediments during the late summer can be related to the longer average HRT (26.9 ± 8.1 days) compared with that in summer. The longer HRT enhanced the settling of solid-bound pesticides from the water column, thus increasing the contact time of the dissolved AMPA−sediment interactions (Figures 7.5-132 and 7.5-133). This result is in agreement with previous studies showing that AMPA is more sorptive than glyphosate and primarily sorbs to the metal (hydro)oxides in clay materials and humic substances. AMPA was also found to be more persistent than glyphosate in soils due to the formation of non extractable residues, which stabilizes AMPA and lowers its bioavailability. In addition, the clay fraction of the fine bed sediment increased by 22 % from day 0 to day 168, which potentially increased the specific surface area for the AMPA-clay metal (hydr)oxide interactions (Figure 7.5-132), thus lowering AMPA bioavailability and degradation during the late summer. The occurrence of a persistent stock of AMPA in the wetland sediments, which can be released during the winter, must be carefully considered in the management of wetland systems receiving pesticide runoff.

Environmental Implications for Wetlands Receiving Pesticide Fluxes

This study represents a first attempt to establish a complete pesticide mass balance in a wetland system under field conditions for assessing dissipation processes. The seasonal change in the partitioning, degradation, and distribution of the pesticides was quantified in a stormwater wetland to evaluate the dynamics of the pesticide sink and source functions. Although wetland field studies are invariably dependent on the system configuration and the study context, our results provide a rational basis for interpreting pesticides dissipation in planted stormwater wetlands collecting contaminated runoff under temperate climates. Our data highlight that the wetland system could act primarily as pesticide sinks from spring to summer. Stormwater wetlands can efficiently remove dissolved and solid-bound pesticides, even when the pesticides are predominately transported in the dissolved phase. The solid-bound molecules were efficiently retained by the wetland whereas mostly dissolved molecules, such as AMPA or fludioxonil, were moderately transported and less retained during the spring and late summer. Plant roots and fine sediments (50 and 250 µm) were the primary contributors to the retention of glyphosate, AMPA and dithiocarbamates. The pesticides did not accumulate in the vegetation except in the vegetative stage during the spring. The wetland vegetation enhanced pesticide degradation in the rhizosphere, and pesticide degradation corresponded to the development of the vegetation. Pesticide mass degradation was maximal during the summer when the vegetation was mature, under prevailing anoxic conditions, and when large pesticide loads entered the wetland. During the spring and late summer, the wetland mostly accumulated pesticides in the fine wetland bed sediments. AMPA accumulation in the fine sediments in late summer raises the issue of the ecotoxicological risk posed by the accumulation and the release of poorly described degradation products from wetland systems. Wetland systems can act not only as pollutant sinks, but also as pollutant sources, which raises concerns on the degradation, retention, and release of pesticides and

degradation products in wetlands intercepting pesticide runoff. This study shows that quantitative understanding of the pesticide sink and source functions can support the evaluation and the management of services provided by wetland ecosystems to improve water quality.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports the pesticide loss and input in a stormwater wetland in an agricultural region in France. Several pesticides were analyzed, among them glyphosate and AMPA. Analytical methods were poorly described in the article but were provided in the supporting information. Mostly dissolved molecules, such as AMPA or fludioxonil, were moderately transported and less retained during the spring and late summer. Plant roots and fine sediments (50 and 250 µm) were the primary contributors to the retention of glyphosate, AMPA. AMPA accumulation in the fine sediments in late summer raises the issue of the ecotoxicological risk posed by the accumulation and the release of poorly described degradation products from wetland systems.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/021
Report author	Norgaard, T *et al.*
Report year	2014
Report title	Leaching of Glyphosate and Aminomethylphosphonic Acid from an Agricultural Field over a Twelve-Year Period
Document No	Vadose Zone J. doi:10.2136/vzj2014.05.0054
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/052
Report author	Ramwell, C. *et al*.
Report year	2014
Report title	Contribution of household herbicide usage to glyphosate and its degradate aminomethylphosphonic acid in surface water drains
Document No	Society of Chemical Industry (wileyonlinelibrary.com) DOI 10.1002/ps.3724
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

It is necessary to understand the extent to which different sources of pesticides contribute to surface water contamination in order to focus preventive measures appropriately. The extent to which glyphosate use in the home and garden sector may contribute to surface water contamination has not previously been quantified. The aim of this study was to quantify the widely used herbicide glyphosate and its degradation product aminomethylphosphonic acid (AMPA) in surface water drains (storm drains) that could be attributed to amateur, non-professional usage alone. Maximum glyphosate and AMPA concentrations in surface water drains were 8.99 and 1.15 µg/L, respectively after the first rain event following the main application period, but concentrations rapidly declined to <1.5 and <0.5 µg/L. The AMPA:glyphosate ratio was typically 0.35. Less than 1 % of the applied glyphosate was recovered in drain water. Glyphosate and AMPA losses from urban areas that arise solely from amateur usage have been quantified. In spite of overdosing occurring, the authors reported that glyphosate concentrations in drain flow were lower than concentrations reported elsewhere from professional use in urban areas.

Materials and methods

A catchment suitable for the investigation of glyphosate in drain flow from purely domestic usage would ideally have the following attributes: no agricultural inputs of glyphosate, separate foul and surface water drains (the latter being reasonably accessible), a mix of hard/impermeable and permeable surfaces and a low probability of vandalism of the monitoring equipment. A small, residential catchment (5.16 ha) where the houses had separate foul sewers and surface water drains was identified in York, England as study site. Two ISCO 6172 automatic water samplers were installed to sample water (120 mL) from the final drain every 5 min, with the water from three consecutive samples being directed to a single bottle, giving one composite sample (360 mL) every 15 min. One sampler was triggered when rainfall exceeded 0.4 mm within 2 h; the other was triggered when the water level in the drain was >0.01 m. This approach was taken to minimise missing a sampling event because of equipment failure. Rainfall was monitored using a tipping-bucket rain gauge (resolution 0.1 mm) sited on top of one of the boxes used to house the water samplers. Discharge was measured using an ISCO 750 area/velocity flow module.

The study was undertaken in early summer (June–July 2009) when herbicide applications in private gardens are common in response to the favourable weather conditions for weed growth. Samples were taken during the first rain event (15 June 2009) after the equipment was installed (22 May 2009) and prior to the survey of the residents in order to monitor any 'background' levels of glyphosate. After that, samples were collected in response to all rain events until the end of July 2009. Samples were collected within 24 h. Samples were decanted from the glass collection bottles into high-density polyethylene (HDPE) bottles on return to the laboratory and stored in the freezer until dispatched for analysis.

The inputs of glyphosate into the catchment were established by means of a questionnaire. All houses in the catchment were approached by door-to-door visits over a period of 5 days during the day, in the evening and at the weekends. Fast Action Roundup Ready-To-Use (RTU) weedkiller (glyphosate 7.2 g/L MAPP 14481) in either a 1 L trigger sprayer or a 5 L 'pump and spray' container was supplied to those participants who requested it, or participants used products that they already had (n=2; Tesco's own-brand glyphosate and Pathclear – containing glyphosate, oxadiazon + diflufenican). The 1 L bottles were weighed before and after use in order to quantify the amount used. This was not possible with the 5 L RTUs as these were too heavy for field-portable scales.

It was necessary to estimate the amounts applied for 39 % of the residents. Similarly, only the total quantity of glyphosate used per household was known, so the amount used per application was calculated from knowledge of the weed density and area treated, as indicated on their pro forma, in order to distribute the total amount of glyphosate spray solution used between each application date.

Samples were analysed using an existing validated method. Samples were thawed, homogenised by shaking and then left to settle. Samples were not filtered in order to avoid potential glyphosate losses. Samples were derivatised prior to analysis: an aliquot of sample (50 µL) was transferred by pipette into a 10 mL reactivial, and reagent (2 mL) was slowly added (freshly prepared 2:1 mixture of trifluacetic anhydride and 2,2,3,3,4,4,4-heptafluorbutanol cooled to −20°C). The vial was then sealed and heated to 95°C for 2 h. After cooling, the excess reagents were removed under a stream of nitrogen at 40°C until dry. The sample was then dissolved in ethyl acetate containing 0.2 % citral (1 mL) and transferred to a vial ready for analysis. The limits of detection were 0.002 µg/L for glyphosate and 0.003 µg/L for AMPA, and the limits of quantification were 0.007 and 0.01 µg/L, respectively. All calibration graphs were linear over the standard range, with a typical linear correlation coefficient of 0.999. Recoveries at 0.05 µg/L were 108 ± 31 % for glyphosate and 121±17 % for AMPA.

Measurements of concentration and discharge were used to calculate the total mass of glyphosate leaving the catchment. Discharge measurements were collected every minute, whereas bulk drain water samples were collected every 15 min. It was therefore necessary to extrapolate the chemical data. It was assumed that there was a linear increase or decrease in concentration between successive samples, enabling a concentration per minute to be estimated. In addition, two total masses per rainfall event were calculated. The first was the total load between the first and last measured concentration. However, as this was not always the very first or very last sample generated, because some samples had insufficient volume for analysis, a second calculation was made where a concentration of zero was assumed as soon as the water sampler was triggered, and concentrations up to the first analysed sample were calculated by linear extrapolation as described above. The final total glyphosate loss per event was calculated from the sum of the loads for glyphosate + AMPA, where the final mass of AMPA was calculated from initial mass of AMPA × (molecular weight of glyphosate/molecular weight of AMPA).

Results

Of the 148 houses in the catchment, 82 separate households were interviewed and, of these, 34 agreed to participate in the study. The majority of applications occurred within the first 2 weeks of the study, with a notable 53 g of glyphosate being applied on a single day. More than half of this application could be attributed to a single person who applied 5 L (and therefore 36 g of Roundup) over a period of 2 days primarily to an area of ~10 m^2 that had a high weed infestation rate of >50 % for weeds that were ~10 cm high. Maximum concentrations of 1 µg/L of glyphosate and 0.43 µg/L of AMPA were detected in the 'background' drain samples, and the concentrations dipped to 0.33 and 0.37 µg/L, respectively, 5 h after the start of the rainfall event. The presence of glyphosate in the background sample indicated that there was an incomplete dataset for the total amount of glyphosate applied.

The first rain event after the main application period occurred on 3 July 2009 (2 weeks after the first recorded application), and three further events were monitored. The highest concentrations of glyphosate (8.99 µg/L) and AMPA (1.15 µg/L) occurred during this first rain event, although the concentrations rapidly declined within the first hour to <2 µg/L, with the final sample taken containing <1 µg/L. A short rain event on the following day (4 July 2009) generated further samples (after a further 0.79 g of glyphosate had been

applied in the catchment), with peak concentrations of 2.08 µg/L of glyphosate and 0.66 µg/L of AMPA. Glyphosate concentrations in the last monitored rain event were <1 µg/L, in spite of more than 4 g of glyphosate being applied in the intervening dry period between sampling events. AMPA concentrations ranged from 0.17 to 0.54 µg/L in this last event. These concentrations are the same order of magnitude as the initial 'background' samples. It should be noted that the glyphosate and AMPA concentrations reported here are those measured in the surface water drains, where there is relatively low discharge and therefore low dilution, and they are not representative of concentrations in surface water, where it would be expected that significant dilution would occur. The load of glyphosate is needed in order to estimate concentrations in surface water.

The total mass of glyphosate and AMPA detected in the drain was calculated for each rain event, and the results are presented in Table 7.5-35. Although over 71 g of glyphosate was applied prior to the first monitored post-application rain event, less than 0.5 % of this glyphosate was detected in surface water drain flow, even when accounting for both the glyphosate +AMPA. Samples collected on the next day, the second rain event after application, added very little glyphosate and AMPA to the total loss, such that the accumulated loss as a percentage of amount applied was still <0.5 %. Between 0.56 and 0.81 % (for the measured and extrapolated data, respectively) of the applied glyphosate had been recovered in drain flow by the end of the sampling period. These findings highlight that only a very small percentage of the applied glyphosate is recovered in surface water drains, and it is assumed that the majority of the applied glyphosate is retained in the catchment and/or degraded.

Table 7.5-168: Mass of glyphosate applied and recovered for individual rain events

	Sampling date			
	1 June	3 July	4 July	12 July
Glyphosate applied between sampling events (mg)	1	71 525	792	4197
Glyphosate load – measured (mg)	2.6	291	0.18	59
Glyphosate load – extrapolated (mg)	15	454	0.26	68
AMPA load – measured (mg)	1.1	40	0.06	36
AMPA load – extrapolated (mg)	5.7	61	0.08	40
Accumulated total glyphosate + AMPA loss (% of applied amount using measured data)	–	0.46	0.46	0.56
Accumulated total glyphosate + AMPA loss (% of applied amount including extrapolated data)	–	0.72	0.71	0.81

Extrapolating the known usage from the households surveyed (76.5 g glyphosate used by 34 out of 82 households) to the total number of households in the catchment (n=148) would give a total of 138 g of glyphosate applied. The quantity of glyphosate detected in the drains would then equate to 0.31 or 0.45 % of the amount applied using the measured and extrapolated sampling data respectively.

However, using a directly proportional relationship to augment wash-off to account for the lower-than-average rainfall in the study period gives a glyphosate loss of only 0.69 % and 1.01 % for the measured and extrapolated water sample data, respectively, which, if further extrapolated to account for glyphosate application in the entire catchment, gives glyphosate losses of 0.38 and 0.56 % for the measured and extrapolated water sample data, respectively. The data demonstrate that the loss of glyphosate in the present study (0.6 %) is low compared with other studies, in spite of one of the residents considerably overdosing. In the present study, an equivalent of 14.8 g/ha was applied, which compares to an estimate of 0.16 g/ha in another study having an emission factor of 2 %. It is likely that the lower quantities of glyphosate detected in drain water in the present study reflect the type of 'impermeable' hard surface treated, affecting the pathways of loss/retention mechanisms.

Conclusion

It is acknowledged that several glyphosate sources such as surface drains and wastewater treatment plants may contribute to the concentrations detected in the larger monitoring programmes, but the calculations above using data from the present study indicates that it is unlikely that losses from residential catchments, following proper usage, will contribute significantly to the total glyphosate load in surface waters compared with other urban areas. The findings of this study can therefore assist in ensuring that mitigation against glyphosate inputs to surface waters are targeted at the appropriate source of emission.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the contribution of the household usage of glyphosate to concentrations of the active and AMPA in surface water drains. The set-up of the experiment excluded agricultural use. The sample site was an urban residential area in the UK. Overall, less than 0.6 % of applied glyphosate was recovered from the storm drain outflow. Maximum detected concentrations were 8.99 µg/L and 1.15 µg/L for glyphosate and AMPA, respectively.
Some information missing, e.g. sample storage.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/006
Report author	Székács, *A. et al.*
Report year	2014
Report title	Monitoring and biological evaluation of surface water and soil micropollutants in hungary
Document No	Carpathian Journal of Earth and Environmental Sciences, August 2014, Vol. 9, No. 3, p. 47 - 60
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the soil monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/053
Report author	Daouk, S. *et al.*
Report year	2013a
Report title	The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part I: Method validation in different water matrices
Document No	Journal of Environmental Science and Health, Part B (2013) 48, 717-724
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

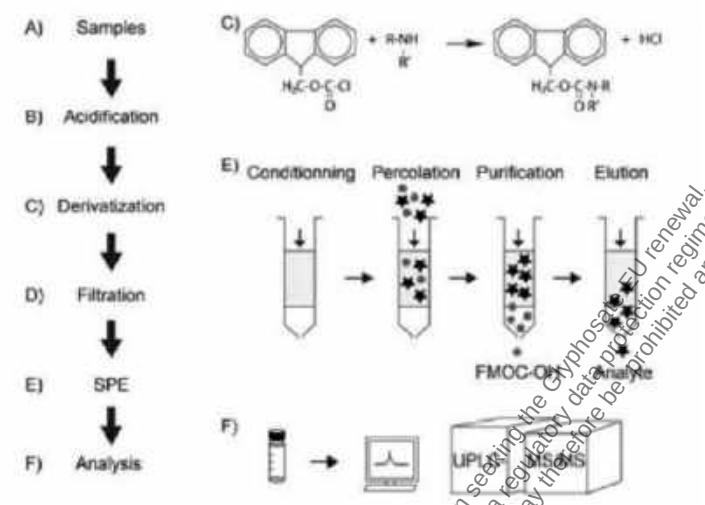
An analytical method for the quantification of the widely used herbicide, glyphosate, its main by-product, aminomethylphosphonic acid (AMPA) and the herbicide glufosinate at trace level was developed and tested in different aqueous matrices. Their derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) was done prior to their concentration and purification by solid phase extraction. The concentrated derivates were then analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Spiking tests at three different concentrations were realized in several water matrices: ultrapure water, Evian© mineral water, river water, soil solution and runoff water of a vineyard. Except for AMPA in runoff water, obtained regression curves for all matrices of interest showed no statistical differences of their slopes and intercepts, validating the method for the matrix effect correction in relevant environmental samples. The limits of detection and quantification of the method were as low as 5 and 10 ng/L, respectively, for the three compounds. Spiked Evian© and river water samples at two different concentrations (30 and 130 ng/L) showed mean recoveries between 86 and 109 %, and between 90 and 133 % respectively. Calibration curves established in spiked Evian© water samples between 10 and 1000 ng/L showed r^2 values above 0.989. Monitoring of a typical vineyard river showed peaks of pollution by glyphosate and AMPA during main rain events, sometimes above the legal threshold of 100 ng/L, suggesting the diffuse export of these compounds by surface runoff. The depth profile sampled in the adjacent lake near a waste water treatment plant outlet showed a concentration peak of AMPA at 25 m depth, indicating its release with treated urban wastewater.

Materials and Methods

Chemicals
Glyphosate (PESTANAL®, 99.7 %), glufosinate-ammonium (PESTANAL®, 99.2 %) and AMPA (99 %) were obtained from Sigma-Aldrich. Glyphosate-FMOC (98.5 %), AMPA-FMOC (97 %), glufosinate-FMOC (94 %) and the internal standards (IS) labeled with stable isotopes 1,2-;$^{13}C_2$,^{15}N glyphosate (98 %) and ^{13}C,^{15}N AMPA (99 %) were obtained from Dr. Ehrenstorfer.

Analytical method
The analytical method was adapted from Hanke *et al.* (see Figure 7.5-134).

Figure 7.5-134: **Main phases of the analytical procedure: a) Samples (80 mL); b) Acidification (1 h); c) Derivatization with FMOC-Cl (2 h); d) Filtration (0.45 µm); e) Solid-phase extraction (SPE); f) Analysis by UPLC-MS/MS**



Method validation in different water matrices

Spiking tests were performed in different water matrices in order to validate the method for further monitoring campaigns. The chosen matrices were: ultrapure water, Evian© water, river water, soil solution and runoff water. Natural water samples were collected close to the Lutrive River in a vineyard area located above the village of Lutry, Switzerland. Spiking tests were performed at three different concentrations (40, 80 and 120 ng/L) in all matrices; in natural waters blank subtraction was performed. In each case, samples were spiked and analyzed in triplicate. The main parameters of the different water samples are presented in Table 7.5-169: dissolved organic carbon (DOC) measurements were realized with a Liquitoc (Elementar©, Hanau, Germany), and water hardness was calculated after Ca^{2+} and Mg^{2+} measurements with an ICS-1100 as following: $[CaCO_3] = 2.5[Ca^{2+}] + 4.1[Mg^{2+}]$. Linear curves were obtained by plotting the ratio of the analyte area to the IS area against the ratio of the theoretical concentration of the analyte to the IS one. The corresponding internal standards were used for glyphosate and AMPA, whereas AMPA IS was used for glufosinate as they are both primary amines. The difference of slopes and intercepts for the curves were tested with the Prism® program. The *P*-values were fixed to 0.05. The accuracy of the method was assessed by calculating mean recoveries between the measured and the spiked concentrations in Evian© and River water, at 30 and 130 ng/L in triplicates. The limits of detection (LOD) and quantification (LOQ) of the method were determined in ultrapure, Evian© and surface water samples as the lowest concentrations with a signal/noise ratio equal or above three and ten respectively.

Table 7.5-169: **Main properties of analyzed water samples: pH, electrical conductivity (EC), dissolved organic carbon (DOC) and hardness, expressed in French degrees [°F]**

	pH	*EC [µS/cm]*	*DOC [mg/L]*	*Hardness [°F]*
Evian© water	7.2	590	<0.5	29.8
River Water	8.2	331	4.5	14.6
Soil Solution	8.5	450	1.5	41
Runoff Water	8.4	110	15	11

Environmental sampling

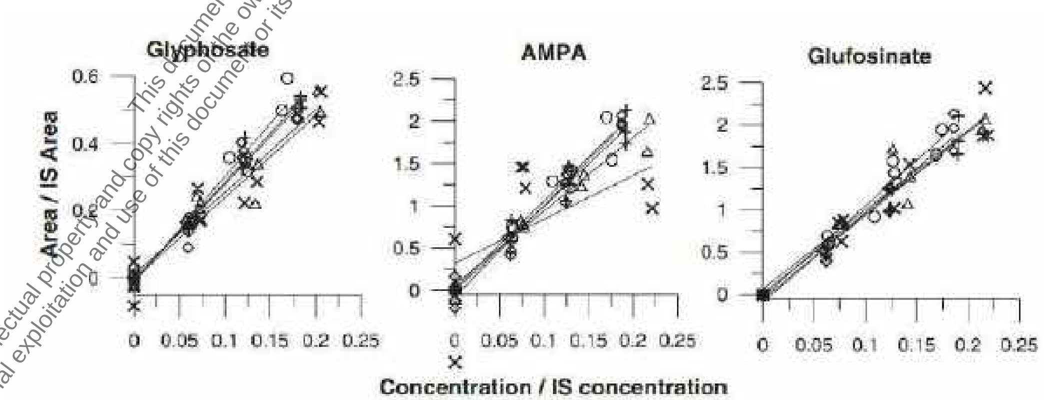
The Lutrive is a local river in the east of the city of Lausanne, at the western limit of the Lavaux vineyard area. Its small watershed (6.4 km^2) is characterized by different land uses: agricultural fields (45 %), of which 4.1 % are vineyards, urban and impervious surfaces (31 %) and forests (24 %). Grab samples were collected in the vineyard area during the growing season of 2010 and during both dry- and wet-weather conditions. Daily precipitations data of the meteorological station of Pully, west of the Lutrive River. Lake Geneva was sampled during dry weather on the 1st of July 2010, in the Vidy Bay near the waste water treatment plant (WWTP) outlet at nine different depths: -2, -5, -10, -15, -18.5, -21, -23, -25 and -29 m.

Results and discussion

Linearity and matrix effect

The response factors, i.e. the ratio area/IS area, for the different concentrations, normalized by IS concentration, showed a good linearity for the three compounds (Figure 7.5-135). Coefficients of determination (r^2) were all above 0.916 except for AMPA in runoff water, which was only 0.324. The slopes were varying between 2.4 and 3.1 for glyphosate, 5.1 and 10 .7 for AMPA and between 9.3 and 10.7 for glufosinate; Intercepts varied between -0.072 to 0.069. Both values, slopes and intercepts, were not significantly different between the different matrices samples for glyphosate and glufosinate. For AMPA however, a significant difference with the others was observed for the runoff sample with a slope of 5.1. The same was observable for the intercept that is higher than the others (0.32). These poorer results for AMPA in runoff samples can be explained either by substantial AMPA content in the spiked sample or by the high DOC concentration in this kind of sample (cf. Table 7.5-169). Nevertheless, in general the results confirm the ability of internal standards to compensate signal losses due to the matrix effect, which was stronger for runoff samples and soil solution. Indeed, both showed considerable discrepancies in slopes when compared with ultrapure, Evian© or river water samples before normalization with IS. Thus, with the exception for AMPA in runoff water, the results show the applicability of the method for the monitoring of several types of environmental samples: surface water, soil solution and runoff samples. Moreover, they confirm the suitability of Evian© water as calibration matrix. Indeed and surely due to its mineral content, Evian© water showed more similar slopes to environmental matrices than ultrapure water, making it more suitable for building calibration curves.

Figure 7.5-135: Performance of the developed method for the five water types tested: triplicates of spiked water samples of three concentrations (40, 80 and 120 ng/L) normalized by internal standards (IS) labeled with stable isotopes, with the different matrices: Ultrapure water (◦), Evian© water (+), River water (◊), Soil solution (Δ) and Runoff water (×); blank subtraction were performed for soil solution and runoff water samples



Precision and accuracy

Calibration curves in spiked Evian© water samples were generated from 10 up to 1000 ng/L. They showed a linear behavior with the following equations and coefficients of determination (r^2): glyphosate=1.222x +

5.204, r^2 = 0.991; AMPA = 1.325x + 1.707, r^2 = 0.989; glufosinate = 1.249x + 0.372, r^2 = 0.995. The inter-day variation of standards responses showed a good reproducibility with relative standard deviations of 9, 17 and 9 % for glyphosate, AMPA and glufosinate respectively at 50 ng/L and of 8, 4 and 9 % at 1000 ng/L; standard deviations of calibration curve slopes varied with 3, 1.6 and 6.5 % respectively. Despite elevated response variations for river water spiked at low concentrations (30 ng/L), the method showed a good accuracy with mean yields of spiked Evian© samples varying from 86 to 109 %, whereas for spiked river water samples they varied from 90 to 133 % (Table 7.5-170). This variability is substantially reduced at higher concentration (130 ng/L) and can thus be related to blank subtraction.

Table 7.5-170: Mean recoveries of spiked water samples (n = 3) [%, (SD%)]

Sample	*Concentration [ng/L]*	*Glyphosate*	*AMPA*	*Glufosinate*
Evian© water	30	109.1 (26.9)	86.3 (28.4)	88.0 (17.9)
Evian© water	130	101.1 (12.1)	105.1 (9.2)	104.0 (20.0)
River water	30	102.9 (133.8)	116.6 (33)	90.3 (47.8)
River water	130	115.9 (9.0)	133.1 (19.6)	109.8 (30.8)

Limits of Detection (LOD) and of Quantification (LOQ)

The limit of quantification (LOQ) in ultrapure and Evian© water samples was 7 ng/L, with a signal/noise ratio (S/N) equal or above 10 for the three compounds, whereas for river water samples S/N was lower. However, at 14 ng/L the S/N ratio was higher than 10. As the concentration of the first standard used to build the calibration curves is 10 ng/L, the LOQ can thus be fixed at this level. Spiked Evian© water at lower concentrations showed S/N ratios above 3 at 5 ng/L. In surface water sample S/N ratios above three were observed at 7 ng/L. Thus, the method LOD and LOQ were fixed at 5 and 10 ng/L respectively.

Environmental samples

Samples taken in the Lutrive River exhibited concentrations between the detection limit and maximum values of 800 ng/L and 300 ng/L for glyphosate and AMPA respectively (Figure 7.5-136). These concentration peaks are well above the legal threshold value defined for pesticides in Switzerland (100 ng/L). This implicates that glyphosate and AMPA may be hazardous for surface waters. These values are in the range of previous results obtained with occasional sampling in different other Swiss rivers. Glyphosate shows a typical pattern for chemicals applied in agriculture, with elevated concentrations during rain events, suggesting the transfer of these compounds from fields to surface water as already shown for other herbicides. The concentration pattern of AMPA also exhibits peaks, suggesting a similar transport pathway than for glyphosate. Results of the depth profile from the Vidy bay of Lake Geneva in July 2010 (Figure 7.5-137) showed glyphosate concentrations in general below the LOQ. Glufosinate and AMPA were detected in higher concentrations reaching a maximum of 26 and 67 ng/L, respectively, suggesting possible other sources than for glyphosate. For AMPA, the highest concentrations were found at 25 m depth, at which depth dissolved organic carbon (DOC) and major ions measurements show also a concentration peak. In a recent publication, Bonvin *et al*. highlighted the influence of the WWTP outlet and the release of treated wastewater at this specific depth, as confirmed by temperature and conductivity anomalies. This may explain the increase in concentrations of the metabolite AMPA and major ions at this depth as shown for other micropollutants such as pharmaceuticals. It has been suggested that the degradation of phosphonic acids in detergents was also an important source of AMPA in wastewater, especially during dry periods.

Figure 7.5-136: **Results for the Lutrive River from April to September 2010: Concentrations of glyphosate (•), AMPA () and glufosinate (◊); daily precipitations from the Pully meteorological station (Source: MeteoSwiss, histograms); threshold of the federal ordinance on water protection (Oeaux) for pesticides (100 ng/L; -).**

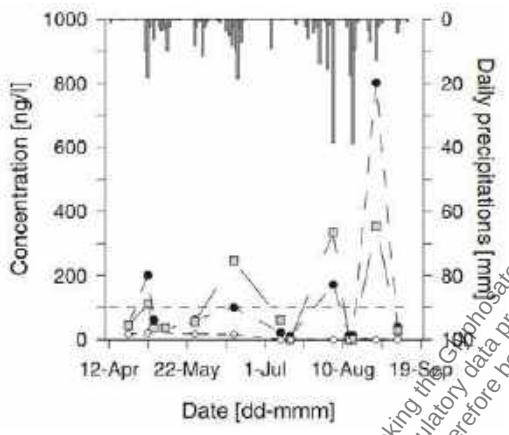
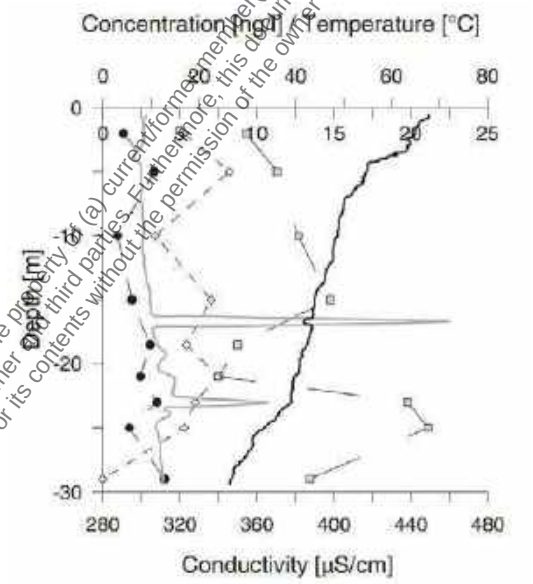


Figure 7.5-137: **Results for the lake depth profile sampled above the WWTP outlet in Vidy Bay, Lake Geneva, the 1st of July 2010; glyphosate (•), AMPA () and glufosinate (◊) concentrations; temperature (black line) and conductivity (grey line) profiles**



Conclusion

The validation of the method to quantify the herbicide glyphosate, its metabolite AMPA and the herbicide glufosinate at trace level in several types of natural waters was successful and allows following these potential hazardous molecules in the environment. Further investigations to better understand their behavior in soils after their application and their transport to surface water will be possible. Preliminary results of field studies show that river water samples exhibit a frequent pollution by the studied herbicides, which finally end up in Lake Geneva. Several samples showed concentrations above the legal threshold of 100 ng/L. This highlights the importance of monitoring these substances in the aquatic system.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The main focus of the article is the validation of an analytical method in different water matrices. The measured values for glyphosate and AMPA from natural sites can be used for monitoring purposes. They represent a vineyard area in Switzerland.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/007
Report author	Daouk, S. *et al.*
Report year	2013b
Report title	The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part II: The role of infiltration and surface runoff
Document No	Journal of Environmental Science and Health, Part B (2013) 48, 725–736
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the soil monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/054
Report author	Houtman, C. *et al.*
Report year	2013
Report title	A Multicomponent Snapshot of Pharmaceuticals and Pesticides and in the River Meuse Basin
Document No	Environmental Toxicology and Chemistry, Vol. 32, No. 11, pp. 2449-2459
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

The river Meuse serves as a drinking-water source for more than 6 million people in France, Belgium, and The Netherlands. Pharmaceuticals and pesticides, both designed to be biologically active, are important classes of contaminants present in this river. The variation in the presence of pharmaceuticals in time and space in the Dutch part of the Meuse was studied using a multicomponent analytical method for pharmaceuticals combined with univariate and multivariate statistical analyses of the results. Trends and variation in time in the presence of pharmaceuticals were investigated in a dead-end side stream of the Meuse that serves as an intake point for the production of drinking water, and 93 % of the selected compounds were detected. Highest concentrations were found for the antidiabetic metformin. Furthermore, a spatial snapshot of the presence of pharmaceuticals and pesticides was made along the river Meuse. Principal component analysis was successfully applied to reveal that wastewater-treatment plant effluent and water composition at the Belgian border were the main factors determining which compounds are found at different locations. The Dutch part of the river basin appeared responsible for approximately one-half of the loads of pharmaceuticals and pesticides discharged by the Meuse into the North Sea. The present study showed that multicomponent monitoring in combination with principal component analysis is a powerful tool to provide insight into contamination patterns in surface waters.

Materials and Methods

Chemicals
All chemicals were bought commercially.

Sampling
Grab-water samples were taken in prerinsed bottles of green glass every 4 wk from August 2010 to August 2012 (27 analyses) at the intake site for drinking-water production in the dead-end side stream of the river Meuse.

Analysis of pharmaceuticals with the ultra-HPLC/MS-MS multicomponent method
The analysis method contained 41 pharmaceuticals. In the selection of compounds, specific attention was given to pharmaceuticals with large consumption volumes. Eleven of the 20 most-sold pharmaceuticals were included. Other selection criteria were previous detection, ecotoxicological relevance (e.g., cytostatics, antibiotics, and nonsteroidal anti-inflammatory drugs), and representation of different therapeutic classes. The method was validated by calculating the recovery and standard deviation in surface-water samples from 8 different locations and sampled on different days spiked with pharmaceuticals. The average recovery was 91±14 %. Most (n = 32) compounds had a minimum reporting limit of 5 ng/L or lower, of which 18 compounds had a minimum reporting limit between 0.1 ng/L and 1 ng/L. The highest minimum reporting limit was obtained for clofibrate (85 ng/L).

Statistical analyses
Box plot figures representing minimum, first quartile, median, third quartile, and maximum concentrations were made in Excel for pharmaceuticals that were detected in at least 5 samples (20 % of the samples). Concentrations less than the minimum reporting limit were artificially set at 25 % of the individual minimum reporting limit. The significance of long term time trends and seasonal variation was tested using the statistical software package Trendanalist. For this purpose, the obtained data set was complemented with archived monitoring results for those pharmaceuticals that had also been monitored with enough sensitivity with LC/MS and gas chromatography (GC)/MS methods at the same location from 2005 to 2010 (the test requires results of a period of at least 4.5 yr). Long-term time trends were tested with linear regression (in case of normally distributed data), and the Mann-Kendall test corrected for seasonal effects (if data were not normally distributed). Seasonal variation was tested with Kruskal-Wallis tests.

Spatial snapshot of pharmaceuticals along the Meuse

Sampling locations

Water from 16 locations was sampled to generate a snapshot of the chemical water quality of the Dutch part of the rive Meuse. Samples were taken either from the main stream of the river Meuse or from rivers feeding the Meuse (Dommel and As) or from points along the Meuse or Waal nearer the entrance to the North Sea. Sampling points included locations near waste water treatment plants and drinking water abstraction points.

Sampling
Grab samples were collected from the 16 locations in a single sampling campaign between 13 and 16 September 2010. This month had some rain and a low to moderate flow in the river of, on average, 6.8 E6 m^3/d at the Belgian border. From 2 locations (1 and 12) additional samples were taken 1 wk prior (week 1, 9 September) and 1 wk after (week 3, 23 September) the sampling campaign (week 2, 13-16 September) to enable calculation of loads (see section Loads discharged into the North Sea) and to gain an understanding of variation in measured concentrations in the semi-long term. Samples were stored at 4°C and processed within 48 h.

Multicomponent analysis of pharmaceuticals and pesticides
Pharmaceuticals were analyzed on ultra-HPLC/MS-MS as described above. Concentrations of bisoprolol and propranolol were not included in the snapshot study due to uncertainty in the quantification in some samples caused by matrix effects (ion enhancement). The pesticides were analyzed by Aqualab Zuid, according to their own validated protocols. In short, pesticides were analyzed using a multicomponent method for 65 polar pesticides on ultra-HPLC/triple-quadrupole-MSMS. A total number of 140 less polar and more volatile pesticides were analyzed with a multicomponent method by means of GC-mass selective detection. The herbicide glyphosate and its metabolite aminomethylphosphonic acid were derivatized and analyzed by HPLC combined with fluorescence detection.

Statistical analysis
A principal component analysis was performed to cluster activities in the river basin according to contamination patterns using XLStat2008 software. Only compounds detected in at least 20 % of the measurements were included (10 water quality parameters, 19 pesticides, and 29 pharmaceuticals). All concentrations less than the minimum reporting limit were artificially set at 0. First, all concentrations were standardized ([concentration at individual location - average concentration]/standard deviation). A matrix was constituted with the 20 samples (16 locations plus the 2 additional samples at both locations 1 and 12) as loadings and filled with the standardized concentrations of general water-quality parameters, pharmaceuticals, and pesticides as observations. Replicates were included to investigate if these measurements would give factor loadings more similar to each other than measurements at other locations. Principal component analysis was performed to check the cumulative variance explained by the first principle component and then repeated with Varimax rotation to reduce the projection of the variance from projection on 20 components to projection on 3 components.

Loads discharged into the North Sea
Daily loads of pharmaceuticals and pesticides passing through the Meuse were calculated from the measured concentrations using flow data at locations 1, 2, 4, 12, and 16, because flow data for these locations could be provided by the Dutch Ministry of Infrastructure and Environment and the Water Board Aa and Meuse. Single measured concentrations for each individual compound were available for locations 2, 4, and 16. Loads for these locations were calculated using the average flow between 2 and 30 September 2010 as follows

$$\text{Load} = Q_{4\text{ wk average}} \times c$$

where Q represents the flow and c represents the compound concentration. Three weekly measured concentrations were available for locations 1 and 12. For these locations, average loads were calculated more precisely using the averaging estimators approach with the formula

$$\text{Load} = \left[\sum (c_i \times Q_i) / \sum (Q_i)\right] \times Q_{4\text{ wk average}}$$

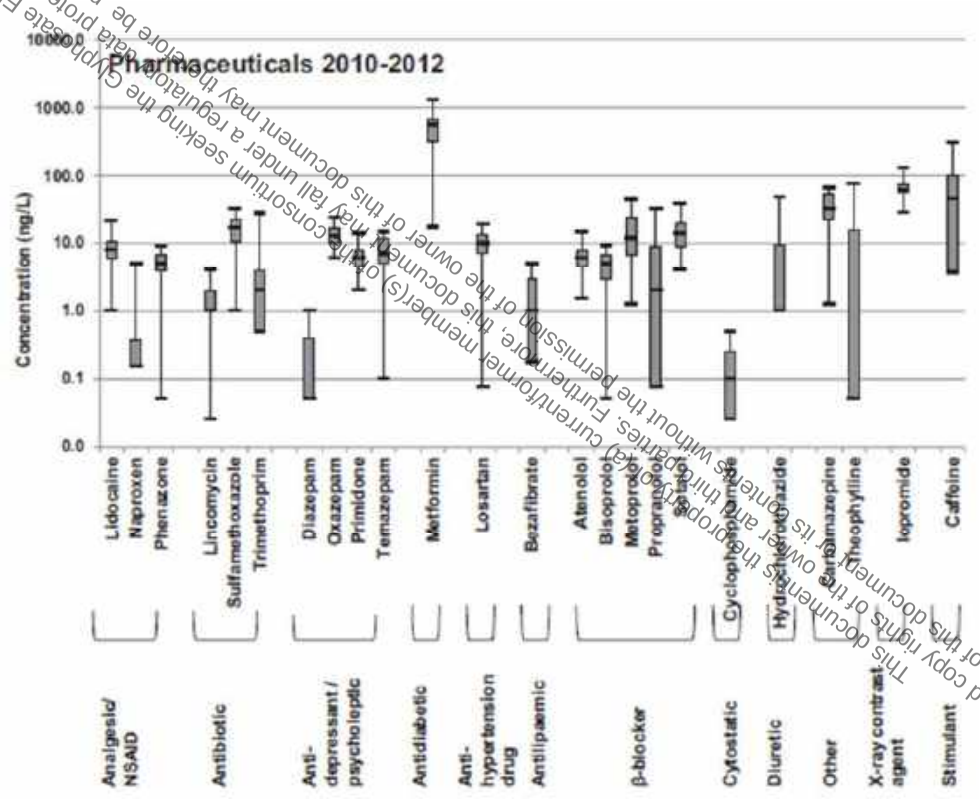
where Qi represents the flow on day i and ci represents the individual compound concentration on day i.

Results and Discussion

Variation and trends of pharmaceuticals in time

Presence of pharmaceuticals
Surface water from the enclosed branch of the Meuse (location 11) was analyzed every 4 wk from August 2010 to August 2012. Thirty-two compounds were detected at least once in the enclosed Meuse, and 20 compounds were detected in >50 % of the samples. Most compounds had median concentrations on the order of 10 ng/L, and variations of concentrations in time were seen in orders of magnitude. Figure 7.5-138 provides the concentration characteristics of those pharmaceuticals detected in at least 20 % of the samples, represented as a box plot.

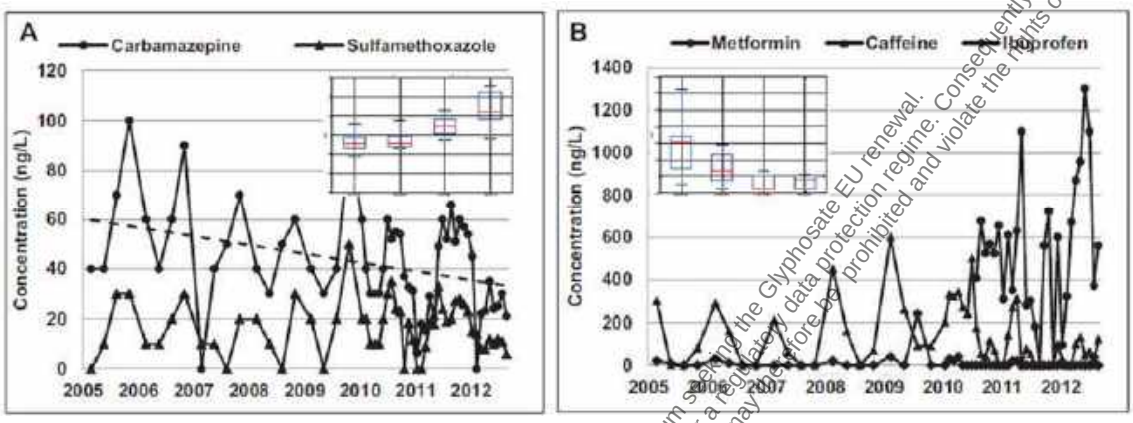
Figure 7.5-138: Box plot diagram summarizing the median, minimum, maximum, and 25th and 75th percentile concentrations of 4-wk measured concentrations of pharmaceuticals in the enclosed Meuse between August 2010 and August 2012. NSAID: nonsteroidal anti-inflammatory drug



Representatives of all investigated therapeutic classes were found during the 2 yr of measurements. Although most individual pharmaceuticals were found in concentrations around 10 ng/L, their combined concentration was between 0.3 µg/L (August 2011) and 1.6 µg/L (May 2012).

By far, the highest concentrations (on average 0.6±0.3 µg/L) were found for the antidiabetic drug metformin (Figure 7.5-139B). Because more than 80 % of Dutch diabetes type II patients are treated with this drug with daily doses up to 3 g to lower their serum glucose levels, this drug is number 5 in the top list of most prescribed drugs in The Netherlands (http://www.gipdatabank.nl/); and will probably also be among the top prescribed drugs in Belgium and France.

Figure 7.5-139: Concentration patterns of pharmaceuticals in surface water from the enclosed Meuse between 2005 and 2011. The dotted line represents the measured trend for carbamazepine. Inserted panes show box-whisker plots of seasonal variations in the concentration of carbamazepine (A) and caffeine (B) in the 4 periods of January to March, April to June, July to September, and October to December



The 2 other compounds that were present in concentrations ≥100 ng/L were the stimulant caffeine and the X-ray contrast agent iopromide. Both compounds were found with median concentrations (46 ng/L and 60 ng/L, respectively) comparable to those previously found for other European rivers (72 ng/L and 100 ng/L, respectively). Six analgesics and nonsteroidal anti-inflammatory drugs were detected. Most prevalent were phenazone and lidocaine present in 96 % to 100 % of the samples. This is in line with previous findings. Ibuprofen, although belonging to the high-consumption volume compounds, was detected only once (40 ng/L), probably due to its relatively high minimum reporting limit (32 ng/L) and its almost complete removal (99 % removed during wastewater treatment.

Of the cholesterol synthesis inhibitors, only atorvastatin was detected once, possibly due to its high removal rate in wastewater treatment (85-90 %).

All investigated antidepressants/psycholeptics were detected. The benzodiazepines diazepam, oxazepam, and temazepam (psycholeptics) were included in the method because of their high consumption volumes. The highest concentration was found for oxazepam (24 ng/L). Of the cytostatics, cyclofosfamide was detected more frequently (52 %) than ifosfamide (11 %). Both were present at very low concentrations (maximum 1 ng/L) and could be detected only because of a rather low minimum reporting limit in our method for these compounds. The investigated antibiotics clearly divided into 3 (chloramphenicol, oxacillin, sulfaquinoxalin) that were (almost) never found and 3 (lincomycin, sulfamethoxazole, and trimethoprim) that were detected in almost every sample.

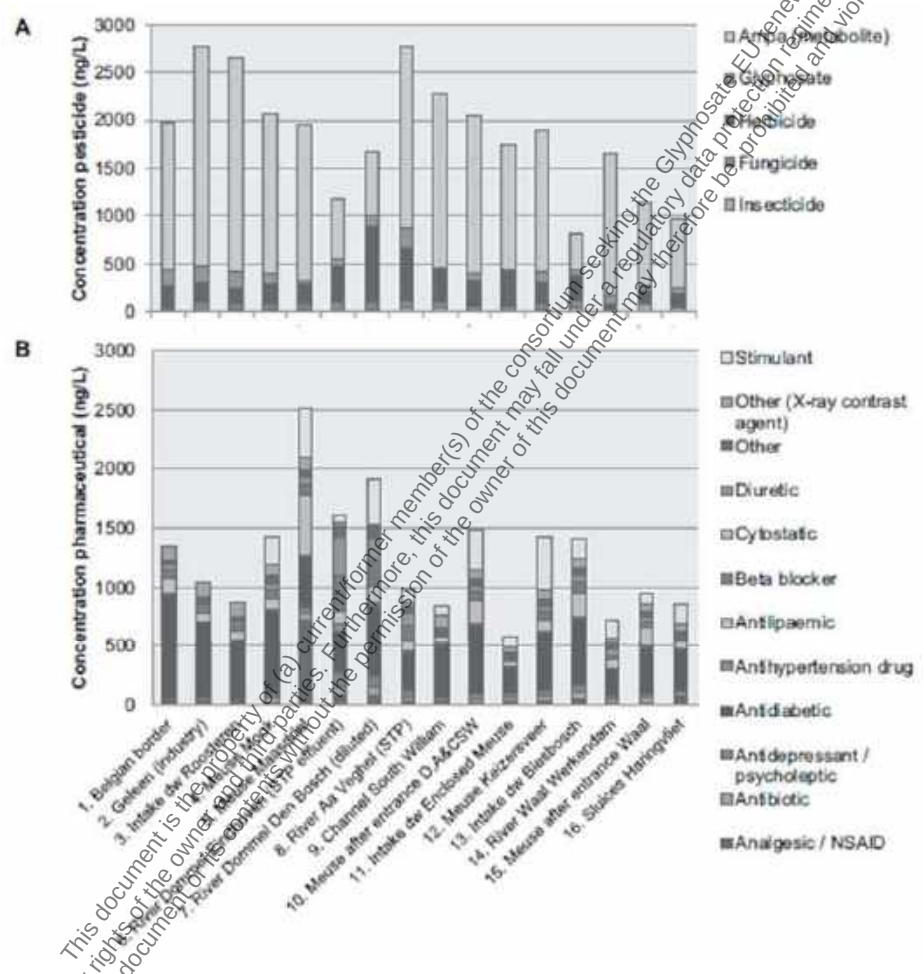
Antihypertension drugs, b-blockers and diuretics, the antiepileptic carbamazepine, and theophylline (drug against chronic obstructive pulmonary disease and asthma) were also structurally detected, with frequencies of 89 % for losartan and 67 % to 100 % for all 5 investigated b-blockers.

Carbamazepine was the only compound for which a significant temporal trend was found (Figure 7.5-139A). The concentration decreased by an average of 7.5 % (3 ng/L) per year. To investigate if the absolute amount of carbamazepine present in the enclosed Meuse had decreased, calculation of loads is necessary. Unfortunately, suitable flow data were not available for this location.

The concentrations of caffeine (p <0.2 %), carbamazepine (p <0.1 %), ibuprofen (p <0.1 %), and sulfamethoxazole (p <1 %) varied significantly between seasons. Carbamazepine and sulfamethoxazole (Figure 7.5-139A) showed highest concentrations in fall. Caffeine and ibuprofen (Figure 7.5-139B) showed highest concentrations (up to 600 ng/L) in winter and spring. Thirty-five pharmaceuticals were detected

during the sampling campaign in the Meuse (Figure 7.5-140B). Remarkably, a high concentration of 442 ng/L of unknown cause of the antilipemic pravastatin was detected in the Meuse at Maasdriel.

Figure 7.5-140: **Pesticides (A) and pharmaceuticals (B) in 20 water samples taken in September 2010 in the Dutch part of the Meuse River basin. Combined concentrations of all pharmaceuticals and pesticides are shown according to their class per location. Strictly speaking, glyphosate is a herbicide; however, because its concentration is so high and as such so determinative for the total concentration of herbicides, it is shown separately. NSAID: nonsteroidal anti-inflammatory drug; DW= drinking water**



Twenty-eight pesticides were detected. Concentrations varied between less than the minimum reporting limit (10 - 20 ng/L for most pesticides) to 1.3 µg/L for aminomethylphosphonic acid at location 2 (Figure 7.5-140A). Pesticides have long been the most important group of contaminants of concern to drinking-water companies using the Meuse as a water source. In contrast to pharmaceuticals, which are generally of point-source origin to watersheds (e.g. via WWTP outfalls), herbicides are mostly of non-point-source origin because they are applied directly to the land for agricultural purposes. The fact that only 14 % of 205 analyzed pesticides were detected might be partly explained by the fact that the multicomponent methods used for pesticides contained many pesticides that are not frequently found in Dutch surface waters anymore but for which monitoring is still obligatory according to European Union or national legislation. Only 4 insecticides were detected: diazinone, bromophos-ethyl, dichlofenthione, and N,N-diethylmeta-toluamide. All were found once, except N,N-diethyl-metatoluamide, which was found in 60 % of the samples. The main use of N,N-diethyl-meta-toluamide is not in agriculture but as an

insect-repellent by the public. Two fungicides were detected: carbendazim and 2,6-dichlorobenzamide. Both were present in more than 75 % of the samples. Nineteen detected pesticides belong to the class of herbicides. Among them were glyphosate and aminomethylphosphonic acid (its degradation product). They are notorious contaminants in the river Meuse. The main emission pathways to the Dutch part of the Meuse are runoff from pavements. Glyphosate is not well degraded in WWTPs. Degradation to aminomethylphosphonic acid takes place mainly in the environment. Glyphosate and aminomethylphosphonic acid were the only pesticides found in all samples. Relatively high concentrations of pharmaceuticals and pesticides were found in samples from the WWTP effluent receiving rivers feeding the Meuse.

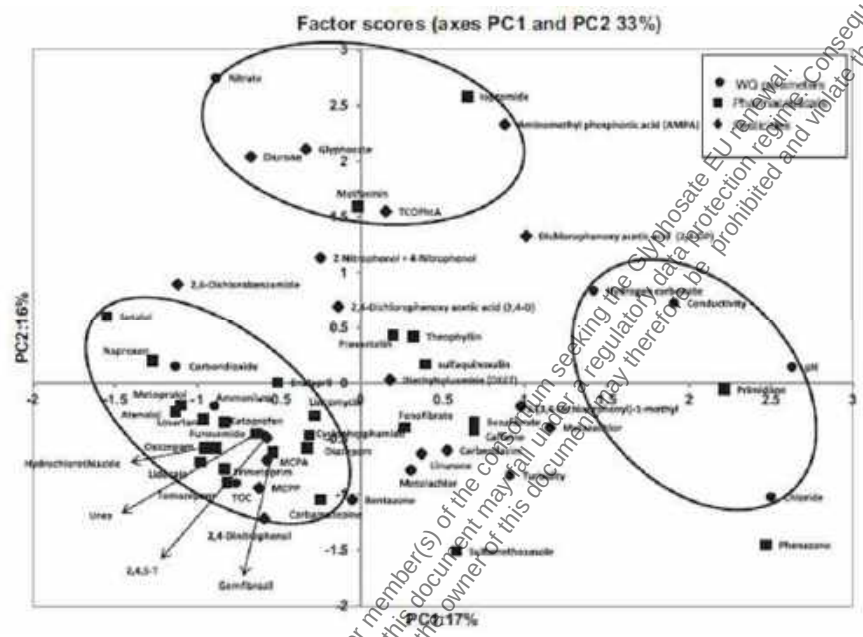
Principal component analysis-factor loadings

Principal component analysis was performed with a data matrix consisting of 20 samples (locations) as variables and 58 parameters as observations (10 water-quality parameters, 29 pharmaceuticals, and 19 pesticides that were detected in at least 20 % of the measurements). The analysis showed that of the 20 principle components, the first accounted for 17 % of the total variance, the second for 16 %, and the third for 14 % of the total variance of the data set. Collectively, the first 3 components could thus explain 47 % of the total variance. Locations with a positive score on principal component 1 are less influenced by WWTP effluent due to strong dilution (locations 14-16 are situated in the large river Waal and in wide parts of the Meuse) or environmental degradation (e.g., the residence time of water in the enclosed Meuse is about 6 wk). Principal component 2 groups samples mainly according to their geographical location in the river basin. A positive loading is found for locations in the first part of the river basin downstream from the Belgian border. No clear trend was observed in the loadings on principal component 3. This principal component apparently reflects projection of a combination of diffuse factors that could not be straightforwardly interpreted. Therefore, interpretation of scores was done only for principal components 1 and 2.

Principal component analysis-factor scores

Figure 7.5-141 shows the factor score plot for principal component 1 versus principal component 2. It gives an impression of the extent to which types of locations are predictors of the compounds found somewhere. The components belonging to the group of pesticides have factor scores most to the center of the plot and are scattered throughout the plot. This indicates that contamination with pesticides as a group occurs throughout the Meuse River basin and is not very location-specific within or is not projected enough on the first 2 components of the principal component analysis to elucidate a specific clustering of individual pesticides. Water-quality parameters and pharmaceuticals, however, do show distinct clustering and separation. On the left in Figure 7.5-141, the water-quality parameters (circles) CO_2, NH_4^+, TOC, and urea are found. Indeed, NH_4^+, TOC, and urea are known to be markers for WWTP effluent, especially during rainy periods and sewer overflows. In addition, the majority of pharmaceuticals detected in the present study (18, 62 %) are found in this same cluster. This is in agreement with the fact that WWTPs are important sources of pharmaceuticals in surface waters. Besides lack of persistence, for some compounds, such as sulfamethoxazole, sulfaquinoxalin (used in veterinary pharmaceuticals), and iopromide (only used in hospitals), scores outside the cluster can be explained because they have emission routes other than WWTPs. The score of caffeine, also not in the cluster, agrees with its high water solubility and low persistence, which make it a suitable marker for anthropogenic influence but not specific for WWTP effluent. Conductivity, HCO_3^-, pH, and chloride cluster positively on principal component 1.

Figure 7.5-141: **Factor score plot of measured parameters of the snapshot study on principal components 1 and 2 (PC1 and PC2, respectively) after Varimax rotation by principal component analysis. The factor scores indicate how the processes projected on the first and second principal components predict the contamination pattern of individual parameters (compounds). WQ = water quality; MCPP = 2-(2-methyl-4-chlorophenoxy) propanoic acid; MCPA = (4-chloro-2-methylphenoxy) acetic acid**



A decrease of HCO_3^- thus leads to higher concentration of CO_2, which was indeed found on the negative part of principal component 1. The highest pH and chloride were measured at locations in the delta area due to influence of intruding seawater and mixing with water from the river Waal.

Principal component 2 was found to represent the water composition of the Meuse at the Belgian border. In the upper part of the score plot, a remarkably high positive score on principal component 2 is found for nitrate and for some pesticides (glyphosate and its metabolite aminomethylphosphonic acid and diurone). This may be explained by leaching of these compounds from the sandy soils in the province of Limburg, which are used for intensive chicken and pig farming and treated with manure.

Calculated loads

The snapshot study was performed in September at low-flow conditions, just before the seasonal rise of flow in the river Meuse occurred. Water flows at the Belgian border were comparable during the first 2 sampling weeks (respectively, 8.2 E6 m^3/d and 8.4 E6 m^3/d) and much lower in the third sampling week (3.4 E6 m^3/d). Therefore, it was important to use all the replicate samples for the calculation of loads. Concentrations did not decrease proportionally (Figure 7.5-140), however, so loads of 18.3 kg/d (6.7 t/yr) of pharmaceuticals and 25.6 kg/d (9.2 t/yr) of pesticides are found at Meuse Keizersveer, indicating an increase in The Netherlands by a factor of 2.0 and 2.6, respectively, between the Belgian border and the Meuse at Keizersveer. In the delta area between Keizersveer and Haringvliet Sluices, a further increase in loads was observed. However, as water in Haringvliet consists of an average 1:4 mixture of water from the rivers Meuse and Waal, concentrations measured here are more representative for the Waal than for the Meuse. The calculated contribution of The Netherlands is higher than expected based on the area of the river basin (23 % of the area is situated downstream from the Belgian-Dutch border) and on the population density (40 % in The Netherlands). A possible explanation could be a higher consumption of pharmaceuticals and pesticides in The Netherlands in comparison with upstream countries. Another explanation might be that compounds emitted in the French and Belgian parts of the river basin have more

time for environmental degradation before they reach the Belgian border and, as such, concentrations in the upper part are less clearly related to emission than those downstream.

Conclusion
Multicomponent methods were successfully applied to investigate the presence of pharmaceuticals in time and space in the river Meuse. Among the detected compounds were those included in the method because of their large consumption volumes and those that were not investigated in the Meuse basin previously, such as metformin and benzodiazepines, confirming the relevance of consumption volume as a selection criterion for analysis of pharmaceuticals in the aquatic environment. It can - ideally, if combined with data on metabolism and degradation - serve to anticipate what can be expected to penetrate into surface waters and thus escape the pattern of focusing environmental monitoring only compounds previously detected (such as carbamazepine). The principal component analysis applied in this snapshot study revealed that emission of WWTP effluent and the composition of Meuse water as it enters The Netherlands at the Belgian border were the most important factors predicting the presence of compounds at locations in the Dutch part of the Meuse River basin. Multicomponent monitoring in combination with principal component analysis thus proved to be a powerful tool to provide insight into the relation between locations (activities in river basin) and compounds. However, pesticides especially occurred throughout the river basin and behaved mutually very differently in the principal component analysis. Therefore, it is not possible without considerable loss of information to select only 1 or a few compounds for monitoring that could represent a large group of environmental contaminants. Monitoring a broad range of compounds thus remains essential to investigate the quality of surface waters, especially if the water functions in the production of drinking water.

Several studies have concluded that measured traces of individual pharmaceuticals in water are too low to give rise to concern. Nevertheless, the structural presence of low concentrations of multiple pharmaceuticals in water abstracted for drinking-water production is an issue requiring further attention. A toxicological risk assessment of the mixture of compounds detected in water sources is the next step of our work. Pharmaceuticals and pesticides were found throughout the Meuse River basin. Because rivers often run through several countries, upstream activities can influence surface-water quality in other countries downstream. A good quantitative view of discharges was lacking for the Meuse. Our study showed that it is not appropriate to speak of the Dutch delta as Europe's "sewage drain," because approximately one-half of the discharged pesticides and pharmaceuticals appear to be added in The Netherlands itself. This result stresses the necessity of international collaboration in the protection of water quality in rivers crossing national boundaries.

Glyphosate concentrations in the range of 0.02 to 0.21 µg/L and AMPA concentrations between 0.38 and 2.28 µg/L were reported.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the results of a monitoring exercise at the river Meuse in the Netherlands, where concentrations of 29 pharmaceuticals and 19 pesticides were reported from a multisite sampling campaign to evaluate the status of the Meuse. Glyphosate concentrations in the range of 0.02 to 0.21 µg/L and AMPA concentrations between 0.38 and 2.28 µg/L were reported.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/055
Report author	Imfeld G. *et al*.
Report year	2013
Report title	Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland
Document No	Chemosphere 90 (2013) 1333–1339
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Glyphosate is an herbicide used widely and increasingly since the early 1990s in production of many crops and in urban areas. However, knowledge on the transport of glyphosate and its degradation to aminomethylphosphonic acid (AMPA) in ecosystems receiving urban or agricultural runoff is lacking. Here we show that transport and attenuation of runoff-associated glyphosate and AMPA in a stormwater wetland differ and largely vary over time. Dissolved concentrations and loads of glyphosate and AMPA in a wetland receiving runoff from a vineyard catchment were assessed during three consecutive seasons of glyphosate use (March to June 2009, 2010 and 2011). The load removal of glyphosate and AMPA by the wetland gradually varied yearly from 75 % to 99 %. However, glyphosate and AMPA were not detected in the wetland sediment, which emphasises that sorption on the wetland vegetation, which increased over time, and biodegradation were prevailing attenuation processes. The relative load of AMPA as a percentage of total glyphosate increased in the wetland and ranged from 0 % to 100 %, which indicates the variability of glyphosate degradation via the AMPA pathway. Our results demonstrate that transport and degradation of glyphosate in stormwater wetlands can largely change over time, mainly depending on the characteristics of the runoff event and the wetland vegetation. We anticipate our results to be a starting point for considering degradation products of runoff-associated pesticides during their transfer in wetlands, in particular when using stormwater wetlands as a management practice targeting pesticide attenuation.

Materials and Methods

Description of the vineyard catchment

The 42.7 ha vineyard catchment is located in Rouffach, Alsace, France. The study was carried out between 23 March and 30 June 2009, 2010 and 2011 because glyphosate use mainly proceeds in spring, from the end of March (bud-breaking of grapevine) to June (fruit-setting of grapevine). The detailed use of glyphosate in commercial preparations is provided in Table 7.5-171. The use of glyphosate was estimated based on yearly surveys addressed to the vine-growers (surveys covered at least 80 % of the vineyard area). The mean precipitation from March 23 to June 30 is 204 ± 70 mm (1998–2011). Rainfall-runoff events do not generate permanent stream in the catchment and statistically occur every week. During rainfall-runoff events, contaminated runoff converges at the outlet of the catchment where it is collected by the stormwater wetland. Surface runoff constitutes the main route of pesticide entry in the wetland.

Table 7.5-171: Glyphosate commercial preparations and amounts of glyphosate used at the vineyard catchment (Rouffach, Alsace, France) from March 23 to June 30 2009, 2010 and 2011. Values are given in grams of glyphosate

Commercial formulation	2009	2010	2011
Agave	0	0	2175
Amega max	0	363	0
Catamaran	0	250	0
Glifax	0	0	158
Glyfos	0	0	1954
Prologue	0	0	349
Roundup	715	170	191
Roundup flash	1112	0	540
Touchdown S4	2053	518	0
Total	**3881**	**1303**	**5370**

Description of the stormwater wetland

The wetland was constructed in 2002 to control flooding into the urban area. The stormwater wetland has a surface area of 319 m^2 and a total volume of 1500 m^3. It is composed of a naturally planted forebay (215 m^2). The mean hydraulic retention time was 11.0 ± 8.3 h during the periods of investigation. The water storage capacity of the wetland forebay was 50 m^3. Water depth in the forebay varied from 0.1 to 0.5 m during the investigation periods, depending on the runoff volume entering. A secondary small inflow also contributed to the volume entering the wetland from March to May. The budget of water volumes entering and outflowing the wetland was balanced when direct rainfall and evapotranspiration volumes were included (data not shown). Due to the clayey wetland bed (permeability (k_s) < 10^{-10} m/s) and based on the water balance, water losses by vertical infiltration were negligible.

The chemical composition of wetland sediment was (mean ± SD%; n = 5): organic carbon 15.0 ± 0.9, SiO_2 49.6 ± 0.5, Al_2O_3 10.4 ± 1.1, MgO 2.2 ± 0.1, CaO 11.6 ± 1.1, Fe_2O_3 4.5 ± 0.5, MnO 0.1 ± 0.0, Na_2O 0.6 ± 0.1, K_2O 2.4 ± 0.2 and P_2O_5 0.4 ± 0.1. The sediment texture was (%): clay 44, fine silt 33, coarse silt 10, fine sand 5, and coarse sand 8. The pH value was 8.1. Sediments were removed from the wetland forebay on February 2008. Glyphosate and AMPA were analysed in the wetland sediment in 2009 and 2011, as described previously (Maillard *et al.*, 2011). In 2009, the vegetation cover (*Phragmites australis, Juncus effusus and Typha latifolia*) in the wetland forebay was <1 % of the area in March and April, 10 % in May, and 50 % in June. In 2010 and 2011, the same plant species were present and the vegetation covered 100 % of the forebay area from April to June. *P. australis* (Cav.) represented 90 % of the total vegetation cover through the investigation period. No algal growth was observed.

Runoff discharge measurement and sampling procedure

Runoff discharges entering and outflowing the wetland were continuously monitored from 23 March to 30 June 2009, 2010 and 2011. The water depth was measured using bubbler flow modules combined with a Venturi channel at the wetland inlet and a V-notch weir at the outlet. Flow proportional water samples were collected at the inlet using a 4010 Hydrologic automatic sampler and at the outlet using a 6712FR ISCO Teledyne automatic sampler. Water samples (300 mL) were collected in jars, stored in the dark at 4°C after each runoff event, and placed on ice during transportation to the laboratory for chemical analysis. The series of discrete flow proportional water samples taken over a runoff event were combined in a single composite sample prior to analysis.

Chemical analysis

Conductivity, pH, dissolved oxygen and redox potential were directly measured in the field using WTW multi 350i portable sensors. Concentrations of dissolved organic carbon (DOC), total suspended solids, total phosphorus and PO_4^{3-} were determined by FR EN ISO standards and laboratory procedures. Glyphosate and AMPA were analysed according to the NF XPT 90-210 at the Pasteur Institute of Lille (France), which is accredited by the French National Accreditation Authority, and recognised by the European Cooperation for Accreditation. Water samples were filtered through 1 µm glass fiber filters and solid-phase extracted. Glyphosate and AMPA were extracted from sediment samples by ultrasonic and

methanol extraction. Quantification of glyphosate and AMPA was performed after derivatisation with fluorenemethoxycarbonyl. Both compounds had a quantification limit of 0.10 µg/L and 10 µg/kg in water and sediment samples, respectively. Extraction efficiencies of pesticides were obtained for each water sample set by spiking with surrogates. Relative standard deviation was 16 % for both compounds. Recovery efficiency was 86 % for glyphosate and 81 % for AMPA. Further quality control was achieved by using a blank for each set of samples.

Data analysis and calculation
Hydrological and hydrochemical variables were compared using the paired nonparametric Wilcoxon signed rank and the Spearman rank correlation tests. When glyphosate and AMPA concentrations were lower than the quantification limit, the concentrations were set to zero for calculating the occurrence and loading. For quantifying the transport of the total glyphosate loadings in the wetland, AMPA, as a glyphosate-derived compound, was expressed on a glyphosate mass equivalent. The mass equivalent load of glyphosate (MELgly) was calculated according to:

$$MEL_{gly} = Load\ (Glyphosate) + \left\{ Load\ (AMPA) \left[\frac{MW_{gly}}{MW_{AMPA}} \right] \right\}$$

where MW_{gly} = molecular weight of glyphosate (0.16907 kg/mol), and MW_{AMPA} = molecular weight of AMPA (0.11104 kg/mol).

For quantification of the total seasonal glyphosate load as a percentage of the seasonal applied amount of glyphosate on the vineyard catchment, a seasonal export coefficient of glyphosate (SEC_{gly}) was calculated:

$$SEC_{gly} = \frac{MEL_{gly}\ (mass\, season^{-1})}{Glyphosate\ \ application\ \ (mass\, season^{-1})} 100$$

The relationship between AMPA and glyphosate was evaluated by calculating the %AMPA as a percentage of total loads of glyphosate and AMPA:

$$\%AMPA = \frac{[AMPA]}{([Glyphosate] + [AMPA])} 100$$

where [AMPA] and [glyphosate] are their respective molar loadings in water. A %AMPA equal to zero indicates either that both AMPA and glyphosate were below the quantification limit or that only AMPA was above it.

Results

Hydrological characteristics and glyphosate export
Climatic and hydrological characteristics from 23 March to 30 June 2009, 2010 and 2011 are summarised in Table 7.5-172 and Figure 7.5-142. Comparison of climatic characteristics revealed that temperature, solar radiation and evapotranspiration values were significantly lower in 2009 compared to those in 2010 and 2011 ($p \leq 0.05$). Runoff events that generated volumes lower than 50 m^3 accounted for more than 80 %, indicating that small and moderate runoff events prevailed. The analysis of climatic and hydrological conditions revealed that conditions and rainfall-runoff patterns globally were similar in 2009, 2010 and 2011, although monthly variation occurred.

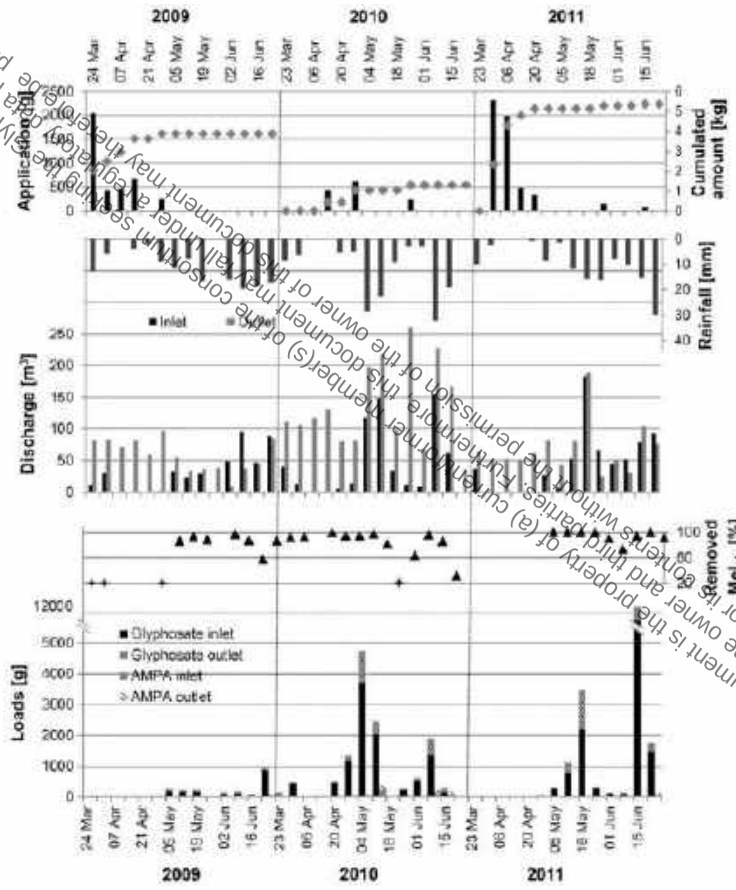
Table 7.5-172: Hydrology, hydrochemistry and glyphosate at the stormwater wetland (Rouffach, Haut-Rhin, France) from 23 March to June 30, 2009, 2010 and 2011. Values are provided as the mean and ranges

			2009		2010		2011	
Hydrology	Rainfall	(mm)	140		144		130	
	Runoff coefficient	(%)	0.82 (0.05–2.6)		0.80 (0.01–1.98)		1.18 (0.11–2.39)	
	Inflowing runoff volume	(m^3)	408		609		645	
	Number of runoff events	(–)	19		33		24	
	Quiescent period	(day)	11 (0.1–28)		7.4 (0.15–11)		10 (0.06–28)	
	Glyphosate use	(kg)	3.881		1.303		5.370	
	SEC_{gly}	(%)	0.07		0.2		0.06	
Hydrochemistry	Temperature	(°C)	16.6 (4.9–24)		11 (3.5–16)		14 (8.5–18)	
	pH	(–)	7.3–8.1		6.2–8.1		7.4–8.1	
	Redox potential	(mV)	153 (−9–260)		−50 (−216–142)		249 (106–334)	
	Dissolved oxygen	(mg L^{-1})	6.9 (1.4–13)		9.7 (3.6–12)		2.9 (0.5–8.5)	
	Total suspended solids	(mg L^{-1})	15 (0.6–97)		20 (7.2–34)		30 (4.9–96)	
	Dissolved organic carbon	(mg L^{-1})	14 (3.6–22)		5.2 (1.3–9.5)		7.2 (4.6–10)	
	Total phosphorus	(mg L^{-1})	0.11 (n.d.–0.31)		0.05 (n.d.–0.39)		0.48 (0.38–0.84)	
	Orthophosphorus	(mg L^{-1})	0.41 (n.d.–1.86)		0.26 (n.d.–1.23)		n.d.	
	Vegetation cover	(%)	1–25		100		100	
			Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
Glyphosate and AMPA	Glyphosate concentration	(µg L^{-1})	3.6 (0.2–11.0)	0.1 (n.d.–0.7)	30 (0.1–110)	0.2 (n.d.–1.7)	26 (n.d.–150)	0.1 (n.d.–0.8)
	AMPA concentration	(µg L^{-1})	1.1 (0.1–2.3)	0.4 (n.d.–0.7)	5.7 (n.d.–19)	0.3 (n.d.–0.9)	3.1 (n.d.–7.0)	0.1 (n.d.–1.1)
	MEL_{gly}	(g)	2.32	0.61	14	1.49	21	0.27
	%AMPA	(%)	35 (11–50)	79 (0–100)	27 (0–92)	73 (0–100)	34 (5–100)	36 (0–100)
	MEL_{gly} removal	(%)	75		89		99	
	Glyphosate load removal	(%)	92		95		100	
	AMPA load removal	(%)	30		76		95	

Note: n.d. = Not detected.

Yearly patterns of glyphosate use are provided in Figure 7.5-142. Most glyphosate is applied in late March and April. There were small applications in May (2010) and two in June (2011). Runoff events generating volume larger than 50 m^3 mainly occurred in May and June and influenced the seasonal pattern of both concentrations and apportionments of both glyphosate and AMPA in runoff entering the wetland. In contrast, MEL_{gly} that entered the wetland in March and April 2009 and 2011 was lower than 70 mg, likely due to the occurrence of less intense rainfall-runoff events. The SEC_{gly} was 0.07 in 2009, 0.2 in 2010 and 0.06 % in 2011, which indicates relatively low MEL_{gly} export. Although 3–5 times less glyphosate was used in 2010, MEL_{gly} export was larger compared to 2009 and 2011. This can be explained by more frequent and intense rainfall-runoff events following the applications and lower quiescent period (dry period between two rainfall-runoff events).

Figure 7.5-142: Temporal changes of glyphosate use, hydrological condition in the vineyard catchment (Rouffach, France) and total mass equivalent loads of glyphosate (MEL_{gly}) at the stormwater wetland from March to June 2009, 2010 and 2011. Stars represent negative removed MEL_{gly}



Occurrence and concentration of glyphosate and AMPA in the wetland

Concentrations and loadings of glyphosate and AMPA in the wetland are summarized in Table 7.5-172 and in Figure 7.5-142. 98 % of water samples (n = 46) collected at the inlet of the wetland through the three investigation periods had glyphosate and AMPA concentrations above the quantification limits. In contrast, only 52 % and 83 % of water samples (n = 64) collected at the outlet of the wetland had quantifiable concentrations of glyphosate and AMPA, respectively, which indicates that transport through the wetland reduced the occurrence of glyphosate and AMPA.

Glyphosate concentrations entering the wetland ranged from 0.1 to 150 µg/L. Mean inlet concentration (mean ± SD µg/L) was 3.6 ± 3.6 in 2009, 30 ± 30 in 2010 and 26 ± 48 in 2011, whereas that of AMPA was

1.1 ± 0.7 in 2009, 5.7 ± 4.9 in 2010 and 3.1 ± 2.6 in 2011. The mean concentration of glyphosate in 2009, 2010 and 2011 decreased by 36, 150 and 263 times from the inlet to the outlet of the wetland, respectively, whereas that of AMPA only decreased by 3, 19, 31 times, respectively. This indicates that concentration reduction by the wetland increased over year, although attenuation of glyphosate always was larger than that of AMPA on the seasonal time scale (Table 7.5-172). Concentrations of glyphosate and AMPA in the wetland sediments were below the detection limits in 2009 and 2011, which indicate no significant transfer of dissolved pesticides from the water column to the bed sediments or degradation of glyphosate and AMPA bond to sediment during the study period.

Transport and attenuation of MEL_{gly} in the wetland

In order to quantify the transfer and attenuation of glyphosate and AMPA in the wetland, the MEL_{gly} was evaluated at the wetland inlet and outlet (Figure 7.5-142). The total MEL_{gly} entering the wetland in 2009, 2010 and 2011 was 37.26 g, and that outflowing was 2.29 g, which corresponds to an overall MEL_{gly} removal efficiency of 94 %. The seasonal MEL_{gly} removal efficiency increased over time (75 % in 2009, 90 % in 2010, and 99 % in 2011). Interestingly, the MEL_{gly} entering the wetland also increased over time (2.38 g in 2009, 14.10 g in 2010 and 20.79 g in 2011), proportionally to the MEL_{gly} removed by the wetland (1.78 in 2009, 12.61 in 2010 and 20.52 g in 2011). This underscores the absence of threshold at which MEL_{gly} removal by the wetland would decrease at larger loading, which is further supported by a positive correlation between the inlet discharge, runoff-associated MEL_{gly} and MEL_{gly} removal by the wetland on the seasonal time scale ($p < 0.001$). Hence, the stormwater wetland very likely was not saturated by large input of glyphosate and AMPA during the study period, which may be due to the relatively low runoff coefficient at the study site. On a weekly basis, the MEL_{gly} removal efficiencies generally ranged between 80 % and 100 %, indicating that the wetland maintained its capacity to attenuate varying runoff-associated MEL_{gly} through the investigation period. When no storm event occurred and the wetland still was releasing water from previous storms, the weekly MEL_{gly} exported by the wetland ranged ranged from 18 to 60 mg. In these cases, the outflowing MEL_{gly} was larger than that at the inlet, thus yielding negative MEL_{gly} removal by the wetland.

Transport and attenuation of AMPA in the wetland

From 23 March to 30 June 2009, 2010 and 2011, the total load of AMPA entering and outflowing the wetland was 5.558 and 1.047 g, respectively. This corresponds to a total removal efficiency of 81 %, and underscores that possible degradation of glyphosate to AMPA did not result in larger amount of AMPA at the outlet compared to the inlet during the study period. The seasonal AMPA removal efficiency (28 % in 2009, 76 % in 2010, and 95 % in 2011) and the amount of AMPA removed by the wetland (0.188 g in 2009, 2.007 g in 2010 and 2.386 g in 2011) both increased over time. Globally, AMPA removal was lower than that of MEL_{gly} and glyphosate. The accumulation of AMPA following glyphosate degradation in the wetland was evaluated based on the relative proportion of AMPA as a percentage of total glyphosate and AMPA loadings (%AMPA). The %AMPA generally exceeded 60 % at the outlet, whereas AMPA rarely prevailed at the inlet. The mean %AMPA through the investigation periods was 32 ± 23 % at the inlet and 63 ± 40 % at the outlet, which clearly emphasises that the AMPA fraction increased during transport through the wetland. However, %AMPA ranged from 0 % to 100 % both at the inlet and the outlet of the wetland, which underlines the temporal variability of the AMPA portion in the MEL_{gly}.

Discussion

Several attenuation processes may simultaneously and synergistically control the transfer of dissolved glyphosate and AMPA in wetlands. The transfer and attenuation of glyphosate and AMPA in the wetland is expected to mostly vary according to their partitioning between the aqueous and solid phases, and the biodegradation activity. The partitioning and biodegradation of glyphosate and AMPA are themselves controlled by the runoff characteristics, the apportionment of runoff-related glyphosate, the extent of sediment sorption, as well as climatic and hydrochemical variables. In particular, the gradual increase of MEL_{gly} removal over time and the increase of %AMPA in the wetland suggest an initial fast attenuation of glyphosate entering the wetland driven by sorption to the wetland sediment and the temporal development of the vegetation, followed by a slower attenuation phase controlled by biodegradation.

The gradual increase of MEL_{gly} removal correlated with the larger cover of wetland vegetation (from <1 % in March 2009 to 100 % in June 2011), which suggests that vegetation also contributed to glyphosate and AMPA attenuation. Owing to large spatial and temporal variations in the vegetal biomass and species in the studied wetland, the contribution of vegetation in glyphosate and AMPA attenuation could not be quantified.

A gradual adaptation of wetland microorganisms for the use of various phosphorus sources, including glyphosate and AMPA may explain the gradual increase of seasonal MEL_{gly} removal. Since the quiescent period (i.e. time between two runoff events) apparently increased when the MEL_{gly} removal decreased, regular and transient runoff passing through the wetland did not seem to result in lower MEL_{gly} removal.

Biodegradation of AMPA generally is slower than that of glyphosate. The %AMPA reflects temporal changes in the glyphosate degradation efficiencies in the wetland. As glyphosate degradation occurred, the amount of dissolved glyphosate available for transport through the wetland decreases, whereas the amount of AMPA relatively increases. Consequently, AMPA may accumulate in the wetland when its degradation efficiency is significantly lower than that of glyphosate.

Conclusion
This quantitatively evaluates the transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland receiving runoff from a vineyard catchment with respect to the hydrological and hydrochemical conditions. The results indicate that the transport of dissolved glyphosate and AMPA through the wetland differed and largely varied both on seasonal and yearly time scales. Attenuation of glyphosate and AMPA loadings by the wetland generally was larger than 80 % and gradually increased over time, which correlated with larger vegetation cover, and possibly with gradual adaptation of glyphosate-degrading microorganisms. However, the fraction of AMPA generally was larger at the wetland outlet, which emphasises the persistence of AMPA and varying efficiencies of glyphosate degradation. Therefore, the transfer of degradation products of runoff-associated pesticides through wetland systems, and in particular those used as a management practice targeting pesticide attenuation, should be carefully considered.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports concentration measurements for glyphosate and AMPA residues in an artificial stormwater wetland in France receiving runoff from a vineyard catchment with respect to the hydrological and hydrochemical conditions. Specific analytical methods were used and the limits of quantification were stated. The maximum glyphosate concentration entering the wetland was 150 µg/L. However, the maximum AMPA concentration was 19 µg/L.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/022 CA 7.5/023 (Translation)
Report author	Martin, J. *et al*.
Report year	2013
Report title	Sugar Cane, Herbicides And water Pollution in Reunion Island: Achievements and Perspectives at the End of the First Decade of monitoring
Document No	Conference paper: 22nd Conference of COLUMA - International Days on Weed Control, Dijon, France, December 10-12, 2013 pp.641-651 ref.13
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/024
Report author	Mörtl, M. *et al*.
Report year	2013
Report title	Determination of glyphosate residues in Hungarian water samples by immunoassay
Document No	Microchemical Journal 107 (2013) 143–151
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (Central Food Research Institute, Hungary)
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/056
Report author	Vialle, C. *et al*.
Report year	2013
Report title	Pesticides in roof runoff: Study of a rural site and a suburban site
Document No	Journal of Environmental Management 120 (2013) 48 – 54
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

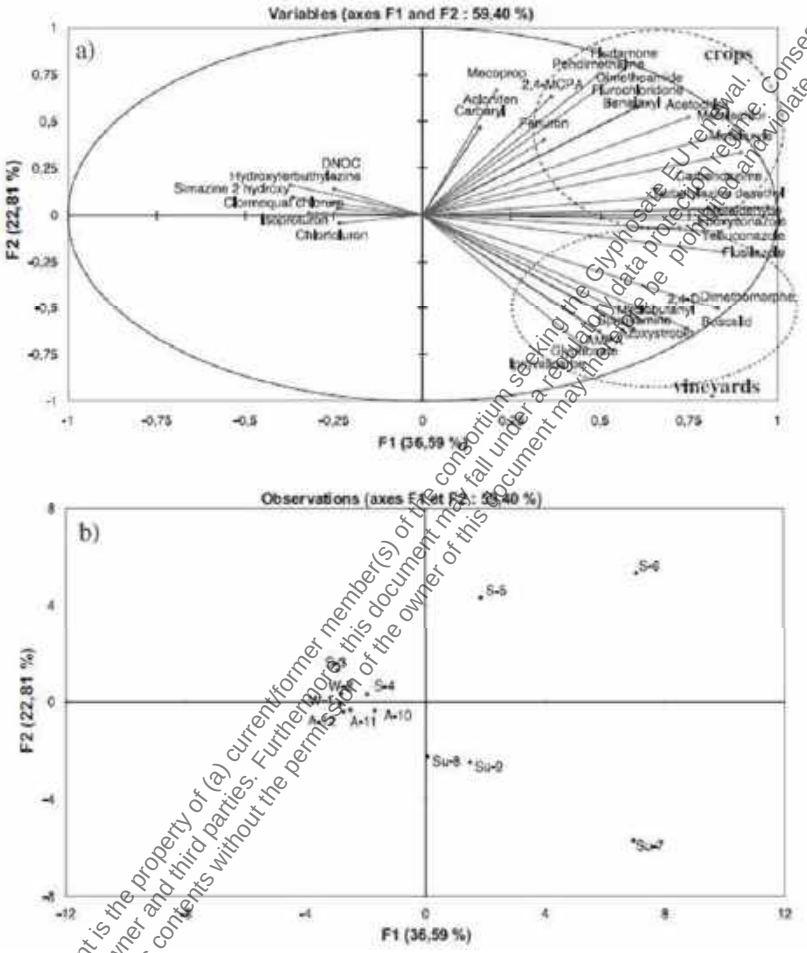
The quality of stored roof runoff in terms of pesticide pollution was assessed over a one-year period. Two tanks, located at a rural and suburban site, respectively, were sampled monthly. The two studied collection surface were respectively a tile slope roof and a bituminous flat roof. Four hundred and five compounds and metabolites were screened using liquid and gas chromatography coupled with various detection systems. Principal Component Analysis was applied to the data sets to elucidate patterns. At the rural site, two groups of compounds associated with two different types of agriculture, vineyard and crops, were distinguished. The most frequently detected compound was glyphosate (83 %) which is the most commonly used herbicide in French vineyards. At the suburban site, quantified compounds were linked to agriculture rather than urban practices. In addition, all samples were contaminated with mecoprop which is a roof-protecting agent. Its presence was attributed to the nature of roofing material used for rainwater collection. For both sites, the highest number and concentrations of compounds and metabolites were recorded at the end of spring and through summer. These results are consistent with treatment periods and higher temperatures.

Materials and Methods

Sampling site
Two sites in south-western France were selected to install commercially available domestic rainwater collection systems. Rainwater is first collected from the roof area and then channeled via gutters through pipes to an underground PEHD storage tank in order to be reused later. The first site was a private house surrounded by cultivated fields. The annual average rainfall in this region is 760 mm, and the average temperatures range from 7.9 to 18.3 °C. Agriculture in this area is characterised by the vineyards of Gaillac and crops such as wheat, maize and colza. The second site was the research building of an engineering school located in the suburban area of Toulouse, which has an urban population of around 860 000 inhabitants. This site is 12 km from the city centre. The annual average rainfall is 668 mm, with average temperature ranging from 8.6°C to 18.1°C. The area is near a well-travelled road and 70 ha of experimental cultivation fields

Sample collection
Stored roof runoff sampling was carried out monthly from January 2009 to December 2009 for site 1 and between November 2009 and October 2010 for site 2. Grab samples of stored roof runoff were taken around 10 cm under the surface water in the tank.

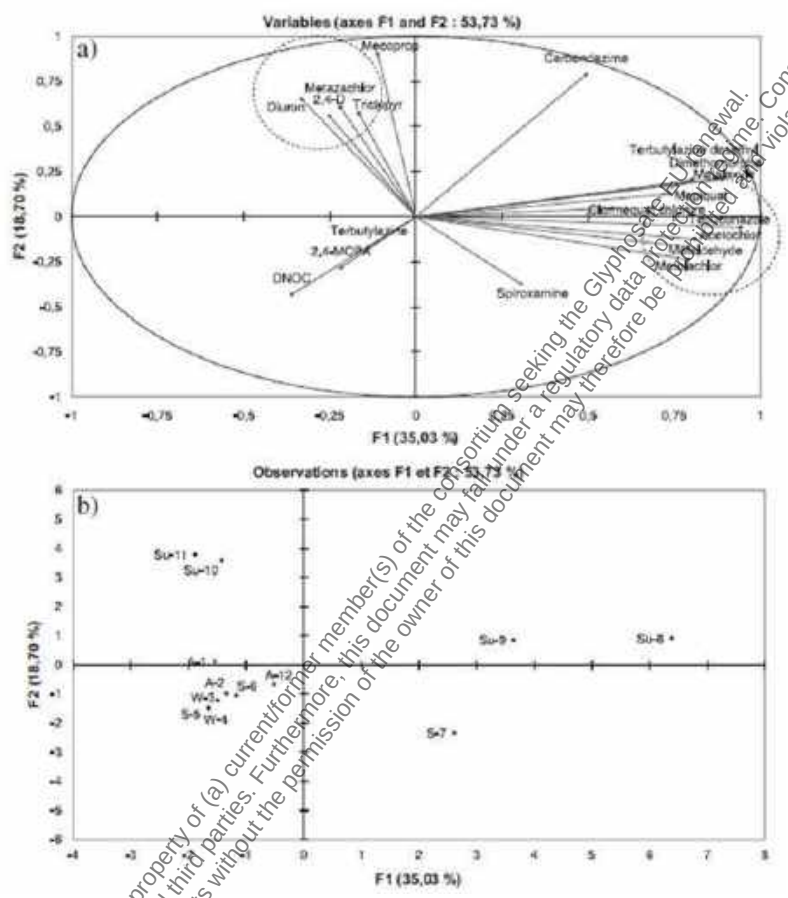
Figure 7.5-143: **a) The square cosines for all detected pesticides at site 1 (rural) in components F1 and F2 account for approximately 59 % of the total variance. b) A two-dimensional plot of the 12 observations at site 1 (rural) in F1 and F2. The letters indicate the sampling season and the number precises the sampling month (Su = Summer; A = Autumn, W = Winter, S = Spring; 1 = November; 2 = December; 3 = January; 12 = October)**



Pesticide analysis

Water samples were screened for 405 compounds. Extracts were simultaneously analysed by liquid chromatography (HPLC) and gas chromatography (GC) with systematic multidetection: with diode array detector (HPLC-DAD), coupled with tandem mass spectrometry (HPLC-MS-MS), with an electron capture detector and a nitrogen phosphorus detector (GC-ECD-NPD), or coupled with mass spectrometry (GC-MS). Other sample aliquots were analysed by HPLC after a derivation, or by headspace with GC-MS. Some compounds were quantified by direct injection and analysis by HPLC-MS-MS.

Figure 7.5-144: **a) The square cosines for all detected pesticides at site 2 (suburban) in components F1 and F2 account for approximately 55 % of the total variance; b) A two-dimensional plot of the 12 observations at the suburban site in F1 and F2. The letters indicate the sampling season and the number precises the sampling month (Su = Summer; A = Autumn, W = Winter, S = Spring; 1 = January; 2 = February; 11 = November; 12 = December)**



Results and discussion

Loadings for the two first components and square cosines are presented in a circle (Figure 7.5-143 and Figure 7.5-144a). A variable is increasingly well represented by a component as the corresponding square cosine nears unity. Graphically, this is represented as the variable nearing the edge of the circle. To elucidate the seasonal influence on concentrations of compounds, different observations were also represented in planes F1 versus F2 (Figure 7.5-143 and Figure 7.5-144b).

Rural site

At the rural site, the most frequently detected compounds were glyphosate (83 %), DNOC (75 %), AMPA (58 %), metolachlor (R + S) (58 %), carbendazim (50 %), and 2,4-MCPA (50 %). Analysis revealed that the highest concentrations measured were for glyphosate (6 µg/L). In addition, concentrations of several hundreds of ng/L were measured for AMPA, metolachlor, DNOC and metaldehyde in order of decreasing concentrations. Types of compounds detected are consistent with the agricultural practices in the region. In rural zones, herbicides are predominantly used, with fungicides being the next most common. Insecticides are used only to a minor extent. The presence of compounds at the end of spring and in the summer is illustrated in Figure 7.5-143b. Some summer samples are well represented in the first group, corresponding to vineyard pesticides, and the spring sampling is well represented in the second group, corresponding to

crop pesticides. As a result, the distinction of samples of the same season is obviously due to agricultural uses. The ambient temperature may also have an influenced.

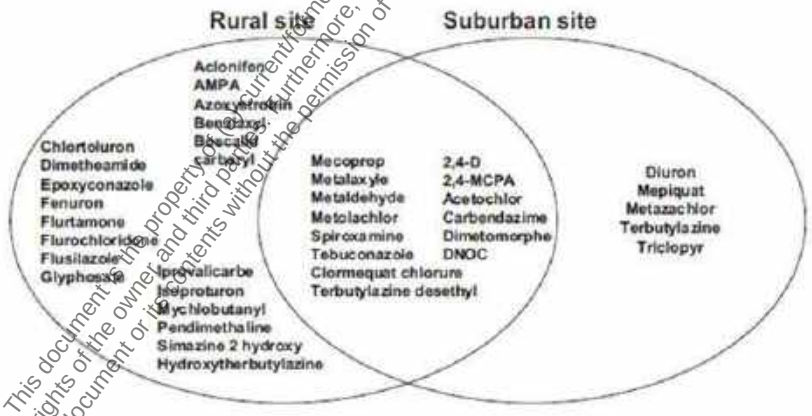
Suburban site

At the suburban site, the most often detected compounds, which appeared in at least 50 % of the suburban samples, were mecoprop (100 %) and DNOC (75 %). The compound with the highest measured concentrations was mecoprop (4.8 µg/L). Up to hundreds of ng/L were quantified for DNOC, metaldehyde, 2,4-MCPA, and metolachlor. The percentage of occurrence of mecoprop in roof runoff at the suburban site was 100 %. Mecoprop is a roof-protecting agent. Thus, in this study, this compound comes from the roofing material itself. The release appeared predominantly when the ambient temperature was high. Thus, the maximum concentration was observed in the summer. The suburban site studied seems to be influenced by nearby agriculture pesticide use rather than urban pesticide practices.

Comparison of the two sites

Of the 405 pesticides and metabolites analysed, 34 were detected more than once in the roof runoff samples collected at the rural site, of which 26 were above the limit of quantification at least once. At the suburban site, 15 pesticides were quantified, and only 4 were detected more than once over the twelve samples. The majority of compounds found were herbicides; the next most common compounds found were fungicides. Metabolites were the third most common class of compounds found. Concerning the spatial variation, compounds detected in the tanks are different for the two sites. There were 14 compounds detected at least once at both of the two sites; 20 compounds were found only in the rural zone, and 5 were detected exclusively in the suburban area (Figure 7.5-145). Considering only the number of compounds detected, a greater diversity of compounds was observed in the rural zone. Concerning the seasonal variation of the number of compounds detected, conclusions are identical for the two study sites. The most complex mixtures of compounds were sampled at the end of spring through summer at both sites (Figure 7.5-146).

Figure 7.5-145: Pesticides detected according to sampling site

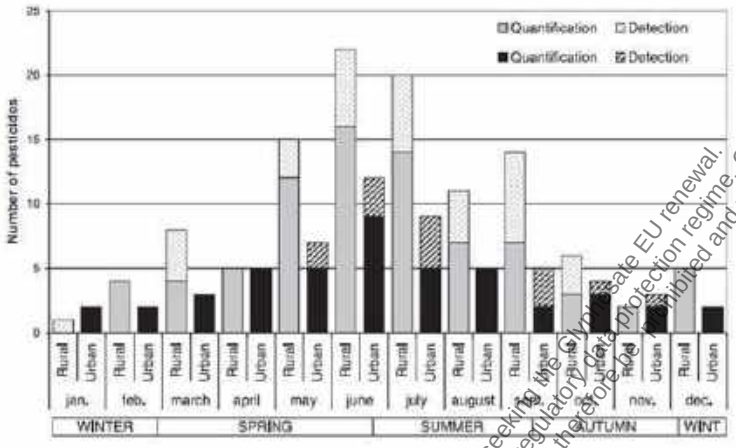


Conclusions

This study presents results concerning the quality of stored roof runoff in terms of pesticide contamination. No less than 405 compounds or metabolites were screened over a year for both a rural and a suburban site in south-west France. Even if this study is based on a limited data set, an effort was made to extract more information from the data set through the use of multivariate analysis techniques. At the rural site, PCA permits distinguishing compounds according to the type of surrounding agriculture, i.e., vineyard and crops. At the suburban site, the presence of compounds seems to be influenced more by local agriculture than by urban practices. Both sites at the end of spring through the summer were identified as particularly sensible seasons for compounds concentration and diversity. High concentrations of a roof-protecting agent were quantified in roof runoff from a bituminous flat roof. In the context of rainwater harvesting, which is becoming a common practice, this study reveals the importance of collected roof runoff pollution in terms

of pesticides concentrations. Not only seasonal but also spatial variability of this contamination over the year was monitored.

Figure 7.5-146: Number of pesticides detected or quantified over the year at the two sites



3. Assessment and conclusion

Assessment and conclusion by applicant:
The article reports the concentrations of glyphosate and AMPA among some other hundreds of substances in the roof runoff from two experimental sites in France, one in a rural area, the other one in a suburban area. At the rural site, two groups of compounds associated with two different types of agriculture, vineyard and crops, were distinguished. The most frequently detected compound was glyphosate (83 %) which is the most commonly used herbicide in French vineyards. At the suburban site, quantified compounds were linked to agriculture rather than urban practices. The measured maximum concentrations of glyphosate and AMPA were 6 µg/L and 0.9 µg/L, respectively.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/057
Report author	Botta F. *et al*.
Report year	2012
Report title	Phyt'Eaux Cités: Application and validation of a programme to reduce surface water contamination with urban pesticides
Document No	Chemosphere 86 (2012) 166–176
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

This paper presents first results of Phyt'Eaux Cités, a program put in place by the local water supply agency, the SEDIF (Syndicat des Eaux d'Ile-de-France), in collaboration with 73 local authorities, private societies and institutional offices (365 km^2). The challenges included: measurement of the previous surface water contamination, control of urban pesticide applications, prevention of pesticide hazard on users and finally an overall reduction of surface water contamination. An inquiry on urban total pesticide amount was coupled with a surface water bi-weekly monitoring to establish the impact of more than 200 molecules upon the Orge River. For 2007, at least 4400 kg and 92 types of pesticides (essentially herbicides) were quantified for all urban users in the Phyt'Eaux Cités perimeter. At the outlet of the Orge River (bi-weekly sampling in 2007), 11 molecules were always detected above 0.1 µg/L. They displayed the mainly urban origin of pesticide surface water contamination. Amitrole, AMPA (Aminomethyl Phosphonic Acid), demethyldiuron, diuron, glyphosate and atrazine were quantified with a 100 % of frequency in 2007 and 2008 at the Orge River outlet. During the year, peaks of contamination were also registered for MCCP, 2,4 MCPA, 2,4 D, triclopyr, dichlorprop, diflufènican, active substances used in large amount in the urban area. However, some other urban molecules, such as isoxaben or flazasulfuron, were detected with low frequency. During late spring and summer, contamination patterns and load were dominated by glyphosate, amitrole and diuron, essentially applied by cities and urban users. Both isoproturon and chlortoluron were quantified during autumn and winter months according to upstream agricultural practices. In conclusion, 3 years after the beginning of this programme, the cities reduced the use of 68 % of the total pesticide amount. An improvement on surface water quality was found from 2008 and during 2009 for all pesticides. In particular, glyphosate showed a decrease of the load above 60 % in 2008, partly related to the Phyt'Eaux Cités action.

Materials and methods

Samplings were conducted by Aspect Environmental Consulting (Ennery, France) and Veolia Water (Paris, France). Manual sampling of surface waters were carried out from bridges in the middle of the water bed with glass grab bottles and samples were stored in 1 L glass bottles. Water samples were transported at 4°C and analyzed within a period of no longer than 1 week.

One hundred eighty nine molecules (active substances and metabolites) in 2007 and 212 in 2008–2009 (implementation after inquiry), were analyzed by the Chemisches Untersuchungslabor (Offenburg, Germany), a laboratory accredited by the German Accreditation Council (DAR). The substances investigated were chosen in accordance with three parameters: molecules with non-agricultural or double uses (from data collected by SIVOA and Phyt'Eaux Cités), molecules detected in urban rivers and molecules followed in other regional pesticide monitoring. Analytical methods were summarized according to extraction method, chromatographic equipment and LQ (limit of quantification). Except for amitrole, all the analytical methods are certified (ISO, DIN or EPA).

To estimate the annual load, discharge data were exported from the database HYDROBANQUE (http://www.hydro.eaufrance.fr/). For the point ''Orge upstream'', (basin area of 112 km^2) concentrations were quantified at Sermaise (no. 4 in Figure 7.5-147) near the associated discharge point (basin of 114 km^2). For the downstream point of the Orge (basin of 936 km^2), Athis-Mons sampling point (no. 1 in Figure 7.5-147) was coupled with Morsang-sur-Orge discharge data (922 km^2). For the Yvette River, pesticide concentrations were registered at Epinay-sur-Yvette (no. 2 in Figure 7.5-147, 279 km^2) and discharge values at Villebon-sur-Yvette (224 km^2). At discharge stations that are not far from sampling stations, it was considered that discharge at the sampling sites can be correlated to the basin size changes. Over the January–December period, daily pesticide fluxes were calculated by multiplying the pesticide concentration of the collected samples from the continuous (bi-weekly) samples by the mean daily flow during that day. The sum of the 24 d load was compared to the average annual stream flow to obtain an annual load according to the equation below (Eq. (1)). Concentrations below the LQ were set to half of the LQ for these statistical calculations.

$$\text{Annual load} = \frac{\sum_{j=2}^{365}([C]j.cont. \times Qjsample.cont.)}{\sum_{j=1}^{365} Qjsample.cont.} \times Qtot \qquad (1)$$

Figure 7.5-147: Phyt'Eaux Cités area and monitoring stations in the Orge basin: no.1 Athis-Mons, no.2 Epinay-sur-Orge, no.3 Chevreuse and no.4 Sermaise

Results

Inquiries about public users were performed in the first semester of 2007. Fifty-seven of the above mentioned 73 local authorities answered to this investigation. The investigated cities declared having used in 2007 at least 167 commercial products with a total of 92 molecules. Totally active ingredient used by cities was 2053 kg year^{-1} in 2007 for the 57 inquired cities (mean of 36 kg year^{-1} for each city) A molecule was chosen as a tracer of this group and used in the following data analysis. First group included molecules essentially used by cities, where glyphosate was chosen as the main applied compound in urban areas. Agricultural applications of glyphosate on this basin were limited. The second group included molecules used by other users (national and regional railways, airport or golfs) in very large amount, most of the time largely applied as compared to city applications. Amitrole was chosen as tracer for group B. Main other users of pesticides were the national and the regional railways companies (846 kg year^{-1} of applied pesticides). Railway spraying is carried out on a surface of 4.93 km^2. Only herbicides were applied (glyphosate essentially, followed by 2,4 D and amitrole). The third group included molecules essentially used by agricultural weed control. No data on agricultural amount were available on agricultural applied amount. The choice of molecules for this group was based on three levels: results of an inquiry on an upstream sub-basin called Remarde (Botta, 2009), water analyzes of Sermaise (agricultural sampling station) samples and on databases on pesticide national homologation by uses. Isoproturon was chosen as tracer for this group. The fourth group included molecules that display mixed sources, such as diuron and mecoprop, homologated as pesticide but also used as biocides. Diuron was chosen as tracer for group D. Herbicides were in all cases the most used family of pesticides. Total urban uses were estimated at 4400 kg for 2007, 1575 kg of which is glyphosate.

In this study, 49 of the 212 active substances and metabolites analyzed during 2007, 2008 and 2009 were detected at the four sampling stations. The sampling campaign for the year 2007 was focused on 189 substances (171 active substances and 18 metabolites). At the outlet of the Orge Basin, 33 substances (29 active substances and 4 metabolites) were quantified and 6 displayed 100 % frequencies (glyphosate and its metabolite, diuron and its metabolite, amitrole and atrazine).

Urban substances mainly used by cities
Glyphosate and its degradation product, AMPA, were by far the most detected molecules in the Orge River basin. Very high concentration peaks were registered at Epinay-sur-Orge (no. 2 in Figure 7.5-147) and at Athis-Mons (no. 1 in Figure 7.5-147) during summer periods. In the upstream stations was detected from March to December but an increase in concentrations was found during the summer. Positive outliers and extreme values were mainly detected for glyphosate during its application period for urban weed management. The result was in accordance with the pesticide inquiries. The inquiry documented that 52 local authorities used this herbicides and also 6 of the other public users settled in the Phyt'Eaux Cités area. The maximal recorded concentration of AMPA was 5.1 µg/L in 2007.

Urban substances mainly from other users
Amitrole was by far the most applied one by the National Railways Society (in 2004 more or less a rate of 2700 g/ha) and in particular during the spring months. The origin of amitrole in the Phyt'Eaux Cités perimeter can be also related to cities application (19 quotations) and to the other public users, especially by the national railways, where amitrole represents 10 % of herbicides amount. Herbicides 2,4 D and 2,4 DP were detected during the first semester of 2007 at very low concentrations in all the monitoring stations.

Substances mainly used by agriculture and analyzes of upstream sampling point
Isoproturon and chlortoluron, are used essentially in wintercrops. They were detected during winter months at the Orge upstream point (concentration level of 1 µg/L). The highest isoproturon concentration was registered in Sermaise (no. 4 in Figure 7.5-147) during the campaign of December 17, 2007 (1.2 µg/L). Highest chlorotoluron concentrations were observed in December 2007 at the upstream stations (Sermaise and Chevreuse) (1.5 µg/L). During the rest of the year, concentrations were between 0.5 and 0.8 µg/L. At the downstream sampling stations they were detected at low concentrations until June.

Substances with different uses (urban application, biocides and agricultural uses)
Diuron showed 100 % of detection frequency in 2007 and 2008. The diuron degradation product, the demethyldiuron was often measured at the Orge stations and in the downstream point of the Yvette River (Epinay-sur-Orge, no. 2 in Figure 7.5-147). Diuron concentrations were fluctuating between 0.5 µg/L and 1 µg/L during May, June, July and August. This herbicide was widely used by municipalities inside the Phyt'Eaux Cités action area (quoted 24 times) and by other users (quoted three times).

Change in pesticide occurrence following implementation of the Phyt'Eaux Cités program
A comparison between concentrations median, quantification frequency and loads between the years 2007, 2008 and 2009 is discussed in this section. The objective was to establish if a real decrease of pesticides concentration was registered in surface water during these 3 years.

Glyphosate (agricultural and urban applications) was always detected in the Orge and Yvette downstream stations. One hundred percent of detection frequency in 2007 and of 87.5 % in 2008 was noted for glyphosate at the outlet of the Orge River (Athis- Mons). In 2009 a decrease was noted and detection frequency was 66.6 %.

The median concentrations decreased between 2007 and 2008, from 0.61 to 0.43 µg/L. In 2009, glyphosate was still detected in all the four sampling stations. Glyphosate and AMPA still represented the two major contaminants at the end of the third year of the action. The highest load was measured for glyphosate that increased significantly between the upstream point and the downstream point. It was followed by its degradation product AMPA, diuron and amitrole. For glyphosate the estimated annual load was 1.7 kg year^{-1} at the upstream point. The same compounds displayed a 179 kg year^{-1} load at the outlet of Orge catchment. AMPA had an annual load of 156.8 kg year^{-1} at the Orge outlet and 1.7 kg year^{-1} in the upstream point. For the Yvette River annual loads were estimated to be 92.3 kg year^{-1} for glyphosate and 52.8 kg year^{-1} for the AMPA. Yvette loads represented 50 % of glyphosate, 30 % of AMPA and 70 % of chlortoluron of total loads of the Orge River.

Finally the annual load of the group A (Urban application) was compared for 2007, 2008 and 2009. Loads at the outlet of Orge River were considered. Glyphosate load decreased in both streams, Yvette and Orge. At the Orge outlet, the load decreased from 126.6 kg year^{-1} in 2007 to 50.5 kg year^{-1} in 2008, with a

diminution of 62 %. In the Yvette, a higher decrease is registered (-85 %) in 2008 as compared to 2007. A reduction of loads (30 %) is also registered for its degradate AMPA. The load decreased more in the Yvette River as compared to the Orge River, probably due to a difference of water discharge volume between 2007 and 2008, higher on the Yvette River.

The Yvette impact on the Orge contamination was mainly due to agricultural pesticides, such as chlortoluron and isoproturon. A particular characteristic of the Orge River catchment is that at least 80 % of the urban area is located between the upstream and the downstream point. For chlortoluron and isoproturon, a load increase was observed for 2008, with higher value at the Orge downstream site. For diuron the annual load downstream was 30 times larger than the Orge upstream flow. In the downstream point of the Orge River (Athis-Mons), annual concentration trend was similar to the one in the upstream point (Sermaise, no. 4 in Figure 7.5-147) but concentrations were 10 times lower.

MCPP (mecoprop) was the only molecule that displays a constant detection frequency during the three year and not a significant decrease. Median values were quite similar in 2007, 2008 and 2009. It was difficult to verify an effect of Phyt'Eaux Cités program because MCPP has different sources (agricultural uses, urban uses or biocides). Release of mecoprop will be primarily from its application as a herbicide, but also potentially from its manufacture, transport and storage.

Compared to 2007 data, this load variation might have different interpretations. Hydrological conditions were partly different and rainfall events were less frequent in 2008. To determine the reason for decrease, the glyphosate and diuron loads were divided into dry weather load and wet weather loads based upon the day of sampling. During both years, 13 samples among 24 were collected during a rainy day. The mean discharge for all the rainy days in 2008 (4.37 m3 s^{-1}) was similar to the one measured in 2007 (4.12 m^3 s^{-1}) and the total amount of rainfall during the sampling days was similar for both years as compared to total annual amounts (5.81 % in 2007 and 5.71 % in 2008). The only load during dry weather days was 4.3 kg $year^{-1}$, lower in 2007 than in 2008, whereas the average concentrations were 0.47 µg/L in 2007 and 0.57 µg/L in 2008. If the rainfall load was separated from the dry weather one, the difference between the two loads was sensible. In this case the rainfall load is three times higher in 2007 as compared to 2008. This tendency was not related to a difference of hydrological conditions but rather to highest average concentrations in 2007 (1.7 µg/L) compared to 0.65 µg/L in 2008. Consequently, Phyt'Eaux Cités appears likely to play a part in surface water quality improvement during 2008. However, data on pesticide loads were only collected for 2 years and data are scarce to certify that this load decrease was only related to the program impact.

Conclusion

Use of pesticides by municipalities generally decreased from 2007 to the end of 2009. In some cities, chemical treatments were also replaced by other type of weed-control (thermal, mechanical, etc.). The impact of pesticides used in urban settlements on surface water quality was confirmed during campaigns of 2007 and 2008. The urban uses impact on surface water quality was confirmed by coupling the results of investigation and the surface water campaigns. Eighteen of the applied pesticides in urban areas were frequently detected in the four sampling stations and in particular high concentrations were registered for glyphosate, amitrole, diuron, MCPP and 2,4-MCPA. Considering the period between May and July (maximum of application), the pesticide sum frequently exceeded the limit of 5 µg/L at Athis-Mons (no. 1 in Figure 7.5-147).

The elevated urban pesticide concentrations observed during 2007–2008 justify the Phyt'Eaux Cités action and also the intervention area chosen by the SEDIF. Multivariate analysis using PCA was applied to explain and confirm the main pattern of pesticide distribution. In the Orge River, detected pesticides that were applied in agricultural and urban areas display essentially urban origins. The inquiries displayed a decrease in pesticide use during the program from 2007 (95 kg city $year^{-1}$) until 2009 (35 kg city $year^{-1}$), also in term of kg/ha (from 2.5 to 0.8 kg/ha). The sustainable planning was carried out by 28 cities, while four reached at least 75 % of the planned BMP by the Phyt'Eaux Cités action and two decided to stop all type of pesticide applications. With those results a decrease of transfer through urban surface water was expected to occur.

The improvement of the program was related with decrease of pesticide detection in surface water. Some substances were not quantified in 2009, whereas they were in 2007–2008. This pattern was observed for molecules frequently used by cities (dicamba and propiconazole) or by other urban applicators, like bromacil. A more important decrease was observed for molecules applied essentially by cities, such as glyphosate. The total load at the outlet of the Orge Basin displayed a spectacular decrease (more than 50 %). Phyt'Eaux Cités was a new approach to reduce the contamination of surface water by pesticides. The more knowledge and mobilization of the local authorities could improve the reduction of pesticides use. The programme suggested to city staff specific pest management strategies and general alternative controls. The objective was to reduce overall pesticide use by the end of 2010.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes monitoring data (surface water) for glyphosate among other pesticides for an urban area in France. No agricultural area is considered. Glyphosate and AMPA concentrations are presented as Figures. The maximum recorded concentration of AMPA was 5.1 µg/L.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/058
Report author	Coupe, R. *et al.*
Report year	2012
Report title	Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins
Document No	Society of Chemical Industry (wileyonlinelibrary.com) DOI 10.1002/ps.2212
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Glyphosate [N-(phosphonomethyl)glycine] is a herbicide used widely throughout the world in the production of many crops and is heavily used on soybeans, corn and cotton. Glyphosate is used in almost all agricultural areas of the United States, and the agricultural use of glyphosate has increased from less than 10 000 Mg in 1992 to more than 80 000 Mg in 2007. The greatest intensity of glyphosate use is in the midwestern United States, where applications are predominantly to genetically modified corn and soybeans. In spite of the increase in usage across the United States, the characterization of the transport of glyphosate and its degradate aminomethylphosphonic acid (AMPA) on a watershed scale is lacking. This publication included results from an investigation carried out in a catchment in Rouffach, France, and this summary will focus on this investigation.

In the French catchment, glyphosate and AMPA were detected in almost every sample: the maximum glyphosate concentration was 86 µg/L, minimum was <0.1 µg/L, and the median was 4.7 µg/L. For AMPA, the maximum concentration was 44 µg/L, the minimum was 0.2 µg/L, and the median was 1.9 µg/L. This catchment could be considered as a worst case, in that glyphosate was used in the catchment almost continuously, and the area, climate and agricultural practice were favourable for runoff.

Glyphosate use in a watershed results in some occurrence in surface water; however, the watersheds most at risk for the offsite transport of glyphosate are those with high application rates, rainfall that results in overland runoff and a flow route that does not include transport through the soil.

Materials and methods

This paper explores the transport of glyphosate and AMPA in seven streams in agricultural basins located in four different environmental settings (Table 7.5-173). Water samples were collected over a 2-year period from two sets of nested basins (Mississippi and Iowa). Water samples were also collected during storm events in Indiana (1 year) and near Rouffach, France (4 years), and the latter investigation will be the focus of this summary.

Table 7.5-173: Study basins and subbasins with basic hydrological and agricultural characteristics, data collection period, basin size, mean daily stream flow for 2007 and 2008 and 1997 – 2006 mean daily streamflow

Location	Basin[a]	Subbasin[a]	Data collection period	Basin size (km^2)	Mean daily flow ($m^3 s^{-1}$) (2007) **(2008)**[b]	1997–2006 mean daily flow ($m^3 s^{-1}$)[b]	Water yield	Basin in agriculture (%)	Percentage of agriculture by major crop type (2007) **(2008)**				
									Soybean	Corn	Cotton	Rice	Grapes
Mississippi, USA	Bogue Phalia		January 2007–November 2008	1250	9.71	21.58	25	>80	45	11	13	13	0
					22.94				**51**	**12**	**4**	**16**	**0**
		Tommie Bayou	April 2007–September 2008	15.2	0.18	n/a			32	0	0	64	0
					0.51				**32**	**0**	**0**	**64**	**0**
		Napanee	April 2007–September 2008	2.20	0.04	n/a			50	0	0	50	0
					0.08				**100**	**0**	**0**	**0**	**0**
Iowa, USA	SFIR New Providence		February 2007–September 2008	570	11.3	3.82	25.1	>85	29	70	0	0	0
					11.33				**34**	**64**	**0**	**0**	**0**
		SFIR Blairsburg	April 2007–September 2008	31.1	0.48	n/a			32	68	0	0	0
					0.65				**34**	**65**	**0**	**0**	**0**
Indiana, USA	Sugar Creek		Two storm events in May 2004	249	n/a	n/a	34.5	75	~50	~50	0	0	0
		Leary Weber Ditch		7.22	n/a	n/a		87	47	39	0	0	0
		Overland Flow Site		0.42	n/a	n/a		~100	100	0	0	0	0
France	Rouffach		58 storm events March–September 2002–2006	0.42	n/a	n/a	1.48	68	0	0	0	0	100

[a] SFIR: South Fork Iowa River.
[b] n/a: not applicable.

Study site

Rouffach, France
The Rouffach basin is located in eastern France in the Alsace region south of Strasbourg on the slopes overlooking the Rhine River Valley. The Rouffach basin is small in size, about 0.42 km^2, with an average slope of about 150 m/km. Streamflow is ephemeral, occurring only during rainfall events. Only rainfall events that generated a runoff volume greater than 8 m^3 were monitored. Land use for about 68 % of the contributing basin is vineyard.

Data collection, analysis and quality assurance
Water samples from the Rouffach basin were collected using an automatic sampler from March to October.

Water sample collection and processing in the United States followed USGS protocols. Water samples were filtered and analyzed for glyphosate and AMPA using online solid-phase extraction and analysis by HPLC/MS. Water samples collected from the Rouffach basin in France were filtered and analyzed using similar methods, with a reporting level of 0.1 µg/L. The results presented here will only represent the portion of glyphosate and AMPA that is dissolved in water, and not the portion attached to sediment.

Glyphosate application and loads
For the Rouffach basin, annual surveys were sent to the 28 farmers in the basin, asking for information on pesticide application methods, timing and amounts.

When glyphosate or AMPA concentrations were reported as less than the reporting limit, the concentrations were set to zero for percentage detection values and load calculations.

To gain a better understanding of the fate and transport of pesticides, it is often insightful to examine the relation between pesticide degradates and the parent compound. Here, the %AMPA as a percentage of total glyphosate (glyphosate + AMPA) was calculated:

$$\% \text{ AMPA} = \frac{[\text{AMPA}]}{[\text{Glyphosate}] + [\text{AMPA}]} \times 100$$

For the site in France, a load was calculated for each event by multiplying the concentration (using linear interpolation between measured concentrations) by the instantaneous flow for each minute and then summing over the entire event. The annual load was calculated by summing the individual event loads for each year.

The annual load as a percentage of use (LAPU) was calculated to compare the behavior of glyphosate across scales and between study areas. It was calculated thus:

$$\text{LAPU} = \frac{\text{annual stream load of glyphosate from that basin (kg year}^{-1})}{\text{annual glyphosate use in that basin (kg year}^{-1})} \times 100 \qquad (2)$$

Additionally, for proper quantification of the total glyphosate load as a percentage of use (TGLAPU), the load of AMPA must be expressed on a glyphosate mass equivalent basis and added to the load of glyphosate.

Results

France

Fifty-eight runoff events from March to September 2003–2006 were sampled, and 303 samples were collected from the Rouffach basin. All but one sample had concentrations of glyphosate above the reporting level of 0.1 µg/L (Table 7.5-174). Every sample had detectable levels of AMPA with maximum concentrations of glyphosate and AMPA of 86 and 44 µg/L (median concentrations: 4.7 and 1.9 µg/L). Generally, the LAPU values for glyphosate (0.009–0.029 %) for the Rouffach basin were an order of magnitude less than at the other sites (Table 7.5-175).

Table 7.5-174: The sampling period, number of samples collected, maximum, minimum and median concentrations of glyphosate, AMPA and %AMPA at each sampling site and the percentage of samples below the reporting limit

Basin	Subbasin[a]	Sampling period	Constituent[b]	Units	Number of samples	Maximum	Minimum	Median	Percent of samples below reporting level (0.02 µg L^{-1})[c]
Bogue Phalia, MS		October 2006 – November 2008	Glyphosate	µg L^{-1}	62	73	0.08	0.96	0
			AMPA			28	0.48	2.6	0
			%AMPA	%		96	14	72	n/a
	Tommie Bayou	April 2007 – September 2008	Glyphosate	µg L^{-1}	34	6.2	0.04	0.82	0
			AMPA			5.9	0.12	1.5	0
			%AMPA	%		94	20	67	n/a
	Napanee	April 2007 – September 2008	Glyphosate	µg L^{-1}	73	41	0.03	1.2	0
			AMPA			9.7	0.27	1.6	0
			%AMPA	%		94	18	53	n/a
SFIR New Providence, IA		February 2007 – September 2008	Glyphosate	µg L^{-1}	34	1.6	<0.02	0.07	41
			AMPA			1.2	<0.02	0.15	3
			%AMPA	%		100	0	76	n/a
	Subsurface Drain	February 2007 – November 2008	Glyphosate	µg L^{-1}	64	290	<0.02	0.87	19
			AMPA			400	<0.02	0.58	1.6
			%AMPA	%		100	0	52	n/a
	SFIR Blairsburg	February 2007 – November 2008	Glyphosate	µg L^{-1}	79	5.7	<0.02	0.18	28
			AMPA			2.9	<0.02	0.32	8
			%AMPA	%		100	0	57	n/a
Sugar Creek, IN		19–21 May 2004	Glyphosate	µg L^{-1}	5	1.0	0.15	0.32	0
			AMPA			0.36	0.07	0.14	0
			%AMPA	%		48	9	38	n/a
		30 May–2 June 2004	Glyphosate	µg L^{-1}	6	1.6	0.37	0.66	0
			AMPA			0.74	0.19	0.39	0
			%AMPA	%		59	12	35	n/a

Table 7.5-174 – continued

Basin	Subbasin[a]	Sampling period	Constituent[b]	Units	Number of samples	Maximum	Minimum	Median	Percent of samples below reporting level (0.02 µg L^{-1})[c]
	Leary Weber Ditch	19–21 May 2004	Glyphosate	µg L^{-1}	6	2.1	0.16	0.90	0
			AMPA			0.23	0.08	0.19	0
			%AMPA	%		47	10	10	n/a
		30 May–2 June 2004	Glyphosate	µg L^{-1}	7	5.5	0.47	1.1	0
			AMPA			0.62	0.07	0.31	0
			%AMPA	%		37	6	16	n/a
	Overland Flow Site	19–21 May 2004	Glyphosate	µg L^{-1}	6	430	21.5	380	0
			AMPA			29.0	24.0	26.0	0
			%AMPA	%		8	6	7	n/a
		30 May–2 June 2004	Glyphosate	µg L^{-1}	7	49.0	22.0	34.0	0
			AMPA			16.0	3.7	8.2	0
			%AMPA	%		18	15	17	n/a
Rouffach, France		March–September: 2003–2006	Glyphosate	µg L^{-1}	303	86	<0.1	4.7	0.3[d]
			AMPA			44	0.2	1.9	0
			%AMPA	%		60	6	31	n/a

[a] SFIR: South Fork Iowa River.
[b] AMPA: aminomethylphosphonic acid; %AMPA: ([AMPA]/([AMPA] + [Glyphosate])) × 100.
[c] n/a: not applicable.
[d] A reporting level of 0.1 µg L^{-1} was used for data from Rouffach.

Table 7.5-175: Comparison of glyphosate application and glyphosate and AMPA loads, glyphosate LAPU values and the mass equivalent total glyphosate LAPU between the basins studied

Sampling site[a]	Year	Glyphosate: Total mass applied to watershed ($kg\ km^{-2}\ year^{-1}$)	Glyphosate: Load ($kg\ year^{-1}$, except where noted)	Glyphosate: LAPU[b] (%)	AMPA[c] load as AMPA ($kg\ year^{-1}$, except where noted)	TGLAPU[d] (%)
Bogue Phalia, MS	2007	78	319	0.33	726	1.5
	2008	105	739	0.56	1025	1.8
Tommie Bayou, MS	2007	199	4.2	0.14	7.1	0.49
	2008	185	10.6	0.37	20.5	1.5
Napanee, MS	2007	188	2.3	0.56	1.3	1.0
	2008	301	5.7	0.86	4.9	2.0
SFIR New Providence, IA	2007	51.7	70	0.24	100	0.75
	2008	63.4	22.7	0.06	59.8	0.44
SFIR Blairsburg, IA	2007	47.6	2.6	0.18	4.0	0.59
	2008	55.3	3.3	0.19	5.8	0.70
Rouffach, France	2003	54.3	6.6 $g\ year^{-1}$	0.029	2.5 $g\ year^{-1}$	0.046
	2004	66.9	9.4 $g\ year^{-1}$	0.033	2.7 $g\ year^{-1}$	0.048
	2005	43.9	4.7 $g\ year^{-1}$	0.026	1.1 $g\ year^{-1}$	0.034
	2006	146	5.7 $g\ year^{-1}$	0.009	1.2 $g\ year^{-1}$	0.012

[a] SFIR: South Fork Iowa River.
[b] LAPU: load as a percentage of use.
[c] AMPA: aminomethylphosphonic acid.
[d] TGLAPU: mass equivalent total glyphosate LAPU.

Conclusion

In the French catchment, where the use is almost continuous, glyphosate and AMPA were detected in almost every sample. The annual stream load of glyphosate as a percentage of annual use was much less in the French catchment, even though the French site had detections in almost every sample at relatively high concentrations, because the amount of water that leaves this basin is small compared with the others.

Glyphosate use in a watershed results in some occurrence in surface water; however, the watersheds most at risk for the offsite transport of glyphosate are those with high application rates, rainfall that results in overland runoff and a flow route that does not include transport through the soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article reports concentration measurements for glyphosate and AMPA residues in stream waters in USA and France. Specific analytical method were used and the limits of reporting were stated. The watersheds most at risk for the offsite transport of glyphosate are those with high application rates, rainfall that results in overland runoff and a flow route that does not include transport through soil. For the French catchment, only runoff events with volumes greater than 8 m^3 were monitored between March and October.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/059
Report author	Petersen, J. *et al*.
Report year	2012
Report title	Sampling of herbicides in streams during flood events
Document No	J. Environ. Monit., 2012, 14, 3284
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Eurofins Denmark A/S)
Acceptability/Reliability:	Reliable with restrictions (No relevant endpoint)

2. Full summary

In stream water xenobiotics usually occur as pulses in connection with floods caused by surface run-off and tile drainage following precipitation events. In streams located in small agricultural catchments we monitored herbicide concentrations during flood events by applying an intensive sampling programme of ½ h intervals for 7 h. In contrast to grab sampling under non-flood conditions, clearly elevated concentrations were recorded during the floods, and pulses varying in occurrence, duration and concentration were recorded. Pulses of recently applied herbicides were the most prominent, but also agricultural herbicides used in previous seasons caused pulses in the streams. Asynchronism of chemographs may be related to the characteristics of the compounds as well as their transport pathways and transformation in compartments between the source and the point of sampling in the stream. Thus, the occurrence of chemographs is difficult to predict, which ought to be taken into account when designing a sampling strategy. Even though the chemographs of herbicides and their transformation products (glyphosate and aminomethylphosphonic acid (AMPA) as well as terbuthylazine and desethylterbuthylazine) seem to be synchronous, their occurrence may still be difficult to predict. It is evident that grab sampling under non-flood conditions yields insufficient information on the dynamics of occurrence of herbicides in stream water, both with respect to environmental effects and the calculation of the load to a recipient. In conclusion, the design of a sampling strategy regarding herbicides in stream waters should adequately consider the aim of the investigation.

Materials and methods
Intensive sampling of herbicide pulses (chemographs) in streams was planned for surface run-off events in the 2004 spring spraying season in Denmark (April–June). Precipitation events of 10 mm within 1–2 days would expectedly occur on average 4–5 times during the spring spraying season. Precipitation of this order was converted to an expected rise in the water level of the catchment stream depending on stream characteristics, typically 5–10 cm. A floating contact was adjusted to start an automatic sampler at the estimated rise in the stream water level (flood) to catch the chemographs.

The stream water sampling was carried out in three catchments (A, B and C; Table 7.5-176) at a precipitation driven flood event as indicated in Figure 7.5-148. The sampling device was a stainless steel pipe (10 mm i.d.) with a 90° bend 10 cm from the end installed vertically in the middle of the stream. The horizontal tube-end was placed at a height above the bottom corresponding to about 40 % of the water level with the opening pointing downstream, and the tube being emptied (blown-out by air pressure) before each sampling. During the flood events sampling was carried out using two ISCO-samplers (no. 3700 with 12 glass bottles of 900 mL annealed at 550 °C). The samplers were programmed to take samples every 15 minutes, and they were combined two by two to represent 30 minute intervals for 5 h, except samples no. 23 and 24 which were taken 6 and 7 h after the start of the sampling, respectively. The 15 minute interval

was applied to catch chemograph peak concentrations, and the combination of the samples ensured sufficient material for analysis. In addition, two grab samples (2 L each) were taken on days without preceding precipitation to record concentrations under non-flood conditions in each stream.

The day after receipt at the commercial laboratory (Eurofins Denmark A/S, DK-8464 Galten, DANAK accreditation no. 168), the samples were homogenised, and an internal standard was added. The 1.8 L combined samples and the 2 L grab samples were divided into three subsamples of 500 mL each, and the compounds were extracted and analysed by three methods according to their chemical properties. Owing to the smaller sample volume of sample no. 23 and 24, these were analysed by method 1 only.

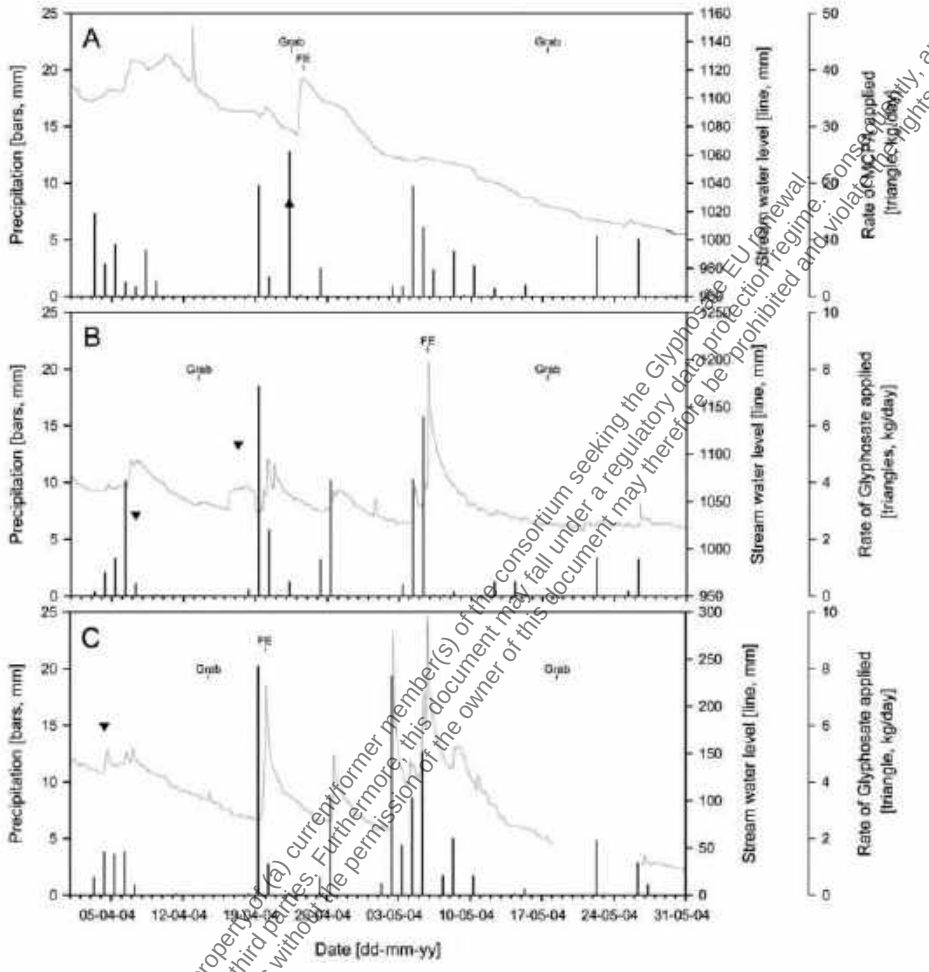
Table 7.5-176: Flood event – catchment key and catchment characteristics

Flooding event	Catchment name	Latitude North	Longitude East	Altitude [m]	Size [ha]	Agriculture [%]	Forest/ nature [%]	Stream flow (average) [$L\ s^{-1}\ km^{-2}$]	Base flow index[a] [%]	Soil type (topsoil)[b]	Soil carbon (interval) [$mg\ g^{-1}$ soil]
A	Højvads Rende	54°12′	11°16′	2–24	980	65[c]	31[f]	5.0	[illegible]	Loamy sand	11 (9–12)
B	Odderbaek	56°46′	9°32′	11–58	1140	86[d]	5	6.4	[illegible]	Sand	27 (15–41)
C	Horndrup Baek	56°7′	9°41′	41–171	550	72[e]	22	9.6	62	Loamy sand	13 (10–17)

[a] BFI calculated by Grant *et al.*[9] for the period 1989–99 according to the method described by Gustard *et al.*[11] [b] The clay content increased to 20–25% in the subsoil of the loamy sand soils. [c] More than 70% are tile drained. [d] About 10% are tile drained. [e] Naturally drained. [f] A large proportion is drained by ditches.

Standards based on Milli-Q water spiked with the respective analytes were processed and analysed in the same way as the samples, and the recovery of the standards was used to correct the concentrations in the samples. The detection limit was 0.01 µg/L with 15 % relative standard deviation for all three methods.

Figure 7.5-148: Daily precipitation (bars), stream water level (line) and daily application of herbicides within the catchment (triangles). Dates of flood events (FE) and 2 L grab samplings under non-flood (Grab) are indicated



Method 1 (LC-MS/MS)
The samples were acidified to pH 4.5 by adding 6 mL 100 % acetic acid and 5 mL 25 % NaOH, and the compounds were concentrated by solid-phase extraction. The columns were dried under a flow of air and eluted using 2 x 5 mL methanol/acetonitrile. Subsequently, 50 µL 1,2-propanediol was added to the elute, which was then evaporated under N_2 flow at 35 °C. The evaporation residue was re-dissolved in 400 µL methanol–water (1 : 1). The analytical column for LC was a Hypersil BDS (Thermo Scientific, 2.1 x 250 mm, 5 µm particle size) and the mobile phase was 5mM ammonium acetate–methanol (Eluent A: 990/10 and B: 100/900, both containing 0.1 % formic acid) in a gradient of: 0 % B (1 min), linearly to 50 % B (2 min), linearly to 100 % B (24 min), 100 % B (3 min), and linearly to 0 % B (3 min). The column temperature was 30°C and the flow rate was 0.2 mL/min.

Method 2 (GC-SIM-MS)
The compounds were concentrated using a Chelex 100 resin column and eluted by 4 x 2 mL 6 M HCl. The elute passed directly into an AG 1-X8 resin column. A subsample of 2 mL was evaporated to dryness and re-dissolved. Trifluroacetic anhydride and 2,2,3,3,4,4,4-heptafluro-1-butanol were used for derivatisation at 90°C. After cooling, the sample was evaporated to dryness under N_2 flow and re-dissolved by 200 µL ethylacetate. The analytical column for GC was a HP-5 (crosslinked 5 % PH ME siloxane, 30 m x 0.25 mm i.d. with a film thickness of 0.25 µm) and the carrier gas was He with a flow rate of 0.9 mL/min. A 2 µL

sample was injected (splittless mode) at 280°C. The oven temperature was 65 °C (2 min) followed by an increase of 20°C/min to 310 °C (1 min) with a post-run (4 min). The mass spectrometer (MS) was kept in Single Ion Monitoring (SIM) mode and the interface temperature was 275°C for detection of glyphosate and aminomethylphosphonic acid (AMPA) (Method M2275, Eurofins Denmark A/S).

Method 3 (GC-SIM-MS)
The samples were acidified to pH <0.5 by adding 7.5 mL concentrated sulphuric acid. Sodium sulphate was added and the samples were extracted with 50 mL methyl-tert-butylether (MTBE) for 30 min. The MTBE phase was re-extracted with MTBE, and the total extract was evaporated to 2 mL. Subsequently, 4 mL 10 % sulphuric acid in methanol was added to the extract which was subsequently heated to 50 °C for 2 h. After cooling, 4 mL saturated sodiumbicarbonate was added and the MTBE phase was removed and evaporated to 200 µL under N_2 flow. The analytical column was a HP-5MS capillary column (30m x 0.25 mm i.d. with a film thickness of 0.25 µm) and the carrier gas was He with a flow rate of 1 mL/min. A 3 µL sample was injected (splittless mode) at 220 °C. The oven temperature was 45 °C (1 min) followed by an increase of 12 °C/min to 130 °C and 30 °C/min to 280 °C with a post-run (5min). The mass spectrometer (MS) was kept in Single Ion Monitoring (SIM) mode and the interface temperature was 280°C for detection of trichloroacetate (TCA) (Method 2276, Eurofins Denmark A/S).

Catchments and use of herbicides
According to the Danish Agricultural Monitoring Programme, we extracted data on the use of the corresponding herbicides. The pulses and occurrence of herbicides and transformation products during flood events were related to (1) herbicides used in the spring season immediately prior to the sampling in 2004 (current season); (2) herbicides used in the previous 6 seasons of the farmer interview period (1998–2003) (average use in the seasons previous to the sampling season); (3) herbicides not used during the interview period but potentially applied before initiation of the farmer interview period in 1998.

Precipitation and stream water level
Meteorological and hydrological recordings were extracted from databases. Based on daily recordings, the Danish Meteorological Institute (DMI) calculates interpolated values for precipitation in 10 x 10 km^2 grids. From the Raingauge Network of The Water Pollution Committee of The Society of Danish Engineers at DMI, we obtained data on precipitation on an hourly basis. The nearest precipitation station was located 10, 31 and 21 km from the centre of catchments A, B and C, respectively. From the database on stream hydrology included in the National Monitoring and Assessment Programme for the Aquatic and Terrestrial Environment, we obtained interpolated hourly values on the stream water level.

Two simple parameters were calculated. Firstly, the amplitude was calculated for each compound as the maximum–minimum concentration ratio during the flood events. Secondly, the recorded concentrations during pulses were normalised for each compound relative to the maximum concentration of the pulse.

Results
Two herbicides were used in the spring before sampling – MCPA in catchment A and glyphosate in catchments B and C. Pulses of glyphosate and AMPA were recorded 3–4 h after the start of the sampling during flood event C. The amplitudes of glyphosate and AMPA during flood event C were 90 and 9, respectively. In contrast, the pulses during flood event B were observed within 1–2 h, and the amplitude of both glyphosate and AMPA was 30. A pulse of MCPA was observed 3–7 h after a short and intensive precipitation event during flood event A, where the maximum concentration was 45 times the minimum concentration. However, agricultural use of glyphosate was not recorded in the current season prior to flood event A, but elevated concentrations were observed during the flood. The clear glyphosate pulse recorded at flood event A had a maximum concentration in the same order as for B and C, but the concentration of the transformation product aminomethylphosphonic acid (AMPA) was doubled–tripled compared with the grab sampling.

The maximum concentrations of glyphosate and AMPA are well below the non-lethal concentrations of 12 000 µg/L (acute 7 days EC value). Even though low concentrations were recorded during flood events, these compounds were often found (>0.1 mg/L) in drain pipe water and soil water extracted by suction cups

installed at 1 m depth under Danish conditions. This means that a more or less constant and recurring contribution to streams may be expected at drainage events.

The physical/chemical properties indicate a fast degradation rate of glyphosate compared to the more persistent AMPA. However, detailed adsorption and degradation studies underpin that the transport of these compounds is complex due to the potential interaction with binding sites in the soil matrix, and the leakage of glyphosate and AMPA was recorded 1 and 2 years, respectively, after application. Thus, the slightly elevated concentrations of AMPA at flood event A (without recorded agricultural use of glyphosate in the current season) indicate leakage of residuals in consequence of former use, in particular the application of 86 kg glyphosate in August–October of the preceding season, illustrating the persistent character of AMPA ($DT_{50\text{-soil}}$ = 151 days).

Typical pulse shapes were recorded at all three flood events. The 10 times greater amplitude of glyphosate compared with the transformation product AMPA indicates a relatively direct leakage of glyphosate applied 2 weeks prior to sampling at C, avoiding adsorption in the soil matrix. The precipitation pattern shows that flood event C was caused by a first flush, whereas B was caused by a third flush. The two flushes preceding flood event B, which did not trigger the automatic samplers, may have facilitated some transportation of glyphosate from the soil phase to the water phase, potentially resulting in an intervening decomposition of glyphosate owing to a shorter 'half-life' of glyphosate in water than in soil ($DT_{50\text{-soil}}$ (typical) = 12 days, $DT_{50\text{-water phase}}$ = 3 days). Thus, the distribution in space and the difference in decomposition rates may explain the similarity in the amplitude of glyphosate and AMPA at event B compared to C. The distinct glyphosate pulse without a concurrent AMPA pulse at flood event A indicates a direct transport of glyphosate applied within a few days prior to the precipitation recorded on the 23 April. However, other sources may also be involved and the glyphosate pulse might be due to non-agricultural use, for instance in spraying of paved driveways and yards, including farm yards, as glyphosate is a very popular herbicide to control weeds in these areas. The distinct pulses (chemographs) of glyphosate and MCPA during floods (hydrographs) seem to be clearly related to their use in the current season.

The intention of this programme was to take into account the intra-annual dynamics of streams and occurrences of pesticides by using grab sampling for monitoring the long-term changes. However, our results indicate that the duration of concentration peaks is short (<2 h) and that peaks are most likely asynchronous. Therefore, it is very difficult to catch the peaks even when using the stratified sampling scheme for flood events, implicating that maximum concentrations may be underestimated.

Conclusion

A number of compounds occur within the same hydrograph when analysing stream water samples from small agricultural catchments under Danish conditions. Herbicides applied within the spring season prior to sampling lead to clear pulses (chemographs), but also herbicides applied in the past cause pulses or elevated concentrations compared with grab sampling under non-flood conditions. The recorded chemographs are not synchronous, except for pairs of a herbicide and its transformation product, and the chemographs are narrow with a typical duration of 1½–4 h. Elevated concentrations of herbicides not recently applied contribute to the total toxicity and are assumed to recur at repeated floods driven by precipitation events. In consequence, detailed studies on the occurrence, fate and transport of herbicides in streams require short sampling intervals, in particular when farmers' use of herbicides is unknown, both in the past and in the future. It is very difficult to catch the short-lived chemograph peaks in long-term monitoring programmes, even when using a stratified grab sampling approach.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes an experiment in a Danish agricultural area, where glyphosate concentrations were measured during stream flood events. The development of concentrations levels after precipitation events were investigated. Different analytical methods were described. Maximum concentration of 2.8 µg/L for glyphosate and 0.54 µg/L for AMPA.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/060
Report author	Zgheib, S. *et al*.
Report year	2012
Report title	Priority pollutants in urban stormwater: Part 1 – Case of separate storm sewers
Document No	Water research 46 (2012) 6683-6692
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Organic and mineral pollutants have become part of today's urban environment. During a rain event, stormwater quality as well as the corresponding contaminant loads is affected by both atmospheric deposition and the various types of impervious surfaces (roads, rooftops, parking lots etc.) on which runoff occurs. This study provides results on stormwater pollution in Paris and its suburbs from three separate storm sewers (n = 20 samples). These results show that the stormwater had been contaminated by 55 chemical substances out of the 88 investigated. A particular attention was given to stormwater particle contamination. Concentrations are provided for: metals, PAHs, PCBs, organotins, alkylphenols, phthalates, pesticides, and VOCs. Our findings are among the first available in the literature since the relevant analyses were all conducted on both the particulate (P) and dissolved (D) phases. For most substances, particles from the three storm sewers were more heavily contaminated than dredged sediments and settleable particles from the Seine River. As a consequence of this finding, the release of untreated stormwater discharges may impact the receiving waters and contribute to sediment contamination.

Materials and Methods

Sampling site
Stormwater quality was monitored on three catchments, all located in Paris and its suburbs. The sites differed however in terms of land development and housing density. Sucy-en-Brie (SEB) is a residential area (with 90 % of individual dwellings) with an impervious surface coefficient (ISC) of 0.27. Noisy-le-Grand (NLG) is an urbanized zone (ISC: 0.65), its catchment is typical of a dense urban area with a

population of 59,000 inhabitants. ZAC Paris Rive Gauche (PRG) is a high density urbanized area with a mixed residential and commercial use area. These three watersheds are served by a separate sewer and storm drain. Polluted stormwater is discharged in an untreated state into local watercourses. Our sampling points were located at the storm sewer outlet of each watershed prior to discharge into the receiving waters.

Sampling procedure
Twenty storms were followed between February 2008 and March 2009: 10 for SEB, 6 on NLG and 4 on PRG. However, due to technical problems, only 16 were analysed for stormwater priority substances. The entire sampling procedure has already been described in Zgheib *et al*. (2008). In brief, once collected, the samples were filtered to separate the dissolved phase (D) from the particulate phase (P). Analyses were carried out within 24 h for the dissolved phase, while the suspended particulate matter was deep-frozen then lyophilised and analysed after 48 h.

Experimental procedure
Routine water quality parameters - Each stormwater sample was analysed for routine water quality parameters (Table 7.5-177), such as pH, conductivity, suspended particulate matter (TS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorus (P_{tot}). These parameters were measured on the bulk water sample, or total phase (T), in accordance with French standards.

Table 7.5-177: Stormwater quality parameters (Minimum - Maximum [median])

Site		SW for this study	A	B	C	D	NSQD
N		20	12	30	4	4	3765
pH	pH	6.99–7.87 [7.43]	N/A	N/A	7.70–7.65 [7.58]	7.55–7.85 [7.68]	[7.50] (1665)
Conductivity	µS/cm	166–1316 [350]	N/A	N/A	989–1062 [1027]	1056–1572 [1361]	[121] (685)
TS	mg/L	11–430 [106]	120–500 [220]	12.9–874 [413]	68–220 [111]	180–420 [325]	[58] (3390)
COD	mg/L	14–320 [89]	117–367 [illegible]	78–1455 [105]	250–400 [305]	380–910 [715]	[53] (2751)
TKN	mg/L	<2–16 [2.8]	6.9–18.4 [8.95]	6.6–23.1 [12.1]	37–48 [42]	46–101 [75.5]	[1.4] (3192)
P_{tot}	mg/L	0.30–3.52 [0.87]	1.21–3.20 [1.98]	2.4–13.7 [7.6]	4.50–6.00 [4.85]	6.1–12.4 [9.23]	[0.27] (3285)

N/A: data not available; SW: data for stormwater from this study; A: data from the database of DSEA 94 for 12 stormwater samples collected between November 2005 and April 2006; B: stormwater collected from an urban area (ISC = 0.75) (Lee and Bang, 2000); C: wastewater collected from combined sewers in Paris during a dry weather period (Zgheib, 2009); D: wastewater collected from separate sewers in suburban Paris during a dry weather period (Zgheib, 2009); NSQD, national quality stormwater database (data from Robert Pitt, Alex Maestre and Renee Morquecho, available at http://rpitt.eng.ua.edu/Research/ms4/Paper/recentpaper.htm, 2004); in brackets, the number of observations for the given parameter.

Stormwater priority substances - The 88 stormwater priority substances (SPS) consisted of 3 organotins, 16 polycyclic aromatic hydrocarbons (PAHs), 8 polychlorobiphenyls (PCBs), 12 volatile organic compounds (VOCs), 5 chlorobenzenes, 2 chlorophenols, 5 alkylphenols (APs), 3 polybromodiphenyl ethers (PBDEs), 24 pesticides, chloroalkanes (sum of C10 - C13), Di(2-ethylhexyl) phthalate (DEHP), 8 metals (i.e., Cd, Cr, Cu, Hg, Ni, Pb, Pt and Zn). All SPS, except for metals and VOCs, were analysed on both the dissolved and particulate fractions for each sample. Metals were evaluated on the total and dissolved fractions, whereas VOCs were only analysed on the total fraction. When a substance provided concentrations below the limit of detection for the two phases, it was considered as not detected. Hence, the total concentration has been calculated as follows:

D + P (µg/L) = D (µg/L) + P (µg/L), with P (µg/L)
= P*(µg/g dw) x TS (g/L)

When a substance was observed in just one of the two phases however, (D + P) was calculated in a way to maximize its concentration by substituting the concentration of the substance by its limit of quantification (LOQ) in the phase where the substance was observed to lie below this LOQ. Maximization referred to the fact that no null concentration was attributed to the phase where a substance was observed below LOQ. Moreover, maximization of the concentration was decided because 23 substances exhibited LOQs less than or equal to their EQS both for the dissolved and the particulate phases. When LOQs were greater than EQS,

for all those substances excepted organotins the LOQ/EQS ratio was in the 1.5 - 10 range for the dissolved phase and in the 1.5 - 5 range for the particulate phase.

Results and Discussion

In a previous study, Zgheib *et al*. (2011a) investigated the relationships between land use and stormwater quality for these three catchments (on the basis of total concentrations). They reported that the statistical analysis of available SPS data did not reveal any significant differences for most substances in any of the three watersheds that could be explained by land use ($\alpha = 0.05$, $p > 95\ \%$). As a matter of fact, SPS concentrations were relatively homogeneous from one watershed to the next, thus suggesting that land use in these urban residential areas would not exert a predominant impact on the levels measured, especially when the land uses of the watersheds were contrasted much less than expected, being too close to Paris conurbation. In fact, the temporal variability was greater than the spatial variability. This finding was supported by previous results from the National Stormwater Quality Database (NSQD), which recorded some 3700 storms throughout the United States. The NSQD provided data for routine water quality parameters, a few metals, methylene chloride and DEHP.

Table 7.5-178: Detected and undetected substances in stormwater

Never detected in stormwater on any of the sites (33 SPS)	Cd, Hg, Ni, Pt, dichloroethane, trichlorobenzenes (3), pentachlorobenzene, hexachlorobenzene, carbon tetrachloride, isopropylbenzene, hexachlorobutadiene, hexachlorocyclohexane, trichloroethylene, chloroform, benzene, endosulfan (2), alachlor, isodrin, lindane, chlorpyrifos, trifluralin, atrazine, chloroalkanes, DDT (2), PCB 194, octaBDE, penta-BDE, Deca-BDE
Detected in stormwater samples on at least one site (17 SPS)	Cr, 4-para-nonylphenol, 4-n-octylphenol, aldrin, chlorfenvinphos, desethylsimazine, simazine, endrin, ethylbenzene, toluene, xylene, tetrachloroethylene, methylene chloride, pentachlorophenol, dieldrin, desethylatrazine, TBT
Detected in stormwater on all sites (38 SPS)	3 metals (Pb, Cu, Zn) 6 pesticides (diuron, isoproturon, metaldehyde, aminotriazole, glyphosate, AMPA) 2 organotins (DBT, MBT) 3 alkylphenols (nonylphenol, para-tert-octylphenol, 4-ter-butylphenol) 16 PAHs (N, Acen, Acy, F, P, A, Fluo, Pyr, B(a)A, Chry, B(a)P, B(b)F, B(k)F, D(ah)A, BP, IP) 7 PCBs (28, 52, 101, 118, 138, 153 and 180) DEHP

(number): number of compounds. For the meaning of abbreviation see Table 3.

Routine water quality parameters

Table 7.5-177 presents the results obtained for routine water quality parameters. This table also provides data found in other databases, either (A) from stormwater networks used in a previous monitoring survey carried out on the residential watershed of Sucy-en-Brie between November 2005 and April 2006 (data provided by the watershed managing entity, DSEA 94) and (B) on an urban area with an ISC equal to 0.75, or from two types of sewer networks (i.e. the Parisian combined sewer (C) and separate sewers (D) in suburban Paris), both during dry weather. Data from NSQD are also given in Table 7.5-177. Routine water quality parameters provide key information on stormwater quality. In general, except for the data from NSQD, most of the routine parameters relative to the three investigated storm sewers exhibited the lowest concentrations: TS ranged from 11 to 430 mg/L, with a median of 106 mg/L. Conductivity varied between 166 and 1316 µS/cm (median: 350 µS/cm). COD ranged between 14 and 320 mg/L (median: 89mg/L), which is comparable to the quality of stormwater collected on the Marais urban catchment, although this represents half the value of wastewater from combined sewer networks. This latter finding suggests that the three storm sewers were not contaminated by infiltration from sewerage, a point reinforced by the fact that stormwater from the three watersheds all contained rather low concentrations of COD, TS, TKN and P_{tot} when compared either to discharge from combined sewer overflows in the Paris network or to wastewater during dry weather flow from a separate sewer and a combined sewer.

Stormwater priority substances

SPS occurrences

SPS occurrences have been already reported (Zgheib *et al*., 2010). Detailed examination of our results showed the total number of substances regardless of the site was comparable and that our samples of stormwater contained 55 different individual substances (Table 7.5-178). 21 SPS were observed across all samples: 15 PAHs, two metals (Cu, Zn), one pesticide (diuron), one organotin (MBT), DEHP, and nonylphenols. Some chlorophenols and VOCs were less commonly observed and seemed to show greater site dependence due to either a local source or a mix of sources that still need to be identified (Table 7.5-178). Besides, 33 substances were never quantified (see Table 7.5-178 for the entire list of these substances). Their concentrations always remained below the limit of detection (LOD) in both fractions. Several explanations for this finding can be forwarded. Samples were in fact only collected at the end of storm events, hence increasing the risk of losses. VOCs are known to be highly volatile, so they were sometimes observed because of the presence of numerous local sources, which compensated losses. For pesticides, many reasons are available to explain the non-detection of some of these products, though the main reason remains the cessation of their use. Most of these pesticides are in fact now banned from use in France. Furthermore, some LODs were set too high to quantify certain substances (i.e. Cd, Ni, PBDEs). This last consideration constitutes one of the main drawbacks to working with accredited laboratories. These LODs appeared to be too high for some substances, in comparison with levels generally determined by research centres. Since in many countries, however, regulation imposes sewer network managers to work with accredited laboratories, the managers must be able to face such constraints.

Table 7.5-179: Concentrations of stormwater priority substances at the outlets of the three storm sewers both in water (DDP) and in the particulate phase (P*)

Substance	DDP						P* (µg/g dw)		
	Unit	n	Occ	Min	Med.	Max	Min	Med.	Max
Lead (Pb)	µg/L	16	82%	<10	27	129	<	283	1000
Chromium (Cr)	µg/L	16	38%	<10	4.5	45	<	<	100
Copper (Cu)	µg/L	16	100%	30	55	220	217	550	4049
Zinc (Zn)	µg/L	16	100%	130	270	520	1087	1865	11,818
Tributyltin (TBT)	ng/L	16	21%	<10	<10	78	<	<	0.18
Dibutyltin (DBT)	ng/L	16	79%	<10	72	516	<	0.19	0.43
Monobutyltin (MBT)	ng/L	16	100%	14	101	572	0.15	0.35	1.2
∑16 PAH	ng/L	16	–	677	1327	6477	3.54	9.26	17.39
Naphthalene (N)	ng/L	16	100%	5	82	490	<	0.1	0.37
Acenaphthene (Ace)	ng/L	16	100%	9	15	63	<	0.04	0.27
Acenaphthylene (Acyl)	ng/L	16	96%	<20	24	126	<	0.07	0.2
Fluorene (F)	ng/L	16	100%	10	28	106	0.03	0.11	0.2
Phenanthrene (P)	ng/L	16	100%	45	140	726	0.22	0.87	2.92
Anthracene (A)	ng/L	16	100%	2	23	104	0	0.12	1.2
Fluoranthene (Fluo)	ng/L	16	100%	23	134	945	0.11	1.2	2.4
Pyrene (Pyr)	ng/L	16	100%	19	177	3254	0.43	1.5	5.82
Benzo[a]anthracene (B(a)A)	ng/L	16	100%	12	53	298	0.08	0.58	0.83
Chrysene (Chry)	ng/L	16	100%	17	104	655	0.19	1.1	1.8
Benzo[a]pyrene (B(a)P)	ng/L	16	100%	11	66	315	0.32	0.71	1.3
Benzo[k]fluoranthene (B(k)F)	ng/L	16	100%	16	52	230	0.17	0.49	0.78
Benzo[b]fluoranthene (B(b)F)	ng/L	16	100%	26	134	656	0.46	1.4	2.1
Dibenzo[a,h]anthracene (D(a,h)A)	ng/L	16	100%	12	30	109	0.08	0.2	0.54
Benzo[g,h,i]perylene (BP)	ng/L	16	100%	14	100	569	0.35	0.93	1.7
Indeno[1,2,3-cd]pyrene (IP)	ng/L	16	100%	12	80	354	0.19	0.69	1.5
∑7 PCB	ng/L	16	–	<10	259	727	<	0.11	0.28
PCB28	ng/L	16	73%	<10	32	104	<	0.02	0.06
PCB52	ng/L	16	67%	<10	31	104	<	0.01	0.09
PCB101	ng/L	16	73%	<10	33	104	<	0.01	0.04
PCB118	ng/L	16	73%	<10	33	104	<	0.01	0.04
PCB138	ng/L	16	87%	<10	48	108	<	0.02	0.06
PCB153	ng/L	16	87%	<10	48	111	<	0.03	0.06
PCB180	ng/L	16	80%	<10	37	108	<	0.02	0.04

Table 7.5-179 – continued

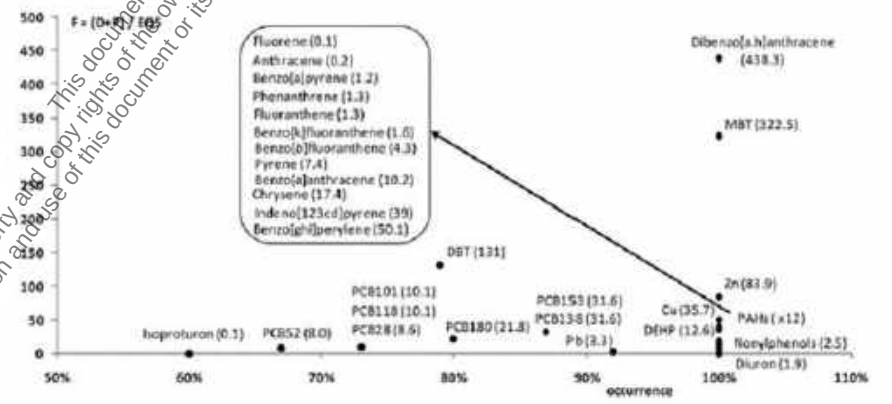
Nonylphenols (NP)	ng/L	14	100%	300	750	9170	1.1	3.75	22
Para-tert-octylphenol (PT-OP)	ng/L	14	86%	<50	110	260	<	0.06	0.38
4-tert-Butylphenol (4-TBP)	ng/L	14	86%	<50	110	200	<	<	0.15
Di-(2-ethylhexyl)-phthalate (DEHP)	µg/L	14	100%	3	22	58	55	98.5	2
Aldrin	µg/L	15	7%	<0.02	<0.02	0.04[a]	<	<	0.02
Endrin	µg/L	15	7%	<0.02	<0.02	0.41[a]	<	<	<
Dieldrin	µg/L	15	27%	<0.02	<0.02	0.2	<	<	0.66
Chlorfenvinphos	µg/L	15	7%	<0.05	<0.05	0.12[a]	<	<	0.21
Desethylatrazine (DEA)	µg/L	15	20%	<0.03	<0.03	0.03	<	<	<
Desethylsimazine (DES)	µg/L	15	7%	<0.04	<0.04	0.04[a]	<	<	<
Simazine	µg/L	15	33%	<0.01	<0.01	0.15	<	<	<
Diuron	µg/L	15	100%	0.03	0.37	1.75	<	<	0.21
Isoproturon	µg/L	15	60%	<0.01	0.09	0.14	<	<	<
Metaldehyde	µg/L	15	60%	<0.02	0.06	0.58	<	<	<
Aminotriazole	µg/L	15	80%	<0.03	0.13	3.25	<	<	1
Glyphosate	µg/L	15	93%	<0.03	1.11	232	<	0.1	8.3
AMPA	µg/L	15	93%	0.14	0.64	9.33	<	0.28	4
Ethylbenzene[b]	µg/L	14	7%	<0.5	<0.5	1.1			
Toluene[b]	µg/L	14	7%	<0.5	<0.5	1.1			
Xylenes[b]	µg/L	14	7%	<0.5	<0.5	1.1			
Methylene chloride[b]	µg/L	14	44%	<1	<1	1.3			
Tetrachloroethylene[b]	µg/L	14	25%	<0.02	<0.02	1.3			

n = number of storms; Occ.: occurrence rate of this substance on each site, in percentage; % min = minimum concentration; med. = median concentration; max = maximum concentration. "<": below LOQ (see Supporting Table 7 for LOQ values).
a Concentration evaluated on one sample only.
b Analyses conducted on the total phase.

SPS concentrations in stormwater particles

Table 7.5-179 presents, for all the 55 detected SPS, the (D + P) event mean concentrations (in µg/L), along with occurrence rates (in %) and particulate contamination levels (in µg/g dw). As previously mentioned, it was observed that (D + P) concentrations for the three watersheds were not significantly different, allowing the pooling of all data. The same observation held true for the particulate concentrations (P*), since statistical ANOVA did not find any significant differences for all tested substances ($\alpha = 0.05$, p> 95 %, data not shown). For this reason, results have been discussed by considering a global approach for interpretation, based on particle contamination followed by a comparison with sediments and settleable particles of the Seine River basin. To our knowledge, such a comparison has never been conducted so far.

Figure 7.5-149: Dilution factor (F), obtained by comparing total concentration (D + P) for stormwater with French EQS, expressed as a function of occurrence



Metals
Metal contents were calculated from the results of the analysis carried out on the bulk sample and on the dissolved phase. The difference was then normalized to TS content. Metals were detected either above LOQ or below LOD, never in between. Stormwater was contaminated by Zn (270 µg/L, median concentration), Cu (55 µg/L), Pb (27 µg/L) and Cr (4.5 µg/L). These concentrations were twice as high as those for stormwater in London: Zn (82 µg/L), Cu (35 µg/L), Pb (10 µg/L), and Cr (3 µg/L). The presence of these metals in stormwater is caused by: i) vehicle brake emissions for Cu, ii) tire wear for Zn, and iii) atmospheric deposition for Cu and Pb.

Cu was observed at 550 µg/kg dw, with a range extending from 217 to 4049 µg/kg dw. These values are similar to the median concentrations typically reported in the literature. The value estimated for Cu in the NQSD equaled about 138 µg/g dw. This estimation was derived using the concentrations of total and filtered fractions, as well as the TS content provided by the database. Pb exhibited a different trend: Pb was evaluated at 283 µg/g dw, a level similar to our estimation in NSQD (241 µg/g dw). For Zn, we measured a level of 1865 µg/g dw (Zn-NQSD:1120 µg/g dw), which equalled the level reported in the QASTOR database, i.e. 1629 µg/g dw. The discharge of untreated stormwater may impact the receiving waters and contribute to sediment contamination with regards to metals.

Polycyclic aromatic hydrocarbons
As can be seen from the Table 7.5-179, the 16 PAHs were observed in almost 100 % of the samples. Stormwater concentrations of the Σ16 PAHs ranged from 677 to 6477 ng/L (median: 1327 ng/L). The composition pattern of PAHs showed a distribution dominated by Pyrene, followed by Fluoranthene, Phenanthrene, and Chrysene. These high molecular-weight PAHs (containing between 4 and 6 aromatic rings) indicate inputs of pyrolytic origin tied to the high density of combustion sources within Paris and its suburbs, such as gasoline-powered vehicles and residential heating. Moreover, the PAH loads varied from 3.5 to 17.4 µg/g dw (median: 9.26 µg/g dw). In contrast, lift station sediments in Paris contained 23.5 µg/g dw (range:14 - 45 µg/g dw) for Σ16 PAHs. It is therefore likely that these findings resulted from the high traffic density in Paris compared to the densities of the three investigated watersheds. The comparison with dredged sediments (6.7 µg/g dw) and settleable particles from the Seine River (2.01-17.31 µg/g dw) has confirmed the severe contamination of stormwater particles in the Paris region by PAHs, which contribute during storm events to the contamination of watercourses.

Table 7.5-180: Comparison of median particulate contents for all three storm sewers with Canadian sediment guidelines

	PEL (µg/g dw)	P*/PEL
		This study
Lead	91.3	3.10
Chromium	90	–
Copper	197	2.79
Zinc	315	5.92
Naphthalene	0.9	0.11
Acenaphthene	0.9	0.04
Acenaphthylene	0.13	0.54
Fluorene	0.14	0.79
Phenanthrene	0.52	1.67
Anthracene	0.25	1.48
Fluoranthene	2.36	0.51
Pyrene	0.88	1.70
Benzo[a]anthracene	0.39	1.49
Chrysene	0.86	1.28
Benzo[a]pyrene	0.78	0.91
Dibenzo[a,h]anthracene	0.14	1.43
∑PCBs	0.28	0.39

PEL: Probable Effect Level, according to the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment, 1999). P*: the particulate median content in µg/g dw.

Polychlorinated biphenyls

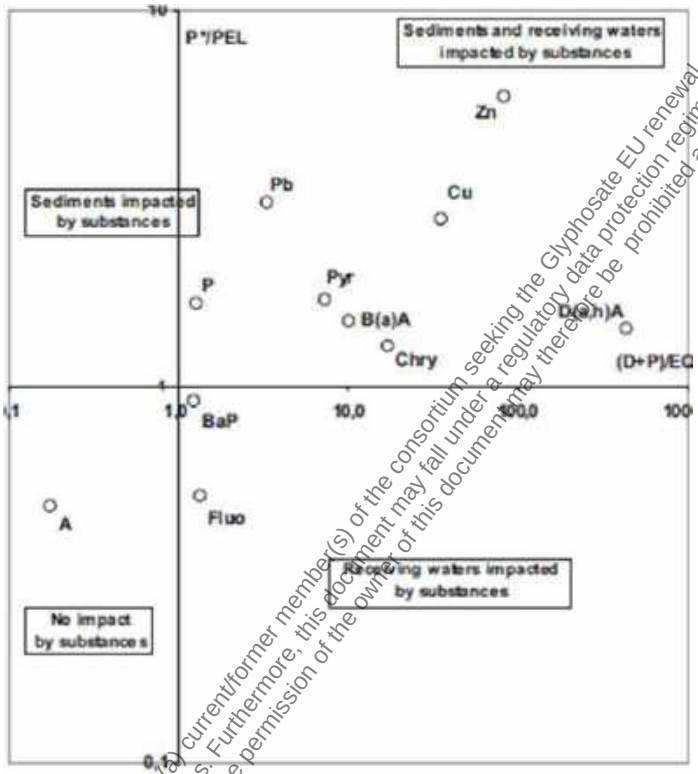
Despite their ban in France since 1970, 7 congeners out the 8 investigated, namely PCB 28, 52, 101, 118, 138, 153 and 180, were detected (Table 7.5-179). The PCB distribution in stormwater revealed that 7-Cl (PCB 180) congener accounted for 14 %, 6-Cl (PCB138 + PCB153) for 29 %, 5-Cl (PCB101 + PCB118) for 27 %, 4-Cl (PCB 52) for 11 %, and 3-Cl (PCB 28) for 14 %. This distribution, comparable to that observed for stormwater in Switzerland, is quite similar to that of the industrial mixture Arochlor but differs from that reported for total atmospheric deposition in Paris. The main sources of PCBs in water resources remain however atmospheric deposition and runoff on urban surfaces. PCBs were particle-bound at 100 % and the ΣP7 PCBs ranged from <0.005 to 0.280 µg/g dw, with a median of 0.110 µg/g dw. These levels were comparable to those observed for a stormwater sediment trap in Norway: 0.0004 - 0.704 µg /g dw.

Organotins

Three organotin compounds, namely monobutyl (MBT), dibutyl (DBT) and tributyl (TBT), were monitored; they all presented contrasted behaviour, since MBT was observed in 100 % of stormwater samples, while TBT and DBT were observed in just 21 % and 79 % of the samples, respectively. Observations were mainly recorded in the particulate phase at the following levels: <10 - 78 (median: <10), <10 – 516 (72) and 14 - 572 (101) ng/L for TBT, DBT and MBT, respectively. Similar ranges of concentrations in stormwater have been measured in two Norwegian harbours: 9 - 185, 8 -140 and 9 -85 ng/L for TBT, DBT and MBT, respectively. The organotin contents of stormwater particles were: 0.35 µg/g dw for MBT, 0.19 µg/g dw for DBT, and below the LOD for TBT. These levels were all higher than those measured in Norwegian stormwater, i.e. from 0.009 to 0.045 µg/g dw for MBT, 0.008 to 0.041 µg/g dw for DBT, with an exception for TBT, whose contents were similar (0.007 - 0.032 µg/g dw). On the other hand, contents were lower than those observed in stormwater particles from an industrial area in Norway (0.1 - 2.3 µg/g dw for DBT, 0.2 - 11 µg/g dw for TBT), except for MBT, whose levels were comparable (0.06 - 1.3 µg/g dw). It is generally agreed that the levels of MBT and DBT in stormwater exceed those of TBT.

Since sediment did not accumulate in any of our three storm sewers, TBT degradation can be neglected and the levels of MBT and DBT may be due to their release from either organotin-stabilized PVC (e.g., in packaging material, piping, window frames.) or the local use of biocides).

Figure 7.5-150: Comparison of the environmental risk assessment for sediments, according to Canadian sediment quality guidelines (P*/PEL), with that for receiving waters, using environmental quality standards ((D+ P)/EQS)



Volatile organic compounds

Amongst the VOCs monitored, only methylene chloride (in 44 % of samples, between <1 and 13 µg/L) and tetrachloroethylene (25 % of samples, <0.02 - 1.3 µg/L) were observed in samples collected from the dense urban areas of PRG and NLG, while they were never detected in the residential area. As previously stated, our sampling strategy was not suitable for VOC analysis.

Pesticides

Data from Table 7.5-179 show that six pesticides were ubiquitous regardless of either the storm event or the watershed, meaning that they displayed an occurrence rate of at least 60 %: diuron (100 %), glyphosate (93 %), amino methyl phosphonic acid or AMPA (93 %), aminotriazole (80 %), isoprotuon (60 %), and metaldehyde (60 %). All these pesticides except metaldehyde are herbicides. This finding was not surprising since herbicides represent 90 % of all pesticides applied in urban areas. AMPA is the major metabolite of glyphosate; as would be expected therefore, the level of AMPA has increased along with that of glyphosate. Our findings are in good agreement with Botta *et al*. (2009), whose results suggested that contamination of the Orge River urban watershed by glyphosate was essentially of an urban origin (road and railway applications). The stormwater is thus contaminated by herbicides through the leaching of impervious urban surfaces. As a consequence, pesticides were able to reach receiving waters mainly through the storm sewer during a storm event. Moreover, the pesticide content in stormwater differed from one compound to another, lying between 0.04 and 0.92 µg/g dw. Among the pesticides listed as priority substances by the WFD, aldrin and chlorofenvinphos were quantified on a single sample with values at 0.62

and 0.21 µg/g dw, respectively. For aminotriazole, the maximum level equaled 1 µg/g dw, while the value for diuron was 0.21 µg/g dw and for dieldrin 0.66 µg/g dw. For glyphosate and AMPA, these levels were respectively 8.30 (median: 0.1) and 4 µg/g dw (median: 0.3). The data presented herein are original because the pesticide contents of particles are rarely reported in urban areas. For the remaining pesticides, particle contents were below LOD. Further research should be conducted to investigate a potential seasonal effect during urban pesticide application (looking closely at spring and fall).

Di(2-ethylhexyl) phthalate
DEHP was measured in all samples between 3 and 58 µg/L. Such levels were higher to those previously reported for stormwater in Sweden (5 µg/L) and in London (0.75 - 1.25 µg/L). The DEHP content in stormwater has ranged between 55 and 260 µg/g dw, with a median concentration of 99 µg/g dw. Surprisingly, Björklund *et al*. (2009) reported that DEHP was never detected in deposits from Norwegian storm sewers; however, their LOD was quite high (approx. 50 µg/g dw).

Alkylphenols
Overall, nonylphenols were ubiquitous in stormwater with a median concentration of 0.75 µg/L. These levels are average levels compared to previous results reported for stormwater. Their presence in stormwater is due to leaching from urban paint and cleaning products, as well as from pesticide residues. Data records for alkylphenols in stormwater particles are rare. For the three investigated watersheds, the levels of nonylphenol in stormwater lie in the range of 1.10 - 22 µg/g dw, with a median of 8.12 mg/g dw (Table 7.5-179). The SEB watershed, in the suburban area, and the NLG watershed, in the dense urban area, posted significantly higher levels for nonylphenols: 5.22 and 17.75 µg/g dw respectively, when compared to PRG watershed (2.85 µg/g dw). These levels exceed those measured in storm sewer deposits (0.72 - 1.5 µg/g dw) in Sweden and in urban stormwater: 3.7 µg/g dw. For the other alkylphenols, particulate contents were as follows: para-tert-octylphenol varied between <LOD and 0.38 µg/g dw, 4-tert-butylphenol between <LOD and 0.15 µg/g dw, and lastly 4-n-octylphenol between <LOD and 0.17 µg/g dw. For octylphenols, Bressy *et al*. (2011) observed a value of 0.27 µg/g dw for urban stormwater.

Environmental risk assessment
The European Commission has established environmental quality standards (EQS) so as to limit the quantity of certain chemical substances in receiving waters in the European Union. As stated in the Directive 2008/105/EC of the European Parliament, Member States must verify that the concentration of substances concerned does not increase significantly in sediments and/or the relevant biota. As a consequence, an environmental risk assessment was carried out according to Zgheib *et al*. (2011b), despite the simplicity of the method. For a given substance, its (D + P) concentration was compared to its corresponding EQS, as established by either the European Commission (Directive 2008/105/EC) or the French government (Circular 2007/23). This approach gave an indicative dilution factor for the stormwater discharge by the river flow to avoid the increase of the concentration of the priority substances in the watercourse beyond their EQS. As shown in Figure 7.5-149, many substances needed a dilution factor between 10 and 50 (the flow of the discharge should be the tenth or the fiftieth of the river flow to comply with regulation), whereas the dilution factor for 5 other substances had to exceed 50, i.e. dibenzo[a,h]anthracene (438), MBT (322), DBT (131), benzo [g,h,i]perylene (50) and Zn (84). This study produced results which corroborate the findings of the previous work on the watershed of Noisy-le-Grand. We have demonstrated for most substances that particles from the three storm sewers were more contaminated than dredged sediments and settleable particles from the Seine River. A consequence of the discharge of contaminated particles can result in sediment contamination. To evaluate to what extent this might occur, SPS particulate content (P*) was compared to the Canadian sediment quality guidelines (Canadian Council of Ministers of the Environment, 1999). According to these guidelines, the probable effect level (PEL) defines the level above which adverse effects are expected to occur frequently. As shown in Table 7.5-180, 8 substances (namely, Pb, Cu, Zn, phenanthrene, pyrene, benzo[a]anthracene, chrysene and dibenzo[a,h] anthracene) exceeded the guideline threshold, thus implying potential adverse biological effects on freshwater organisms. These results also point out that PAHs and metals in stormwater particles constitute a potential risk to the receiving waters. Finally, Figure 7.5-150 establishes a comparison of the trends observed for the environmental risk assessment using both approaches, for substances having thresholds defined both for sediments (PEL) and receiving waters (EQS). Though no mathematical

correlation could be established between P*/PEL and (D + P)/EQS, it can be seen that the 8 substances exceeding the guideline threshold (i.e., P*/PEL > 1) displayed a dilution factor greater than one ((D + P)/EQS > 1). Therefore, this means that each approach led to the same result: these substances represent a threat to both media. As a consequence, they should be included into monitoring programs. For the remaining substances, two different situations were observed the substance impacts the receiving waters but not the sediments (i.e., benzo[a]pyrene and fluoranthene), or no impact was observed whatever the media (i.e., anthracene and fluorene). The remaining situation, namely the substance impacts the sediments but not the receiving waters, was not encountered.

Conclusion

The aim of this research has been to assess the potential presence of 88 stormwater priority substances in three watersheds located within the Paris metropolitan area with respect to particle contamination. A good number of findings have been derived from our results:

- Among the 55 substances observed at least once, 21 were present in all samples: 15 PAHs, two metals (Cu, Zn), one pesticide (diuron), one organotin (MBT), DEHP and nonylphenols.
- The levels of contamination of particles for the three watersheds were not significantly different.
- For most pollutants (metals, PAHs, PCBs, etc.), particles from the three storm sewers were more contaminated than dredged sediments and settleable particles from the Seine River. Consequently, the release of untreated stormwater discharge may impact receiving waters and contribute to sediment contamination. This point has been confirmed by comparing particulate concentrations with the Canadian Sediment Quality Guidelines, which have shown that metals and PAHs in stormwater particles constitute a potential risk to receiving waters.

A special effort should therefore be made to treat or remove as much of the particulate fraction of stormwater as possible, as this step will significantly reduce the impact on receiving waters given that most stormwater priority substances are particle-bound. To supplement our assessment of stormwater in the urban environment, a comparison of stormwater quality from separate storm sewers with the quality from combined sewer overflows is discussed in details in Gasperi *et al*. (2012).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article reports the contamination of stormwater with organic and mineral pollutants in the urban region of Paris. Among other substances, glyphosate and AMPA were measured and identified. The detected concentrations derive from atmospheric deposition and surface runoff from the urban environment, i.e. agricultural uses are not in the focus. Maximum glyphosate concentration of 232 µg/L in water (dissolved and particulate phases) and 8.3 µg/g dw (particulate phase). Maximum AMPA concentrations of 9.37 µg/L in water (dissolved and particulate phases) and 4 µg/g dw (particulate phase). The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/061
Report author	Birch H. *et al*.
Report year	2011
Report title	Micropollutants in stormwater runoff and combined sewer overflow in the Copenhagen area, Denmark
Document No	Water science and technology (2011) Vol. 64, No. 2, pp. 485-93.
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Eurofins Miljø A/S)
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Stormwater runoff contains a broad range of micropollutants. In Europe a number of these substances are regulated through the Water Framework Directive, which establishes Environmental Quality Standards (EQSs) for surface waters. Knowledge about discharge of these substances through stormwater runoff and combined sewer overflows (CSOs) is essential to ensure compliance with the EQSs. Results from a screening campaign including more than 50 substances at four stormwater discharge locations and one CSO in Copenhagen are reported in the paper. Glyphosate and AMPA were found in all samples at similar levels (glyphosate 0.043 – 1.3 µg/L; AMPA 0.06 – 1.3 µg/L). The highest concentrations were found in the combined sewer overflow; all these sources would result in direct input into streams without any form of treatment.

Materials and methods

Sampling and sampling locations
Five sampling locations in the greater Copenhagen area were selected for this study and a total of 10 samples were analysed (see Table 7.5-181). Two of the sites (SS1 and SS2) were located in Tårnby, situated on the island Amager, and the remaining three (CS1, SS3 and SS4) were located in Gentofte, Albertslund and Glostrup, respectively. The sites varied in size, catchment type and treatment method and different events were sampled using different sampling methods. None of the rain events sampled were extreme rain events and all had return periods below 0.5 yr^{-1}. Samples were stored at 5°C and in darkness before analysis, which was started within 24 h of sampling.

Substances and sources
Substances for analysis were primarily selected from the WFD list, as illustrated in Figure 7.5-151, but earlier Danish runoff studies and a risk assessment for one of the catchment areas were also considered. Furthermore, industrial intermediates not used in Denmark, available analytical packages and prices affected the final choice of analysed substances.

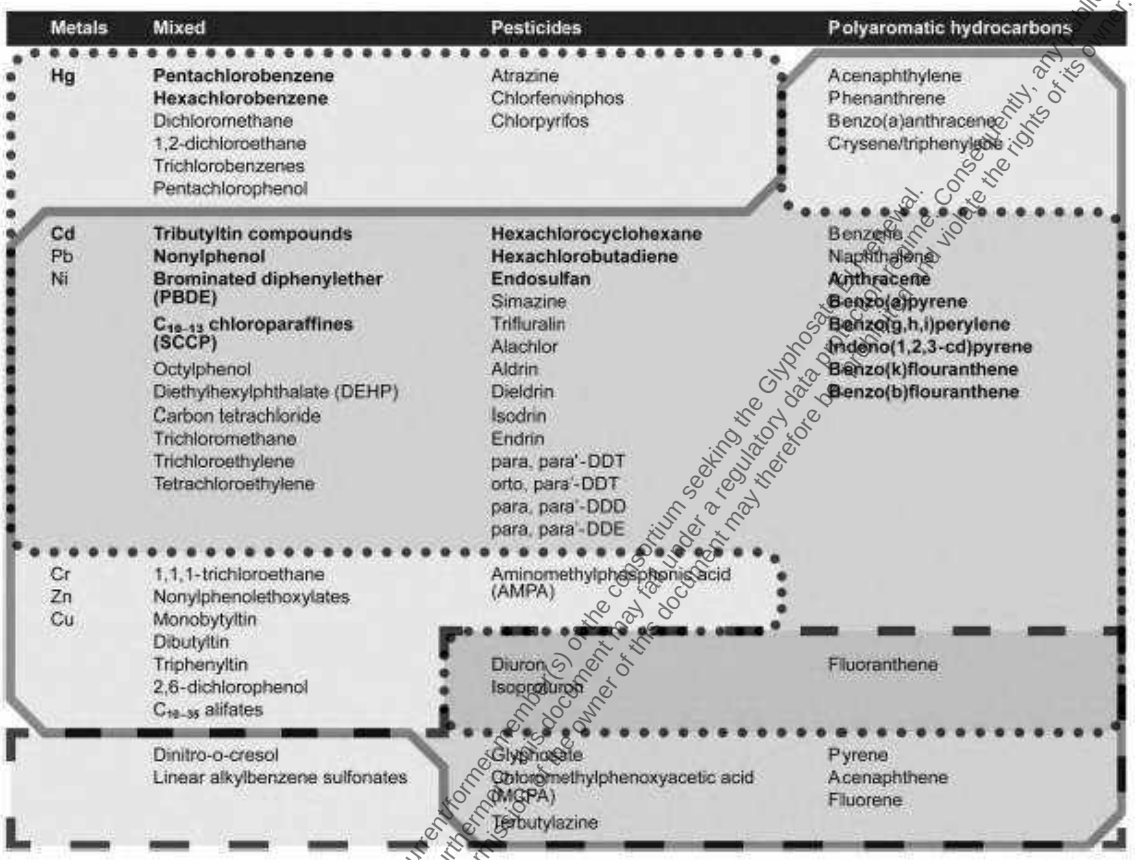
Table 7.5-181: Description of the sites, samples and rain events

Sites	Byparken		Digevej	Fabriksparken	Ejby mose					Scherfigsvej
Sewer type	Storm sewer		Storm sewer	Storm sewer	Storm sewer					CSO[a]
Sample	SS1-1	SS1-2	SS2	SS3	SS4-1	SS4-2	SS4-3	SS4-4	SS4-5	CS1
Impervious area	1.3 ha		9.4 ha	56 ha	13 ha	60 ha	5 ha	–	–	1,100 ha
Catchment type[b]	Roads		Res. and metro	Ind. & res.	Res.	Ind. & road	Res. & road	In the bog[c]	Outlet of the bog	Res. & roads & drains
Treatment	Grit chamber Inlet to DPF[e]	Outlet from DPF[e]	Grit chamber	Oil sep.[d]	Oil sep.[d]	Oil sep.[d]	Oil sep.[d]			–
Sample type	Grab		Grab	Grab	Precipitation dependant[f]			Grab	Grab	Volume proportional
Date (mm/dd)	10/15 2008		11/18 2008	11/18 2008	09/02–09/03 2009					09/30 2008
Rain depth[g]	2.3 mm		1 mm	5.7 mm	11 mm					6.4 mm
Duration[g]	3 h		30 min	3 h	16 h					23 h
Antecedent dwp[h]	9 days		36 h	36 h	36 h					7 days

[a]Combined Sewer Overflow, [b]Res. = residential, Ind. = industrial, [c]natural wetland, [d]separator, [e]DPF = Dual porosity filtration, description can be found in Jensen & Bisballe (2009), [f]Samples are taken depending on precipitation measured in a rain gauge at the site, [g]For grab samples this depth and time of rain before the sample was taken, not total rain event depth and time, [h]dwp = dry weather period.

Pesticides originate from public and private use as well as atmospheric deposition and leaching from building materials and paints during rain events.

Figure 7.5-151: **EU PS and PHS are shown in the dotted shape. Substances found in an earlier risk assessment of a catchment in Copenhagen are shown in the stippled shape. Substances selected in the present study are shown in the solid shape**



Analysis

Eurofins Miljø A/S (Denmark) performed all analyses of more than 50 micropollutants, except heavy metals in the samples from SS1, SS2, SS3 and CS1 which were analysed at DTU Environment's own laboratories. For all analyses total concentrations were measured.

Results

After a discussion of the sampling method, the following paragraphs present and discuss the findings for glyphosate and AMPA only (see Table 7.5-182).

Table 7.5-182: Presence in µg/L of the micropollutants found in water samples from the sampling locations SS1-SS4 and CS1

Substance	SS1-1	SS1-2	SS2	SS3	SS4 1-3	SS4-4	SS4-5	CS1	Danish literature stormwater[a]	Expected range in CSO in Denmark[b]	AA-EQS	MAC-EQS
PAHs												
Naphthalene	<0.01	<0.01	0.072	0.021	na	na	na	1.4	<0.02–0.59	0.65–5	2.4	
Acenaphtylene	<0.01	<0.01	0.039	0.028	0.013–0.024	<0.01	<0.01	0.029	<0.01–0.23			
Acenaphthene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01–0.054	0.01–1		
Fluorene	<0.01	<0.01	0.028	0.014	na	na	na	0.13	<0.01–0.27	0.01–1		
Phenanthrene	0.017	<0.01	0.29	0.11	na	na	na	0.82	<0.01–5.1	0.01–0.5		
Anthracene	0.012	<0.01	0.084	0.057	na	na	na	0.22	<0.01–0.38	0.01–0.5	0.1	0.4
Fluoranthene	0.025	<0.01	0.55	0.18	na	na	na	2	0.016–3.9		0.1	1
Pyrene	0.034	<0.01	0.56	0.27	0.044–0.19	0.05	0.019	2.1	0.02–4.1			
Benzo(a)anthracene	<0.01	<0.01	0.21	0.066	<0.01–0.042	<0.01	<0.01	1	<0.01–0.54			
Chrysen/triphenylene	<0.01	<0.01	0.38	0.25	0.052–0.14	0.015	<0.01	0.76	0.016–1.9			
Benzo(b + j + k) fluoranthene	<0.01	<0.01	1	0.26	0.046–0.12	0.016	0.011	3.1	<0.01–2.0	0.01–0.5	0.03[c]	
Benzo(a)pyrene	<0.01	<0.01	0.31	0.06	<0.01–0.064	<0.01	<0.01	1.6	<0.01–0.59	0.01–0.3	0.05	0.1
Benzo(g,h,i)perylene	<0.01	<0.01	0.47	0.16	0.026–0.085	<0.01	<0.01	1.4	<0.01–0.96		0.002	
Indeno(1,2,3-cd) pyrene	<0.01	<0.01	0.39	0.12	0.016–0.044	<0.01	<0.01	2.6	<0.01–0.42	0.02–0.5		
Dibenzo(a,h) anthracene	<0.01	<0.01	<0.01	<0.01	na	na	na	0.19	<0.01–0.086			
Total PAH	0.088	<0.01	4.385	1.576	0.197–0.707	0.061	0.030	17.35				
Pesticides												
Glyphosate	1.2	0.26	0.043	0.17	0.59–0.94	0.33	0.088	1.3	0.1–4.0			
AMPA	0.32	0.42	0.077	0.13	0.06–0.33	0.84	0.95	0.3	0.2–0.9			
Diuron	0.055	0.027	<0.01	<0.01	na	na	na	0.48			0.2	1.8
Isoproturon	0.044	0.035	<0.01	<0.01	na	na	na	0.20	<0.05–0.079		0.3	1.0
Terbutylazine	<0.01	<0.01	<0.01	<0.01	na	na	na	0.20	<0.05–0.16			
MCPA	<0.01	<0.01	<0.01	0.018	na	na	na	<0.01	<0.05–0.13			
Monobutyltin	0.035	0.018	0.048	0.072	na	na	na	<0.01				
Dibutyltin	0.008	<0.005	0.009	0.009	na	na	na	<0.005				
TBT	<0.004	<0.004	<0.004	<0.004	na	na	na	<0.004			0.0002	0.0015

AMPA: Aminomethylphosphonic acid; MCPA: Chloromethylphenoxy acetic acid; TBT: Tributyltin; NP: Nonylphenol; NPEs: Nonylphenol ethoxylates; NPE_2s: Nonylphenol diethoxylates; OPE_ns: Octylphenol polyethoxylates; DEHP: Diethylhexylphthalate; PBDE: Polybrominated diphenylether; na: no analysis; <: concentration below the LOD; CSO: combined seweroverflow; AA: annual average; MAC: maximum allowable concentration; –: not applicable; [a]Kjølholt et al. (1997) and Danish EPA (2006); [b]Arnbjerg-Nielsen et al. (2002); [c]EQS is only for b and k

Sampling methods

When sampling stormwater, the most representative sampling method is to use flow-proportional sampling or volume proportional sampling. Using these methods, event mean concentrations (EMCs) can be evaluated from each rain event. Another method, which is not as accurate as flow-and volume-proportional sampling, is the precipitation dependant sampling method where the input to the autosampler is determined by a rain gauge rather than flow measurements. Since the actual flow is not measured with this method, variation in rain intensity and varying runoff times over the catchment area are sources of uncertainty. Grab sampling is the least representative sampling method, but also the cheapest. The variability of pollutant concentrations in stormwater is very high, both between sites, between events and during events. This means that the variation of grab samples from different sites and events will be higher than the expected variation of EMCs from the same site or event.

In this study grab sampling was used where equipment for volume or precipitation proportional sampling was not available. Different sites were sampled during different events without specific attention to the duration and intensity of the event or the antecedent dry weather period. This means that the results cannot be considered statistically representative, be used to distinguish different pollution sources across sites or be used to calculate EMCs.

Nevertheless, the pattern of identified substances and their concentrations give a valuable first insight into the presence of a large number of micropollutants in stormwater runoff and CSO around Copenhagen and

may be used as a starting point for more detailed monitoring studies targeting urban discharges of PSs in the context of the WFD.

Pesticides
Alachlor, aldrin, para,para'-DDT, orto,para'-DDT, para,para'-DDD, para,para'-DDE, dieldrin, endosulfan, endrin, hexachlorobutadiene, isodrin, lindane, simazin and trifluralin are all regulated under the WFD but were not found in this study. They are all prohibited in Denmark.

The CSO sample contained the highest concentrations of pesticides. Glyphosate and the degradation product aminomethylphosphonic acid (AMPA) were found in all samples. Glyphosate is currently included in the list of candidate substances for the WFD. From the inlet to the outlet of the treatment facility (SS1) as well as from the inlet to the outlet of the bog (SS4) the concentration of glyphosate decreased and the concentration of AMPA increased, indicating degradation throughout the two systems.

Pesticide concentrations in runoff are influenced more by local conditions and specific uses, than traffic related substances such as heavy metals and PAHs. For example, Weston *et al.* (2009) showed that pyrethroid pesticides in an urban creek came from residential runoff. Blanchoud *et al.* (2007) found a range of different pesticides in the Marne stream and showed that urban pesticide uses were important factors because of application on impervious areas resulting in rapid, unimpeded transport to the river during rain. An environmental risk assessment performed for a stream in the greater Copenhagen area, concluded that glyphosate, diuron, isoproturon, terbutylazine and MCPA all pose a risk to the stream's aquatic environment. This study confirms that these specific pesticides are being used in the greater Copenhagen area and that stormwater as well as CSOs contribute to the pesticide pollution load to the stream.

Monitoring
Whether stormwater discharges pose a risk to the aqueous environment depends on local conditions (water baseflow, amount and frequency of discharged water, etc.). Nevertheless, untreated stormwater discharges, especially CSOs, are a considerable source of pollution. For the Danish RBMPs, submitted for revision during spring 2010, the influence of micropollutants on the water quality was only included when monitoring data allowed doing so. There is however a severe lack of data on the presence of micropollutants in Danish surface waters, lakes and streams, for which reason it is difficult to exempt these substances from deteriorating water courses. In preliminary investigations on which Danish RBMPs are based, it is therefore 'anticipated that water courses receiving large amounts of stormwater discharges from roads and/or larger cities will be at risk'.

Conclusion
The present investigation shows that a broad range of EU WFD priority substances and other identified micropollutants including degradation products are found in various stormwater and combined sewage discharges around the greater Copenhagen area. Glyphosate and AMPA were found in all samples at similar levels (glyphosate 0.043 – 1.3 µg/L; AMPA 0.06 – 1.3 µg/L). The highest concentrations were found in the combined sewer overflow; all these sources would result in direct input into streams without any form of treatment.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a monitoring experiment considering storm water from different catchments in the Copenhagen area. Glyphosate and AMPA were measured in the study, and the catchments are classified as mainly urban.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/027
Report author	Bruchet, A. *et al.*
Report year	2011
Report title	Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge
Document No	European Journal of Water Quality 42 (2011) 123-133
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at an officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/062
Report author	Lamprea, K., Ruban, V.
Report year	2011
Report title	Pollutant concentrations and fluxes in both stormwater and wastewater at the outlet of two urban watersheds in Nantes (France)
Document No	Urban Water Journal (2011), Vol. 8, no. 4, pp. 219-231
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (IDAC and IANESCO-CHIMIE Laboratory)
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

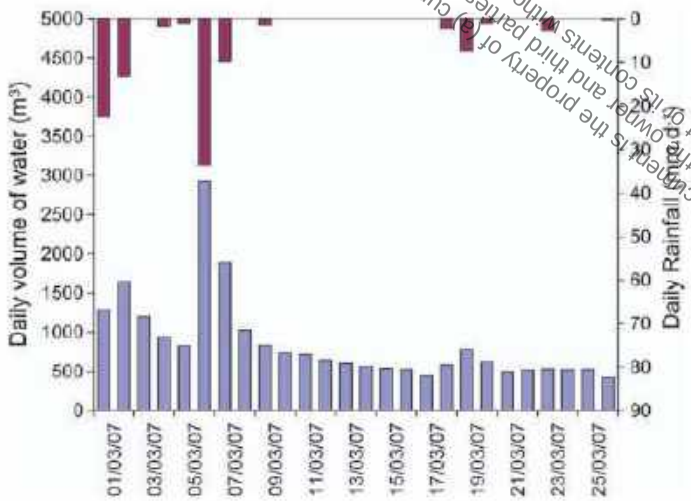
A two-year study of pollutants in both the stormwater and wastewater of urban watersheds was conducted in Nantes (France). The present paper discusses the characteristics of pollutants transported by stormwater and wastewater collection networks in two urban watersheds. A physicochemical characterisation of the effluents was performed, along with an estimation of pollutant fluxes discharged into the Gohards River. Suspended solids (SS), trace metals, polycyclic aromatic hydrocarbons (PAHs) and pesticides were studied. SS, Zn, Cu and glyphosate were the main pollutants in stormwater and wastewater.. Despite a reduction in the use of pesticides in Nantes Metropolitan area, herbicides containing glyphosate were still detected in stormwater. It should be noted that this herbicide is widely used by homeowners, a fact that may explain its occurrence in stormwater.

Materials and methods

Study sites

This study was conducted in the Pin Sec and Gohards watersheds located to the east of the city of Nantes (western France), between the Loire and Erdre rivers. In this area, the urban network was a separate sewer system. Stormwater was collected separately and discharged directly to Gohards River, whereas the wastewater network connected to the combined sewer system of Nantes city centre. The Pin Sec watershed comprised a surface area of 31 ha and encompassed 2500 residents. The type of housing was primarily composed of single-family dwellings and multi-family units. Impervious surfaces accounted for 49 % of the area, mainly roofs, streets, pavements and parking lots. Roof surfaces represented 18 % of the total watershed surface area. The mean watershed slope was approx. 1.1 %. The stormwater network had a total length of 4 km and the diameter pipe at the outlet is was 1200 mm. The total length of wastewater network was 7.3 km and the diameter at the outlet pipe was 600 mm. The Gohards watershed contained a total surface area of 174 ha; land use was mixed, with both residential and commercial zones. This watershed was located between thoroughfares carrying moderate traffic loads (9300 vehicles per day) and crossed by a highway with an average traffic load of 44,200 vehicles per day. The impervious surfaces, which represented 38 % of this watershed, consist mainly of: roofs, streets, pavements and parking lots. Roof surfaces accounted for 14 % of the total surface area, while streets and parking lots made up 24 % of the total. The total length of the stormwater network was 14.3 km and the diameter at the outlet pipe was 1600 mm. Although separate sewer systems were conceived to be selective, extraneous water inflow was observed. Extraneous water includes groundwater infiltrations and inappropriate connections (stormwater directly introduced to the wastewater network or wastewater collected by stormwater pipes). In the stormwater network of Pin Sec watershed, it was reported elsewhere that there was a strong relation between dry periods base flow and seasonal variation of the groundwater level. These authors report leaks as the main cause of infiltration. In the wastewater network, the inflow of stormwater has been observed in this study (Figure 7.5-152). These observations are in good agreement with other studies conducted in separate sewer systems and show that the networks are were not perfectly water tight. The behaviour of this separate wastewater network during wet periods is was similar to those observed in combined sewer systems.

Figure 7.5-152: Evolution of water volume in the wastewater sewer during wet weather



Sampling campaigns

So as to characterize quality and pollutant substances transported by stormwater, dry and wet weather conditions were studied in both stormwater and wastewater networks. The campaigns were carried out from September 2007 to October 2008 for stormwater, and from April 2007 to December 2008 for wastewater. During wet periods, sampling was flow dependent. The flow was monitored continuously and measured by

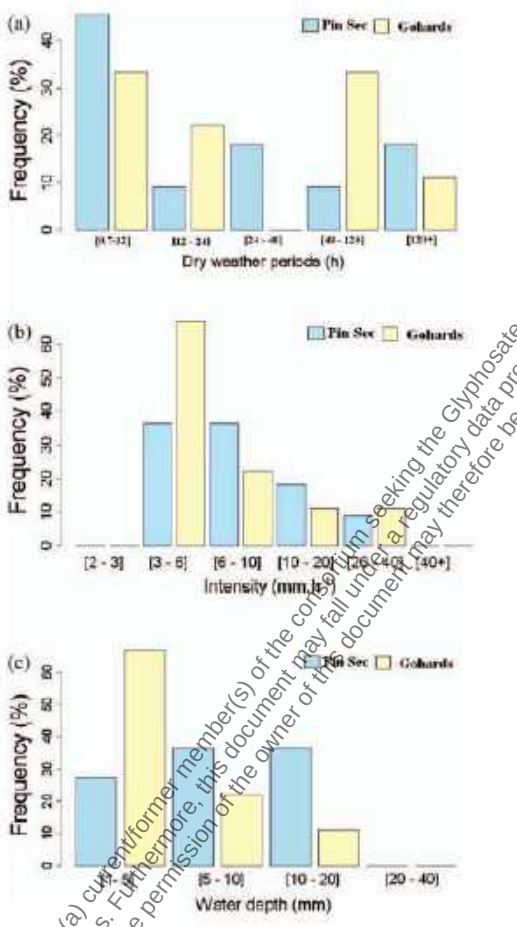
ISCO or SIGMA flowmeters associating water level and velocity sensors. The base flow was used as reference to start sampling. Samples were collected by automatic samplers and stored in polyethylene bottles of 1L capacity. In dry periods, samples collected in stormwater networks were performed by instantaneous samples taken manually. Samples of 4.5 L were collected and stored in glass or polyethylene bottles depending on the type of analysis. In waste-water sewer system, samples of 120 mL were collected each 10 minutes over 24 hour periods. 24 mean hourly samples were collected for each campaign. In the laboratory, a mean daily flow-proportional sample was then prepared.

Stormwater
In order to characterise dry weather conditions, six sampling campaigns were carried out in the Pin Sec watershed and four in Gohards. Recordings were collected for 11 rainfall events at the Pin Sec watershed outlet and for nine events at the Gohards outlet.

Wastewater
Eight dry weather campaigns were conducted at the Pin Sec wastewater network outlet. In order to determine the variation in pollutant concentration throughout the day, five of the eight days sampled were selected and analysed. In each case, 24 mean hourly samples were analysed for suspended solids (SS) and chemical oxygen demand (COD). Individual hourly time segments were also determined according to the variations in daily water flow, as well as in SS, VM (volatile matter) and COD concentrations. From these results, the day was divided into four time segments: 7 am–1 pm, 1 pm–7 pm, 7 pm–1 am, and 1 am–7 am. In addition, eight wet weather samples were collected, with sampling once again being flow-dependent.

Characteristics of rainfall events
Figure 7.5-153 presents the characteristics of these sampled rainfall events. In the Gohards watershed, 6 3 % of events displayed low intensity (3–6 mm/h), with 56 % of the events producing a rainfall depth ranging from 1 mm to 5 mm. Dry weather periods lasting less than 24 hours were observed 56 % of the time. For the Pin Sec watershed, rainfall event characteristics were more diverse, with 27 % of events producing a rainfall depth (H) lying between 1 mm and 5 mm, 36 % with a depth of 5 mm < H < 10 mm and 36 % with 10 mm < H < 20 mm. The maximum intensity (I_m) was moderate, i.e. 36 % of precipitation within the interval of 3 mm/h < I_m < 6 mm/h. For 54 % of events, the antecedent dry period (ADP) lasted less than 24 hours. The return period of these events, as well as the comparison of characteristics between sampled events and all events recorded in Nantes over the 2007–2008 period, shows that the sampled events were frequent and representative of Nantes rainfall in the Pin Sec and Gohards watersheds.

Figure 7.5-153: Characteristics of sampled rain events in the Pin Sec and Gohards watersheds. (a) Dry weather periods. (b) Intensity. (c) Water depth



Analyses

pH and conductivity were measured in situ and in the laboratory. Before analysis, samples were sieved through a 2 mm mesh and analysed to obtain the concentrations of suspended solids (SS) according to French and European NF EN 872 standards. Bulk parameters and trace metals were analysed 24 h after the campaigns. Polycyclic aromatic hydrocarbons (PAHs) and pesticides were analysed by IDAC and IANESCO-CHIMIE Laboratory, respectively. For these analyses, the samples were stored in glass bottles in the dark at 4 °C until analyses. pH, conductivity, suspended solids (SS) and total organic carbon (TOC) were all determined according to the French standards for water analysis. Chemical oxygen demand (COD), biological oxygen demand (BOD_5), volatile matter (VM), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were only measured in the wastewater samples, in accordance with French analytical standards. 15 of the 16 polycyclic aromatic hydrocarbons (PAHs) recommended by the Environmental Protection Agency (US EPA), i.e. naphthalene (Np), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (An), fluoranthene (Flu), pyrene (Py), benzo(a)anthracene (B[a]An), chrysene (Chry), benzo(b)fluoranthene (B[b]Fl), benzo(k)fluoranthene (B[k]Fl), benzo(a)pyrene (B(a)Py), indeno(1,2,3-c,d)pyrene (I[1,2,3-c,d]Py), dibenzo(a,h)anthracene (D(ah)An) and benzo(g,h,i)perylene (B[g,h,i]Pe), were analysed by the IDAC Laboratory as per the NF EN ISO 17993 protocol. The quantification limits for PAHs was 2.0 ng/L, with the exception of Np, Fl, Phe and An (10 ng/L). Pesticide analyses were performed by the IANES-CO-CHIMIE Laboratory in Poitiers. Glyphosate and aminomethyl phosphonic acid (AMPA) were evaluated using HPLC with a fluorimetric detection. Prior to analysis, the homogenised sample was derived with 9-fluorenyl methyl chloroformate (FMO-Cl) at pH 9. For diuron, 250 mL of the sample were

extracted (liquid/solid extraction). The extract was then analysed using HPLC coupled with a double mass spectrometer (GC/MS/MS). Quantification limits were 0.05 ug/L for glyphosate and AMPA, and 0.1 ug/L for diuron. Trace metals (Zn, Ni, Cd, Cr, Cu, Pb) were studied at the LCPC Environmental and Chemical Laboratory by means of atomic absorption spectrometry, according to Standard NF EN ISO 15586. The quantification limits used for this analysis were: 0.10 ug/L for Cd, 1.0 ug/L for Pb, 2.0 ug/L for Cu, 0.5 ug/L for Cr, 8.0 ug/L for Zn, and 1.0 ug/L for Ni.

Table 7.5-183: Median, maximum and minimum values of pH, conductivity (µs/cm), suspended solids (SS, mg/L), total organic carbon (TOC, mg/L), trace metals (µg/L), PAHs (µg/L) and pesticides (µg/L) in stormwater at the Pin Sec and Gohards watersheds (Nantes, France)

	Pin Sec		Gohards	
Parameter	Dry weather	Wet weather	Dry weather	Wet weather
pH	7.3 (6.6–7.7)	6.6 (6.4–7.0)	7.5 (7.3–8.0)	6.6 (6.5–7.2)
Conductivity	475 (212–606)	145 (92–218)	533 (395–596)	146 (98–250)
SS	13 (2–45)	69 (17–413)	6 (2–16)	75 (30–152)
TOC	3.8 (2.0–45)	9.8 (1.0–46)	2.8 (1.8–9.3)	5.6 (2.7–19)
Zn	41 (5.7–58)	146 (64–536)	52 (33–87)	209 (145–388)
Pb	9.0 (3.0–47)	21 (9.5–71)	4.3 (1.0–4.6)	14 (3.8–33)
Cu	7.5 (3.3–12)	31 (13–123)	4.6 (3.1–6.8)	24 (18–43)
Cr	4.6 (2.1–8.0)	7.5 (2.1–14)	4.9 (1.2–8.9)	6.3 (2.3–11)
Ni	6.4 (3.5–19)	5.0 (2.2–32)	7.9 (2.0–11)	6.2 (3.0–9.8)
Cd	0.1 (0.1–0.8)	0.7 (0.1–3.9)	0.2 (0.1–0.3)	0.3 (0.1–0.6)
Σ 15 PAHs	0.06 (0.01–0.14)	0.11 (0.04–0.27)	<Q.L (<Q.L- 0.05)	0.86 (0.09–4.71)
Glyphosate	0.23 (<0.10–0.29)	3.27 (1.06–71)	0.58 (<0.10–0.70)	2.15 (<0.10–3.84)
AMPA	0.46 (<0.10–0.46)	0.35 (0.16–1.45)	<0.10 (<0.10–0.45)	0.23 (<0.10–0.37)
Diuron	0.10 (0.10–0.16)	0.21 (0.10–0.78)	0.16 (<0.05–0.18)	0.10 (0.07–0.13)

Results and Discussion

Stormwater quality

Bulk parameters

The bulk parameters concentrations are listed in Table 7.5-58. Stormwater pH and conductivity values measured in situ and in the laboratory were similar. pH ranged from 6.4 to 7.2; these values lie close to those measured in the collector during dry weather periods. Conductivity values were similar in the Pin Sec and Gohards watersheds, ranging between 92 µs/cm and 250 µs/cm. These values were three to four times less than those recorded during dry weather periods, a finding that can be explained by lower ion concentrations in runoff water as well as by a dilution during rainfall events. Regarding SS, 90 % of the concentrations exceeded the maximum value of 35 mg/L set by the European directive on urban wastewater Directive 91/271/EEC. Median concentrations equal 69 mg/L at Pin Sec and 75 mg/L at Gohards; in both cases, these values were well above those measured during dry weather periods (Table 7.5-58). It needs to be pointed out that two values recorded for Pin Sec are exceptionally high (315 mg/L and 413 mg/L), most likely due to an accidental pollution incident that occurred in September and October 2008 related to the civil engineering works taking place upstream of the network. SS concentrations remained similar in both watersheds (Wilcoxon test with α = 0.05). On the other hand, total organic carbon (TOC) concentrations were twice as high as those measured during dry weather, with median concentrations of 9.8 mg/L for Pin Sec and 5.6 mg/L for Gohards. High concentrations of TOC in stormwater reflected urban runoff impact.

Table 7.5-184: Comparison of pollutant concentrations in stormwater - (suspended solids (SS) in mg/L, trace metals, PAHs and pesticides in µg/L). Concentrations used for this comparison were 10th and 90th percentiles for metals and PAHs, min and max values for pesticides

	This study				Surface water SEQ-eau			
Parameter	Pin Sec	Gohards	Ruban et al. (2005)	Rossi (1998)	Good	Fair	Poor	Decree 2001–1220*
SS	52–299	54–103	35–238	10–204	25	38	50	25
Zn	82–373	158–295	99–262	52–541	2.3–14	23–140	52–330	3 000–5 000
Pb	11–58	6.6–31	5.5–28	19–170	2.1–10	21–30	50	25
Cu	17–75	19–42	8.5–35	41–197	0.17–2.7	1.7–27	2.5–40	2000
Cr	2.7–11	3.6–9.9	2.0–8.5	2.0–68	0.4–3.6	3.6–36	50	50
Ni	3.2–11	3.9–8.5	3.0–17	–	2.5–12	20–26	40	20
Cd	0.5–1.5	0.2–0.6	0.1–0.6	0.5–3.8	0.01–0.09	0.1–0.85	0.37–3.0	5
Σ 15 PAHs	0.06–0.13	0.66–1.06	0.09–0.8	0.3–9.2	–	–	–	0.1**
Glyphosate	1.1–71	<0.10–3.8	0.2–3.0	–	0.4	–	2	0.1
AMPA	0.2–1.5	<0.1–0.4	0.1–0.8	–	–	–	–	0.1
Diuron	0.1–0.7	0.1–0.13	0.5–3.0	–	0.2	–	2	0.1

Note: *Decree of 20 December 2001 on surface water used for drinking water production. **Value correspond to addition of B[b]Fl, B[k]Fl, I[1,2,3-cd]Py and B[g,h,i]Pe concentrations.

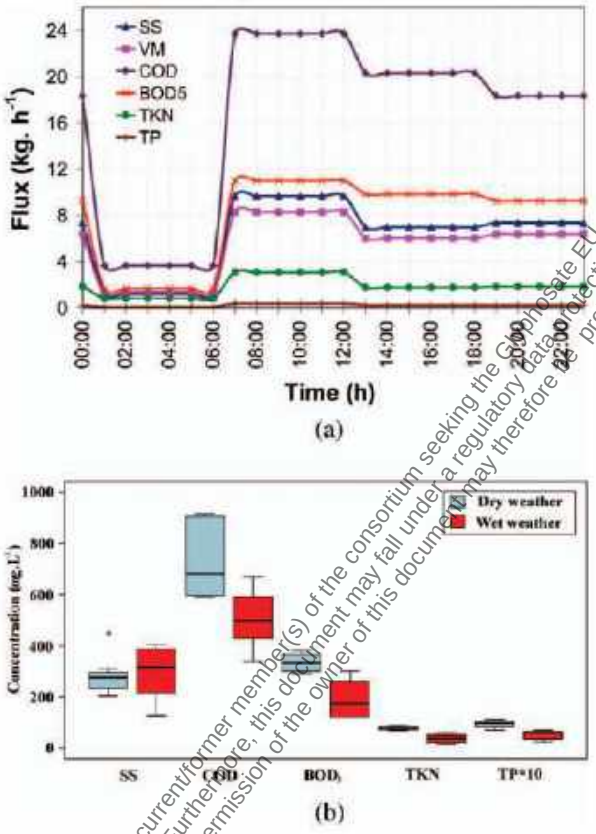
Pesticides

During wet weather periods, diuron and AMPA concentrations in the Gohards watershed wereclose to the quantification limits and similar to dry weather measurements. For glyphosate, wet weather concentrations were three times higher than the dry weather values (Table 7.5-58). In Pin Sec, most wet weather diuron and AMPA concentrations were similar to the dry weather values, with a maximum of 0.73 µg/L and 1.45 µg/L for diuron and AMPA, respectively. As regards glyphosate, stormwater concentrations were always higher than the dry weather values, with a median concentration of 3.27 µg/L and a maximum of 71 µg/L. The occurrence of these pesticides in stormwater can be explained by their application for cleaning unwanted grass and weeds from impervious surfaces and open spaces. The use of glyphosate has been reported by the Nantes municipality; this herbicide was also being widely used by homeowners.

Table 7.5-185: Median, minimum and maximum values of pollutant masses generated per active surface in the Pin Sec and Gohards watersheds - suspended solids (SS) in mg/m², trace metals, PAHs and pesticides (Σ glyphosate + AMPA + diuron) in µg/m²

Parameter	Pin Sec	Gohards	Parameter	Pin Sec	Gohards
SS	246 (36–2359)	153 (62–702)	Cr	16 (2.3–73)	14 (4.3–43)
TOC	25 (22–146)	13 (4.8–141)	Ni	16 (2.5–83)	10 (5.6–63)
Zn	481 (92–2850)	403 (191–1862)	Cd	2.4 (0.2–6.6)	1.0 (0.2–2.4)
Pb	70 (7.4–530)	26 (11–140)	Σ 15 PAHs	0.1 (0.05–2.0)	1.7 (0.6–5.6)
Cu	96 (13–594)	62 (28–278)	Pesticides	11 (1.7–440)	3.1 (0.2–4.8)

Figure 7.5-154: **Daily flux variations (a) and mean daily concentrations (b) for global parameters measured at the outlet of Pin Sec wastewater network. (Suspended solids (SS), volatile matter (VM), chemical oxygen demand (COD), biological oxygen demand (BOD5), total kjeldahl nitrogen (TKN) and total phosphorus (TP))**



Influence of rainfall characteristics

The influence of rainfall characteristics on pollutant concentrations in stormwater was studied by introducing Pearson correlation coefficients. The targeted variables were: SS, Cd, Cr, Cu, Ni, Pb, Zn, the sum of PAH concentrations, rainfall depth (H), total antecedent dry period (ADP), and maximum 5 min intensity of rainfall (Imax - 5 min). The Pearson coefficients however did not display any significant linear correlation between rainfall characteristics and pollutant concentrations.

Stormwater quality and comparison with other studies

The stormwater concentrations measured in the Pin Sec and Gohards watersheds were also compared to both the SEQ-Eau regulatory values (i.e., the French standard for surface water quality) and Decree 2001–1220 (2001) relative to the quality of surface water used for drinking water production. Concentration values used for this comparison were 10th and 90th percentiles for metals and PAHs, min and max values for pesticides (Table 7.5-184). In the two watersheds, nickel concentrations are in the range of 'good quality' water, as defined by SEQ-Eau. Chromium and PAH concentrations lie in the 'fair quality' category, while cadmium, copper and zinc concentrations vary from 'fair' to 'very poor' quality. Cadmium, chromium, copper, nickel and zinc concentrations are all below the reference values set for drinking water production (Table 7.5-184). In contrast, lead, pesticide and PAH (B[b]Fl, B[k]Fl, I[1,2,3-c,d]Py and B[g,h,i]Pe) concentrations often exceed the corresponding threshold values. Such is the case for Pb in 36 % of the samples; also, 50 % (Pin Sec) and 83 % (Gohards) of PAH concentrations surpass the maximum value of 0.1 µg/L. Pesticide concentrations also lie above the threshold; it should be noted that such is the case for dry weather concentrations as well. Based on these results, it would appear that stormwater quality

in the studied watersheds is poor. Our results have been compared to those of analyses carried out in residential areas equipped with separate sewer systems (Table 7.5-184). Furthermore, most references relative to stormwater systems are old; we then choose to present the most recent and relevant references. This comparison is not straightforward since many factors vary from one study to the other (site, meteorological conditions, sampling techniques, analysis, etc.). With results from the St. Joseph watershed located north-east of the two monitored herein and the Swiss study, the 10th and 90th percentile values were also used.

- SS, Cd, Cr and Ni concentrations are similar in the watersheds studied;
- Cu, Pb and Zn concentrations are higher in the Pin Sec and Gohards watersheds, likely as a result of higher traffic density;
- PAH concentrations in Pin Sec and St. Joseph are similar, while at Gohards they prove to be higher, again due to traffic density;
- Diuron concentrations are 4–23 times lower than those measured at St. Joseph, a finding that can be explained by a reduction in the use of this herbicide. Glyphosate and AMPA concentrations are comparable in Gohards and St. Joseph, whereas glyphosate is much more heavily con-centrated in Pin Sec than in St. Joseph. Glyphosate is widely used as a herbicide in the Pin Sec watershed area, which underscores this difference.

With regard to the Swiss study, SS, heavy metals and PAHs concentrations are similar to those measured at Pin Sec and Gohards watersheds.

Pollutant fluxes

For each watershed, the mass of pollutants released via active surfaces for each rainfall event along with the corresponding fluxes were examined. The objective of this estimation was to compare, for a given rainfall event, the pollution generated in each watershed and then derive an annual estimation of the pollutant flux likely to be discharged into the Gohards River, which is the watercourse that receives effluent from both the Pin Sec and Gohards watersheds. The following equation was used to calculate the pollutant mass generated during rainfall events:

$$M_{ac} = \frac{C \; x \; V}{S_{ac}}$$

with:

M_{ac} = mass per active surface (in mg/m^2 or µg/m^2)

C = concentration measured for each sampling campaign (mg/L or µm/L)

V = total water flow in the collector (L)

S_{ac} = active surface area of the watershed (m^2).

As mentioned above, no correlation was observed between metal concentrations and either rainfall depth, max I_{5min} or ADP. Each rainfall event selected in 2009 was thus multiplied by the experimental runoff coefficient determined for Pin Sec (0.25) and Gohards (0.29) and by one of the concentration values obtained during the sampling period and then chosen at random. The sum of all masses corresponds to the mean annual flux; this operation was repeated 1000 times in order to yield the mean annual flux and its confidence interval (the ‘‘bootstrap method’’). For organic micro-pollutants, this estimation proved impossible due to the correlations observed between PAHs concentrations and ADP, as well as to the seasonal occurrence of pesticides. Only the per-event masses were therefore calculated for PAHs and pesticides.

Masses generated for a rainfall event:

Table 7.5-185 gives the pollutant masses generated at the rainfall event scale for all events collected on the two studied watersheds. Metal, SS and TOC concentrations are similar in both watersheds, although total precipitation is higher in Pin Sec; consequently, pollutant masses are greater in this watershed. When the common rainfall events are considered, however, the masses observed at Gohards turn out to be higher, which can be explained by the more intensive commercial traffic activity and the prevalence of zinc roofs found in this watershed. The PAH mass generated at Gohards is greater, as already observed for concentrations and explained by the heavier vehicle traffic loads. Due to the higher pesticide concentrations

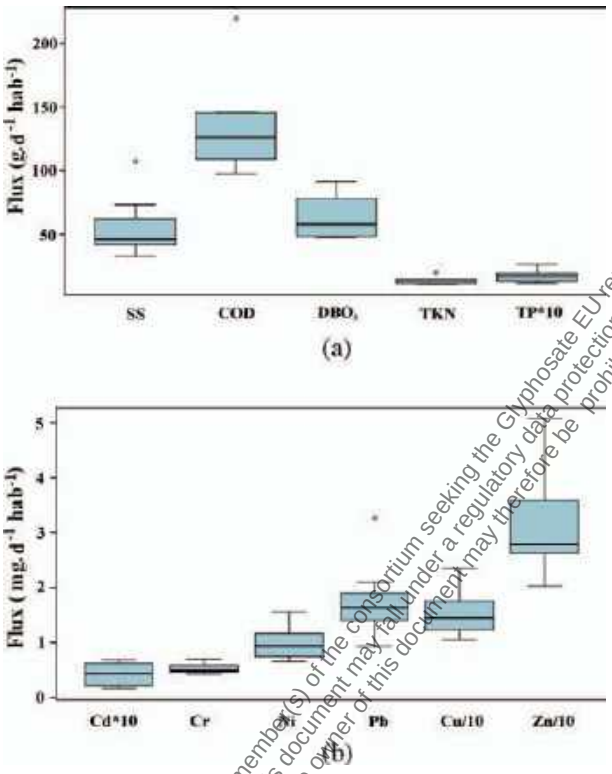
measured in Pin Sec, the mass measurements there are 3.5 to 100 times greater than those in Gohards.

Wastewater quality

Global parameters

In the Pin Sec watershed, dry weather pH values range from 7.3 to 8 (median: 7.6) and conductivity is between 960 µs/cm and 1150 µs/cm (median: 1096 µs/cm). During wet weather periods, median pH values drop slightly to 7.1; conductivity is also lower, with a median of 589 µs/cm. This difference can be explained by a dilution of effluents during rainfall events. The ion concentration of stormwater is indeed less than that of wastewater. Daily variations in SS, VM, COD, BOD_5, TKN and TP are similar to the flow variations. Figure 7.5-154a shows daily flux variations. Minimum values are observed during the early morning hours (1 am to 7 pm), whereas maximum values appear between 7 am and 1 pm, which corresponds with a daily time segment of greater human activity. The 1 pm-to-7 pm and 7 pm-to-1 am concentrations remain roughly the same. Similar observations have been reported in other studies. From these values, mean daily concentrations were estimated. Concentrations are presented in Figure 7.5-154b. The median values of mean daily SS, VM, COD, BOD_5, TKN and TP concentrations are 275, 241, 681, 333, 78 and 9.6 mg/L, respectively. The high concentrations of COD, BOD_5 and TKN attest to the rich organic matter content of these effluents, which may be explained by the upstream location of the sampling station; at this site, the degradation in organic matter is negligible, as demonstrated by the presence of toilet paper, faeces and food residue. It should be noted that the biodegradability of effluents evaluated as COD/BOD_5 displays a median of 2.04. Median SS, COD and TKN concentrations are similar to those reported for the St. Joseph watershed (220 mg/L (SS), 518 mg/L (COD) and 72 mg/L (TKN)). SS concentrations are also similar to measurement results down-stream of the Nantes combined sewer system during dry weather periods (200–400 mg/L). These values exceed those cited in other studies also conducted in dry periods but in combined sewer systems (100–243 mg/L for SS, 231–535 mg/L for COD and 31–73 mg/L for TKN). As previously mentioned for the high organic matter content, this finding could be explained by the upstream location of the Pin Sec outlet. Except for COD, the variability of wet weather concentrations is greater than that observed for dry weather values (Figure 7.5-154b). Moreover, in all cases, the wet weather COD, BOD_5, TKN and TP concentrations are lower than the dry weather recordings, with median values of 500/ 681 mg/L for COD and 37/ 78 mg/L for TKN. Effluent dilution during wet weather periods offers a possible reason for this difference.

Figure 7.5-155: Daily fluxes per inhabitant in global parameters (a) and heavy metals (b) estimated for Pin Sec watershed wastewater



Organic micropollutants

Glyphosate was not detected in any of the dry weather samples, and AMPA could not be analysed due to interference. The presence of AMPA in wastewater has been reported in the literature as a result of degradation of phosphonic acids present in detergents such as EDTMP (Ethylene Diamine Tetra Methylene Phosphonic acid) and DTPMP (Diethylene Triamine Penta Methylene Phosphonic acid). Glyphosate and AMPA were detected in both spring and summer wet weather samples; concentrations varied between 0.3–49 µg/L, with a maximum observed in June 2008. The presence of glyphosate in wastewater probably indicates storm-water infiltration into the collector, which corroborates our results on stormwater effluent, given that a concentration of 71 µg/L was measured over the same period. These observations are in agreement with other works conducted in separate sewer networks.

Influence of meteorological conditions

The influence of meteorological conditions on SS, metal and PAHs concentrations was studied through the use of Pearson coefficients. As was the case for stormwater, no significant linear correlation could be observed.

Pollutant fluxes

Under dry weather conditions, the daily pollutant mass generated per inhabitant is considered to be the flux. Such fluxes are shown for global parameters and trace metals in Figure 7.5-155a and b. As previously highlighted for pollutant concentrations, dry and wet weather pollutant fluxes are highly variable, especially during wet weather periods. The median dry weather fluxes (in g/inhabitant/day) are: 46 (SS), 127 (COD), 58 (BOD_5), 14 (TKN), and 1.8 (TP). During wet weather, the median global parameter masses (in g/m^2) are: 0.36 (SS), 0.57 (COD), 0.21 (BOD_5), 0.05 (TKN), and 0.01 (TP).

Comparison between stormwater and wastewater

A detailed study of dry and wet weather concentrations and fluxes within the stormwater and wastewater

of both the Pin Sec and Gohards watersheds yields the following observations (see Table 7.5-186); Median stormwater concentrations for glyphosate varied from 0.2 µg/L in dry weather, to 3.3 µg/L in wet weather (<0.1 and 0.4 µg/L for AMPA). In wastewater, glyphosate was not detected during dry weather (and AMPA could not be determined because of interference), while during wet weather median glyphosate concentrations reached 49 µg/L and AMPA 2 µg/L:

- The median glyphosate concentration in stormwater and wastewater was higher during wet weather periods.;
- Higher glyphosate concentrations during wet weather (in both stormwater and wastewater) can be attributed to the washout of impervious surfaces; this situation also enhances stormwater infiltration into wastewater pipes;

Table 7.5-186: Comparison of pollutant median concentrations obtained in stormwater (Pin Sec and Gohards watersheds) and wastewater (Pin Sec watershed). Suspended solids (SS) in mg/L, trace metals, PAHs and pesticides in µg/L

Parameter	Dry weather			Wet weather		
	Stormwater		Wastewater	Stormwater		Wastewater
Wastewater	Pin Sec	Gohards	Pin Sec	Pin Sec	Gohards	Pin Sec
SS	13	6	275	69	75	317
Zn	41	52	150	146	209	280
Pb	9.0	4.3	7.9	21	14	14
Cu	7.5	4.6	79	31	24	72
Cr	4.6	4.9	3	7.5	6.3	2.3
Ni	6.4	7.9	5	5.0	6.2	4.8
Cd	0.1	0.2	0.3	0.7	0.3	0.4
Σ 15 PAHs	0.06	<QL	0.2	0.1	0.9	0.3
Glyphosate	0.2	0.6	–	3.3	2.2	0.3 – 49
AMPA	0.5	<0.1	–	0.4	0.2	0.3–2.0
Diuron	0.1	0.2	–	0.2	0.1	–

Conclusion

The study of the quality of effluents transported by separate stormwater and wastewater networks in the Pin Sec and Gohards watersheds reveals that during wet weather periods the concentrations of suspended solids, organic matter, metals, PAH and pesticides are higher than those measured in dry weather. These results are in agreement with the literature and reflect the impact of urban runoff on stormwater and wastewater quality. Most of the time during wet weather, high variations of pollutant concentrations and fluxes are observed in stormwater and wastewater samples. This variability cannot be explained by any of the rain characteristics taken into account in this study. The use of pesticides in these watersheds (homeowners and municipality) appear to be the main sources of those organic pollutants during wet weather periods. High concentrations of glyphosate are still detected in stormwater and wastewater samples despite the reduction in the use of pesticides by Nantes metropolitan authorities. Our results demonstrate that pollutant transport via separate sewer system effluent is far from being negligible, therefore effluent from both the Pin Sec and Gohards watersheds discharged directly to Gohards River can contribute to the deterioration of this river.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a monitoring campaign in an urban area in the region of Nantes / France. Among others, glyphosate is measured. However, agricultural land use does not contribute significantly to the measured concentrations as the study area is described as an urban area. Median stormwater concentrations for glyphosate varied from 0.2 µg/L in dry weather, to 3.3 µg/L in wet weather (<0.1 and 0.4 µg/L for AMPA). In wastewater, glyphosate was not detected during dry weather (and AMPA could not be determined because of interference), while during wet weather median glyphosate concentrations reached 49 µg/L and AMPA 2 µg/L. Hence, urban use of glyphosate can generate significant residues in both stormwater and wastewater. The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/063
Report author	Litz, N.T. *et al.*
Report year	2011
Report title	Comparative studies on the retardation and reduction of glyphosate during subsurface passage
Document No	Water research (2011), Vol. 45, No. 10, pp. 3047-54
Guidelines followed in study	None (for filter experiments)
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (German UBA, German KompetenzZentrum Wasser)
Acceptability/Reliability:	Reliable

2. Full summary

The herbicide Glyphosate was detected in River Havel (Berlin, Germany) in concentrations between 0.1 and 2 µg/L (single maximum outlier: 5 µg/L). As the river indirectly acts as drinking water source for the city's 3.4 million inhabitants' potential risks for drinking water production needed to be assessed. For this reason laboratory (sorption and degradation studies) and technical scale investigations (bank filtration and slow sand filter experiments) were carried out. Batch adsorption experiments with glyphosate yielded a low K_F of 1.89 (1/n = 0.48) for concentrations between 0.1 and 100 mg/L. Degradation experiments at 8°C with oxygen limitation resulted in a decrease of glyphosate concentrations in the liquid phase probably due to slow adsorption (half life: 30 days). During technical scale slow sand filter (SSF) experiments glyphosate attenuation was 70-80 % for constant inlet concentrations of 0.7, 3.5 and 11.6 µg/L, respectively. Relevant retardation of glyphosate breakthrough was observed despite the low adsorption potential of the sandy filter substrate and the relatively high flow velocity. The VisualCXTFit model was applied with data from typical Berlin bank filtration sites to extrapolate the results to a realistic field setting and yielded sufficient attenuation within a few days of travel time. Experiments on an SSF planted with *Phragmites australis* and an unplanted SSF with mainly vertical flow conditions to which glyphosate was continuously dosed showed that in the planted SSF glyphosate retardation exceeds 54 % compared to 14 % retardation in the unplanted SSF. The results show that saturated subsurface passage has the potential to efficiently attenuate glyphosate,

favourably with aerobic conditions, long travel times and the presence of planted riparian boundary buffer strips.

Materials and methods

In all experimental settings – laboratory batch, enclosure and SSF tests- the same filter material was used. The texture of the applied sandy substrate can be characterized as follows: on average 2 % fine sand (0.1-0.2 mm), 43 % medium sand (>0.2-0.5 mm), 49 % coarse sand (>0.5-2.0 mm) and 6 % fine gravel (>2 mm), no clay or silt with only traces of organic matter and an effective porosity of 0.38-0.4 % (Table 7.5-187). The pH value of the percolated water was ~7.7. Solid glyphosate produced by Sigma-Aldrich with a purity degree of 98.7 %, dissolved in a 0.01 M $CaCl_2$-solution, was used for the experiments. Glyphosate concentrations were analyzed according to the German Standard DIN 38407-22 (2001). The quantitative determination of AMPA and glyphosate was done using a Waters HPLC system with a fluorescence detector and two Knauer 64 as reagent pumps. The analytical column for glyphosate was a Supelco SAX column (25 x 4 mm), for the quantification of AMPA a cation exchange column (Pickering) was applied (15 x 4 mm), because in field samples the AMPA peak was interfered by matrix peaks. The run conditions were: 0.4 mL/min, isocratic, phosphate buffer pH 2.05 ± 0.1 at 50°C. Retention time for glyphosate was 13.6 min on the anion exchange column and for AMPA 13.9 min on the cation exchange column. The detection limits were 0.02 µg/L and 0.005 µg/L, the quantification limit 0.07 µg/L and 0.02 µg/L for glyphosate, for AMPA, respectively. The two analytes AMPA and glyphosate were detected after a 2-step post-column derivatization. The first step was an oxidation with a phosphate buffer containing sodium hypochlorite (0.4 mL/min) in a 10 m reaction coil of PEEK tubing (i. d. 0.25 mm, volume 500 µL) at 50°C, the second a transformation into fluorescing compounds by reaction with phthaldialdehyde and 2-mercaptoethanol in an alkaline borate buffer (0.3 mL/min) in a 2 m reaction coil of PEEK tubing (i.d. 0.25 mm, volume 100 µL) at ambient temperature. The excitation wavelength of the resulting compounds was 390 nm and the emission wavelength 450 nm. All solutions were degassed and filtered through 0.45 µm prior to use. Samples of the filter substrate were extracted according to methodology reported elsewhere: 10 g of the sample were brought into contact for 30 min with 25 mL of 1 M NaOH. Subsequently the mixture was centrifuged for 15 min at 3000 rpm. The supernatant was abstracted with a pipette and the extraction was repeated. 4.2 mL concentrated HCl was added to the combined supernatants. After dilution of the sample with deionized water to a volume of 200 mL the analytes glyphosate and AMPA were determined as described above. The cleanup of the water samples was also performed according to the abovementioned German standard method DIN 38407-22. Water samples obtained from laboratory-, and enclosure experiments (typically 100-500 mL) were filtrated through glass fiber filters and adjusted with hydrochloric acid to pH 2 ± 0.1. The filtrate was applied to a column filled with a cation exchange resin which had been loaded with Fe^{3+} ions. Subsequently the column was rinsed with 20 mL water and 40 mL 0.02 M HCl. The analyte-iron complex was eluted with 10 mL 6 M HCl and 4 mL 32 % HCl were added to the eluate. This solution was applied to an anion exchange column. By elution of the column with 6 M HCl the iron was retained on the column.

Table 7.5-187: Characterisation of the enclosure filling material

Characteristics	Clogging layer	Filter substrate	Drainage stratum
Soil type	n.a.	mS, gS, fg	fG, mg
Thickness [m]	0.05[b]	1	0.25
CU[a]/CG[a]	n.a.	3.2/0.7	2.0/1.0
Fe(ox) [mg/kg]	605	275	n.a.
Mn(ox) [mg/kg]	68	11	n.a.
$C_{org}/C_{an.org}$ [%]	0.343/1.4	0.022/0.12	n.a.

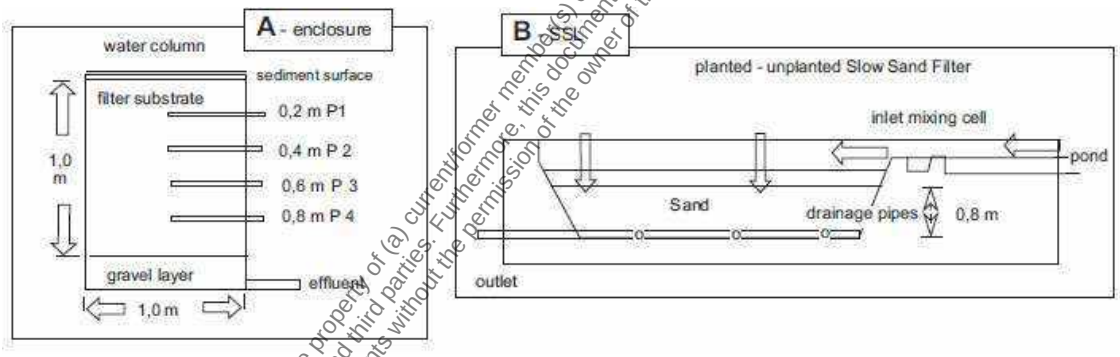
a Parameters for classification of non-structured sediments (uniformity coefficient, coefficient of gradation).
b Clogging layer is situated in the upper layer of the filter substrate, n.a. = not analyzed.

Laboratory experiments

Batch experiments
The batch experiments were conducted according to OECD 106 using the filter substrate and deionized water with glyphosate concentrations of 0.1 mg/L, 1 mg/L, 10 mg/L and 100 mg/L and a soil/water-ratio of 1:2, shaking the mixture for 4 h to establish an equilibrium. The chosen concentrations were applied in three parallels. After centrifugation the supernatant was carefully extracted and prepared for measurement. The Freundlich adsorption isothermal model was used to describe the nonlinear water/sediment distribution relations (K_F) over the total concentration range. The equation's first differentiation was used to describe also the linear distribution coefficient (K_D) and to estimate retardation factors (R_F).

Degradation experiment
Degradation studies were carried out by taking a defined sediment sample of 450 g wet material and mixing it with 10 mg glyphosate per kg filter substrate. The vessels were stored in the dark at a temperature of around 8°C for a period of up to 73 days to allow for biological degradation processes to take place. The airtight stoppers of the vessels sealed the sample from the atmosphere. During the experiment the vessels were left undisturbed. The redox potential, oxygen content, pH value and the temperature in the supernatant were determined after the respective vessels were opened and sampled. At intervals (7, 14, 21, 28 and 73 days) two experiment vessels were opened at a time. This experimental arrangement was intended to simulate naturally deposited filter substrate under partly reducing conditions, as it would be expected in slowly flowing groundwater.

Figure 7.5-156: Schematic cross section and location of sampling ports in enclosures (A) and slow sand filter - infiltration site with inlet and outlet device (B)



Technical scale experiments

Enclosure experiments
Water production pre-treatment via bank filtration or/and slow sand filtration is commonly used if drinking water is produced from surface water. In enclosure experiments the attenuation of compounds can be determined simulating conditions that occur during slow sand filtration or within the first meter of infiltration. The enclosures are three metal cylinders with an area of 1 m^2 and a height of 1.85 m (filtration length 1.00 m) (see Figure 7.5-156A). They are situated within an infiltration pond (area: 90 m^2) in order to be exposed to natural environmental conditions. Three different concentration levels of glyphosate were continuously dosed to the supernatant of the enclosures over a time period of 14 d from 20 October to 6 November 2007, yielding average inlet concentrations of 0.7, 3.5 and 11.6 µg/L. Water samples for glyphosate and AMPA analysis were taken for 34 days from the supernatant, from sampling points within the filter material and from the filter effluent. The flow rate was set at 50 cm/d and was controlled by adjustable pumps connected to the enclosure outlets. The depth of the supernatant was kept constant by siphoning the water out of the infiltration pond into the enclosure without additional pumping. The water in the infiltration pond originates from a large storage pond (volume of 7000 m^3) with relatively high mineralization (average electrical conductivity: 1000 µS/m) but low nutrient status (nitrate < 1 mg/L,

orthophosphate <1 mg/L, DOC 3-4 mg/L) thus representing oligotrophic surface water.

Slow and filter (SSF) experiments
The SSF experiments were conducted at two vertical-flow experimental SSFs: (Figure 7.5-156B) one without vegetation cover (average area 60 m^2, filter depth 0.8 m, filter volume 48 m^3) and the other with a 3 year old vegetation cover of *Phragmites australis* (average area 68 m^2, filter depth 1.2 m, filter volume 81.6 m^3) to simulate processes in grown planted bank filtration sites along rivers or surface water lakes. Due to the arrangement of inflow, water reservoir and drainage pipes, water flow through the SSFs was assumed to be predominantly vertical simulating conditions that occur during the first meter of bank filtration. The water fluxes of the unplanted and the planted SSF were regulated at the outlet and were regularly controlled by discharge measurements. Their yield amounted in average to approximately 0.41 and 0.45 m^3/h, respectively (corresponding to a filtration velocity of 0.16 and 0.18 m/d). Physico-chemical parameters of the water (pH, redox potential, and temperature) as well as DOC, PO_4^{3-} and NO_3^- concentrations were also monitored to gain insights into controlling processes. After an equilibration phase of 1 month during which nitrate and phosphate were dosed to target 10 mg/L N and 1 mg/L PO_4^{3-} in the supernatant, glyphosate was additionally applied for 22 days with a target concentration of 20 µg/L.

Results and Discussion

Batch experiments
Glyphosate exhibits under different site conditions a complex adsorption behavior in the environment which is influenced by pH and by variation of soil constituents and the chemical glyphosate species. In order to determine the distribution coefficient of glyphosate, degree of adsorption in the filter substrate batch experiments were conducted. The resulting linear regression with a Freundlich sorption coefficient (K_F) of 1.90 and a Freundlich exponential of 0.48 confirms the poor adsorptive characteristics of the sandy material and indicates beginning saturation at higher concentrations (Table 7.5-188). With sorption data from different concentration ranges a calculation of the adsorption coefficients (K_D-value) was carried out for different concentration ranges. Due to lower adsorption at high concentrations the K_D-values decrease by 3 orders of magnitude when regarding the complete range of concentrations from 0.1 to 100 mg/L. This is in agreement with comparable experiments of with sandy material reported elsewhere, which is comparable to the one used in this study, where a K_D-range of 1.5-2.9 L/kg was determined. Compared to other studies on glyphosate adsorption with soils showing K_D values that range from 62 to 410 L/kg these values are quite low. This is most probably due to the low content of clay, iron and aluminum oxide or organic matter content in the filter material. Only some iron and organic matter content may have influenced the sorption in the filter material and should be responsible for slightly elevated adsorption coefficients (5.4 L/kg) at least with low glyphosate concentrations (0.1-1 mg/L).

Table 7.5-188: Estimated retardation of glyphosate in the filter substrate on the basis of Freundlich distribution equation

Concentration (c_{aq}) [mg/L]	Gradient from first differentiation (G)[a] [L/kg]	Retardation factor (R_f)[b]
100	0.08	1.4
10	0.28	2.2
1	0.9	5
0.1	3	14
0.02	7	31

a $G = 1/n \times K_F \times (c_{aq})^{1/n-1}$ with K_F: 1.9 $mg^{1-1/n} \times L^{1/n} kg^{-1}$ and 1/n: 0.48.
b $R_f = 1 + (\rho_b/n_e) \times G$, with an effective porosity (n_e): 0.37 and bulk density (ρ_b): 1.59 kg/L.

Degradation experiment

It is well known that glyphosate degrades more easily under aerobic conditions compared to anaerobic conditions. Figure 7.5-157 shows the residual glyphosate concentrations, obtained from the analysis of the solvent samples in the batch degradation experiment under anaerobic conditions. As it is not clear, if the reduction of concentrations was due to degradation or adsorption, the term dissipation will be used in the following. The development of the redox potential and oxygen content during the degradation experiment showed that oxygen-free conditions were partially achieved. The oxygen in the supernatant was almost completely consumed (data not shown) whereas the pH value remained constant at around 7.7. Dissipation of 50 % (DT_{50}) of the glyphosate in the supernatant was calculated to be achieved after 30.5 days yielding a rate of dissipation of 0.0227/ d. A mass balance approach was carried out taking into account the initially applied amount of glyphosate, the concentrations measured in solution and the adsorbed fraction. During the first 30 days the decrease in dissolved concentration is due to a continuous adsorption in this time (data not shown). Degradation must therefore be initially negligible. Similar findings in anoxic substrate have been reported elsewhere. The results of laboratory degradation studies differed from the findings in the outside enclosure experiments, which were carried out under more aerobic and temperate conditions.

Enclosure experiments

By simulating slow sand filter conditions, enclosure experiments can help to verify the risk for groundwater pollution by contaminants entering from surface waters. Glyphosate and AMPA concentrations in enclosures II and III for the time of the experiment (34 days) are given in Figure 7.5-158 and Figure 7.5-159. Glyphosate was continuously dosed for 14 days to both enclosures reaching average concentrations of 3.5 and 11.6 µg/L, respectively, with a standard deviation of 20 %. The two concentrations reflect medium and maximum levels generally observed in surface water. In enclosure II the glyphosate concentrations at the outlet reached a maximum value of 0.7 µg/L towards the end of the experiment (after 34 days). Since the experiment was terminated before the concentrations decreased again the point in time for the peak value could only be estimated. A break-through curve was observed in enclosure III, to which the highest glyphosate concentration was applied. The maximum outlet concentration for glyphosate of 2.7 µg/L occurred after 23 days. After 8 days (enclosure III) and after 17 days (enclosure II) nearly all observed glyphosate concentrations exceeded the European limit for pesticides in drinking water of 0.1 µg/L. AMPA concentrations above 0.1 µg/L were observed since day 6 in enclosure III and since day 12 in enclosure II. An example vertical concentration profile is illustrated for enclosure III in Figure 7.5-160. This shows sthat retardation and degradation processes are distributed almost linearly along the filtration depth as this was also observed in experiments elsewhere. Tracer and glyphosate concentrations at the outlets of enclosures II and III were modeled using the computer program VisualCXTFit. On the basis of the hydrodynamic properties of the filter substrate obtained from the tracer experiment ($R^2 = 0.95$ and 0.93 for enclosures II and III, respectively (data not shown)), it was possible to assess the retardation and degradation capacity of the enclosures for glyphosate. The modeled results of the glyphosate concentrations in enclosures II and III corresponds well compared to the observed breakthrough curves. Based on the recovered concentrations at the outlet the applied glyphosate was reduced by 78-80 %. Modeling yielded a retardation factor of 25 and 18 and a degradation rate of 0.0069/d and 0.092/d in enclosures II and III, respectively. The half-lives derived from the modeled degradation rates, amounted to 10 d (enclosure II) and 7.5 d (enclosure III), respectively, and correspond well to the values mentioned in literature with 2-14 d for aerobic conditions. The slightly higher degradation in enclosure III could be related to the higher glyphosate concentrations in the liquid phase and a resulting better access of microorganisms to glyphosate. With the obtained parameters data it was attempted to predict the necessary depth of filter substrate to ensure an attenuation of glyphosate to values below the European threshold for drinking water starting from source water concentrations of 3.5 µg/L (enclosure II) and 11.6 µg/L (enclosure III). The modeled filtration length for a sufficient attenuation in enclosure II and III would be about 2.75 m and 3.75 m, respectively (Figure 7.5-161). Model calculations assuming conditions occurring at existing bank filtration well fields yielded in all cases no contamination risk for the water used in drinking water production. Similar findings have been published elsewhere.

Figure 7.5-157: Glyphosate partitioning between solid and aqueous phases during degradation batch experiments (points represent samples from 2 replicates for each sampling date)

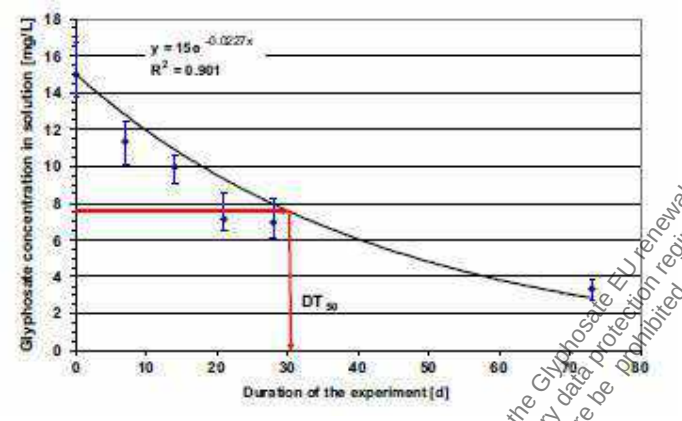


Figure 7.5-158: Glyphosate and AMPA concentrations in the outlet of enclosure II (with an average inlet glyphosate concentration of 3.5 µg/L)

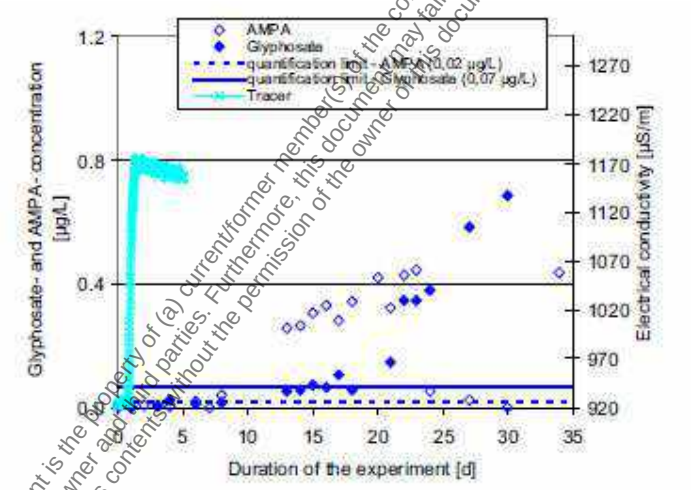


Figure 7.5-159: Glyphosate and AMPA concentrations in the outlet of enclosure III (with an average inlet glyphosate concentration of 11.6 µg/L)

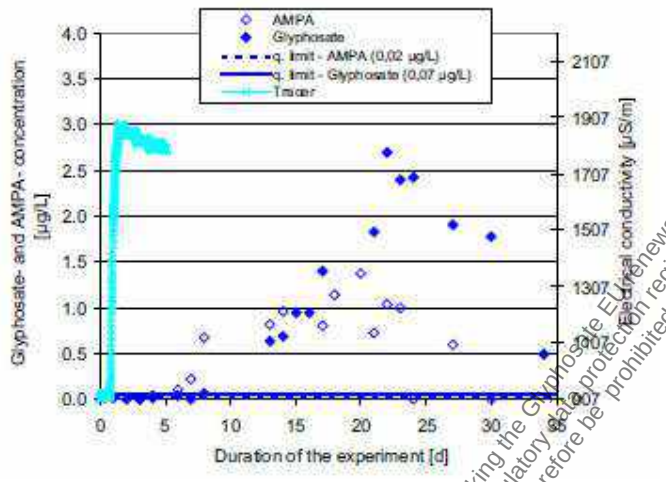
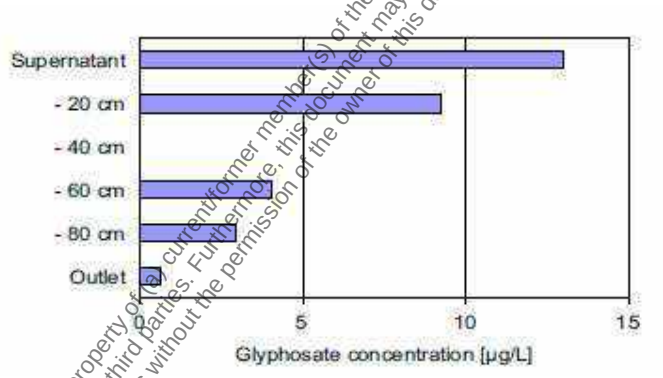


Figure 7.5-160: Vertical distribution of glyphosate concentrations in enclosure III on 05.11.2007 (16 days after dosing commenced)



Slow sand filter experiments

For simulating glyphosate attenuation in a riparian zone, studies with an adapted planted SSF and unplanted SSF were conducted. The hydro-chemical analyses (tracer tests, break-through curves of nitrate) indicated that the planted SSF does not show a homogeneous vertical flow pattern. Thus the planted SSF was divided into two zones (right and left) with different hydraulic and subsequently hydro-chemical characteristics and an estimation of the hydraulically effective surface area was carried out. These estimations showed a reduction in average surface area of the planted SSF to around 67 % of the unplanted SSF, confirming that the flux in the planted SSF seems to be partly inhibited. The lowering to around 67 % of the average surface area could be explained by collimation due to high production of biomass which at constant hydraulic head results in a decrease of pore velocities or even blocking of pore volume. The concentrations of glyphosate measured in the mixing cell, in the supernatant, in 40 cm depth and in the outlet of the planted SSF (left site) are given in Figure 7.5-162. In the mixing cell of the planted SSF the average glyphosate concentration of 21.2 µg/L was slightly higher than the targeted level of 20 µg/L. In the left zone of the planted SSF only little reduction was observed in the water reservoir above the SSF surface (19 µg/L in average). In 40 cm depth the maximum concentration of glyphosate was retarded by 11 days and reduced to approximately 7 µg/L (63 % of the average concentration in the supernatant). In the right zone (data not shown) the concentrations decreased by more than 50 % between mixing cell and surface water of the SSF. Glyphosate was completely removed from solution in 40 cm depth, which seems to be due to lower inlet concentrations,

higher residence times and therefore higher efficiency of reduction. In the combined outlet (left and right zone) the fluxes of all sampling sites rejoined and resulted in a maximum concentration of 1.4 µg/L. The final measurements at the end of the experiment showed a reduction of about 93 % of the applied glyphosate compared to the inlet concentration. While the planted SSF had to be divided into two zones the unplanted SSF can be regarded as homogenous (Figure 7.5-163). The inlet concentrations of the unplanted SSF did not reach the targeted level of 20 µg/L. In average it was lower and characterized by strong fluctuations probably due to degradation processes in the stock solution (17.6 µg/L in average). The concentration gradient between the level of glyphosate in the mixing cell corresponds well to the concentrations measured in the supernatant. In contrast to the planted SSF where an increase in 40 cm depth was found only after 10 days, low concentrations of glyphosate were observed here from the very beginning in the unplanted SSF. This is clearly a result of enhanced attenuation and could be interpreted as retardation by the biomass of the root zone. Maximum glyphosate concentrations decreased to 9 µg/L after 40 cm of the filter passage (49 % reduction of average supernatant concentration). The concentration in the outlet did not reach the climax of the breakthrough curve. The maximum concentration detected here was 4.5 µg/L. Comparing the concentrations in 40 cm depth and in the effluent of the unplanted SSF with those of the left zone as representative for the planted SSF there was slightly higher glyphosate reduction in the planted SSF (63 % in 40 cm depth, compared to 49 % in the unplanted filter), although the inlet concentrations were slightly higher and the residence time was lower. The higher reduction rate of glyphosate in the planted SSF could be due to the strong biological activity, which was concluded from the lower oxygen contents. The redox potential at 40 cm depth varied strongly in both SSFs and amounted to an average of -200 eV in the left zone as representative for the planted and +235 eV in the unplanted SSF. The decisive factor seems to be the availability of organic carbon, due to vegetal growth. The influence of *phragmites* buffer strips along surface water on glyphosate retardation has not been not studied by other experts before. Studies elsewhere on glyphosate attenuation during artificial recharge bank filtration have been carried out. Comparison of the results, demonstrated a high natural variability of subsurface mobility for glyphosate depending on site characteristics.

Figure 7.5-161: Modeled length of the filter substrate (from left to right: 1.25; 2.0; 3.0; 3.5 and 3.75 m) in order to ensure a reduction of the glyphosate concentrations below the European threshold for drinking water of 0.1 µg/L (enclosure III)

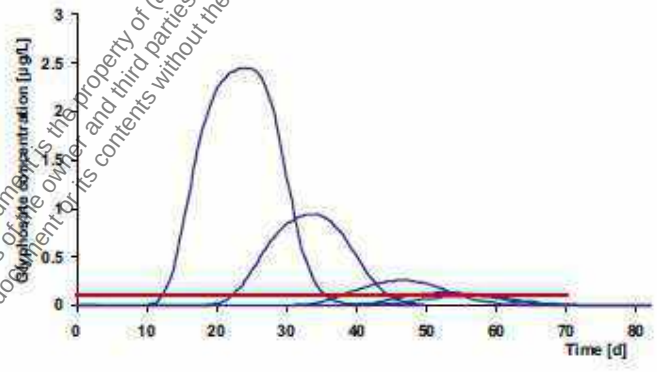


Figure 7.5-162: Glyphosate distribution in the left zone of the vegetated SSF

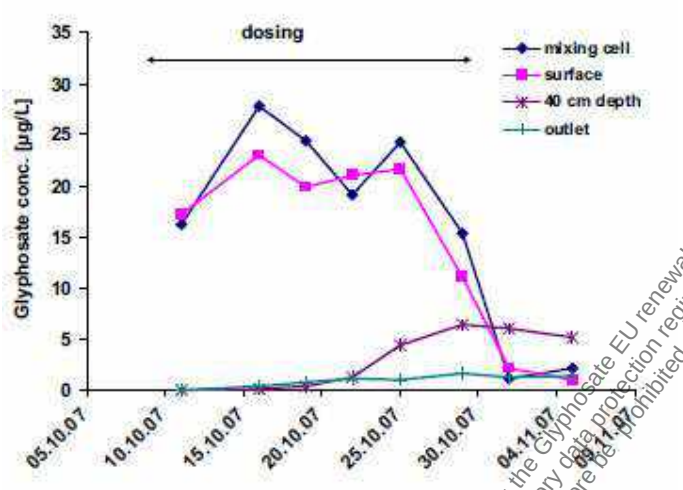
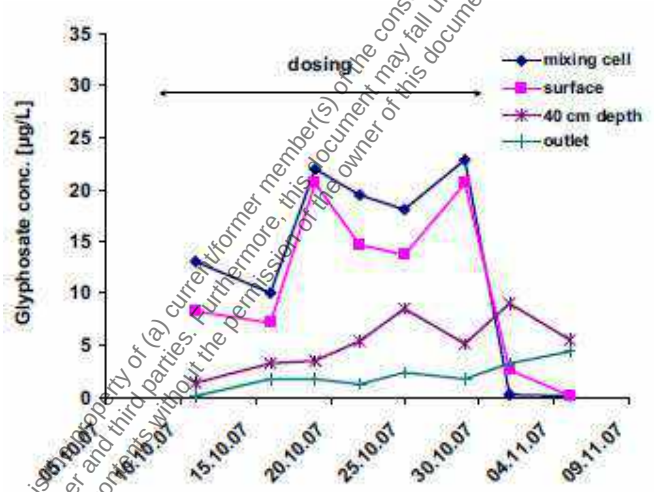


Figure 7.5-163: Glyphosate distribution in the unplanted SSF



Conclusion

Laboratory studies were conducted to characterize the substrate of the enclosures and the slow sand filters with regard to glyphosate removal processes. Batch adsorption studies yielded a very low adsorption capacity for glyphosate with a K_F of 1.9 in the sandy material. This is presumably due to the low organic matter content compared to studies carried out with soils, especially with those of a higher iron and aluminum oxide content. Anaerobic dissipation studies under laboratory conditions at 10 °C resulted in a half-life of 30.5 d with dissipation rate of 0.023/ d in the solvent phase. However, it could not be proven, that degradation is the main removal process for short subsurface passage as complete recovery was achieved from the solid phase after 30 d. In the further course of the experiment, however, significant degradation was observed. In the enclosure experiments a rapid degradation was observed due to the aerobic conditions and higher temperatures with a half-life of 7.5-10.5/ d, with lower initial concentrations (3.5-12 µg/L) compared to the lab experiments. The enclosure experiments showed that between 78 and 80 % of continuously applied glyphosate (3.5 µg/L or 11.6 µg/L in average) can be attenuated despite of low adsorption capacity of the filter substrate and high filtration velocity. The necessary length of the filter substrate in order to ensure a reduction of the glyphosate concentrations below the European threshold for

drinking water of 0.1 µg/L was modeled with VisualCXTfit and must exceed 2.75 or 3.75 m for an initial glyphosate concentration of 3.5 µg/L (enclosure II) or 11.6 µg/L (enclosure III), respectively. In the SSF experiments the SSF covered with *P. australis* showed a 2-5 times higher removal capacity (57 %) for glyphosate than the one without reed cover (14 %). Thus, the following conclusions can be drawn for the attenuation of glyphosate during subsurface passage: At low concentrations adsorption may play an important role, however, degradation needs to be considered as the main process for glyphosate attenuation. Favourable for glyphosate removal at bank filtration sites are oxic conditions, planted sediment surfaces and travel times of more than 10 days.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes experiments on subsurface passage of river water using so-called enclosures and semi-technical scale vertical slow sand filters (SSFs) to investigate the behavior of glyphosate and AMPA during bank filtration for drinking water supply. The filter experiments were supported by batch adsorption and degradation experiments with the filter material. Overall, the results showed that saturated subsurface passage has the potential to efficiently attenuate glyphosate, with aerobic conditions, long travel times and the presence of riparian boundary buffer strips. The main filter experiments and the analytical methods are well described and reported with sufficient details.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/064
Report author	Maillard, E. *et al*.
Report year	2011
Report title	Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment
Document No	The Science of the total environment (2011), Vol. 409, No. 11, pp. 2317-24
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Pasteur Institute of Lille (France))
Acceptability/Reliability:	Reliable

2. Full summary

Wetlands can collect contaminated runoff from agricultural catchments and retain dissolved and particle-laden pesticides. However, knowledge about the capacity and functioning of wetland systems with respect to the removal of pesticides is very limited. Here we show that stormwater wetlands can efficiently remove pesticides in runoff from vineyard catchments during the period of pesticide application, although flow and hydrochemical conditions of the wetland largely vary over time. During the entire agricultural season, the inflowing load of nine fungicides, six herbicides, one insecticide and four degradation products was 8.039 g whereas the outflowing load was 2.181 g. Removal rates of dissolved loads by the wetland ranged from

39 % (simazine) to 100 % (cymoxanil, gluphosinate, kresoxim methyl and terbuthylazine). Dimethomorph, diuron, glyphosate, metalaxyl and tetraconazole were more efficiently removed in spring than in summer. More than 88 % of the input mass of suspended solids was retained, underscoring the capability of the wetland to trap pesticide-laden particles via sedimentation. Only the insecticide flufenoxuron was frequently detected in the wetland sediments. Our results demonstrate that stormwater wetlands can efficiently remove pesticide mixtures in agricultural runoff during critical periods of pesticide application, although fluctuations in the runoff regime and hydrochemical characteristics can affect the removal rates of individual pesticides.

Materials and methods

Description of the vineyard catchment and stormwater wetland

The studied wetland is located at the outlet of a 42.7 ha vineyard catchment in Rouffach (Alsace, France; 47°57′9 N, 07°17′3 E). The characteristics of the catchment and agricultural practices have already been described (Gregoire et al., 2010). Application of pesticides typically takes place from mid-April (bud breaking of grapevine) until August (grapevine maturity). Nine fungicides, six herbicides, one insecticide and four degradation products were selected for the present study because of their widespread use as well as their high frequency of application and detection revealed in previous studies (Gregoire et al., 2010). The studied compounds belong to 12 different chemical groups and largely differ with respect to their physico-chemical properties. Rainfall–runoff events do not generate permanent streams in the catchment and statistically occur every week through the year. Runoff converges at the outlet of the catchment and is collected by the stormwater wetland, which is sized for a 100-year return period of rainfall.

The stormwater wetland has a surface area of 319 m^2 and a total volume of 1500 m^3 and was initially constructed to control flooding in the downstream urban area (Figure 7.5-164). The wetland is composed of two main zones in series. The first zone is a sediment deposition pond (234 m^2) that collects suspended solids. The water storage capacity of the sediment deposition zone was 40 m^3. Hence, runoff water mixes with water stored during quiescent period. Water depth in the sediment deposition zone varied from 0.05 to 0.5 m from April to September. Physico-chemical characteristics of wetland sediments were (%): clay 44, fine silt 33, coarse silt 10, fine sand 5, coarse sand 8; organic carbon 14.8; SiO_2 50, Al_2O_3 9.5, MgO 2.2, CaO 11.6, Fe_2O_3 4.1, MnO 0.1, Na_2O 0.7, K_2O 2.5 and pH 8.1 (in water) (n= 5). A gabion barrier is used to enhance the dispersion of water ahead of the gravel filter. The second zone is a 13 m long, 8 m wide and 0.6 m deep gravel filter (saturated hydraulic conductivity, $K=10^{-3}$ m/s) that increases the hydraulic retention time in the wetland, and thus the capacity of contaminant removal. Detailed characteristics and hydraulic functioning of the wetland and gravel filter have been studied previously (Wanko et al., 2009) and detailed hydrological characteristics of the wetland that correspond to the investigation period are provided in Table 7.5-189. Due to the clay liner on the wetland bed ($K_s < 10^{-10}$ m/s) and based on the water balance, water losses by vertical subsurface infiltration between the sediment/gravel and the clay liner were negligible. The bottom slope of the stormwater wetland was 2.8 %. The vegetation cover in the sediment deposition zone, mainly formed of *Phragmites australis, Schoenoplectus lacustris* and *Typha latifolia*, was <1 % of the area in April, 5 % in May, 25 % in June, 60 % in July, 70 % in August and 85 % in September. *Phragmites australis* ranged between 70 % and 80 %of the total vegetation cover through the investigation period. The vegetation in the gravel filter, mainly formed by *Lolium perenne* and *P. austalis*, varied, respectively, from 20 to 30 % and from 5 to 15 % of the area throughout the investigation period. Algae, mainly *Chara vulgaris*, appeared in the sediment deposition zone since August and covered more than 70 % of the area in September.

Figure 7.5-164: **Schematic of the storm water wetland (Rouffach, Alsace, France) and sampling locations (●) in the sediment deposition zone (SDZ), the gabion barrier (G) and the gravel filter (GF)**

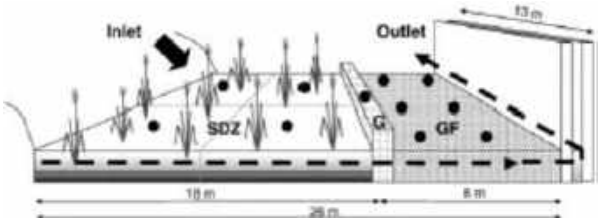


Table 7.5-189: **Climatic and hydrological conditions in the vineyard catchment (Rouffach, Haut-Rhin, France) and the stormwater wetland in spring (06 April to 15 June 2009) and in summer (15 June to 29 September 2009). Values are provided as the median and ranges.**

		Spring (April 06 to June 15, 2009)	Summer (June 16 to September 29, 2009)	p*
Temperature	[°C day^{-1}]	16.1 (9.6 - 26.7)	19.3 (12.5 - 27.9)	***
Solar radiation	[joules cm^{-2} day^{-1}]	1978 (471 - 2909)	1720 (431 - 2897)	n.s.
Evaporation	[mm]	280.5	390.4	-
Rainfall	[mm]	88.8	162.2	-
Number of rainfall events	[-]	32	45	-
Rainfall amount	[mm]	1.7 (0.2 - 15.4)	1 (0.2 - 24)	n.s.
Rainfall duration	[hs]	1.9 (0.1 - 16.7)	1.1 (0.1 - 13.9)	n.s.
Mean rainfall intensity	[mm h^{-1}]	1.4 (0.2 - 6.7)	2 (0.1 - 21)	n.s.
Maximum rainfall intensity	[mm h^{-1}]	4 (2 - 38)	2 (2 - 46)	n.s.
Number of runoff events	[-]	13	17	-
Discharge	[m^3]	4 (0.4 - 83)	8.4 (0.3 - 141.8)	n.s.
Quiescent period	[day]	4 (0.1 - 17)	3.6 (0.3 - 22.4)	n.s.
Runoff coefficient	[%]	0.2 (0.0 - 1.5)	0.6 (0.02 - 1.5)	n.s.
Inlet flow rate	[m^3 h^{-1}]	0.28 (0 - 13.5)	2.5 (0 - 158.7)	n.s.
Outlet flow rate	[m^3 h^{-1}]	0.28 (0 - 4.2)	0.002 (0 - 11)	***

* Wilcoxon test: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s. =not significant

Sampling procedure

Daily rainfall and evapotranspiration were measured at a weather station located on the catchment (Meteo France, station no. 68287003). Samples were collected from the inlet, the sediment deposition zone, the gravel filter, and the outlet of the wetland (Figure 7.5-164) from 01 April through 29 September 2009, corresponding to the period of pesticide application. Runoff discharges were continuously monitored by measurements of water depth using bubbler flow modules (Hydrologic, Sainte-Foy, Québec, Canada) combined with a Venturi channel at the inlet and a V-notch weir at the outlet. Water samples were collected every 6 m^3 at the inlet of the wetland using a 4010 Hydrologic automatic sampler (Hydrologic, Sainte-Foy, Québec, Canada) and at the outlet using a 6712FR ISCO Teledyne automatic sampler (Lincoln, Nebraska, US). The detailed procedure of sample collection and storage ensuring reliable pesticide measurements was previously tested and discussed (Domange and Gregoire, 2006). Briefly, water samples (100 mL) were collected in glass jars, stored in the dark at 4 °C until collection after each runoff event, and placed on ice during transportation to the laboratory. A series of discrete water samples taken over a runoff event were combined in a single composite sample. Suspended solids were obtained from continuously operating samplers consisting of 2 mm and 50 µm sieves in series and installed at the inlet and outlet of the wetland. The samplers were emptied every week throughout the investigation period. In order to ensure representative and reliable measurements, pesticide concentrations in suspended-solids were measured only when the mass of collected material reached 20 g or more. In parallel, 10 sampling campaigns were performed every two weeks during quiescent period (i.e. in the period between two runoff events) on day

21 (21 April 2009), 35, 49, 63, 76, 91, 111, 128, 141 and, after harvesting grapevine, on day 182 (29 September 2009) to collect water and sediment samples within the wetland. At each sampling campaign, grid-cell sampling was performed in the sediment deposition zone by dividing the zone in four equal rectangular cells (9 × 6 m) (Figure 7.5-164). Four water samples (collected from 0 to 10 cm depth from the water surface) and four surface sediment grab samples (collected from 0 to 5 cm depth from the sediment surface) were separately collected at the center of each cell. Pore water samples were also collected in the gabion barrier from one PVC well and in the gravel filter from six PVC wells (Figure 7.5-164). To ensure representative sampling, the wells were purged using a pump to replace the equivalent of one volume of the tube. Dissolved oxygen, pH, conductivity, redox potential and temperature were directly measured in the field using WTW multi 350i portable sensors (WTW, Weilheim, Germany). Water samples were dispensed into 100 ml glass and plastic vials for pesticide analysis (headspace free) and 1 L acid washed HDPE bottles (10 % HCl and rinsed with distilled water) for hydrochemical analysis. Water and sediment samples were placed on ice and directly transported to the laboratory for chemical analysis. A chemical analysis of water samples was performed within 2 days of collection. Sediment samples were kept at −20°C until chemical analysis, for a maximum of 30 days.

Analysis of water and sediment samples

Eighteen hydrochemical parameters (TIC, DIC, NPOC, DOC, TKN, PO_4^{2-}, P_{tot}, NO_3^-, NO_2^-, NH_4^+, Mn_2^+, Fe_2^+, Fe_{tot}, SO_4^-, Mg_2^+, Na^+, Cl^-, and K^+) were determined by FR EN ISO standards and laboratory procedures. Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC). For international quality control purposes, the COFRAC calibration certificate is recognized by other European calibration services (EA — European Cooperation for Accreditation). Briefly, water samples were filtered through 1 µm glass fiber filters, solid–liquid extracted before analyzing the subsequent extracts. The 16 pesticides and four degradation products were quantified using liquid chromatography coupled to tandem mass spectrometry (LC–MS–MS). Quantification of glyphosate, AMPA and glufosinate was performed after derivatization with fluorenemethoxycarbonyle (FMOC). Limits of pesticide quantification in water samples ranged from 0.02 to 0.1 µg/L. Quantification of pesticide residues in sediment samples was performed by LC–MS–MS measurements following ultrasonic and methanol extraction. Limits of quantification ranged from 2 to 10 µg/kg. Extraction efficiencies of pesticides from water and sediment samples were estimated for each sample set by spiking with surrogates. Surrogate recovery for water samples ranged from 70 to 89 % and those of sediment from 68 to 85 %. Further quality control was achieved by using a blank for each set of samples. Detection and quantification limits, relative standard deviation (RSD) and recovery efficiencies for each studied pesticide are provided for both water and sediment samples in Table 7.5-190.

Table 7.5-190: Detection and quantification limits, as well as relative standard deviation (RSD) and recovery efficiency for the investigated pesticide in both water and sediment samples. Values are provided as the median and ranges

Compound	Water samples				Sediment samples			
	LOD [µg L^{-1}]	LOQ [µg L^{-1}]	RSD [%]	Recovery [%]	LOD [µg kg^{-1}]	LOQ [µg kg^{-1}]	RSD [%]	Recovery [%]
Azoxystrobin	0.02	0.05	15	83	1	2	10	71
Cyazofamid	0.02	0.05	15	70	1	2	13	70
Cyprodinil	0.01	0.02	20	89	2	5	13	81
Carbendazim	0.02	0.05	25	80	1	2	10	75
Dimethomorph	0.02	0.05	30	76	1	2	16	72
Diuron	0.01	0.02	23	85	1	2	9	69
DCPU	0.02	0.05	26	82	1	2	10	72
DCPMU	0.02	0.05	25	79	1	5	9	73
4-chlorobenzamide	0.03	0.10	25	75	5	10	13	76
Flufenoxuron	0.02	0.05	24	81	1	2	10	75
Glyphosate	0.03	0.10	25	85	5	10	14	72
Glyphosate	0.03	0.10	16	81	5	10	10	76
AMPA	0.03	0.10	16	86	5	10	10	72
Isoxaben	0.02	0.05	15	89	1	2	9	71
Kresoxim-methyl	0.03	0.10	28	81	5	10	18	70
Metalaxyl	0.02	0.05	17	86	1	2	10	72
Pyrimethanil	0.02	0.05	22	89	1	2	12	81
Simazine	0.01	0.02	22	85	1	2	29	82
Terbuthylazine	0.01	0.02	20	90	1	2	11	80
Tetraconazole	0.02	0.05	25	86	1	2	29	71

Data analysis

Dissolved pesticide concentrations found at the inlet and outlet of the wetland were compared using the paired nonparametric Wilcoxon Signed Rank test. Correlations between hydrological variables and pesticide metrics were tested by the rank-based Spearman's test. Hydrochemical data were subjected to principal component analysis (PCA), which were performed on the basis of the correlation matrix. In turn, the numerical data matrices were converted using the program R (R: Copyright 2005, The R Foundation for Statistical Computing, Version 2.1.1). Principal component analysis (PCA) is an ordination method that allows summarizing large data sets and exploring the spatial and temporal trends in the data. Reduction of pesticide concentration, RC (%), was calculated for each runoff event as the reduction of mean concentrations at the outlet relatively to the mean concentrations at the inlet of the wetland. A nondetect (n.d.) was treated as zero. The RC (%) in a given period was the average of all runoff event RC (%) values for this period. Pesticide event loads at the inlet and the outlet of the wetland were obtained by multiplying the mean pesticide concentrations by the corresponding runoff volume. Removal rates of pesticide load RL (%) were calculated for each runoff event as the reduction of the load at the outlet relatively to the load at the inlet of the wetland using Eq. (1).

$$R_L(\%) = \left[1 - \frac{M_{out}}{M_{in}}\right] \times 100 = \left[1 - \frac{C_{out} V_{out}}{C_{in} V_{in}}\right] \times 100 \qquad (1)$$

where M_{in} and M_{out} are the influent and effluent pesticide loadings, V_{in} and V_{out} are the influent and effluent volumes, and C_{in} and C_{out} the inlet and outlet mean concentrations, respectively. Load (mg) at the inlet or outlet of the wetland was calculated from the integral sum of all event loads in a given period (i.e., between 2 sampling campaigns or in a season).

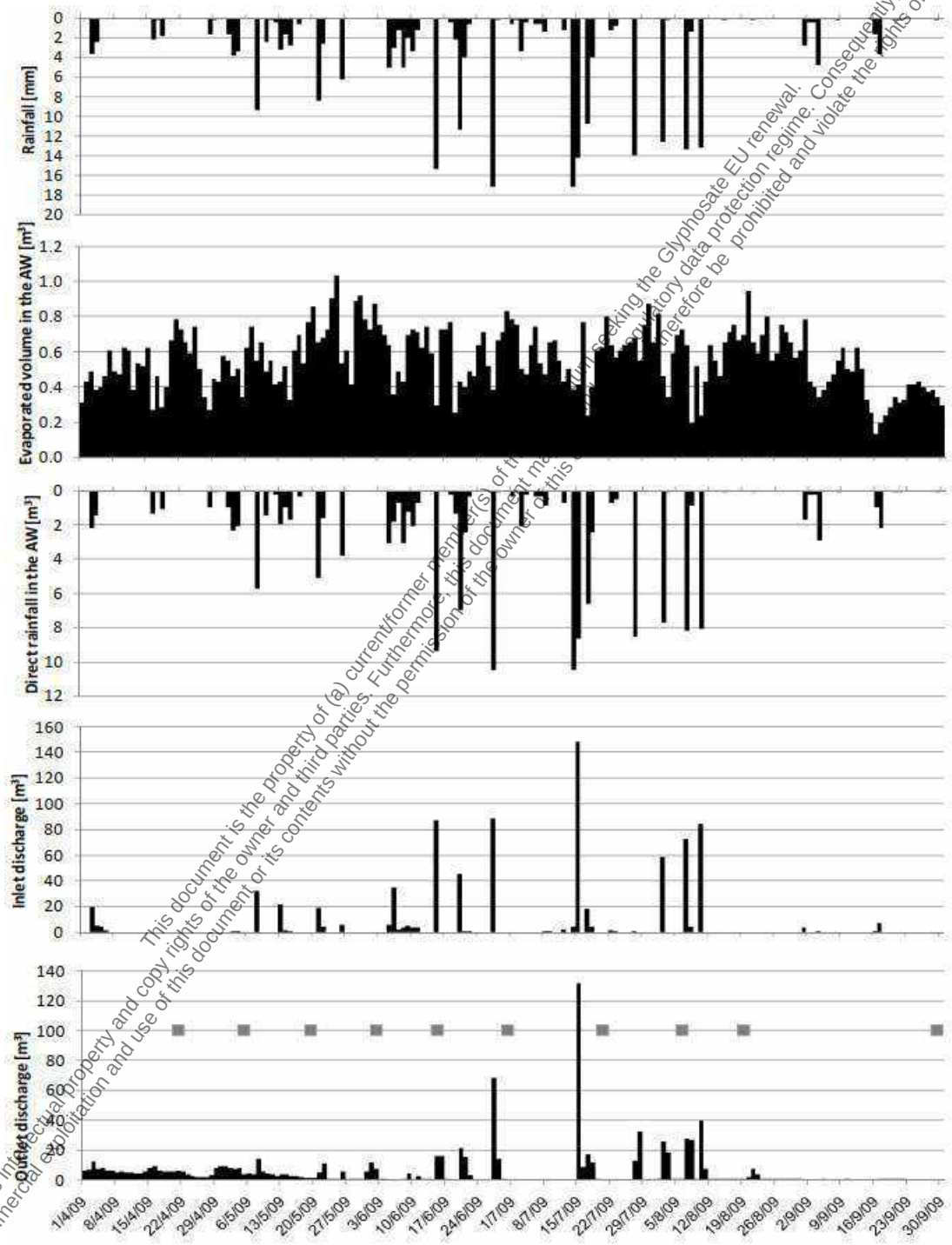
Results

Hydrological and hydrochemical characteristics of the wetland

Detailed climatic and hydraulic data from 01 April through 29 September is provided in Figure 7.5-165 and in Table 7.5-189. Rainfall amount, duration, mean and maximal intensities, as well as the duration of the period between two rainfall events did not significantly differ between spring and summer ($p > 0.37$). Rainfall on the vineyard catchment amounted to 251 mm between 06 April and 29 September, and the direct rainfall input on the wetland was 153 m^3. Waterloss resulting from evaporation was 99 m^3. Thirty

runoff events ranging from 0.3 to 141.8 m^3 occurred during the investigation period, generating a total volume of 730 m^3. The mean quiescent period between two runoff events ranged from 2.4 h to 27 days during the investigation period and did not significantly differ between spring and summer ($p > 0.61$). The budget of water volumes inflowing and outflowing the wetland was balanced when direct rainfall and evapotranspiration volumes were included. Flow rates at the wetland inlet ranged from 0 to 158.7 m^3/h (mean±SE: 6.3 ± 9.6 m^3/h) during the investigation period. Inlet flow rates in spring (2.1 ± 2.7 m^3/h) and summer (12.2 ± 11.8 m^3/h) did not significantly differ ($p > 0.09$), although larger and more variable flow rates were observed in summer. In contrast, outlet flow rates significantly differed between spring (0.3 ± 0.8 m^3/h) and summer (0.2 ± 1.0 m^3/h) ($p < 0.001$), which strongly suggests that larger vegetation cover in summer reduced the flow rate. During the investigation period, the hydraulic retention time (HRT) of the wetland ranged between 6.7 and 14 h (mean±SE: 10.8 ± 2.6 h) for runoff events exceeding 40 m^3, whereas smaller runoff events could be stored in the wetland. The duration of runoff events ranged between 0.78 and 15 h. However, only one runoff event lasts longer than 12 h and likely completely flushed the stormwater wetland. The PCA ordination plot (Figure 7.5-166) shows for each of the 10 sampling campaigns the replicate samples collected from the sediment deposition zone and the gravel filter as well as the hydrochemical variables. Symbols in the plot lying close together display similar hydrochemical patterns. The principal component analysis of hydro-chemical data revealed that hydrochemical conditions changed in the wetland over time. Water samples collected from the first (21 April) to the fifth sampling campaigns (15 June) clustered together and were clearly separated from those collected from the sixth (30 June) to the tenth sampling campaign (29 September), which indicates distinct hydrochemical profiles between the two periods corresponding to spring and summer. On the variables plot (Figure 7.5-166), scores of PC1 correlated positively to cations (Ca^{2+}, Mg^{2+}, and Na^{+}), anions (Cl^{-}, NO_2^{-}, and NO_3^{-}), redox potential, as well as organic (DOC and NPOC) and inorganic carbon (TIC and DIC). In addition, they correlated negatively to temperature, showing that these hydrochemical variables considerably changed in the wetland between spring and summer. Samples corresponding to the tenth sampling campaign (29 September) were associated with higher concentrations of ferrous iron, manganese and ammonium, indicating the prevalence of reducing conditions in the wetland. Mean water temperature and pH across all sampling points and campaigns was 19.0 ± 4.3 °C and 7.6 ± 0.3, respectively. In spring, oxic conditions prevailed in the wetland, as inferred from mean values of redox potential larger than 50 mV, concentrations of ferrous iron lower than 1 mg/L, and concentrations of dissolved oxygen higher than 2.9 mg/L in the sediment deposition zone. In summer, lower concentrations of dissolved oxygen and negative values of redox potential indicated the prevalence of an anoxic milieu. In spring, Fe^{2+} concentrations were one order of magnitude lower than those of total iron, suggesting the prevalence of the ferric form. In contrast, larger Fe^{2+} concentrations (up to 6.0 mg/L) attested the occurrence of anoxic conditions in summer. The analysis of both hydrological and hydrochemical data revealed that conditions in the wetland differed between spring and summer. Therefore, pesticide removal by the wetland in spring and summer is compared.

Figure 7.5-165: **Daily rainfall [mm] in the catchment area, evaporated volume (m^3), direct rainfall in the wetland [mm], and daily discharges (m^3) at the inlet and outlet of the stormwater wetland (Rouffach, Haut-Rhin, France) during the investigation period (06 April to 29 September 2009) that corresponded to the wine growing season and the period of pesticide. Grey squares indicate water and sediment sampling in the wetland**



Occurrence and concentration reduction of pesticides in the wetland

Detailed data of pesticide concentrations in water, in suspended solids and wetland sediments as well as reduction of pesticides based on inlet and outlet concentrations are provided in Table 7.5-69. Mean concentrations of dissolved pesticides generally decreased between the inlet, the sediment deposition zone, the gravel filter and the outlet of the wetland (Figure 7.5-167A and B). Temporal variation of pesticide concentrations in runoff reflects both timing of pesticide applications in the catchment and changes in rainfall–runoff patterns over time, as previously shown (Gregoire et al., 2010). Degradation products of diuron (DCPU, DCPMU and 1,3-dichloroaniline) were systematically below the detection limit, suggesting that diuron was not subject to aerobic degradation or that degradation products were readily degraded in the wetland. In spring, reduction in mean concentrations from inlet to outlet ranged from 71 (AMPA) to 100 % (cymoxanil, dimethomorph, gluphosinate, kresoxim methyl, terbuthylazine and tetraconazole). In summer, concentration reductions were lower compared to those observed in spring, and ranged from 0 (tetraconazole) to 100 % (azoxystrobin, cyprodinil, isoxaben, kresoxim methyl and terbuthylazine). Concentrations from inlet to outlet significantly differed for cymoxanil, diuron, glyphosate, AMPA, isoxaben, metalaxyl, simazine, terbuthylazin and tetraconazol in spring and for glyphosate in summer ($p < 0.05$). Pesticide concentrations in water from the sediment deposition zone and the gravel filter were smaller in spring compared to those measured in summer, although concentrations found in the inflowing runoff were similar. Altogether, the results indicate lower efficacy of the wetland in reducing pesticide concentrations in summer. Patterns of pesticide concentrations associated with suspended solids and the wetland sediments also differed between spring and summer (Figure 7.5-167C). Flufenoxuron, dimethomorph, and cyprodinil concentrations associated with suspended solids in inlet samples increased over time and then decreased. However, mean concentrations of pesticides and degradation products in the wetland sediments were close to or below the detection limits, except for flufenoxuron. The results indicate no significant transfer of dissolved or particle-laden pesticides from the water column to the bed sediments, and thus no accumulation or persistence of pesticides in the wetland sediments.

Figure 7.5-166: **PCA ordination plots of hydrochemical characteristics of water samples collected in the storm water wetland (Rouffach, Alsace, France) between day 0 (06 April 2009) and day 182 (29 September 2009)**

Values on the axes indicate the % of the total variation explanation by the corresponding axis (PC 1, principal component axis 1; PC 2, principal component axis 2). The first and second principal components accounted for 52.7 % of the variance in the data set. Objects are labeled according to the section of the wetland they were collected from (▲, sediment deposition zone; ◊, gabion barrier and gravel filter) and numbered according to their sampling date: day 0 (06 April 2009), 21, 35, 49, 63, 76, 91, 111, 128, 141, and 182 (29 September 2009). Description vectors correspond to: T °C, temperature; P_{tot}, total phosphorus; Fe^{2+}, ferrous iron; Mn^{2+}, manganese; Fe_{tot}, total iron; NH_4^+, ammonium; TKN, total kjeldahl nitrogen; TIC, total inorganic carbon; DIC, dissolved inorganic carbon; NPOC, non-purgeable organic carbon; DOC, dissolved organic carbon; EC, electric conductivity; Ca^{2+}, calcium; NO_2^-, nitrite; Mg^{2+}, magnesium; Cl^-, chlorine; SO_4^-, sulfate; Eh, redox potential; Na^+, sodium; NO_3, nitrate; PO_4^{3-}, orthophosphate; K^+, potassium.

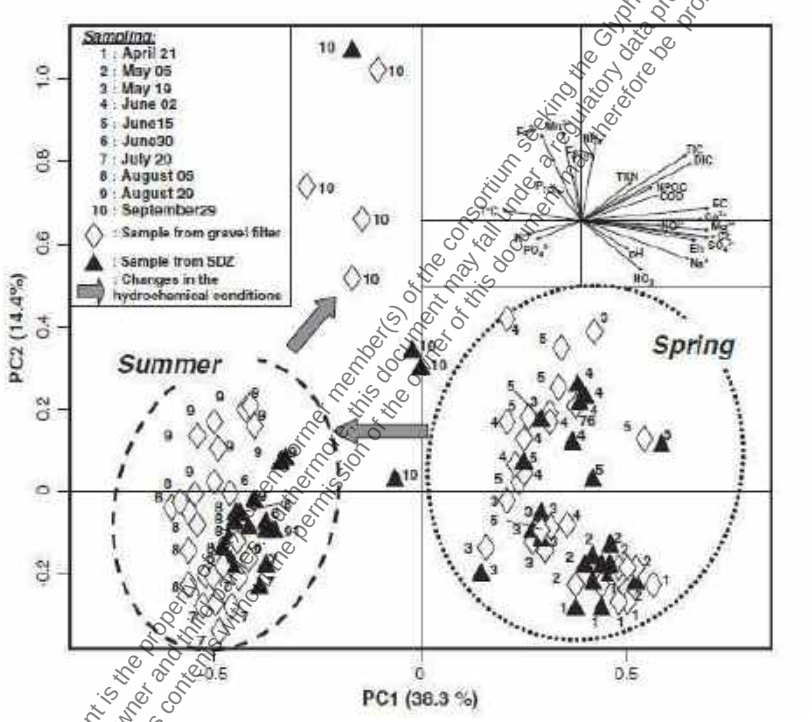


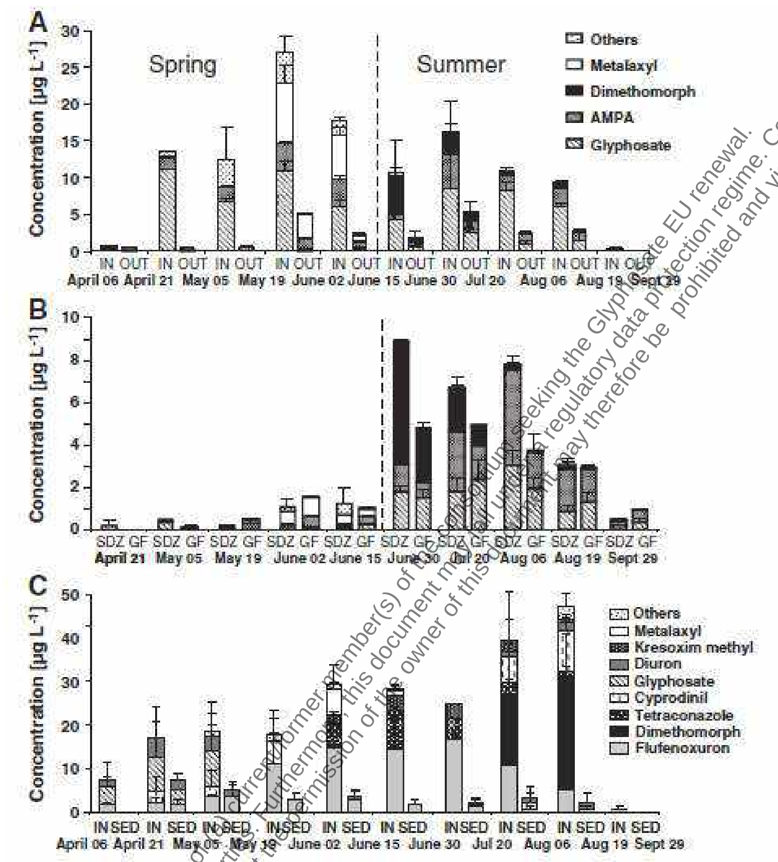
Table 7.5-191: Mean concentrations and ranges of dissolved and particle-bound pesticides in the inlet, the sediment deposition zone (SDZ), the gravel filter (GF) and the outlet of the storm water wetland (Rouffach, Haut-Rhin, France) in spring (06 April to 15 June 2009) and in summer (15 June to 29 September 2009). Reduction in mean concentrations from inlet to outlet are given in percent (RC %). n.d.: not detected

	Spring							Summer						
	Dissolved pesticides [µg L^{-1}]					Particle-bound pesticides [µg kg^{-1}]		Dissolved pesticides [µg L^{-1}]					Particle-bound pesticides [µg kg^{-1}]	
Compound	Inlet (n = 10)	SDZ (n = 20)	GF (n = 10)	Outlet (n = 17)	R_C [%]	Inlet (n = 7)	SDZ (n = 16)	Inlet (n = 18)	SDZ (n = 20)	GF (n = 10)	Outlet (n = 9)	R_C [%]	Inlet (n = 9)	SDZ (n = 20)
Azoxystrobin	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	0.02 (n.d.-0.12)	0.01 (n.d.-0.07)	n.d.	< LOQ (n.d.-0.04)	100	< LOQ (n.d.-2.0)	n.d.
Cymoxanil	0.15 (n.d.-0.96)	0.03 (n.d.-0.60)	n.d.	n.d. [a]	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
Cyprodinil	n.d.	n.d.	n.d.	n.d.	n.a.	0.71 (n.d.-5.0)	n.d.	0.02 (n.d.-0.14)	0.01 (n.d.-0.04)	0.01 (n.d.-0.03)	n.d.	100	30.8 (n.d.-145)	< LOQ (n.d.-7.0)
Carbendazim	n.d.	0.01 (n.d.-0.11)	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	0.04 (n.d.-0.20)	n.d.	n.d.	n.a.	< LOQ (n.d.-2.0)	n.d.
Dimethomorph	0.03 (n.d.-0.18)	n.d.	n.d.	n.d.	100	< LOQ (n.d.-1.0)	n.d.	2.22 (n.d.-10.0)	1.62 (n.d.-5.80)	0.69 (n.d.-2.0)	0.50 (n.d.-2.20)	78	11.3 (n.d.-53)	n.d.
Diuron	0.16 (0.11-0.32)	0.04 (n.d.-0.06)	0.01 (n.d.-0.05)	0.02 [a] (n.d.-0.04)	90	< LOQ (n.d.-7.0)	< LOQ (n.d.-4.0)	0.02 (n.d.-0.16)	0.01 (n.d.-0.03)	0.01 (n.d.-0.02)	0.01 (n.d.-0.04)	46	2.1 (n.d.-2.5)	< LOQ (n.d.-4.0)
DCPU	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
DCPMU	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	< LOQ (n.d.-2.0)	n.d.
3,4-dichloroaniline	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
Flufenoxuron	n.d.	0.04 (n.d.-0.59)	n.d.	n.d.	n.a.	8.5 (1.5-18.5)	2.75 (n.d.-6.0)	n.d.	n.d.	n.d.	n.d.	n.a.	6.1 (1.5-16.0)	< LOQ (n.d.-3.0)
Gluphosinate	0.85 (n.d.-5.30)	0.11 (n.d.-1.40)	n.d.	n.d.	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
Glyphosate	4.13 (0.30-11.0)	0.10 (n.d.-0.40)	0.07 (n.d.-0.30)	0.05 [a] (n.d.-0.3)	98	< LOQ (n.d.-10.0)	< LOQ (n.d.-6.0)	0.44 (0.01-1.70)	1.61 (n.d.-3.90)	1.40 (0.20-3.0)	1.28 [a] (0.80-3.90)	79	11.1 (n.d.-45.0)	n.d.
AMPA	1.37 (0.20-2.30)	0.08 (n.d.-0.30)	0.27 (n.d.-0.60)	0.39 [a] (n.d.-0.70)	71	n.d.	n.d.	2.03 (n.d.-21.0)	2.05 (n.d.-4.80)	1.85 (0.50-2.30)	1.00 (0.50-1.80)	57	5.8 (n.d.-21.0)	n.d.
Isoxaben	0.11 (n.d.-0.23)	0.02 (n.d.-0.10)	0.04 (n.d.-0.18)	0.01 [a] (n.d.-0.15)	89	< LOQ (n.d.-2.0)	n.d.	< LOQ (n.d.-0.06)	n.d.	n.d.	n.d.	100	< LOQ (n.d.-1.0)	n.d.
Kresoxim methyl	0.01 (n.d.-0.05)	0.02 (n.d.-0.10)	n.d.	n.d.	100	1.0 (n.d.-4.0)	n.d.	< LOQ (n.d.-0.40)	n.d.	n.d.	n.d.	100	< LOQ (n.d.-3.0)	n.d.
Metalaxyl	1.43 (n.d.-5.80)	0.21 (n.d.-0.64)	0.23 (n.d.-0.92)	0.25 [a] (n.d.-1.20)	80	1.7 (n.d.-10.0)	n.d.	0.05 (n.d.-0.35)	n.d.	n.d.	0.04 (n.d.-0.37)	10	n.d.	n.d.
Pyrimethanil	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	0.02 (n.d.-0.08)	0.01 (n.d.-0.06)	0.01 (n.d.-0.05)	0.01 (n.d.-0.05)	65	1.5 (n.d.-4.5)	n.d.
Simazine	0.05 (0.04-0.18)	n.d.	0.02 (n.d.-0.03)	0.02 [a] (n.d.-0.03)	80	n.d.	n.d.	< 0.02 (n.d.-0.02)	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.
Terbuthylazine	0.06 (0.06-0.07)	n.d.	n.d.	n.d. [a]	100	n.d.	n.d.	< 0.02 (n.d.-0.04)	n.d.	n.d.	n.d.	100	n.d.	n.d.
Tetraconazole	0.02 (n.d.-0.09)	0.01 (n.d.-0.07)	n.d.	n.d. [a]	100	[illegible] (n.d.-0.03)	n.d.	0.01 (n.d.-0.07)	n.d.	n.d.	0.01 (n.d.-0.03)	0	1.1 (n.d.-3.0)	< LOQ (n.d.-2.0)

Removal of dissolved pesticides by the wetland

During the investigation period, the load of the 20 pesticides and degradation products entering the wetland was 8.039 g whereas 2.181 g passed through the wetland (Table 7.5-189). Inflowing load in summer (6.819 g, i.e. 85 % of the total dissolved load) was larger compared to that of spring (1.219 g, i.e. 15 % of the total dissolved load). This reflects both the seasonal change of runoff regime as underscored in Section *Hydrological and hydrochemical characteristics of the wetland* and pesticide applications in the vineyard catchment (31 %of the total applications occurred in spring and 69 % in summer, data not shown). Glyphosate, AMPA, dimethomorph and the other compounds accounted for, respectively, 51.7, 20.4, 21.1, and 6.8 % of the total inflowing load. According to the removal rates calculated from the difference between loads at the outlet and the inlet of the wetland during the entire period of investigation, pesticides can be classified as (i) efficiently retained (removal rates between 80 and 100 %; i.e. azoxystrobin, cymoxanil, gluphosinate, kresoxim methyl and terbuthylazine); (ii) moderately retained (removal rates between 50 and 80 %; i.e. cyprodinil, dimethomorph, diuron, glyphosate, AMPA, isoxaben, metalaxyl, pyrimethanil and tetraconazole); and (iii) poorly retained (removal rates lower than 50 %; i.e. simazine). Summing seasonal loads of all compounds, very similar removal rates were found for spring and summer (i.e. 72 and 73 %, respectively), indicating that seasonal changes of pesticide loadings did not affect the removal capacity of the wetland. This was supported by the absence of significant correlation between pesticide loadings in runoff and pesticide removal rates ($p > 0.1$) throughout the investigation period, suggesting no threshold at which pesticide removal would decrease at larger loads. However, removal rates of individual compounds largely varied between spring and summer (Table 7.5-189).

Figure 7.5-167: Mean concentrations of pesticides (A) in the inflowing runoff (IN) and the outlet (OUT), (B) within the sediment deposition zone (SDZ) and the gravel filter (GF), and (C) associated with inflowing suspended solids (IN) and sediment (SED) of the stormwater wetland (Rouffach, Alsace, France). Error bars show the standard deviation



Retention of particle-laden pesticides

The role of sedimentation in pesticide removal was evaluated based on analysis of total suspended solids (TSS) and dissolved organic carbon (DOC). Detailed loads of pesticide associated with suspended-solids entering the wetland are provided in Table 7.5-193. The pesticide load associated with suspended solids in inflowing runoff was 198 mg for the entire investigation period, which represents 2.4 % of the total load. The trifling contribution of the solid load to the total load of pesticides is due to low fractions (<1 %) of glyphosate, AMPA and dimethomorph associated with suspended solids, while these compounds accounted for 93 % of the total dissolved pesticide load. Nevertheless, partition coefficient K_d in inflowing runoff ranged between −4.22 (glyphosate) and 1.07 (diuron), reflecting large variations of the partitioning patterns among pesticides and seasons (see Table 7.5-193 for detailed values of K_d). Pesticide concentrations in suspended solids at the outlet of the wetland could not be obtained on a runoff-event basis because the amount of material collected in the sieve of the suspended solid samplers was too low (<5 g of sediment) to enable reliable measurements. Therefore, rates of pesticide removal attributable to retention by the wetland of pesticides associated with suspended solids could not be calculated using a mass balance approach. Nevertheless, average sedimentation rates estimated from discharge measurements and TSS values were 2.7 kg/day (99 % of the input mass) in spring and 7.0 kg/day (88 % of the input mass) in summer, indicating that the wetland can act as a sink for particle-laden pesticides. Since the pore size of the filter paper used for separating TSS from DOC was 0.45 µm, only finer particles were included in the DOC mass balance analysis. Mass balance of DOC between the inlet and the outlet showed that the output

mass (12.9 kg) exceeded by 34 % the input mass. This indicates that pesticide removal cannot be attributed to the retention of the DOC-bound fraction in the wetland. Additionally, re-suspension of particles from the wetland bed to the water column during higher flow regime and plant material, as well as sediment re-suspension by the aquatic fauna and proliferation of algae likely reduced the removal of pesticides associated with TSS and DOC by the wetland.

Table 7.5-192: Load estimates (mg) of dissolved pesticides and load reduction, RL (%) by the storm water wetland (Rouffach, Alsace, France) in spring (06 April to 15 June 2009), in summer (15 June to 29 September 2009) and during the wine growing season (06 April to 29 September 2009). Degradation products are shown in italics

Compound	Spring			Summer			Wine growing season		
	Inlet	Outlet	R_L [%]	Inlet	Outlet	R_L [%]	Inlet	Outlet	R_L [%]
Azoxystrobin	0	0	n.a.	18.1	1.3	93	18.1	1.3	93
Cymoxanil	12.3	0	100	0	0	n.a.	12.3	0	100
Cyprodinil	0	0	n.a.	18.6	4.3	77	18.6	4.3	77
Carbendazim	0	0	n.a.	0	0	n.a.	0	0	n.a.
Dimethomorph	3.4	0	100	1693	409	76	1696	409	76
Diuron	26.5	7.4	72	13.2	5.7	57	39.7	13.1	67
DCPU	0	0	n.a.	0	0	n.a.	0	0	n.a.
DCPMU	0	0	n.a.	0	0	n.a.	0	0	n.a.
3,4-dichloroaniline	0	0	n.a.	0	0	n.a.	0	0	n.a.
Flufenoxuron	0	0	n.a.	0	0	n.a.	0	0	n.a.
Glufosinate	93.0	0	100	0	0	n.a.	93.0	0	100
Glyphosate	585	56.3	90	3571	811	77	4156	867	79
AMPA	217	196	10	1421	585	59	1637	781	52
Isoxaben	17.0	6.1	64	7.4	0	100	24.5	6.1	75
Kresoxim methyl	1.0	0	100	15.7	0	100	16.7	0	100
Metalaxyl	277	65.1	73	355	156	56	272	80.8	70
Pyrimethanil	0	0	n.a.	12.6	5.3	57	12.6	5.3	57
Simazine	13.0	8.4	36	2.1	0.8	60	15.1	9.2	39
Terbuthylazine	10.4	0	100	3.8	0	100	14.2	0	100
Tetraconazole	3.8	0	100	7.1	3.6	50	11.0	3.6	67
Total pesticides	1219	339	72	6821	1842	73	8039	2181	73

n.a. not applicable

Table 7.5-193: Load estimates [mg] of dissolved pesticides and suspended-solid associated pesticides entering the storm water wetland in spring (06 April to 15 June 2009), in summer (15 June to 29 September 2009) and during the entire investigation period (06 April to 29 September 2009). Solid to dissolved load ratio [%] are also provide. Degradation products are shown in italics

Compound	Spring			Summer			Wine growing season		
	Dissolved load	Solid load	LogK_d	Dissolved load	Solid load	LogK_d	Dissolved load	Solid load	LogK_d
Azoxystrobin	0	0	n.a.	18.1	0.03	-2.73	18.1	0.03	-2.73
Cymoxanil	12.3	0	n.a.	0	0	0	12.3	0	0
Cyprodinil	0.00	0.01	n.a.	18.6	11.27	-0.22	18.6	11.28	-0.22
Carbendazim	0	0	n.a.	0	0	n.a.	0	0	n.a.
Dimethomorph	3.4	0.01	-2.62	1693	11.97	-2.15	1696	12.0	-2.15
Diuron	26.5	0.03	-2.93	13.2	156	1.07	39.7	155	0.59
DCPU	0	0	n.a.	0	0	n.a.	0	0	n.a.
DCPMU	0	0	n.a.	0	0.34	n.a.	0	0.34	n.a.
3,4-dichloroaniline	0	0	n.a.	0	0	n.a.	1.3	0	0
Flufenoxuron	0	1.24	n.a.	0	3.96	n.a.	0	5.19	n.a.
Gluphosinate	93	0	n.a.	0	0	n.a.	93	0	0
Glyphosate	585	0.04	-4.22	3571	6.66	-2.73	4156	6.69	-2.79
AMPA	217	0.00	n.a.	1421	3.77	-2.58	1637	3.77	-2.64
Isoxaben	17	0.33	-1.71	7.4	0.02	-2.69	24.5	0.35	-1.85
Kresoxim methyl	1	0.08	1.10	15.7	0.41	-1.59	16.7	0.49	-1.54
Metalaxyl	237	0.15	0.06	35.3	0	n.a.	272.5	0.15	-3.26
Pyrimethanil	0	0	n.a.	12.6	1.28	-0.99	12.6	1.28	-0.99
Simazine	13	0	n.a.	2.1	0.00	n.a.	15.1	0	0
Terbuthylazine	10.4	0	n.a.	3.8	0	n.a.	14.2	0	0
Tetraconazole	3.8	0.18	-1.31	7.3	0.73	-1.00	11	0.92	-1.08
Total	1219	2.07	-2.77	6819	196	2.9	8039	198	-1.61

Discussion

Pesticides in runoff from agricultural catchments typically occur in mixtures. Therefore studies on pesticide mixtures are necessary to understand how mitigation capacities in wetland systems develop over time and can be used for reducing impacts on receiving aquatic ecosystems. Lizotte et al. (2009) observed in a 700 m long backwater wetland in summer a larger concentration reduction (N90 %) for individual pesticides of a mixture of atrazine, S-metolachlor and fipronil than those observed in our study, although no pesticide load removal estimates was provided. The same authors emphasized that factors such as wetland size, sediment characteristics, type and density of vegetation and hydrochemical conditions that prevailed at a particular stage of the wetland lifespan can largely influence the ability to mitigate pesticide mixtures. In stormwater wetlands, removal processes of dissolved and particle-laden pesticides such as sedimentation, photolysis, hydrolysis and degradation are intimately linked with both the prevailing hydrochemistry and the rapid changes of runoff regime. Moreover, their respective contribution strongly depends on the properties of the molecules. Smaller logK_{ow} pesticides (with logK_{ow}< 3) result in loading being predominantly associated with runoff and wetland water, lower partitioning to suspended solids or DOC, and a potentially faster degradation in the dissolved phase owing to higher availability of molecules in abiotic or biotic transformation processes. For less-hydrophobic pesticides included in this study (e.i. azoxystrobin, cymoxanil, carbendazim, dimethomorph, diuron, gluphosinate, glyphosate, AMPA, metalaxyl, pyrimethanil, and simazine) an important hydrochemical characteristic in constructed wetlands is their pH. Azoxystrobin, 3.4-dichloroaniline, and simazine were expected to dissipate through aqueous photolysis that prevailed in the wetland during the entire investigation period, given that their half-life was lower than 6 days. Cymoxanil can be degraded by aqueous hydrolysis at pH 7 which is supported by the complete removal of cymoxanil by the wetland. In contrast, degradation of carbendazim, dimethomorph,

diuron, glyphosate and pyrimethanil via photolysis or hydrolysis was not a dominant removal process (half-life > 40 days). Nevertheless, mean quiescent period (±SD) between runoff events were 5.1 ± 7.3 and 5.2 ± 5.7 days for spring and summer respectively, indicating sufficient time for both biotic and abiotic degradation reactions to occur in the wetland. In spring, runoff events were lower than 40 m^3 and thus could be stored in the wetland and treated until the next runoff event. Moreover, average inlet flow rates were smaller in spring compared to those observed in summer, although the difference was not statistically significant, and outlet flow rate were significantly larger in spring. Small runoff volumes entering the wetland, low flow rates and longer quiescent periods can increase the contact time between runoff water and wetland compartments. In contrast, larger runoff volumes and inlet flow rates, such as those observed in summer, are expected to limit the occurrence of removal processes. Nevertheless, larger vegetation cover in summer compared to that observed in spring can largely enhance pesticide removal efficiencies by increasing both sorption sites and contact time, thus compensating shorter times of contact and degradative reactions during high flow conditions. Further-more, incomplete flushing of the wetland during low to moderate flow conditions (<40 m^3) can also cause longer retention of stable and less-sorptive substances. For instance, Lange et al. (in press) used uranine (DT_{50}-photolysis=11 days) as a reference to mimic photolysis, and sulforhodamine B ($LogK_{ow}$=−2.02) as one to mimic moderate sorption of contaminants in various wetland systems, including our stormwater wetland. Their study simulated a 37.5 m^3 runoff event and indicated favourable conditions for photocatalytic decay (removal of uranine by 57 %) and high sorption capacities (removal of sulforhodamine B by 82 %) in our stormwater wetland. In contrast, shorter circuiting and contact time with sediment and vegetation under high flow or flood conditions is expected to decrease removal of dissolved contaminant via sorption and degradation processes, as previously described (Lange et al., in press; Holland et al., 2004). Besides sorption, larger plant cover and density can also directly affect the removal of pesticides in wetlands. Under anaerobic conditions (prevalent in summer), it is likely that the elimination of chlorinated pesticides (i.e. simazine and terbuthylazine) via reductive dechlorination was also favored by the occurrence of biofilm, sediment, root complexes as well as potential sources of electron donors provided by roots and organic matter in the wetland. Besides, plant uptake cannot be excluded for compounds with a log K_{ow} ranging between 1 and 3. However, due to large variations of the vegetal biomass and type in our wetland on both spatial and temporal scales, the contribution of vegetation and vegetal material to pesticide removal could not be quantified in the present study. Though the comprehensive sampling highlighted major hydro-chemical changes in the wetland during quiescent period between runoff events, transient changes during runoff events may also occur. Intermittent flow regime in stormwater wetland is presumed to enhance the mixing of anaerobic zones in sediments with the adjacent aerobic and anoxic micro-sites in the rhizosphere, leading to temporal variations of hydrochemical conditions. Oxic conditions that prevailed in spring can be related to higher removal of dimethomorph, diuron, glyphosate, metalaxyl and tetraconazole, whereas higher temperatures and anaerobic conditions in summer can be related to the removal of AMPA, isoxaben and simazine. Seasonal changes of the duration and frequency of rainfall–runoff events, vegetal covering and ecotypes, as well as hydrochemical and climate conditions very likely determined the dominant microbial populations present in the wetland, as well as the metabolic pathway that pesticides and their degradation products took. In summer, higher plant density slowed water flows and allowed for particle settling to occur and may have increased degradation rates by favoring oxidative transformation pathways in the rhizosphere. Glyphosate and AMPA that accounted for 72.1 % of the contaminant load entering the wetland are major compounds in our study. Biodegradation of glyphosate in the environment takes place under both aerobic and anaerobic conditions, although biodegradation under anaerobic conditions is normally less than under aerobic conditions. Biodegradation of AMPA is generally slower than that of glyphosate possibly because of AMPA transient capacity to be strongly sorbed through the phosphonate group and thus protected against further biodegradation. Among the compounds studied, glyphosate and AMPA are strongly sorbed by soil minerals, and have been previously observed to rapidly adsorb to wetland sediments, before being gradually removed within 5 to 15 days. This is in agreement with our results showing no accumulation of glyphosate and AMPA in the wetland sediments and efficient degradation of glyphosate into AMPA in the dissolved phase. This was underscored by a larger AMPA to glyphosate ratio at the outlet (3.5) compared to that found at the inlet (0.4) in spring. In summer, AMPA to glyphosate ratio at the outlet was 0.9, which indicates a more effective removal of AMPA in the dissolved phase. Since glyphosate and AMPA were not detected in sediments and the occurrence of abiotic degradation mechanisms is unlikely for these compounds, the results indicate that glyphosate was microbially degraded into AMPA, which in turn was gradually

degraded in the water column of the wetland. Though variable-charge minerals, such as aluminum or iron oxides, can adsorb large amounts of glyphosate and AMPA, competitive adsorption with phosphorus may occur, explaining the absence of significant sorption onto wetland sediments. Ratios of dissolved inorganic phosphorus to glyphosate (µmol/µmol) ranging between 20 and 21,040 indicate that competitive adsorption can hinder the partitioning of glyphosate and AMPA into the wetland sediment. Several studies have shown that a large portion of the removal of hydrophobic chemicals with $\log K_{ow}$ values > 3 in aquatic environments is due to the sedimentation of pesticide-laden solids. However, concentrations in the wetland sediments of flufenoxuron, cyprodinil, isoxaben, kresoxim methyl, tetraconazole and terbuthylazine could not be detected or were one order of magnitude lower than concentrations at the wetland inlet. Although aqueous photolysis of isoxaben and flufenoxuron cannot be excluded (DT_{50} = 6 days, at pH= 7), significant degradation of hydrophobic compounds in the wetland is not expected due to reduced bioavailability. Therefore, a large fraction of these contaminants passed through the stormwater wetland in association with suspended particles. Transport of pesticides-laden sediment through the wetland under high flow regime has been previously suggested to decrease the removal of hydrophobic pesticides by affecting the degree of bottom scouring and re-suspension of settled solids. However, no significant correlation was found between runoff volumes and removal rates of dissolved pesticides (i.e. DOC-laden pesticides and pesticides in the aqueous phase) observed in our study, suggesting no threshold at which removal of dissolved pesticides would be reduced at greater runoff inflow. Nevertheless, positive correlations between runoff volumes and both TSS and DOC loads at the inlet ($p < 0.001$) indicate larger particle mass transport through the wetland during large flow events. This is also under-scored by moderate load removal of cyprodinil and isoxaben, suggesting that hydrophobic compounds associated with DOC, which are taken into account in the mass balance of dissolved pesticides, were transferred through the wetland. It also has to be noted that pesticide concentrations in fall (from 01 October to 30 December 2009) ranged from not detected to 0.85 ± 0.42 µg/L (glyphosate) at the inlet, and from not detected to 0.57 ± 0.13 µg/L (AMPA) at the outlet. No significant release of pesticides with $\log K_{ow}$ value and no release of hydrophobic pesticides could be observed during fall which indicates that the most of the pesticides mass (>99.6 %) entered the wetland and passed from April to September, which correspond to the period of pesticide application (see also Table 7.5-194).

Table 7.5-194: Mean concentrations and ranges and loads estimates of dissolved pesticides at the inlet and the outlet of the stormwater wetland (Rouffach, Haut-Rhin, France) during fall

Compound	Concentration range[a]				Load[b]	
	Inlet (n^c=4)	SE	Outlet (n^c=11)	SE	Inlet (n^c=2)	Outlet (n^c=2)
	[µg L^{-1}]		[µg L^{-1}]		[mg]	[mg]
Azoxystrobin	n.d.	-	n.d.	-	0	0
Cymoxanil	n.d.	-	n.d.	-	0	0
Cyprodinil	n.d.	-	n.d.	-	0	0
Carbendazime	n.d.	-	n.d.	-	0	0
Dimethomorph	0.09 (n.d. - 0.13)	0.06	n.d.	-	1.12	0
Diuron	n.d.	-	n.d.	-	0	0
DCPU	n.d.	-	n.d.	-	0	0
DCPMU	n.d.	-	n.d.	-	0	0
3,4 DCA	0.03 (n.d. - 0.10)	0.05	n.d.	-	1.40	0
Flufenoxuron	n.d.	-	n.d.	-	0	0
Gluphosinate	n.d.	-	n.d.	-	0	0
Glyphosate	0.85 (0.40 - 1.30)	0.42	0.12 (n.d. - 0.30)	0.12	12.2	1.80
AMPA	0.58 (0.50 - 0.60)	0.05	0.57 (0.40 - 0.80)	0.13	14.9	7.20
Isoxaben	n.d.	-	n.d.	-	0	0
Kresoxim methyl	n.d.	-	n.d.	-	0	0
Metalaxyl	n.d.	-	n.d.	-	0	0
Pyrimethanil	n.d.	-	n.d.	-	0	0
Simazine	n.d.	-	n.d.	-	0	0
Terbuthylazine	n.d.	-	n.d.	-	0	0
Tertraconazole	n.d.	-	n.d.	-	0	0

[a] Between October 01 to December 30, 2009
[b] Calculated between October 13 and November 13, 2009 (dissolved loads were not calculated for the whole period (October 01 to December 30, 2009) due to intermittent freezing events starting from November 13 limiting the continuous monitoring of runoff discharge)
[c] Number of analysed samples

Conclusion

Our results provide quantitative field data of pesticide mixtures in runoff and stormwater wetlands, in both the particulate and dissolved phases, that often fail to completely evaluate the potential of best management practices (BMPs) for agricultural stormwater. To the best of our knowledge, this paper is the first investigation that reports detailed concentrations and mass balances of pesticides mixtures in a stormwater wetland collecting agricultural runoff during an entire agricultural season. The results for pesticides and some of their degradation products in this study indicate that stormwater wetlands collecting agricultural runoff have good capacities for retaining, at various flow conditions and loadings, mixtures of pesticides with different physico-chemical properties. Seasonal removal rates of dissolved loads by the wetland ranged from below 60 % (simazine, AMPA and pyrimethanil) to 100 % (cymoxanil, gluphosinate, kresosxim methyl and terbuthylazine). Our findings also underscore the crucial role of vegetation characteristics for retaining pesticides and of dissolved organic carbon for transporting hydrophobic pesticides in stormwater wetlands. Accompanied with careful guidance and planning, stormwater wetlands have the potential to serve as a tool for urban and agricultural stormwater management practices, thus contributing to the improvement of water quality for receiving aquatic ecosystems. However, the use of stormwater wetlands as a management practice targeting pesticide mitigation should not be utilized as a unique solution to treat pesticide runoff, but should rather integrate in the design of holistic approaches to stormwater management.

The present study demonstrates that the runoff regime works in concert with hydrochemical characteristics to mitigate pesticide in runoff, which should be included into design considerations of stormwater wetlands. However, further knowledge about hydrological and biogeochemical processes that alter stormwater wetlands during their lifespan is necessary to improve removal of pesticides in runoff.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a runoff experiment in a vineyard of the Alsatian area in France. The results demonstrate that storm water wetlands can efficiently remove pesticide mixtures in agricultural runoff during critical periods of pesticide application, although fluctuations in the runoff regime and hydrochemical characteristics can affect the removal rates of individual pesticides. Maximum concentrations of glyphosate and its main metabolite AMPA measured at the inlet of the catchment were 15 µg/L and 21 µg/L, respectively.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/065
Report author	Meyer, B. *et al*
Report year	2011
Report title	Concentrations of dissolved herbicides and pharmaceuticals in a small river in Luxembourg
Document No	Environmental Monitoring and Assessment, (2011) Vol. 180, No. 1-4, pp. 127-146
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facility (Department of Environmentand Agro-Biotechnologies (EVA))
Acceptability/Reliability:	Reliable

2. Full summary

Urban and agricultural areas affect the hydraulic patterns as well as the water quality of receiving drainage systems, especially of catchments smaller than 50 km^2. Urban runoff is prone to contamination due to pollutants like pesticides or pharmaceuticals. Agricultural areas are possible sources of nutrient and herbicide contamination for receiving water bodies. The pollution is derived from leaching by subsurface flow, as well as wash-off and erosion caused by surface runoff. In the Luxembourgish Mess River catchment, the pharmaceutical and pesticide concentrations are comparable with those detected by other authors in different river systems worldwide. Some investigated pesticide concentrations infringe current regulations. The maximum allowable concentration for diuron of 1.8 µg/L is exceeded fourfold by measured 7.41 µg/L in a flood event. The load of dissolved pesticides reaching the stream gauge is primarily determined by the amount applied to the surfaces within the catchment area. Storm water runoff from urban areas causes short-lived but high-pollutant concentrations and moderate loads, whereas moderate concentrations and high loads are representative for agricultural inputs to the drainage system. Dissolved

herbicides, sulfonamides, tetracyclines, analgesics and hormones can be used as indicators to investigate runoff generation processes, including inputs from anthropogenic sources. The measurements prove that the influence of kinematic wave effects on the relationship between hydrograph and chemographs should not be neglected in smaller basins. The time lag shows that it is not possible to connect analysed substances of defined samples to the corresponding section of the hydrograph.

Materials and methods

Area under investigation

Luxembourg is divided into two natural regions, the Oesling in the north (225–559 m above sea level) and the Gutland in the south (140–440 m above sea level). Hydrological measurements are conducted in the small Mess catchment in the southwestern part of Luxembourg. It is located in the Gutland region, which is characterised by a cuesta landscape where large gentile sloped valleys occur on marly substrates, contrasting with the deeply cut Luxembourg sandstone. The basin has a total surface area of 32.5 km^2 at the stream gauge. Marls and sandy marls of the sedimentary Paris basin dominate the lithology (93 % Lias bedrock, 7 % alluvials near the stream network). The marly bedrock is considered as being mostly impermeable. Luvisoils, pelosoils, planosoils, fluvisoils and gleysoils are dominating, with a silty–clayey to clayey texture. The land use in the basin consists of grassland (58 %) and arable land (22.7 %); forest is about 9.7 %, urban and industrial areas amount to 8.7 %, 2.3 % contain the road and rail network. The most widespread crops are maize, colza and winter wheat. Runoff from several roads, effluents from small industries and untreated wastewaters from solitary farms and storm drainages of the combined sewer system influence river water quality. A mechanical–biological sewage water treatment plant is located in the small village of Reckange. This purification plant is connected with 3,500 inhabitants (340,000 m^3 sewage per year). Housing areas are drained by a combined sewage water system with several storm-control reservoirs. By passing above the Ardennes massif, the dominating westerly atmospheric fluxes cause annual rainfall totals in Luxembourg exceeding 900 mm. December, January and February are the wettest months (more than 100 mm), while April, August and September are the driest months (less than 70 mm) on average. January is the coldest month (0°C) and July the warmest month (16.9°C). Monthly potential evapotranspiration values vary from 81.8 mm in July to 13.5 mm in December (Local station, 1971–2000). The runoff regime is of pluvial oceanic unimodal type, with high runoff occurring during winter (maximum runoff in February) and low runoff occurring in summer (minimum runoff in September). A meteorological station of the 'Administration des services techniques de l'Agriculture' (Agriculture Administration) is recording the most important hydro-climatological parameters, such as air temperature and humidity (both in 2 m above ground). Rainfall (1 m above ground) is measured in ten minutes intervals with a heated tipping bucket rain gauge (Lambrecht 15188). This station is located about three kilometres north of our stream gauge in the center of the catchment area. The stream gauge (ISCO 4120 flow logger, pressure probe) in the village of Pontpierre registers 15-minute average water levels. Discharge is obtained with level-to-flow conversions applying the Manning equation. In parallel, conductivity is automatically registered in 10-min intervals (WTW 3310). The mean discharge of the Mess was of 261 L/s in the year 2008, with a specific runoff of 8 L/(s km^2). During the same year a total of 253 of 804 mm rainfall had been transformed into discharge. In summer, multi-peaked flood waves, which can be traced to consecutive contributions of tributaries and the rainfall patterns, are characteristic in the catchment. Especially thunderstorms produce runoff events characteristic of a steep gradient and a relatively short outlet. Precipitation events of very small intensities and amount are indicated by small discharge peaks, which result predominantly from the runoff from impervious surface areas. The long-lasting, low intensity winter precipitation events cause singular broad discharge maxima, which are primarily composed of laterally flowing soil water and groundwater. In the Mess basin, during winter runoff events, the largest dilution mostly occurs some hours before the discharge maximum. This dilution is mainly induced by rainwater runoff from paved surface areas like streets or roofage. Furthermore, the spillways of the sewage system storm water retention basins and the sewage water treatment plant deliver larger volumes of rainwater and high quantities of diluted sewerage water.

Sampling

Two ISCO autosamplers with 2-l glass bottles (24 bottles, non-cooled) were connected to the flow logger in order to trigger the sampling after a fixed water level is reached. Subsequently, sampling is performed at

different intervals through-out the duration of the investigated events. Every sample is a spot sample and not a composite one, collected during a certain time span. A representative selection of samples has been chosen for analysis selected according to discharge and electrical conductivity (WTW 197i conductivity meter) or water colour. In total, between October 2006 and January 2010, 29 flood events were analysed with respect to nitrate–nitrogen (NO_3–N), nitrite–nitrogen (NO_2–N), chloride (Cl^-) and sulphate (SO_4^{2-}). Fourteen of these floods were additionally investigated concerning dissolved pharmaceuticals or pesticides. During base-flow conditions, grab samples were taken by hand in brown glass bottles to investigate low flow conditions before and after the flood events under investigation. In addition to the sampling described above, during March 2007 and January 2010, 36 samples were taken from the outflow of the local sewage water treatment plant of Reckange. All samples were stored at 4°C in the dark and processed immediately as described below. Concentrations of Cl^-, NO_2–N, SO_4^{2-} and NO_3–N were determined by ion chromatography (Dionex DX-500).

This investigation focuses on the analysis of four classes of veterinary and human pharmaceuticals (sulfonamides, tetracyclines, analgesics and hormones). The 12 selected pharmaceuticals include four sulfonamides (sulfathiazole, sulfamethoxazole, sulfadimethoxine and sulfamethazine), three tetracyclines (chlortetracycline, tetracycline and oxytetracycline), two analgesics (ibuprofen and diclofenac) and three hormones (estrone, ß-estradiol and 17-α-ethinylestradiol). In addition, the two degradation compounds sulfamethazine-N4-acetyl and 4′-hydroxy-diclofenac are under investigation. Furthermore, 19 herbicides belonging to various chemical classes (phenylureas, chlorotriazines, triazinones, organophosphorus and chloroacetanilides) were analysed. The phenylureas are isoproturon, diuron, linuron, metoxuron, chlorotoluron, monolinuron, metabenzthiazuron and metobromuron. From the triazines group atrazine, simazine, desethylatrazine (DEA), terbutylazine, cyanazine and sebutylazine were investigated. Considered organophosphorus herbicides are glyphosate and its main metabolite aminomethylphosphonic acid (AMPA). Metazachlor and metolachlor were chosen from the chloroacetanilide herbicide group.

Sample preparation and extraction
Surface water and wastewater were successively filtered through 3- and 1-µm glass fibre filters (Pall Corporation, Ann Arbor, USA) to eliminate the coarse suspended matter and then filtered through 0.45 µm cellulose acetate filters (Sartorius, Göttingen, Germany). For the extraction of the pharmaceuticals, the 2 l samples were acidified to pH 4 with diluted sulphuric acid solution (25 %). Afterwards, 3 ml of Na_2-EDTA 0.5 M were added per liter of water and extracted in the following 24 to 48 h to minimise degradation. All target compounds were concentrated by Solid-Phase Extraction (SPE) on polymeric cartridges (Waters Oasis® HLB, 200 mg, 6 mL) using an automated SPE workstation (Caliper Autotrace, Teralfene, Belgium). One liter of the samples was loaded on 200 mg–6 ml HLB at 10 ml/min. The sorbents were previously conditioned using 5 ml of methanol and 5 ml of Milli-Q water at pH 4. After sample loading, the cartridges were rinsed with 5 % of methanol in water (5 ml) and dried with a stream of N_2 for 15 min. The selected compounds were eluted using methanol (2 × 5ml). Extracts were concentrated with a gentle stream of N_2 and redissolved in 1 ml of a water/acetonitrile 75/25 (*v/v*) mixtures before HPLC injection.

Due to their specific chemical properties, glyphosate and its main metabolite AMPA were analysed by derivatisation with Fluorenylmethyloxycarbonyl chloride (FMOC-Cl), off-line SPE and LC-MS/MS. The derivatisation was obtained by adding 5 ml of Borate buffer (120 mM) and 7 ml of FMOC-Cl solution (2.5 mM in acetonitrile) to 50 ml of filtered sample in a 100-ml glass bottle. The mixture was left to react overnight at room temperature, then the derivatisation was stopped by adding 0.5 ml of concentrated phosphoric acid. After a dilution with DI-water, the derivatised analytes were extracted by automated off-line SPE on Waters Oasis HLB cartridges, using the above-mentioned Caliper Autotrace SPE Workstation.

LC/MS–MS analysis
The chromatographic system consisted of an Ultimate 3000 Intelligent LC system (Dionex, Sunnyvale, USA) with a binary high-pressure gradient pump HPG-3200, an automatic injector WPS-3000 and a column oven TCC-3100. For the analysis of the pharmaceuticals and hormones, the chromatographic column was a NUCLEODUR C18 GRAVITY column, 125 × 2 mm internal diameter, 3 µm particle size (Macherey Nagel, Düren, Germany). The MS–MS analyser consisted of a triple quadrupole mass spectrometer API 3200 (Applied Biosystem/MDS Sciex, Rotterdam, The Netherlands) equipped with a

Turbo Ion Spray interface (Electrospray). N_2 was used as nebuliser, curtain and collision gas. Sulfonamides, tetracyclines and diclofenac were analysed in positive electrospray ionisation mode (+ESI) while estrogens and ibuprofen were analysed separately in negative electrospray ionisation mode (−ESI). The API 3200 triple quadrupole mass spectrometer was running under Multiple Reaction Monitoring mode (MRM) for increased sensitivity, with two MRM transitions for each molecule for improved selectivity. Optimal conditions were chosen in each mode. Each compound was analysed separately by flow injection analysis, in positive and negative mode, to find the optimum parameters (voltages and gas flows) for maximum intensities. Calibration curves ranging from 1 to 100 ng/ml were used to quantify the xenobiotics. After the final calculation the majority of the substances were successfully quantified at 1 ng/L except for E2 (3 ng/L) and EE2 (6 ng/L). The choice of a single extraction method on HLB cartridges was a compromise between recovery of extraction and the ease of the method. Our method led to efficient recoveries for sulfonamides (75–85 %), analgesics (80–95 %) and hormones (80–90 %). The recovery of tetracycline group was sufficient. For the pesticides, the analytical column was a Dionex Acclaim C18 (2 × 100 mm, 3 µm particle size) and the mobile phase was a gradient of water and acetonitrile, both containing 0.1 % formic acid. The column temperature was 40 °C and the flow rate was 250 µL/min. The detection and quantification were achieved by positive electrospray MS/MS in Multiple Reaction Monitoring (MRM) mode. Each compound was detected and confirmed by two MRM transitions. The FMOC derivatives were quantified by reverse-phase chromatography coupled to a triple quadrupole. The analytical column was a Macherey-Nagel Nucleodur Gravity C18 and the mobile phase was a gradient of ACN and 10 mM ammonium acetate. The oven temperature was set at 40°C, and the flow rate was 250 µL/min. The detection was achieved in negative electrospray mode, using two transitions for each compound. For the pesticides, the limit of quantification is 1 ng/L.

Results

Concentrations of dissolved pharmaceuticals and herbicides

Despite usage restrictions and the banishment of different toxic compounds, pesticides still represent an issue in water pollution. For the EU-wide banned atrazine, the measured maximum is 118 ng/L (Table 7.5-195). All samples had atrazine concentrations well above the LOQ of 1 ng/L indicating recent use of this herbicide. Due to their broad application fields, determining the main origin of pesticides found in water streams is not always easy. Glyphosate (6,220 ng/L), AMPA (1,118 ng/L), diuron (7,410 ng/L), terbutylazine (4,038 ng/L) and metolachlor (1,140 ng/L) were the pesticides found in the highest concentrations during flood events in the Mess River. Metoxuron, cyanazine, hexazinone, sebutylazine and monolinuron have not been detected in the investigated flood events. According to Skark et al. (2004) the occurrence of herbicides such as chlortoluron, isoproturon and terbutylazine in surface water is due to agricultural application. In Luxembourg, terbutylazine and metolachlor are used in the production of maize, rape, turnip and cabbage. Isoproturon is mainly applied in the cultivation of grain. The occurrence of diuron (house paint and antifouling) and glyphosate (fruit, vegetable, not cultivated land, private gardens, parks and public areas) primarily results from their use in settlement areas. A snapshot sampling in different catchments all over the country supports these assumptions (results not shown). Corresponding distribution patterns appeared to be significantly different depending on the land-use of the river catchments. Glyphosate and AMPA were found in higher concentrations in urban basins, whereas terbutylazine, metolachlor, atrazine and DEA were prominent in rural zones. In addition, Table 7.5-195 illustrates that the pesticide concentrations in the Mess are in the same range than those detected in other river systems.

Table 7.5-195: Measured concentrations of selected dissolved herbicides in three flood events from May/June 2008 in comparison to other studies, detection limits and limits of quantification

Substance	*n*	*n* (>LOQ)	Maximum value (ng l^{-1})	Mean (*n* > LOQ) (ng l^{-1})	St. dev. (*n* > LOQ) (ng l^{-1})	Detection limit (ng l^{-1})	Limit of quantification (ng l^{-1})	Maximum values of other surface water studies (ng l^{-1})
Glyphosate	28	28	6,220	1,650	1,638	0.5	1	820[c]
Diuron	19	19	7,410	683	1,652	0.5	1	310[g], 1,600[b]
AMPA	28	28	1,118	599	293	0.5	1	1,423[c]
Terbutylazine	28	28	4,038	519	1,073	0.5	1	570[g], 2000[d], 240[g]
Metolachlor	28	28	1,140	251	340	0.5	1	6,700[f], 270[g]
Metabenzthiazuron	28	28	990	96	222	0.5	1	
Isoproturon	28	28	1,040	74	195	0.5	1	7,800[a], 0[b], 2,000[d]
Atrazine	28	28	118	9	22	0.5	1	10,100[a], 0[b], 9,300[d], 630[e], 14,170[f], 410[g]
Linuron	28	10	20	9	6	0.5	1	1,800[d]
Simazine	28	17	38	9	11	0.5	1	0[b], 300[d], 294[e], 310[g]
Metazachlor	28	27	35	7	7	0.5	1	5,100[d]
DEA	28	28	6	3	1	0.5	1	600[g]
Chlortoluron	28	4	3	2	1	0.5	1	
Metobromuron	28	1	2	2	–	0.5	1	
Cyanazine	28	0	–	–	–	0.5	1	2,800[d]
Hexazinon	28	0	–	–	–	0.5	1	
Metoxuron	28	0	–	–	–	0.5	1	
Monolinuron	28	0	–	–	–	0.5	1	
Sebutylazine	28	0	–	–	–	0.5	1	

LOQ limit of quantification

[a] Wittmer et al. (2010), Headwater catchment, Switzerland

[b] Gasperi et al. (2008), Combined sewer Paris, France

[c] Botta et al. (2009), Boëlle, France

[d] Kreuger (1999), Vemmenhög, Sweden

[e] Cerejeira et al. (2003), Tejo, Portugal

[f] Ng et al. (1995), Missouri Creek, Canada

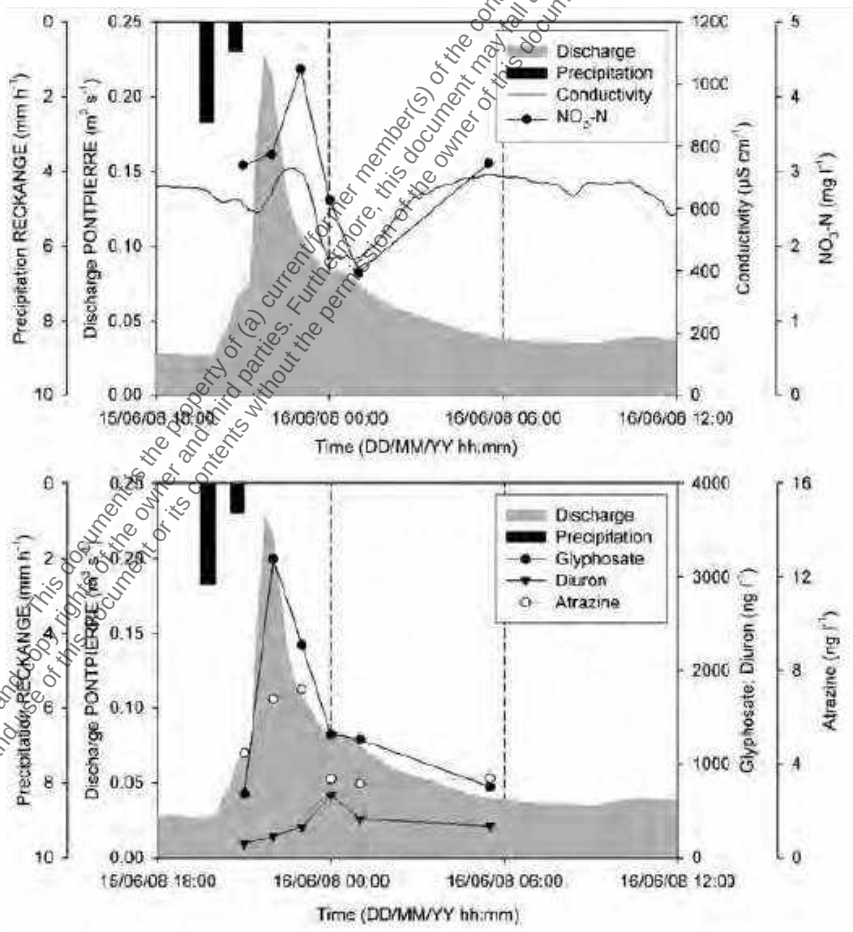
[g] Hildebrandt et al. (2008), Ebro basin, Spain

Chemographs of dissolved herbicides during flood events

Several flood waves with different precipitation intensities and runoff ratios have been investigated and sampled in early summer 2008, a main application period of herbicides in the area under investigation. Three events have been selected according to different precipitation intensities for a further thorough analysis. The following results in Figures 7.5-169, 7.5-170 and 7.5-171 are presented in the order of increasing flood intensity.

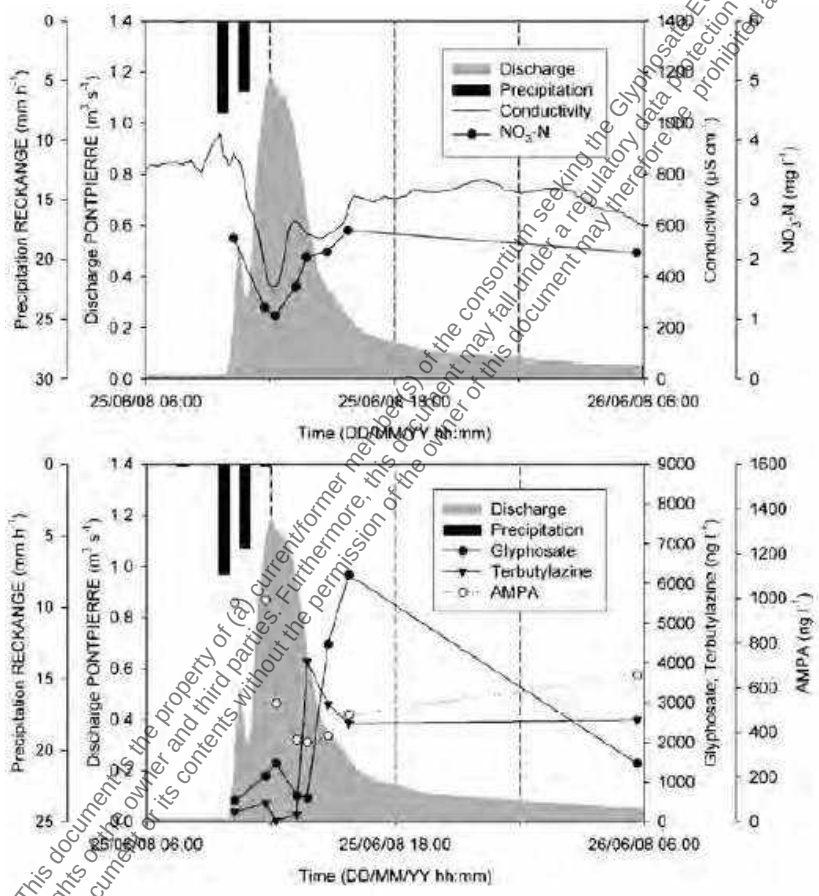
The flood event of 15 June 2008 is characterised by low rainfall (3.5 mm), low precipitation intensities (max. 1.2 mm/10 min) and a small runoff ratio (2.4 %; Figure 7.5-168). At 10 p m., a single peak of dissolved glyphosate (3,000 ng/L) is observed, originating from the vicinity of the gauging station, including the motorway crossing the Mess River approximately 150 m upstream and the village of Pontpierre. The local department of highways, the municipal administrations and private house owners apply this herbicide for weed removal at roadsides. A peak of atrazine (8 ng/L) is registered 1 h later together with increasing NO_3–N (4.5 mg/L) and the maximum of a small conductivity peak. This runoff component from agricultural sources is followed by peaking diuron concentrations (700 ng/L) originating from runoff from the settlement area of Reckange. This peak goes in parallel with declining conductivity, indicating dilution with low mineralised rainwater, which is supposed to be flushed from impervious surfaces in the relevant village.

Figure 7.5-168: Dissolved NO_3–N, glyphosate, diuron, atrazine and conductivity measured during the flood event in the Mess River catchment on 15 June 2008



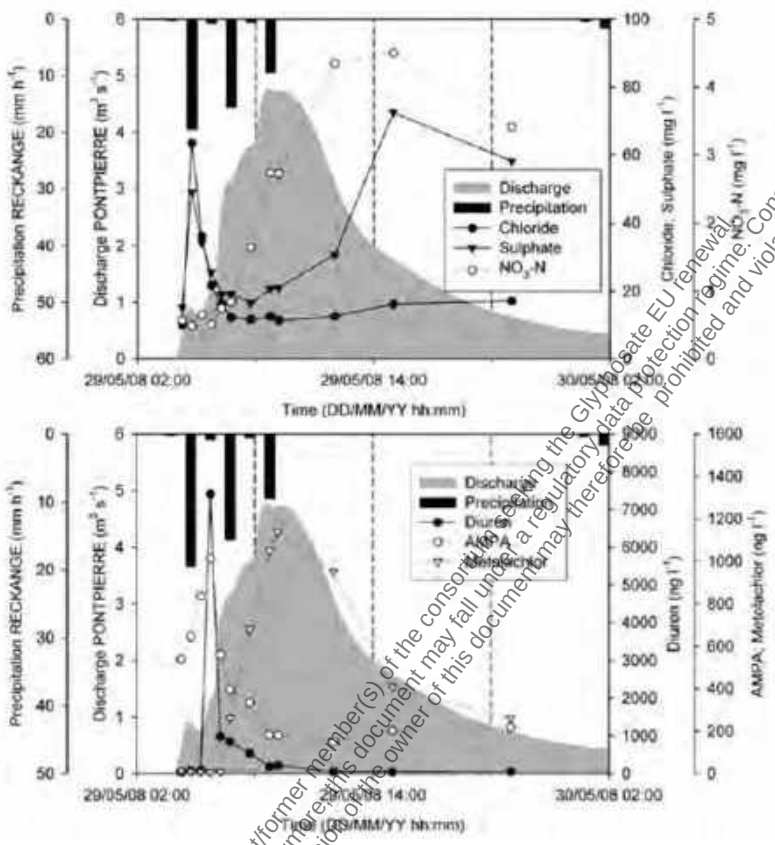
The flood event of 25 June 2008 (Figure 7.5-169) is characterised by a higher rainfall (13.7 mm), higher precipitation intensities (4.3 mm/10 min) and a higher runoff ratio (3.7 %) than the first flood event on 15 June 2008. The first concentration peak of glyphosate (1,500 ng/L) at 12 a m. originates from the vicinity of the stream gauge mainly from the town of Ehlange. Between the flood events on 15 June (Figure 7.5-168) and 25 June (Figure 7.5-169), pesticides have again been applied in the catchment area, which is indicated by a late distinct glyphosate peak (6,000 ng/L) in the falling limb (3 p m.). AMPA shows a dilution curve in parallel to peaking discharge, but this concentration decrease is shifted 2 h after the discharge peak. The highest concentrations of terbutylazine (4,000 ng/L) are measured when AMPA exhibits the biggest dilution; this water mainly originates from the agricultural surroundings of Reckange.

Figure 7.5-169: Dissolved NO_3–N, glyphosate, terbuthylazine, AMPA and conductivity measured during the flood event in the Mess River catchment on 25 June 2008



The flood event of 29 May 2008 (Figure 7.5-170) is characterised by the highest rainfall intensities (10.1 mm/10 min) and the highest runoff ratio (9.6 %) from the selected flood events. It shows a clear succession of different runoff components. The first discharge originates from impervious areas near the stream gauge, shown by a first small discharge peak with high concentrations of dissolved chloride (flushed atmospheric deposition material), sulphate (weathering material) and glyphosate (5,075 ng/L, not shown). In the following rising limb, the sewer overflows of Reckange leads to high AMPA (1,100 ng/L) and diuron (7,000 ng/L) concentration peaks, which are diluted afterward by the main discharge peak. Simultaneously, isoproturon (1,040 ng/L) and atrazine (118 ng/L) concentrations rise. Some hours later, a further runoff component contains surface runoff from arable land highlighted by an increase of the metolachlor concentration up to 1,200 ng/L. A distinct NO_3–N curve indicates the soil water component followed by a late peak of dissolved sulphate representing the final groundwater component. Sulphate originates from gypsum layers and gypsum pockets incorporated in the local bedrock.

Figure 7.5-170: Dissolved chloride, sulphate, NO_3–N, diuron, AMPA and metolachlor measured during a flood event in the Mess River catchment on 29 May 2008



Substance loads and event mean concentrations

The load of different substances has been calculated by multiplying substance concentrations with corresponding discharge values. The load of a single flood is the total of these products and equals the area of the time series plotted against the multiplication results between discharge and substance concentration. The Event Mean Concentration (EMC) is a flow-weighted average of the constituent concentration. For an individual storm runoff event, it is defined as the total pollutant load divided by total runoff volume. Table 7.5-196 shows the loads and the EMC of different compounds calculated for the three flood events. With increasing precipitation amount and intensity, the runoff ratio increases (2.4 %, 3.6 %, 9.8 %). Nutrient loads and loads of sulphate and chloride exhibit a strong relationship to discharged volume. The EMC of chloride decreases with rising runoff ratios, which is an indication of the lower importance of surface runoff from paved areas like rooftops or streets in stronger rainfall runoff events. On the contrary, NO_3–N exhibits the highest EMC in the biggest flood just as the EMC of metolachlor or isoproturon. This indicates a higher proportion of surface runoff from arable land and higher proportions of soil water in general. The EMCs for glyphosate and AMPA are elevated in smaller floods originating mainly from urban storm water runoff, running directly into the brook. High EMC values in this flood event of 25 June are caused by repeated applications of terbutylazine and glyphosate before the event. Furthermore, a smaller AMPA/glyphosate ratio is an indication for "fresh glyphosate sources" with only a small amount of AMPA as the relevant degradation compound. However, the study of Botta *et al.* (2009) suggests that sewage from domestic activities with cleaning agents are likely to be another source of AMPA. Here, further investigations are necessary. In total, the herbicide loads confirm the outcome of investigations by Skark et al. (2004) who concluded that non-agricultural pesticide use contributed more than two thirds of the whole observed pesticide load in the tributaries and at least one third in the River Ruhr.

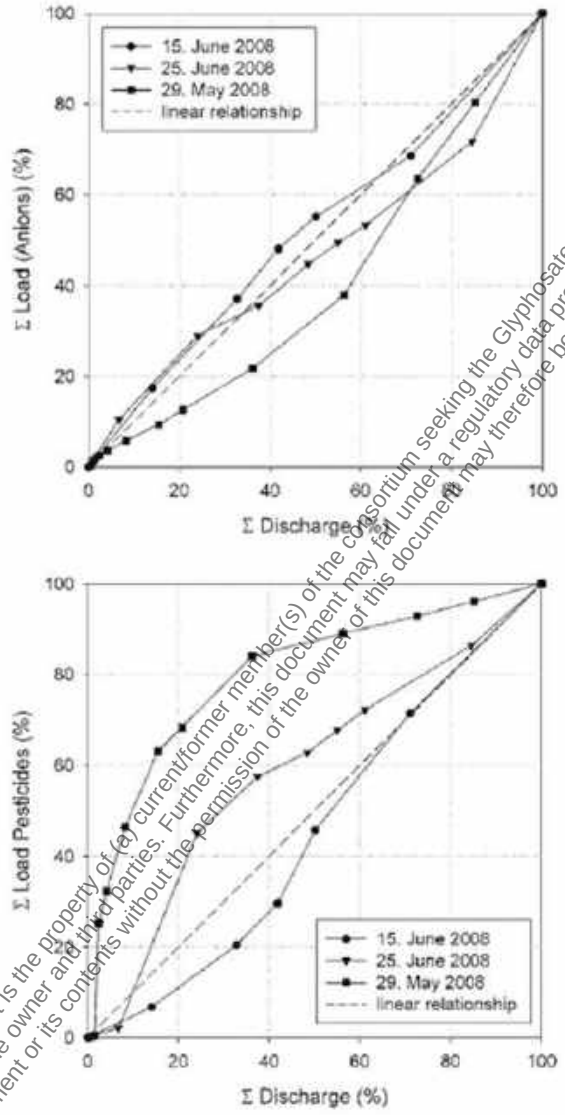
Table 7.5-196: Hydro-climatological characterisation, chemical loads and corresponding event-mean concentrations of three flood events in the Mess River catchment from May/June 2008

	15.6.2008		25.6.2008		29.5.2008	
Precipitation	Rainfall (mm)	Maximum intensity (mm 10 min^{-1})	Rainfall (mm)	Maximum intensity (mm 10 min^{-1})	Rainfall (mm)	Maximum intensity (mm 10 min^{-1})
	3.5	1.2	13.7	4.3	46.2	10.1
Discharge	Runoff (m^3)	Runoff ratio (%)	Runoff (m^3)	Runoff ratio (%)	Runoff (m^3)	Runoff ratio (%)
	2,700	2.4	15,000	3.6	147,000	9.8
Anions	Load (kg)	EMC (g m^{-3})	Load (kg)	EMC (g m^{-3})	Load (kg)	EMC (g m^{-3})
SO_4	134	50	606	40	6,063	41
Cl	97	36	350	23	2,149	15
NO_3-N	8	2.9	28	1.8	483	3
NO_2-N	0.4	0.1	2.6	0.2	12	0.1
Pesticides	Load (mg)	EMC (µg m^{-3})	Load (mg)	EMC (µg m^{-3})	Load (mg)	EMC (µg m^{-3})
Glyphosate	4,038	1,496	37,668	2,502	68,741	467
AMPA	2,068	764	9,182	610	33,792	230
Diuron	897	331	Not meas.	Not meas.	34,004	231
Metabenzthiazuron	261	96	569	38	57,711	392
Terbutylazine	258	95	23,277	1,546	2,658	18
Metolachlor	213	79	4,073	271	91,284	621
Isoproturon	56	21	252	17	7,951	54
Atrazine	12	4	84	6	949	6
Metazachlor	8	3	135	9	1,291	9
AMPA/Glyphosate ratio	0.51		0.24		0.49	

Discussion

The results show that comparable to other studies (Wittmer *et al.* 2010; Pailler *et al.* 2009a), a distinct relationship between discharge and pollutant concentrations does not exist for pharmaceuticals or for pesticides. The variable dependence of xenobiotic concentrations to event specific conditions and processes is discussed in the following sections. Many studies have described the first flush phenomenon as a relatively high load of pollutants in the first part of runoff events. In contrast, the kinematic wave effect results in a postponement of pollutant loads in comparison to associated discharge. Lee and Bang (2000) concluded that the pollutant concentration peak occurs before the flow peak in watersheds with areas smaller than 100 ha, and the pollutant concentration peak is followed by the flow peak in the watersheds with areas larger than 100 ha. The investigation of first flush effects and kinematic wave effects is done by drawing the curve (Figure 7.5-171) that gives the variation of the cumulative pollutant mass divided by the total pollutant mass (dimensionless cumulative pollutant mass) in relation to the cumulative volume divided by the total volume (dimensionless cumulative runoff volume).

Figure 7.5-171: Dimensionless cumulative runoff volume and runoff mass curves for measured anions (Chloride, NO_3–N, NO_2–N, Sulphate) and selected pesticides (isoproturon, atrazine and diuron) supposed to be flushed from impervious surfaces



If the concentration remains constant during the storm event, the pollutant mass is proportional to the volume and the double frequency cumulating curve follows the line of origin with a gradient of one (Line of Identity). If the data for a particular storm lies above this, a first flush is suggested. If the curve falls below the Line of Identity, the main substance load is observed coming after the discharge peak. This can be caused by the kinematic wave effect, the later arrival of compounds originating farer away from the gauging station or a late reaction of deeper soil or groundwater components. Figure 7.5-171 highlights that in the flood event with the lowest precipitation intensity, measured anions are not important and the curve goes along the Line of Identity. In the bigger events, the late soil water component with measured anions is more important. Therefore, this line lies under the Line of Identity. In contrast, the cumulative load curves of the selected pesticides lay about this line. The maximum divergence was used as a measure of the magnitude of the first flush. A significant first flush was considered to have occurred in the biggest event on 29 May 2008. The presence of accumulated materials on the surfaces tends to be responsible for the first flush phenomenon of herbicides.

The results confirm the investigations by Skark *et al.* (2004), who concluded that pathways for pesticide input to the receiving waters were related to both, surface runoff and underground passage. Two thirds of the observed diuron load in the surface water resulted from an input by direct runoff. The corresponding spills cause high but short-lived concentration peaks. The authors interpreted this as a result of total pesticide application to impervious surfaces. As a consequence, the high corresponding concentrations in the tributary infringe current regulations and recommendations. The directive 2008/105/ EC of the European Parliament and of the Council on Environmental Quality Standards in the field of water policy contains environmental quality standard parameters. The maximum allowable concentration for diuron of 1.8 µg/L is exceeded fourfold by measured 7.41 µg/L in the flood event of 29 May 2008. The determination of the impact of storm water runoff from settlement areas can greatly increase the predictive power of models of urban effects on water quality. In addition, the results show that like Hatt *et al.* (2004) demonstrated, very small proportions of impervious area are capable of increasing pollutant concentrations, as long as there is a direct connection between the impervious area and the corresponding stream. Consequently, the aim must be to break the direct linkage between the impervious areas and the receiving water.

Furthermore, it seems that for some compounds the antecedent conditions before flood events, such as precipitation quantities, results in an exhaustion of potential sources, so that less material is available to be washed off in subsequent events. Kim *et al.* (2006) and Krein and Schorer (2000) show similar results for dissolved and particle bound pollutants. An example is the short succession of the three thunderstorms with high precipitation amounts, which induced the flood event in the Mess River on 29 May 2008 (Figure 7.5-170). Areas directly connected to the Mess River are flushed by the first event and the second and the third thunderstorms do not mobilise further dissolved diuron, AMPA or chloride. These com-pounds show distinct peaks after the first rainfall event and no reaction thereafter.

Conclusion

Overall, the pharmaceutical and pesticide concentrations in the Mess are comparable with those detected by other authors in different river systems. Some investigated pesticide concentrations in the tributary temporarily infringe current regulations. The analysis of flood events using rainfall pattern, hydrograph and dissolved xenobiotic chemographs can provide a detailed insight into the temporal structure of flood events. However, the corresponding anthropogenic sources show a temporal and spatial variability, caused by different rainfall patterns and distributions as well as different characteristics (e.g. retention capacities) of the sewer systems. The discharge increase from anthropogenic sources is mainly brought about by overlandflow, the influx of surface water from the road network, as well as from residential areas. It is difficult to postulate that recurring characteristics of the processes control the xenobiotics chemographs, due to highly variable anthropogenic factors. These are the changing amount of pharmaceutical consumption, sewage water treatment plant control programs, pesticide application dates and amounts, or the heterogeneous urban storm water runoff generation. Furthermore, hydraulic processes within current flood waves like kinematic wave effects influence the event structure e.g. time lags between discharge and dis-solved loads. The load of dissolved pesticides reaching the stream gauge is primarily determined by the amount applied to the surfaces within the catchment area. In the Mess River catchment, a characteristic difference between urban and agricultural induced pollution by pesticides exists in the concentration/load relationship. Storm water runoff from urban areas causes short-lived but high-pollutant concentrations and moderate loads in the Mess River, whereas moderate concentrations and high loads are representative for agricultural inputs to the drainage system. Non-agricultural pesticides contribute to a large part to the observed pesticide loads in the Mess.

Generally, kinematic wave effect, accumulation, exchange, dilution and mixture processes modify the flood wave and its composition within the watercourse. The measurements prove that the influence of kinematic wave effects on the relationship between hydrograph and chemographs should not be neglected in smaller basins. The time lag shows that it is not always possible to connect analysed substances of defined samples to the corresponding section of the hydrograph. The different velocities indicate that after the substances have been transported over several hundred meters, there is no relationship between those parameters. Consequently, classification be-tween discharge component and dissolved substances at the sampling points is impeded. These results highlight that simple rating curves between discharge and pollutant loads

intended to calculate the total load by hydrographs are overly simple. At the Mess River, even the position of the gauging station is important, because the time lag between chemical signal and discharge increases over distance.

However, every flood event is unique due to variable rainfall characteristics, changing catchment conditions, as well as anthropogenic activities. The next step is the investigation of long lasting, low intensity winter precipitation events that cause singular broad discharge maxima, which are primarily composed of laterally flowing soil water and groundwater.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a monitoring study in an agricultural area in Luxembourg. The study design and the analytical methods are well described. The highest concentration of glyphosate was 6.22 µg/L and for AMPA was 1.118 µg/L.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/066 CA 7.5/067 (Translation)
Report author	Busetto, M. *et al.*
Report year	2010
Report title	Survey of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province
Document No	Il bolletino 2010/4
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary

During the period 2006-2009 ARPA (the Lambardy Regional Environmental Agency) has been collecting analytical data concerning the presence and concentration of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in the water of the Lambro, Seveso and Terrò rivers in the Brianza region. River flow-rate, COD, BOD_5 and conductivity have also been measured in each sample.

Both AMPA and glyphosate have been found in every sample, with AMPA concentrations always higher than glyphosate concentrations. Larger amounts of herbicide have been detected in water sampled in autumn, with concentrations decreasing in the following months. Our data are consistent with the available information about the use and release of the herbicide during the year.

Materials and Methods

Monitoring in the Lombardy Region and Purpose of the Study
In pursuing the objective envisaged in the "National Plan for the Control of Environmental Effects of Plant Protection Products" to adjust controls on the basis of substances actually used in its territory, the Lombardy Region has included glyphosate and AMPA among the compounds to be periodically monitored in its waters. The ARPA Department of Monza was entrusted with the task of monitoring the possible presence of glyphosate and ammonium methylphosphonic acid in the Lambro and Seveso waterways, in the area around the capital of the province of Monza-Brianza, by relying on measuring and sampling stations assigned as follows:

- Lambro river (Stations of Lesmo and Cologno Monzese);
- Seveso creek (Stations of Lentate sul Seveso and Bresso).

Samples were collected at periodic intervals, in the months of March, June, September and December during the 2007-2009 three-year period. An additional sampling point was added in 2009 along the Terrò creek, near Cesano Maderno, in the proximity of the confluence into the Seveso river. The results obtained are detailed in this article. The concentrations of glyphosate and AMPA, as well as their trends recorded during the three-year observation period detected on the samples analyzed were compared with some parameters (Flow, Conductivity, COD and BOD_5) characteristic of the watercourses under study. In March 2010, the data relating to glyphosate and AMPA became available also for the three waterways relating to the collection points of Lesmo, Lentate and Cesano Maderno.

The Lambro River and the Seveso and Terrò Creeks

The Lambro river originates in the territory of the municipality of Magreglio (Como), continues towards Vallassina and feeds the lake of Pusiano. It reaches Brianza by flowing at the foot of morainic hills, where it collects the waters of numerous streams, irrigation ditches and small lakes of the Brianza area.

It quickly reaches the city of Monza through the homonymous park. It continues its course east of Milan, in the low Lombard plain, until it enters the Po river. In the stretch of river between Lesmo and Cologno Monzese, the Lambro river has relatively constant average flow values between 3 m^3/s (Lesmo) and 5-10 m^3/s (Cologno Monzese); however, frequent flood phenomena related to rainfall may bring about notable flow fluctuations. The analytical findings relating to macro-descriptors, which represent the state of health of stream waters, point to a marked deterioration of the river waters downstream of the city of Monza, with a transition of the environmental quality from sufficient (Lesmo station) to poor (Cologno Monzese station). However, it should be noted that the parameters measured in the latter station are influenced by the water contributions of the purification plant of the Consorzio di Bonifica dell'Alto Lambro, which discharges treated water a few tens of meters upstream of the Cologno Monzese sample collection point.

The Seveso creek originates at the foot of Mount Pallanza (province of Como), near the Swiss border, and dumps into the Naviglio della Martesana within the urban circle of the city of Milan. In the first section of its course, the creek flows through a hilly area, passing through inhabited places of modest size and relatively distant from each other. In the valley area, the Seveso creek seamlessly crosses broad urban centers, consequently behaving much like a sewer. The control stations of Lentate and Bresso are located, respectively, at the end of the mountainous stretch and downstream of the main industrial areas and urban settlements in the western sector of Brianza (Cesano Maderno and Varedo). In this stretch, the flow rates observed are modest, even with respect to the measured values of the Lambro, with average values between 0.5-1.0 m^3/s, and peaks up of about 9.0 m^3/s in periods of swells, without remarkable differences between the stations of Lentate and Bresso. The state of health of the creek is quite compromised: macro-descriptors show a change in the environmental quality from poor (Lentate station) to bad (Bresso station).

The Terrò creek is indebted to the union of several streams in the area of the morainic hills between Cascina Inchigollo and Cascina Cassinazza, collecting rainwater and some springs. After a journey of about 20 kilometers, it flows into Seveso creek. In its terminal part, crossing markedly anthropized and industrialized areas (Mariano Comense and Meda), it undergoes a marked deterioration as it pertains to water quality, which practically becomes sewage. The flow rates are minimal in dry periods, in the absence of rainfall.

AMPA and Glyphosate were determined by HPLC equipped with a fluorescence detector, in accordance with the MTMI604 Rev.0 method.

Results

Table 7.5-197 shows the Flow, COD, BOD_5 and Conductivity values detected in water samples of the Lambro river, collected from the Lesmo and Cologno Monzese stations.

The values obtained do not differ, in terms of average and maximum values, from those published in previous years concerning the health of rivers north of Milan.

Table 7.5-197: Flow rate, COD, BOD_5 and Conductivity values detected in Lambro river at the Lesmo and Cologno Monzese stations, during 2007-2009

Date of sampling	Flow Capacity (m^3/s)		COD (mg/L)		BOD_5[1] (mg/L)		Conductivity (µS/cm)	
	Lesmo	Cologno Monzese	Lesmo	Cologno Monzese	Lesmo	Cologno Monzese	Lesmo	Cologno Monzese
Mar-07	5.2	16.3	70	74	8	6	--	--
Jun-07	5.9	14.4	29	37	<2	12	400	--
Sep-07	0.9	3.8	10	31	8	5	552	975
Dec-07	2.8	5.0	18	42	<2	5	536	869
May-08	3.0	5.3	17	31	<2	3	478	810
Jun-08	13.7	13.9	14	28	2	2	390	468
Sep-08	--	--	16	27	<2	3	509	852
Dec-08	6.5	12.0	15	74	2	8	448	765
Mar-09	4.8	7.5	11	16	<2	2	446	630
Jun-09	2.6	6.5	18	41	2	7	448	665
Sep-09	0.4	4.1	15	75	<2	3	492	924
Dec-09	2.3	6.3	13	48	<2	9	485	731
Value avg	**4.4**	**8.6**	**21**	**44**	**3**	**5**	**471**	**767**

[1] A value of 2 was assumed in calculating the average value where the measured concentration was <2

The increase in COD and BOD_5 values show the deterioration of the environmental quality of the river as it passes through the city of Monza, as a result of the discharge of civil waste, which determines the organic pollution of waters. The increase in conductivity is also remarkable and testifies to a significant contribution of ionic products in the deterioration of water quality. For example, a significant increase in the concentration of nitrates had previously been reported at the point the river transits between the Stations of Lesmo and Cologno Monzese, and had been related to the purification processes that take place upstream of the latter station, in the of Treatment Plant of Upper Lambro.

Taking into consideration the data relating to the individual stations, variances over time of the different parameters do not seem to be influenced by the seasons; in fact, the same months in subsequent years yield differing values. In particular, variances in flow rates are probably not so much related to season effects, which occur with a certain periodicity, but rather by the extent of rainfall recorded during the sampling period.

Likewise, Table 7.5-198 shows the values yielded on the samples of the waters of the Seveso creek collected from the Lentate and Bresso stations. The high concentrations of COD and BOD_5 confirm the poor water

quality, with values comparable to each other throughout the course of the stream monitored by the ARPA of Monza. As regards conductivity, similar parameter values are observed in water samples collected from the Lentate and Bresso stations. This trend testifies to considerable pollution, due to ionic substances, which is greater not only than the values yielded by samples of the Lambro river collected at the Lesmo station, but also than those measured at the Cologno Monzese station. Just like the Lambro, for the Seveso too changes in values with respect to the monitored parameters do not seem to show trends over time linked to seasonal phenomena. The flow rates are much lower than those recorded for the Lambro. Only on particular occasions are the maximum values observed at the Lentate Station comparable to the average values calculated for the Lambro.

Table 7.5-198: Flow rate, COD, BOD_5 and Conductivity values detected in the Seveso river at Lentate and Bresso stations, during 2007-2009

Date of sampling	Flow Capacity (m^3/s)		COD (mg/L)		BOD_5[1] (mg/L)		Conductivity (µS/cm)	
	Lentate	Bresso	Lentate	Bresso	Lentate	Bresso	Lentate	Bresso
Mar-07	0.6	1.0	55	57	<2	8	--	--
Jun-07	2.1	2.3	31	28	6	9	--	--
Sep-07	0.5	1.1	50	37	11	11	1250	894
Dec-07	0.5	1.0	45	33	<2	<2	1151	1082
May-08	0.7	1.2	57	59	2	2	1089	1123
Jun-08	--	1.6	26	26	<2	<2	712	643
Sep-08	0.5	0.5	40	31	<2	5	1559	762
Dec-08	4.0	1.1	24	37	5	8	437	349
Mar-09	1.5	1.4	30	30	3	6	783	769
Jun-09	1.2	--	28	28	7	6	742	492
Sep-09	0.5	0.9	22	31	<2	3	1165	1053
Dec-09	4.5	1.0	22	25	<2	4	924	904
Value avg	**1.5**	**1.1**	**36**	**35**	**4**	**5**	**981**	**807**

[1] A value of 2 was assumed in calculating the average value where the measured concentration was <2

Table 7.5-199 shows the Flow, COD, BOD_5 and Conductivity values found on the samples collected from the Terrò creek, in the proximity of Cesano Maderno in 2009 only, the period in which the ARPA of Monza began its monitoring activities. The values yielded show a relatively constant trend of the parameters over the months in which monitoring was conducted, highlighting a high degree of pollution of organic nature, pertaining to ionic products. The flow rates are very limited, with values at almost zero in the winter months. Regarding the presence of glyphosate herbicide and the AMPA degradation product, the relevant data are detailed in Tables 7.5-199, 7.5-200 and 7.5-201. All the three waterways sampled show average values of concentrations higher than 0.1 µg/L, which are comparable with data relating to the presence of these pollutants reported in the relevant literature.

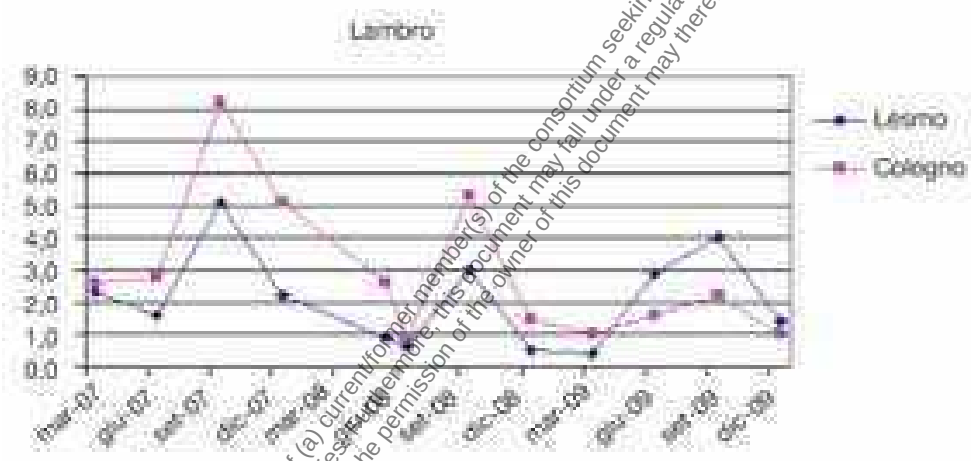
The AMPA/Glyphosate ratio in all the cases under study is skewed in favor of the degradation product, in accordance with the half-life times of the two compounds. Hence the accumulation of aminomethylphosphonic acid in the environment. Figure 7.5-172 shows trends relating to the sum of the concentrations of aminomethylphosphonic acid and the parental product measured on water samples collected from the Lambro river, in the Lesmo and Cologno Monzese stations. Unlike what has emerged for COD, BOD_5 and Conductivity, the trend of concentrations is dependent on the period in which sampling was performed.

Table 7.5-199: Flow rate, COD, BOD_5 and Conductivity values detected in the Terrò creek near Cesano Moderno during 2009

Date of sampling	Flow Capacity (m^3/s)	COD (mg/L)	BOD_5 [1] (mg/L)	Conductivity (µS/cm)
	Cesano Maderno	Cesano Maderno	Cesano Maderno	Cesano Maderno
Mar-09	0.2	25	3	788
Jun-09	0.3	34	4	750
Sep-09	0.2	25	<2	859
Dec-09	0.1	35	2	1002
Value avg	**0.2**	**30**	**3**	**850**

[1] A value of 2 was assumed in calculating the average value where the measured concentration was <2

Figure 7.5-172: Variations in the sum of AMPA and glyphosate concentrations observed on samples of water of the Lambro river, collected from the Lesmo and Cologno Monzese stations, during the 2007-2009 three-year period.



During the three-year monitoring period, maximum values were observed in September, with a subsequent decrease in values in the winter period. Data yielded are consistent with the methods of use and release of the herbicide during the course of the year. Glyphosate is applied to foliage during the growth period of the plant (spring and summer); subsequently, it is released and accumulates in the ground, where it undergoes partial degradation into AMPA. The two compounds are therefore washed out and transported to the waterways by the abundant rainfall that generally occurs in late summer and early autumn.

Sampling also shows greater concentrations of AMPA in samples collected at the Cologno Monzese station, downstream of the city of Monza. This phenomenon could be explained on the basis of a greater use of the herbicide in the area of the homonymous park, which is crossed by the river, and attributed to the purification processes that take place upstream of the Cologno Monzese station, in the Consortium Purification plant of the Upper Lambro (use of phosphonate-based additives). The values measured in September 2009 seem to counter this trend, highlighting an inversion between the concentrations of Lesmo and those of Cologno. The presence of the consortium plant could also be responsible for this anomaly, being the plant able to perform the dual function of removing polluting compounds from waste water, by adsorption by the treatment sludge, and to promote the formation of AMPA from additives used during the cleaning processes.

Depending on the operating mode of the plant, one of the two processes may conceivably prevail, thus altering the concentration trends. Figure 7.5-173 shows trends yielded by the sum of the concentrations of

aminomethylphosphonic acid and the parental product measured on water samples collected from the Seveso creek, at the Lentate and Bresso stations. Similarly to the results yielded by the Lambro river, during the three-year monitoring period, maximum concentration values were observed in the months of September, with subsequent decrease in the winter and spring period. In this case too, there are no correspondences among COD, BOD_5 and Conductivity values. The concentration values are comparable for the entire stretch of the stream monitored by the ARPA of Monza. Pollution by AMPA and glyphosate due to the path of the stream in the hilly area richest in vegetation (upstream of the Lentate station) are not subject to significant changes at the point in which the Seveso creek transits through an area of high urban density and numerous industrial sites (stretch between the Lentate and Bresso Stations). The comparison between the concentrations monitored on the samples of the Lambro river with respect to those collected from the Seveso creek highlights a more marked degree of pollution of the latter. The average values for the sum of the concentrations of the two products are 9.1 µg/L and 7.1 µg/L, respectively in the Lentate and Bresso samples, compared to 2.1 µg/L and 2.9 µg/L for samples collected at the Lesmo and Cologno Monzese stations. The maximum values observed in the months of September fluctuate between 16.2-14.1 µg/L (Lentate) and 15.1-6.1 µg/L (Bresso), against 5.1-3.0 µg/L (Lesmo) and 8.2-2.2 µg/L (Cologno Monzese). Even the minimum values of the concentrations, which for both watercourses are those measured on samples collected at the end of winter or in the spring, are higher for the Seveso Station than for those of the Lambro point of collection. Although the Terrò creek was sampled only during 2009, it can be said that the trends observed (Figure 7.5-174) follow the same evolution as those recorded on the two main waterways: also in this case, the maximum concentrations of the two products are recorded at the end of summer and early autumn, at the end of the period of application of the herbicide.

Figure 7.5-173: Variations in the sum of AMPA and glyphosate concentrations observed in water samples of the Seveso creek, collected at the Lentate and Bresso stations, during the 2007-2009 three-year period

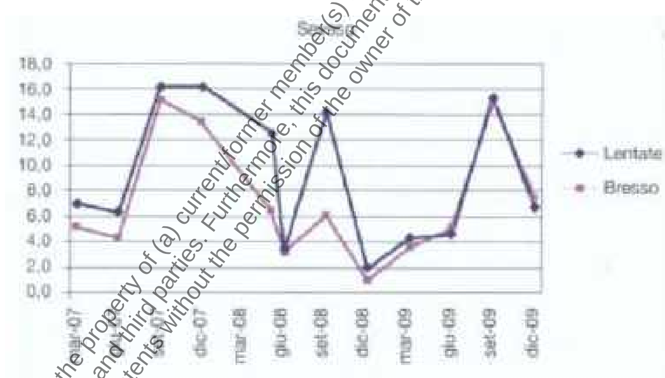


Figure 7.5-174: Variations in the sum of AMPA and glyphosate concentrations detected in the water samples of the Terrò creek, collected near Cesano Maderno during 2009

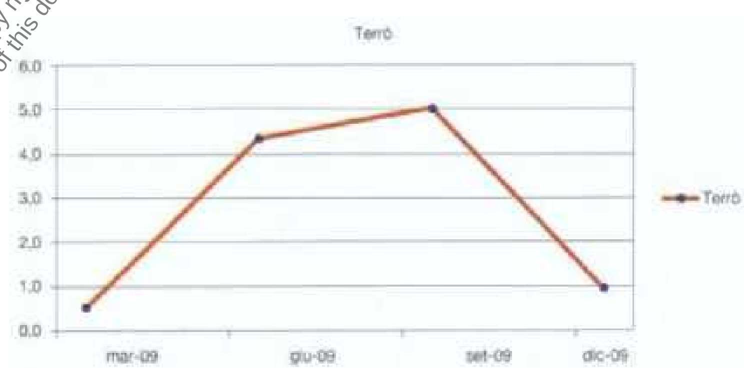


Table 7.5-200: Values relating to the concentrations of AMPA and glyphosate found in the Lambro, at the Lesmo and Cologno Monzese Stations during 2007-2009

LESMO STATION[1]			
Date of sampling	**AMPA (µg/L)**	**Glyphosate (µg/L)**	**Sum (µg/L)**
Mar-07	2.2	0.1	2.3
Jun-07	1.1	0.5	1.6
Sep-07	5.0	<0.1	5.1
Dec-07	1.7	0.5	2.2
May-08	0.7	0.2	0.9
Jun-08	0.5	<0.1	0.6
Sep-08	2.9	0.1	3.0
Dec-08	0.4	<0.1	0.5
Mar-09	0.3	<0.1	0.4
Jun-09	1.7	1.2	2.9
Sep-09	3.3	0.7	4.0
Dec-09	1.3	<0.1	1.4
Value avg	1.8	0.3	2.1
AMPA/Glyphosate ratio		6.0	
COLOGNO MONZESE STATION[a)]			
Date of sampling	**AMPA (µg/L)**	**Glyphosate (µg/L)**	**Sum (µg/L)**
Mar-07	2.4	0.2	2.6
Jun-07	2.3	0.5	2.8
Sep-07	7.7	0.5	8.2
Dec-07	4.7	0.4	5.1
May-08	2.1	0.5	2.6
Jun-08	0.7	0.2	0.9
Sep-08	4.9	0.4	5.3
Dec-08	1.3	0.2	1.5
Mar-09	0.9	<0.1	1.0
Jun-09	1.0	0.6	1.6
Sep-09	1.7	0.5	2.2
Dec-09	0.7	0.3	1.0
Value avg	2.5	0.4	2.9
AMPA/Glyphosate ratio		6.2	

[1] A value of 1 was assumed in calculating the average value and the sums of AMPA and glyphosate concentrations where the measured concentration was <1.

Table 7.5-201: Values relating to the concentrations of AMPA and glyphosate found in the Seveso creek, at the Lentate and Bresso Stations during 2007-2009

LENTATE STATION[1]			
Date of sampling	**AMPA (µg/L)**	**Glyphosate (µg/L)**	**Sum (µg/L)**
Mar-07	6.1	0.9	7.0
Jun-07	6.1	0.2	6.3
Sep-07	16.0	0.2	16.2
Dec-07	16.0	0.3	16.9
May-08	12.0	0.6	12.6
Jun-08	3.3	0.2	3.5
Sep-08	14.0	<0.1	14.1
Dec-08	1.2	0.9	2.1
Mar-09	4.2	0.1	4.3
Jun-09	4.0	0.6	4.6
Sep-09	13.2	2.2	15.4
Dec-09	6.4	0.3	6.7
Value avg	8.5	0.6	9.1

Table 7.5-201: Values relating to the concentrations of AMPA and glyphosate found in the Seveso creek, at the Lentate and Bresso Stations during 2007-2009

AMPA/Glyphosate ratio		14.2	
BRESSO STATION			
Date of sampling	**AMPA (µg/L)**	**Glyphosate (µg/L)**	**Sum (µg/L)**
Mar-07	4.1	1.0	5.1
Jun-07	3.8	0.5	4.3
Sep-07	14.9	0.2	15.1
Dec-07	13.3	0.1	13.4
May-08	6.2	0.2	6.4
Jun-08	2.9	0.2	3.1
Sep-08	6.0	<0.1	6.1
Dec-08	0.7	0.2	0.9
Mar-09	3.4	0.2	3.6
Jun-09	4.0	1.0	5.0
Sep-09	13.3	1.6	14.9
Dec-09	7.2	0.2	7.4
Value avg	6.7	0.5	7.1
AMPA/Glyphosate ratio		13.4	

[1] A value of 1 was assumed in calculating the average value and the sums of AMPA and glyphosate concentrations where the measured concentration was <1.

Table 7.5-202: Values relating to the concentrations of AMPA and glyphosate found in the Terrò creek, at the Cesane Maderno station in 2009

CESANO MADERNO[1]			
Date of sampling	**AMPA (µg/L)**	**Glyphosate (µg/L)**	**Sum (µg/L)**
Mar-09	0.5	<0.1	0.6
Jun-09	3.0	1.3	4.3
Sep-09	4.0	1.0	5.0
Dec-09	0.9	<0.1	1.0
Value avg	2.1	1.2	2.7
AMPA/Glyphosate ratio		1.7	

[1] A value of 1 was assumed in calculating the average value and the sums of AMPA and glyphosate concentrations where the measured concentration was <1.

Conclusions

The analyses carried out in the 2007-2009 three-year period have confirmed the broad presence of AMPA and lesser quantities of glyphosate in all the waterways monitored. The highest concentrations were detected on samples taken at the start of the autumn season, at the end of the period of application of the herbicide, carried out in the previous months during plant growth. The winter and early spring season show decreasing concentration trends. These trends occurred periodically over the three years of observation.

Among the three waterways monitored, the Seveso creek shows the highest degree of pollution, both as an average value of the sum of concentrations of the two compounds and with respect to the maximum values found for this sum, 16.3 µg/L for the Seveso creek on samples collected from the control station downstream of the hilly area with most vegetation, compared to 8.2 µg/L on the Lambro waters sampled at the point where the river has just traversed the center of Monza, and 4.3 µg/L measured for the water samples of the Terrò creek. Data relating to the sampling carried out downstream of the city of Monza seem to be influenced by water contributions from the water purification plant of the Alto Lambro Reclamation Consortium, which dumps treated water near the sampling point. It has been hypothesized that the plant fulfills at once the role of filtering out glyphosate and AMPA contained in treated waters, and of being one of the sources of pollution by AMPA due to the degradation of phosphonate additives used in cleaning processes.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the monitoring results for glyphosate and AMPA from the Lombardy region in Italy. The information relies on official monitoring data of the authorities. The maximum measured concentrations for glyphosate and AMPA in river samples were 2.2 µg/L and 16.0 µg/L, respectively. The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/068
Report author	Gregoire, C. *et al*.
Report year	2010
Report title	Use and fate of 17 pesticides applied on a vineyard catchment
Document No	International Journal of Environmental Analytical Chemistry (2010), Volume 90, Number 3/6, pp. 406-420
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, analyses of samples conducted by officially recognised testing facility (Pasteur Institute of Lille (France))
Acceptability/Reliability:	Reliable

2. Full summary

Non point source (NPS) pollution may degrade water quality and is of concern to water quality managers and environmental risk regulators whose responsibility it is to monitor the status of water bodies. There are many methods of evaluating the impact on a water body from NPS pollution, but one of the most important, effective, and unfortunately expensive methods is to monitor the quality of water flowing from a particular catchment. The flux of 17 pesticides from a small (42.7 ha) agricultural (vineyard) catchment in the Alsatian piemont (France) was systematically monitored over 4 years (2003–2006) from June to September. A metrological station is located within the catchment area and run-off of 58 run-off events was monitored throughout. A water sample for pesticide analyses was collected every 8 m^3 of run-off. Detailed information regarding pesticide application was obtained from voluntary surveys submitted annually to active farmers of the studied catchment. There was considerable climatic variation among years. However, variability of the total load of pesticides exported yearly from the catchment was low. Some 78 % of the total pesticide applications in the catchment were herbicides and glyphosate was the most used herbicide with annual application ranging from 18 to 61 kg. The run-off coefficient was low (less than 2 %), but the frequency of determination was high for some pesticides such as the fungicide dimetomorph (72 %) and the herbicides diuron (98 %) and glyphosate (100 %). The pesticide export coefficients were below 1 % of the applied amount, and often below 0.1 %. Every water sample exceeded the EU drinking water limit of 0.1 ug/L.

Materials and methods

Study site
The studied Hohrain catchment area is located in the Alsatian vineyard (Eastern part of France, latitude 47°57′9 N; longitude 007°17′3 E; altitude 284 m). The area of the catchment is 42 hectares. The minimum and maximum annual precipitation for the period of record was 361 mm (1953) and 867 mm (1999), respectively. The average annual rainfall calculated since 1946 is 600 mm. The mean slope of the catchment is 15 %. Geologically, Würm loamy loess and Oligocene clayey conglomerates and marls, as well as compact calcareous substrate, largely dominate in the upper and lower parts of the catchment, respectively. The main soil type is mostly calcareous clay loams with medium infiltration capacity. Sixty-eight per cent of the hydraulic catchment is covered by vineyards. The land use shows a gradient from mostly forested areas and partly orchard at the upstream of the basin to agricultural and vineyard areas nearer to the outlet. With more than 120 farming plots, it should be noted that the road network is dense, mostly impervious and represents about 6 % of the area of catchment. The catchment can be qualified as 'dry' catchment with no permanent flow. The hydrological functioning can be summarised in three steps: (1) no discharge occurs without rainfall, (2) then, from >0 to 4 mm of rainfall per event only the road network contributes to the discharge, (3) finally, rainfall greater than 4 mm, the number of fields contributing to the discharge increases with both intensity and total rainfall depth (unpublished results).

Sampling and sample collection
The catchment area is equipped with a meteorological station and the outlet of the catchment has been instrumented for 4 years to monitor water, only observed during rainfall-run-off events, and pesticide concentrations. The measurement of the water level was carried out with a Venturi channel (ENDRESS and HAUSER, Huninge, France) and was performed with a surface water level sensor. Flow proportional water samples of 0.9 L were systematically collected every 8 m^3 for measurement of pesticide concentrations by a cooled automatic sampler (Hydrologic, Sainte-Foy, Québec, Canada). Samples were transferred via a polyethylene pipe to glass bottles and stored in the dark at 4°C. Twice a week, samples were collected and subsampled into plastic and glass bottles and analysed for glyphosate and aminomethyl phosponic acid (AMPA) and for the other pesticides.

Then, the samples were frozen until their analysis. According to the quality assurance procedures performed during this work, volatilisation, degradation and adsorption between the sampling and the analysis of the samples is negligible. Water sampling was conducted from 2003 to 2006, during the active wine growing season that corresponds to the major period of pesticide application and where the risk of offsite movement is large, i.e. March to October. Fifty-eight storm events were measured, which include a total of 280 collected water samples for pesticide concentration analyses and transfer quantification.

The variability of pesticide concentration was analysed over the targeted run-off events. Hydrograms and chemograms were available for each storm event from April 2003 to September 2006. Corresponding hyetograms were provided by the Meteo France station.

Estimation of applied pesticides and selection of monitored pesticides
Surveys were sent annually to the 28 farmers active in the Hohrain catchment in order to record the type and amount of pesticides applied. The survey includes the chemical species, their quantities, and their application date. No farmyard or urban area is located within the Hohrain catchment, which minimises the potential for pesticide point source pollution.

The goal of this study is to assess a broad spectrum of pesticides that display various physico-chemical characteristics in order to allow a thorough estimation of contaminant transfer at the catchment scale. The selection of compounds analysed at each sample series was based on preliminary knowledge regarding annual pesticide applications on the Hohrain catchment and on the physico-chemical properties of compounds most likely to move from their application site. According to the monitoring studies of the pesticide fate at the catchment scale, the sorption coefficient normalised to soil organic carbon content (K_{oc}) and the time for 50 % decline of the initial pesticide concentration in soil, i.e. dissipation half-time (DT_{50soil}) are the important physico-chemical properties to explain pesticide fate.

The full list includes 17 molecules (8 herbicides, 8 fungicides and 1 insecticide) and 3 degradation products. The K_{oc} and $DT_{50,soil}$ values of the 17 molecules and the three metabolites are summarised in Table 7.5-203.

Pesticides such as diuron, the triazines, e.g. atrazine, simazine and terbuthylazine, have had their environmental behaviour studied for years; oryzalin and others such as glyphosate and glufosinate (Table 7.5-203) have been studied fewer times. Carbendazim and norflurazon belong to the priority list for groundwater survey in the Alsace area (France) and were included in the list of analyses because of their persistence, even if they are no longer applied (Table 7.5-203). The three degradation products investigated are AMPA (aminomethyl phosphonic acid), glyphosate's degradation product, DCPMU (3,4-Dichlorophenyl-N-methyl urea) and DCPU (3,4-Dichlorophenyl urea), both degrades of diuron.

The application method, i.e. directly onto the soil for herbicides or on the leaves for fungicides and insecticides, represent a key-information to assess the fate of pesticides at the catchment scale. The herbicides, applied directly onto the soil, were *a priori* more available during the run-off process whereas the fungicides and insecticides can be also mobilised by foliar wash-off during rainfall event.

Table 7.5-203: Half-life of pesticide in soil (DT_{50soil}) and sorption coefficient normalised to soil organic carbon content (K_{oc}) for 17 pesticides and 3 degradation products (AMPA: aminomethyl phosphonic acid; DCPMU: 3,4-Dichlorophenyl-N-methyl urea and DCPU: 3,4-Dichlorophenyl urea)

Substances	DT_{50soil} (Field) Day	K_{oc} L kg^{-1}
Fungicides applied		
Azoxystrobin	21	423
Cymoxanil	3.5	44
Dimetomorph	44	348
Kresoxim methyl	16	308
Penconazole	86	2205
Pyrimethanil	30	301
Tetraconazole	61	1039
Herbicides applied		
Diuron	89	1067
Glufosinate	7	755
Glyphosate	12	21699
Isoxaben	123	601
Oryzalin	50	949
Terbuthylazine	46	220
Simazine	90	130
Insecticides applied		
Thiodicarb	18	418
Pesticides not applied		
Carbendazim (*Fungicide*)	18	223
Norflurazon (*Herbicide*)	225	700
Degradation products		
AMPA (from Glyphosate)	151	8027
DCPMU (from Diuron)	–	928
DCPU (from Diuron)	–	694

Pesticide analyses
Suspended sediment was separated from the water phase by filtration through 1 mm glass fibre filters. Aqueous samples were solid-liquid extracted and extracts were analysed. The fungicides azoxystrobin, cymoxanil, dimethomorph, kresoxim methyl, penconazole, pyrimethanil, tetraconazole, carbendazim, the herbicides diuron and its degradation products DCPMU and DCPU, as well as isoxaben, oryzalin, simazine, terbuthylazine, norflurazon, and the insecticide thiodicarb were analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS), according to the French standard. For glyphosate, AMPA, its

degradation product and glufosinate-ammonium, the method of analysis consists of a derivatisation with 9-fluorenylmethyl chloroformate (FMOC-Cl) and detection by LC-MS-MS. The recovery rates ranged between 70 % and 88 % depending on the compound. All the analyses were carried out by the Pasteur Institute of Lille (France) certified by the French Ministries of Health and Environment. Due to this externalisation, no replicates were managed during the study. Therefore, duplicate frozen samples were stored in case of analytical problems with the original sample.

Pesticide use and fate metrics

Various pesticide metrics have been developed to evaluate the transfer of pesticides at the catchment scale. Metrics defined in the following equations (1 to 5), have been calculated and include the estimated values of pesticide use, as well as rainfall, run-off and the concentration of pesticides in water samples collected during each storm event. The selection of these metrics has been based on the balance between the required and available data, the environmental relevance of the information provided by these metrics and the possibility of performing a mass balance between the annual pesticide inputs applied to the fields and the loads detected at the outlet of the catchment.

The run-off coefficient (%) provides essential information about the hydrological behaviour during a rainfall event. Knowledge on the run-off to infiltration ratio is required to assess the potential vulnerability of surface water and groundwater. The run-off coefficient RC is calculated for each event by normalising the total run-off generated during a rainfall event (V_{run},m^3) by the total rainfall amount over a rainfall event (V_{rain},m^3) (Equation (1)).

$$RC = \frac{V_{run}}{V_{rain}} \quad [1]$$

To assess the occurrence of pesticides in the various environment compartments, a widely used metric is the detection rate. This metric is usually performed with the limit of detection (LOD), but it can also be performed with the limit of quantification (LOQ). A frequency of determination (FOD) is calculated by Equation (2):

$$FOD = \frac{n_{sloq}}{n_i} \quad [2]$$

where n_{sloq} is the number of samples during an event i for which the pesticides were detected at a concentration higher than the limit of quantification (LOQ) and n_i is the total number of samples collected during an event i. The frequency of determination (FOD) is mathematically lower or equal to the limit of detection.

Assuming that the water sample is flow proportional, the calculation of the mean concentration for an event is Equation (3):

$$C_{mean\,j} = \frac{\sum_{s=1}^{n_j} C_{js}}{n_j} \quad [3]$$

where n_j is the total number of instantaneous concentrations available for a pesticide j, C_{js} is the instantaneous concentration of the pesticide j.

Because of analytical difficulties in analysing the fraction of pesticides sorbed on sediments, several studies on the fate and transport of pesticide only examine pesticides in the dissolved phase. Unless the pesticide has a very high partitioning coefficient, most of the flux of pesticide will be the dissolved phase. The sampling devices in the Hohrain catchment allow only monitoring pesticide in the dissolved phase.

Furthermore, the pesticide loads in the dissolved phase were calculated with the run-off and the pesticide concentration data. We have assumed a linear change between two successive analysed concentrations and monitored run-off data. We assumed a linear concentration between a null value of concentration at the beginning of discharge and the concentration of the first sample and between the concentration of the last sample and a null value at the end of the discharge. The exported quantities LPj out are calculated with one minute time step according to Equation (4):

$$LP_{j\,out} = \sum_{t=1}^{n} C_{jt} \times Q_t \qquad [4]$$

where C_{jt} is the instantaneous concentration of the pesticide *j*, *n* the duration of run-off event expressed in minutes and Q_t is the instantaneous run-off. To perform a mass balance between applied pesticide amount and pesticide loads, the estimation of the pesticide sorbed both in bedload and suspended matter would be also required. This pesticide amount can either be directly monitored or derived from the pesticide amount in the dissolved phase according to empirical equations. In the Hohrain catchment, the sampling device does not allow to collect enough suspended matter to perform pesticide analyses on the sorbed phase. The empirical equations cannot be applied without calibration in the Hohrain catchment. Therefore, the exported pesticide load (Equation (4)) should be considered lower than the total pesticide loads at the catchment outlet.

A yearly overall export coefficient Ec (%) for each compound by Equation (5) based on the estimates of pesticide application and the pesticides outputs calculated according to the Equation (4). Ec is calculated by comparing $LP_{j\,out}$ (g) the load of the pesticide *j* exported at the outlet of the catchment with $LP_{j\,in}$ (g), the cumulated load of each pesticide applied each year:

$$Ec = \frac{LP_{j\,out}}{LP_{j\,in}} \qquad [5]$$

Focusing on the removal rates calculated by comparing the pesticide inputs and the loads detected at the outlet of a hydro-system, this metric seems to be the most relevant to assess the export of active substances.

These 5 metrics were calculated for the 58 monitored run-off events to analyse the pesticide fate on vineyards in the Hohrain catchment.

Results and Discussion

Hydrology

Over the study period of 4 years, there was a large variability in rainfall amounts ranging between 359 and 730 mm per year (Table 7.5-204). There is no correlation between annual rainfall and the number of events analysed. All the rainfall events which generated a run-off volume higher than 8 m^3 were monitored and the associated pesticide concentrations were analysed according to the sampling method. The main run-off events, i.e. with more than 8 m^3, represented each year only 29 % of the total rainfall amount between March and October (Table 7.5-204). No samples were collected for the run-off events generating less than 8 m^3. The threshold of 8 m^3 had the advantage to focus on the main run-off events with a contribution of vineyard fields on which the pesticides were applied but introduced a bias in the total annual pesticide loads. The mean run-off per event is stable (mean: 4 L/s; standard deviation: 0.9 L/s). The maximum run-off value observed each year is quite variable between 19 and 127 L/s. The run-off coefficients calculated (Equation (1)) are less than 2 % for the 4 years. This low value from an agricultural area can be explained by (1) the medium infiltration capacity of the soil, (2) the vineyard management involving grass cover, which was initially adopted for soil conservation and induces a decrease of surface run-off and (3) the fact that the effective area contributing to run-off is limited with respect to the total catchment area. Therefore, the mean volume generated during rainfall events is relatively low and ranged between 31 m^3 in 2004 and 95 m^3 in 2006 with a maximum value observed in 2006 (250 m^3) (Table 7.5-204). The infiltration process

is predominant during the rainfall events. However, the pesticides in the surface water represent the main threat both for surface water and groundwater regionally. Indeed, the run-off produced from the vineyard catchment rapidly flows into downstream water bodies, which are closely linked to the Rhenan aquifer.

Table 7.5-204: Hydrological metrics: Number of monitored events; total yearly rainfall; rainfall from March to October; rainfall of monitored events; proportion of monitored rainfall/rainfall from March to October; mean and maximum discharge observed during events; minimum, maximum and mean volume generated during events; and the mean Run-off Coefficient (RC) for water associated with run-off events

	Number of monitored events	Yearly rainfall (mm)	Rainfall from March to October** (mm)	Rainfall of *m* monitored events** (mm)	Proportion of monitored rainfall/rainfall from March to October (%)	Discharge		Volume			RC* (%)
						Mean (l/s)	Max (l/s)	Min (m^3)	Max (m^3)	Mean (m^3)	
2003	12	359	265	101	38	4.8	127	2	198	62	1.6
2004	29	669	487	159	33	2.9	67	1	131	31	1.4
2005	8	470	407	105	26	3.6	45	6.7	117	64	1.1
2006	9	730	649	115	18	4.6	66	10.3	250	95	1.8
Mean		557	452	120	29	*4.0*	*76*	*4.8*	*174*	*63*	*1.475*
SD		172	160	27	8.9	*0.9*	*28.8*	*4.5*	*61.8*	*26.1*	*0.3*

*RC: Run-off coefficient.
**This period corresponds to the crop growing season.

Pesticide inputs

The survey response rates, expressed in proportion of the total vineyard catchment's area, are 75 %, 83 %, 57 % and 61 %, respectively, for 2003, 2004, 2005 and 2006. To take into account the missing information, a correcting ratio, i.e. ratio of investigated to total vineyard area, has been applied to estimate the total pesticide applied amount.

The difference of the total quantities of pesticides for 2003 and 2004 was low, i.e. 5 % (Table 7.5-205) in spite of marked climatic variations (Table 7.5-204). This variation was higher for 2005 and 2006. For 2005, the total input decreased by 44 % in comparison with the mean value calculated for 2003–2004. For 2006, we observed an increase of 66 % compared to the mean values for the period 2003–2004, owing to the used of diuron and glyphosate by the Agricultural and Viticultural College of Rouffach (50 % of the vineyard areas). Herbicides are the most used category of pesticides with 78 % of the total amount applied (Table 7.5-205). Glyphosate was the most used herbicide and the yearly applied amount ranged from 18 to 61 kg. The highest input (61.4 kg for 2006) was associated with a very rainy year (730 mm, i.e. 22 % more than the average inter-annual rainfall). In contrast, quantities of insecticides applied are marginal with nearly 1 kg annually. These quantities will continue to decrease due to the use of pheromones. Two hypotheses can be formulated to explain the frequency of determination of simazine banned since 2002: first, simazine was applied illegally on fields after 2002; secondly, the fraction of simazine sorbed on field soil particles has progressively desorbed and transferred during run-off events. The survey results have confirmed the first hypothesis as simazine was been applied until 2004. However, in 2008 on the Hohrain catchment, simazine was systematically detected during the run-off events monitored (non published data). Consequently, the second hypothesis of desorption associated with low degradation kinetics in soil, cannot be excluded, in agreement with previous observations.

Figure 7.5-175 illustrates pesticide used in 2004. These results underline the diversity of compounds applied in 2004 (20 fungicides, 8 herbicides and 6 insecticides). However, three pesticides analysed between 2003 and 2006 were not applied in 2004. Carbendazim and norflurazon were not applied during the studied period (2003–2006), according to the survey results, but analysed in 2003 and 2004. Indeed, these two pesticides belong to the priority list for groundwater survey and they had been applied in the past. The last year of their application was unknown.

Table 7.5-205: Use and fate pesticide metrics with input data from farmer surveys; output flux for 17 pesticides and 3 compounds of degradation (AMPA: aminomethyl phosphonic acid; DCPMU: 3,4-Dichlorophenyl-N-methyl urea and DCPU: 3,4-Dichlorophenyl urea) and the export coefficient (Ec)(‘‘/’’pesticide was not analysed; “n.a.” the pesticide was not applied; and “n.c.” the export coefficient could not be calculated

Pesticides	Total applied amount (g)				Pesticide outputs (g)				Export coefficient (Ec) (%)			
	2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006
Fungicides applied												
Azoxystrobin	671	n.a.	n.a.	n.a.	0.11	0.04	/	/	0.016	n.c.	n.c.	n.c.
Cymoxanil	5846	5187	763	3364	0.02	0.03	/	/	0.000	0.001	n.c.	n.c.
Dimetomorph	767	257	1003	190	0.51	0.26	/	0.11	0.066	0.099	n.c.	0.059
Kresoxim methyl	6039	3129	2608	1995	0.04	0.11	/	0.04	0.001	0.003	n.c.	0.0015
Penconazole	58	n.a.	33	33	0.02	/	0.01	/	0.035	n.c.	0.043	n.c.
Pyrimethanil	3185	2808	1246	n.a.	0.48	0.16	0.16	0.06	0.015	0.006	0.013	n.c.
Tetraconazole	54	68	n.a.	295	0.03	/	0.01	/	0.048	n.c.	n.c.	n.c.
Herbicides applied												
Diuron	289	1499	195	5199	0.31	0.55	0.68	2.71	0.106	0.037	0.035	0.052
Glufosinate	384	496	n.a.	n.a.	/	0.08	/	/	n.c.	0.017	n.c.	n.c.
Glyphosate	22816	28101	18459	61411	6.60	9.40	4.71	5.57	0.029	0.033	0.026	0.009
Isoxaben	42	n.a.	n.a.	n.a.	0.04	/	0.04	0.02	0.09	n.c.	n.c.	n.c.
Oryzalin	1663	2990	311	947	0.04	0.15	/	/	0.002	0.005	n.c.	n.c.
Terbuthylazine	n.a.	386	n.a.	n.a.	0.11	0.05	0.04	0.03	n.c.	0.013	n.c.	n.c.
Simazine	81	168	n.a.	n.a.	0.06	0.50	0.01	0.02	0.077	0.298	n.c.	n.c.
Insecticides applied												
Thiodicarb	1171	42	n.a.	n.a.	/	0.01	/	/	n.c.	0.317	n.c.	n.c.
Pesticides non applied												
Carbendazim (Fungicide)	n.a.	n.a.	n.a.	n.a.	0.02	0.01	/	/	n.c.	n.c.	n.c.	n.c.
Norflurazon (Herbicide)	n.a.	n.a.	n.a.	n.a.	0.08	0.11	/	/	n.c.	n.c.	n.c.	n.c.
Degradation products												
AMPA (from Glyphosate)	n.a.	n.a.	n.a.	n.a.	3.50	0.77	1.06	1.22	n.c.	n.c.	n.c.	n.c.
DCPMU (from Diuron)	n.a.	n.a.	n.a.	n.a.	/	/	/	0.027	n.c.	n.c.	n.c.	n.c.
DCPU (from Diuron)	n.a.	n.a.	n.a.	n.a.	/	/	/	/	n.c.	n.c.	n.c.	n.c.

Frequency of determination

Table 7.5-206 synthesises the results of pesticide fate metrics, i.e. the frequency of determination (FOD) and the maximum C_{max} and mean C_{mean} concentrations calculated for the 58 events between 2003 and 2006. The number of samples analysed is not the same for the different molecules in a same year. Indeed, owing to different technical constraints and timing of application, the numbers of sample for each pesticide can vary from one year to another.

The frequency of determination (FOD) (Equation (2)) was higher for herbicides (62 %) than fungicides (30 %). The rate is very low for the sole insecticide monitored (2 %). The highest FOD have been observed for dimethomorph (74 % on average for 2003–2006), pyrimethanil (67 %), terbuthylazine (97.5 %), diuron (98.5 %) and glyphosate (99.75 %) (Table 7.5-206). AMPA and DCPMU, degradation products of glyphosate and diuron, respectively, were always detected (100 %) (Table 7.5-206). DCPU produced by the degradation of DCPMU could not be detected during the events of 2006.

Figure 7.5-175: **Total of pesticide amounts applied in 2004 (per kg) by distinguishing the pesticides (fungicides, herbicides and insecticides) analysed (black asterisk) or not during run-off events**

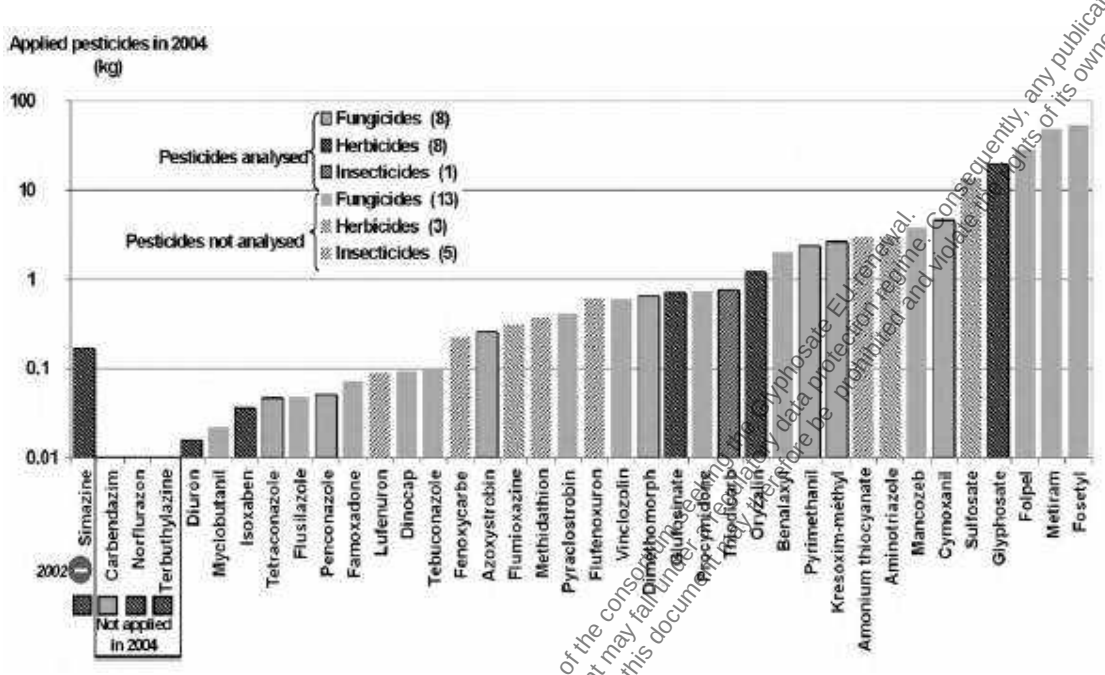


Table 7.5-206: **Pesticide fate metrics for 17 pesticides and 3 degradation products (AMPA: aminomethyl phosphonic acid; DCPMU: 3,4-Dichlorophenyl-N-methyl urea and DCPU: 3,4-Dichlorophenyl urea) for 58 run-off events between 2003 and 2006: limit of quantification (LOQ); number of samples analysed by year; the annual frequency of determination (FOD); maximum concentrations C_{max} and mean concentrations C_{mean}; (''/'' the pesticide was not analysed)**

Pesticides	LOQ ($\mu g L^{-1}$)	Number of samples analysed				Frequency of determination (FOD) (%)				C_{max} ($\mu g L^{-1}$)				C_{mean} ($\mu g L^{-1}$)			
		2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006	2003	2004	2005	2006
Fungicides applied																	
Azoxystrobin	0.05	82	98	0	0	30	41	/	/	3.4	0.36	/	/	0.28	0.06	/	/
Cymoxanil	0.05	82	98	0	58	12	21	/	0	0.3	0.34	/	/	0.08	0.05	/	/
Dimetomorph	0.05	82	98	0	86	98	66	/	57	4.4	5.7	/	0.66	0.68	0.3	/	0.16
Kresoxim methyl	0.1	82	98	0	86	12	12	/	1	0.17	2.2	/	0.07	0.04	0.09	/	0.004
Penconazole	0.05	82	98	37	58	1	0	5	0	0.22	/	0.16	/	0.01	/	0.02	/
Pyrimethanil	0.1	82	98	37	86	48	80	100	39	5.8	1.5	1.8	0.39	0.45	0.2	0.23	0.1
Tetraconazole	0.05	82	98	37	58	21	0	9	0	0.14	/	0.09	/	0.05	/	0.01	/
Herbicides applied																	
Diuron	0.02	82	98	37	86	100	97	100	97	11	14	8.8	32	0.84	0.54	1.2	3.7
Glufosinate	0.1	82	98	0	0	0	16	/	/	/	1.3	/	/	/	0.26	/	/
Glyphosate	0.1	82	98	37	86	100	100	100	99	86	70	63	40	6.76	10.4	7.4	5.6
Isoxaben	0.1	82	98	37	86	4	0	8	2	0.28	/	1.9	0.07	0.04	/	0.15	0.001
Oryzalin	0.1	82	98	0	0	11	14	/	/	2.6	4.4	/	/	0.3	0.17	/	/
Terbuthylazine	0.025	82	98	37	86	100	99	100	91	5.6	0.27	0.22	0.12	0.44	0.05	0.07	0.04
Simazine	0.02	82	98	37	86	100	79	24	42	2.7	10.2	0.05	0.09	0.2	0.22	0.006	0.02
Insecticides applied																	
Thiacloprid	0.05	82	98	0	0	0	4	/	/	/	60	/	/	/	15	/	/
Pesticides not applied																	
Carbendazim (Fungicide)	0.05	82	98	0	0	10	19	/	/	0.19	0.18	/	/	0.03	0.03	/	/
Norflurazon (Herbicide)	0.1	82	98	0	0	24	49	/	/	1.6	4	/	/	0.32	0.2	/	/
Degradation products																	
AMPA (from Glyphosate)	0.1	82	98	37	86	100	100	100	100	23	44	8.5	5.5	2.76	2.9	1.9	1.4
DCPMU (from Diuron)	0.05	0	0	0	28	/	/	/	100	/	/	/	0.31	/	/	/	0.13
DCPU (from Diuron)	0.05	0	0	0	28	/	/	/	0	/	/	/	/	/	/	/	/

The pesticides studied involved a diverse group of chemical substances. Some older types in use, such as simazine, banned in France in 2002, persisted, with FODs of 100, 79, 24 and 42 %, respectively, over the four years.

These frequencies of determination are relatively high with respect to the low run-off coefficient calculated. This could be explained by the hydrological connection of some areas within the catchment. Some vineyard fields located near the outlet may be directly connected to the impervious road network. Consequently, for all the run-off events, they would always contribute to both the discharge and to the pesticide loads.

The mean frequency of determination value for fungicide was about 50 % lower than the herbicides with 28.4 % (standard deviation: 32.3 %) and 61.8 % (standard deviation: 43.9 %), respectively. These values were in agreement with the *a priori* higher availability of herbicides applied directly on soil compared to fungicides directly sprayed on the leaves. With only one export coefficient value (Table 7.5-205), it was not possible to compare the behaviour of insecticide with the one of herbicides and fungicides.

Pesticide concentration
Mean concentration values of herbicides was generally larger (1.7 µg/L on average for the 2003–2006 period) than fungicides concentrations (0.15 µg/L). The largest concentrations were obtained for the herbicide glyphosate (7.5 µg/L mean and 86 µg/L max), the insecticide thiodicarb (15 µg/L mean and 60 µg/L max) and the glyphosate degradation product AMPA (2.9 µg/L mean and 44 µ/gL max).

Concentrations detected in filtrated surface waters were one to three orders of magnitude larger than the drinking water limit (0.1 µg/L) (Table 7.5-206). Although water from the Hohrain catchment is not used directly for drinking water supply, such high pesticide concentrations could cause problems downstream.

Schulz (2004) reported a negative correlation (with a significance of $p = 0.0025$) between the log-transformed maximum insecticide concentration and the catchment size. The high pesticide concentration values obtained in the Hohrain catchment, 42 ha, are in agreement with this correlation. The Koc values of the monitored pesticides range from 44 L/kg (cymoxanil, fungicide) to 21 699 L/kg (glyphosate, herbicide). It may be noted that this range is similar to those mentioned by Schulz, suggesting similar fate processes. These results are of particular importance with regard to the European Water Framework Directive, which currently only covers catchment areas over 10 km^2. As discussed by Schulz, this directive thus excludes aquatic habitats that are potentially at the highest risk of being negatively affected by high pesticide concentrations.

Export coefficient
Knowledge of both pesticide input and output is used to calculate an export coefficient Ec (Equation (4)). The export coefficients calculated at the catchment scale were always less than 1 % and often less than 0.1 % (Table 7.5-206). The pesticides with higher export coefficients were thiodicarb and simazine (0.31 %). The lower ratio is observed for fungicides such as cymoxanil (0.0003 %). Despite these low export coefficients, all water samples were above the drinking water limit (0.1 µg/L). A comparison between the 4 years shows a relative constant export coefficient. No significant relationship can be determined between the export coefficient and (1) the characteristics of rainfall calculated yearly, or (2) the physico-chemical properties of each pesticide.

The export coefficients calculated for the Hohrain catchment were lower than the values obtained in similar studies, e.g. between 0.09 % and 0.87 % for Poissan *et al.*; between 0.2 % and 17.5 % for Blanchoud *et al.* and between 0.26 % and 0.57 % for Baran *et al.*

Considering mean and standard deviation values of export coefficient, no difference of availability can be determined at the catchment scale between fungicides (mean: 0.027 %; standard deviation: 0.03 %) and herbicides (mean: 0.055 %; standard deviation: 0.074 %).

As discussed in the hydrology results Section, the main run-off events, i.e. with more than 8 m^3, represented each year only 29 % of the total rainfall amount between March and October (Table 7.5-204) and so the export coefficient values likely underestimate the total annual pesticide loads.

Conclusion
More than 80 kg of pesticides can be applied annually to the Hohrain vineyard catchment during a growing season. Pesticides studied were a diverse group of chemical substances. Some compounds were frequently detected at the outlet of the catchment for the 2003–2006 period (dimethomorph: 74 %, pyrimethanil: 67 %, diuron: 98.5 % and glyphosate: 99 %). AMPA and DCPMU, degradation products of glyphosate and diuron, respectively, were detected in every sample.

Glyphosate and diuron are the most extensively used pesticides on the Hohrain catchment. Overall, pesticides losses from Hohrain catchment were systematically less than 0.1 %. Surprisingly, considering the high variability of applied amounts and weather conditions, this value (0.1 %) seems to be stable over the study period.

Pesticides and their degradation products were present in the Hohrain catchment with maximum concentrations of 86 µg/L for the herbicide glyphosate and 44 µg/L for its degradation product AMPA.

The results from this 4 year study underscore that pesticide behaviour at the catchment scale varies both over time and according to the type of pesticide considered. Assessing the fate of pesticide in agro-ecosystems based on land use patterns is not a straightforward exercise. Indeed, the quantification of the export coefficient, expressing a mass balance requires also significant investment both to collect information on pesticides application amount and timing as well as to calculate the pesticides loads at the catchment scale.

Because a broad spectrum of pesticides has been detected in natural water, the effect of mixtures should also be taken into account; because the overall toxicity could be higher than the sum of toxicities caused by the concentrations of the individual pesticides.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a monitoring study in a French vineyard catchment where glyphosate and AMPA among other pesticides were measured at the outlet flow of the catchment in water only observed during rainfall runoff events. Information on pesticide application amounts are provided as well as mean and max values of the measured concentrations on a yearly basis. The measured maximum concentration of glyphosate was 86 µg/L. Also, the measured maximum concentration of AMPA was 44 µg/L.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/069
Report author	Hanke I. *et al*.
Report year	2010
Report title	Relevance of urban glyphosate use for surface water quality
Document No	Chemosphere (2010), Vol. 81, No. 3, pp. 422-9.
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facility (Eawag, Swiss Federal Institute of Aquatic Science and Technology)
Acceptability/Reliability:	Reliable

2. Full summary

Relative contributions of agricultural and urban uses to the glyphosate contamination of surface waters were studied in a small catchment (25 km^2) in Switzerland. Monitoring in four sub-catchments with differing land use allowed comparing load and input dynamics from different sources. Agricultural as well as urban use was surveyed in all sub-catchments allowing for a detailed interpretation of the monitoring results. Water samples from the river system and from the urban drainage system (combined sewer overflow, storm sewer and outflow of wastewater treatment plant) were investigated. The concentrations at peak discharge during storm events were elevated throughout the year with maximum concentrations of 4.15 µg/L. Glyphosate concentrations mostly exceeded those of other commonly used herbicides such as atrazine or mecoprop. Fast runoff from hard surfaces led to a fast increase of the glyphosate concentration shortly after the beginning of rainfall not coinciding with the concentration peak normally observed from agricultural fields. The comparison of the agricultural application and the seasonal concentration and load pattern in the main creek from March to November revealed that the occurrence of glyphosate cannot be explained by agricultural use only. Extrapolations from agricultural loss rates and from concentrations found in the urban drainage system showed that more than half of the load during selected rain events originates from urban areas. The inputs from the effluent of the wastewater treatment plant, the overflow of the combined sewer system and of the separate sewer system summed up to 60 % of the total load**.**

Materials and methods

The study catchment is located in the North-East of Switzerland and part of the Lake Greifen catchment where pesticide behavior has been studied in the past. In 2007, the significance of agriculture and urban uses of biocides and pesticides was studied in a small part of the catchment. Based on this study, the behavior of the herbicide glyphosate was examined. The study catchment (Figure 7.5-176) covers 25 km^2, of which 75 % is used for agriculture, whereas 470 ha of the agricultural area are used for arable farming. Climate, soil, and land use are representative for the Swiss Plateau. There are two villages with 10 000 and 2000 inhabitants respectively. The urban sewer system is a mixture of a combined and a separate system (Figure 7.5-176c). In the combined sewer system, wastewater from households and the urban storm water are collected in the same sewer and discharged to the WWTP. In case of intense rainfall these combined sewer systems route excess water via overflows to surface waters. In the separate sewer systems, the urban storm water is collected separately and discharged directly to surface waters. The municipal waste water system lies completely within the hydrological boundaries. To differentiate the sources, the catchment was divided into four hydrological sub-catchments with different land use. The river water at each catchment outlet was sampled separately. The sub-catchments were characterized as follows:

Sub-catchment URB_{north} is highly influenced by water from urban origin since the larger city is situated in this area (site 2). There are two combined sewer overflows (CSO) active during heavy rain events and

several storm sewers (StS) discharging into the small creek. The total catchment size of the CSOs is 120 ha, whereas that of the StSs sums up to 46 ha. The wastewater treatment plant (WWTP), which collects wastewater from the whole catchment, is a conventional treatment plant and discharges into this creek as well. Additionally, to the river water the effluents of the WWTP (site 5) and of one StS with a catchment of 5.7 ha were monitored (site 6).

Sub-catchment AGR is dominated by agricultural uses (site 3). There are no CSO or StS discharging into this creek.

The land use in sub-catchment DRAI is dominated by agriculture. There is also one CSO with a catchment size of 28 ha discharging storm water into the creek; however, this CSO is hardly ever active. At the sampling site at the outlet of this sub-catchment, water from the entire catchment was collected (site 1).

Use in agricultural and non-agricultural applications
In total 100 farmers in the study catchment were interviewed about the application date and amount of pesticides (including products containing glyphosate). The survey covered 85 % of the agricultural area. On the basis of land use data, the authors assumed that the remaining 15 % of the agricultural area which were not considered in the survey received no further glyphosate applications. In order to evaluate the use of pesticides by private garden owners, 61 households out of approximately 1800 households with a garden in the two villages were interviewed to determine their pesticide use (reported elsewhere). Furthermore, other urban sources (e.g. road maintenance) were assessed by inquiries in the catchment. Use data for professional gardening were derived from a nationwide survey of gardeners and market gardens in Switzerland. Glyphosate is also important for weed control on railways; however, in the catchment there was no railway system.

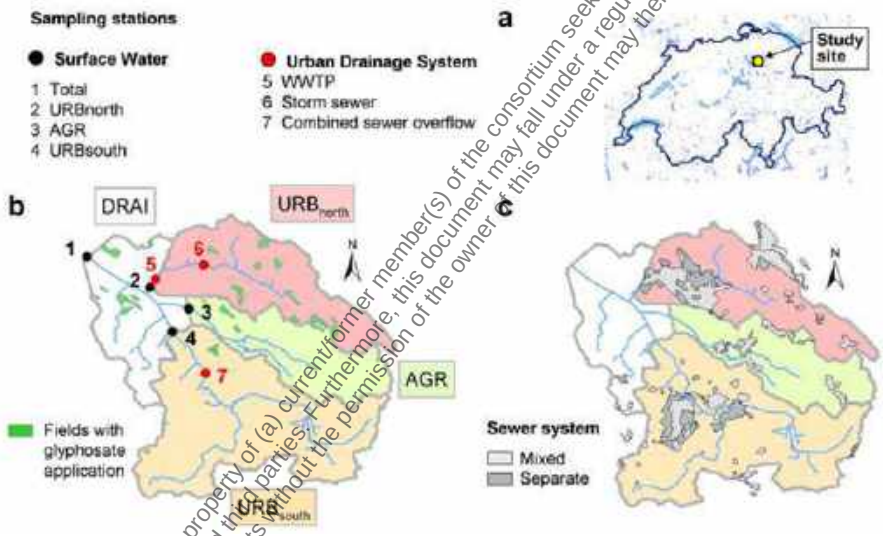
Discharge and precipitation measurements
Discharge was measured at every sampling site. Precipitation was determined by three rain gauges (WWTP, two in sub-catchment URB_{south}). The data procedure is described in detail in Wittmer *et al.* (2010a). The uncertainties of the discharge and the precipitation measurements were in the range of 10–20 %.

Sampling
Surface water and water from the urban drainage system were sampled by automatic devices at every sampling site except for the WWTP, where daily flow proportional composites were used. Samples were taken at high temporal resolution during 16 out of 35 rain events from March to November 2007. The event based sampling was done as follows: Time-proportional 15-min composite samples (three aliquots every 5 min) were collected during the first 6 h of an event, followed by a reduced sampling frequency of one composite sample per hour (four aliquots every 15 min). During dry periods base flow grab samples were taken. In total, 1600 samples were taken and stored in 250 mL glass bottles in the dark at -20 °C. For glyphosate and AMPA no significant losses were detected during sampling and storage. To compare the situation in the study catchment to the situation in Switzerland, grab samples of the river Rhine at the monitoring station at Basel were taken in May, July, and August 2006. The average discharge during this time period was 1250 m^3/s. Based on Swiss agricultural use data, the estimated agricultural use of glyphosate within the catchment of the river Rhine was approximately 50 t.

Analytical procedure
The samples were analyzed according to the method described in elsewhere which is based on a derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) followed by solid phase extraction (SPE) of the derivatized and filtered sample (0.45 µm regenerated cellulose membrane filter) and detection by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Using this method, dissolved glyphosate and complexes of glyphosate with cations can be assessed. Samples with expected high concentrations were diluted with nanopure water. The overflow and WWTP samples were all diluted 1:4. To compensate analyte losses during sample preparation isotope labeled glyphosate and AMPA were spiked to the water samples. The calibration curve was linear over the entire range of 0.02–1.0 µg/L. The limit of quantification (LOQ) was defined by the lowest standard of 0.02 µg/L. A blank (without analytes, but with internal standard) and a double blank (nanopure water) were used to monitor background

concentrations. For glyphosate, no significant background contamination was found. The background concentrations of AMPA were considerably lower than LOQ and could therefore be neglected. The precision was routinely determined by analyzing aliquots of a sample from sampling site 1 and from the outflow of the WWTP, which had been filtrated and spiked with 0.20 µg/L glyphosate and AMPA. The relative standard deviations (RSDs) for the surface water sample were 12 % for glyphosate and 14 % for AMPA (N = 6). The RSDs of the WWTP samples were 5 % for glyphosate and 13 % for AMPA (N = 6). The accuracy was determined by the recovery of a spiked analyte amount in environmental samples (at a level of 0.20 µg/L). The recoveries were in the range of 80–121 % for glyphosate and 90 to 118 % for AMPA. Due to the time-consuming and elaborate analytical method only selected samples could be analyzed (75 samples in total).

Figure 7.5-176: **(a) Location of the study catchment in Switzerland. (b) Study catchment separated into the four sub-catchments (DRAI, URB_{north}, AGR, and URB_{south}) with sampling sites in the river (black, 1–4) and in the urban drainage system (red, 5–7). Furthermore, agricultural fields, which were treated with glyphosate in 2007 are shown in green. (1c) Urban areas with mixed or separate sewer system**



Results and Discussion

Weather conditions and discharge

2007 was the fifth warmest year in Switzerland since 1864. Especially April and October were warmer, sunnier and drier than normal. The annual precipitation was 1112 mm, slightly more than the mean annual precipitation of the last 12 years (1073 mm). However, July and August were very rainy. At the beginning of August (8th and 9th), heavy continuing rainfall (120 mm in 2 d) caused the largest flood event during the study season. The discharge peak at the outlet of the study area reached 28 m^3/s compared to the mean base flow of 0.2 m^3/s (Figure 7.5-177b).

Use of glyphosate

In Switzerland, glyphosate is the pesticide with the highest sales volume, although the cultivation of genetically modified crops is not allowed in Switzerland. On agricultural areas, glyphosate is mainly used on conservation tillage acres to kill weeds or residues of intermediate crop before the main crop is sown. In 2005, 191 t were sold, which was over four times more than the Swiss sales volume of isoproturon (41 t) or atrazine (38 t). However, it is not known which fraction was used in agriculture or for urban weed control, respectively.

In the catchment area, the survey with around 100 farmers showed that glyphosate was, with a total of 88 kg, the second most used pesticide after isoproturon (107 kg) in agriculture. The third and fourth most used pesticides were atrazine with 74 kg and terbuthylazine with 42 kg. In total, 370 fields (470 ha) were used for arable farming. Glyphosate was applied on 32 fields with a total area of 53 ha (Figure 7.5-176b). There were two application periods. In spring, around 25 % (21.5 kg) of the total yearly amount was mainly applied on corn fields for no-tillage farming. The rest (66.5 kg) was used in August and September for preparing the fields for new crop. In urban areas, the applied amounts were more challenging to estimate, since glyphosate was used in different applications and by a variety of people. To determine the total use by owners of private gardens, the results of the survey considering 61 households was extrapolated to all households with a garden in the catchment (approximately 1800). The evaluation of the questionnaires completed by the owners of private gardens in the study area revealed that up to 90 % of them use plant protection products to control weed. Every fifth of the questioned house-holds admitted to spray paved forecourts and streets too, which is not allowed in Switzerland. Based on this survey, the total extrapolated glyphosate amount used in private gardens in the two villages was approximately 0.4 kg. Since the 61 households used 46 different substances, the uncertainty of the extrapolation was high. Based on Monte Carlo simulations, the applied amount was in the range of 0.04–1.3 kg. However, the used amount was considerably lower than the agricultural application amount of 88 kg. More important than the use of glyphosate by private persons was the use by professional gardeners. In 2005/2006, the non-agricultural use of glyphosate in horticulture and by professional gardeners in Switzerland was assessed on a national scale with information based on 10 % of all registered private and public companies in this sector. Total use of glyphosate was found to be 14 t per year which accounted for around 7 % of the total sales volume in Switzerland. The extrapolation of the Swiss use to our catchment revealed that around 18 kg were used in professional gardening. The maintenance of roads and roadsides often requires the use of pesticides. However, the public services and the street maintenance authority did not use glyphosate in the catchment according to interviews with the responsible persons. The amount of glyphosate used in agriculture could thus be determined accurately regarding the application date as well as the spatial distribution of treated fields. However, knowledge about urban use was scarce and no detailed information about the date of urban glyphosate applications was known.

Seasonal pattern at the outlet of the catchment

Concentration Dynamics

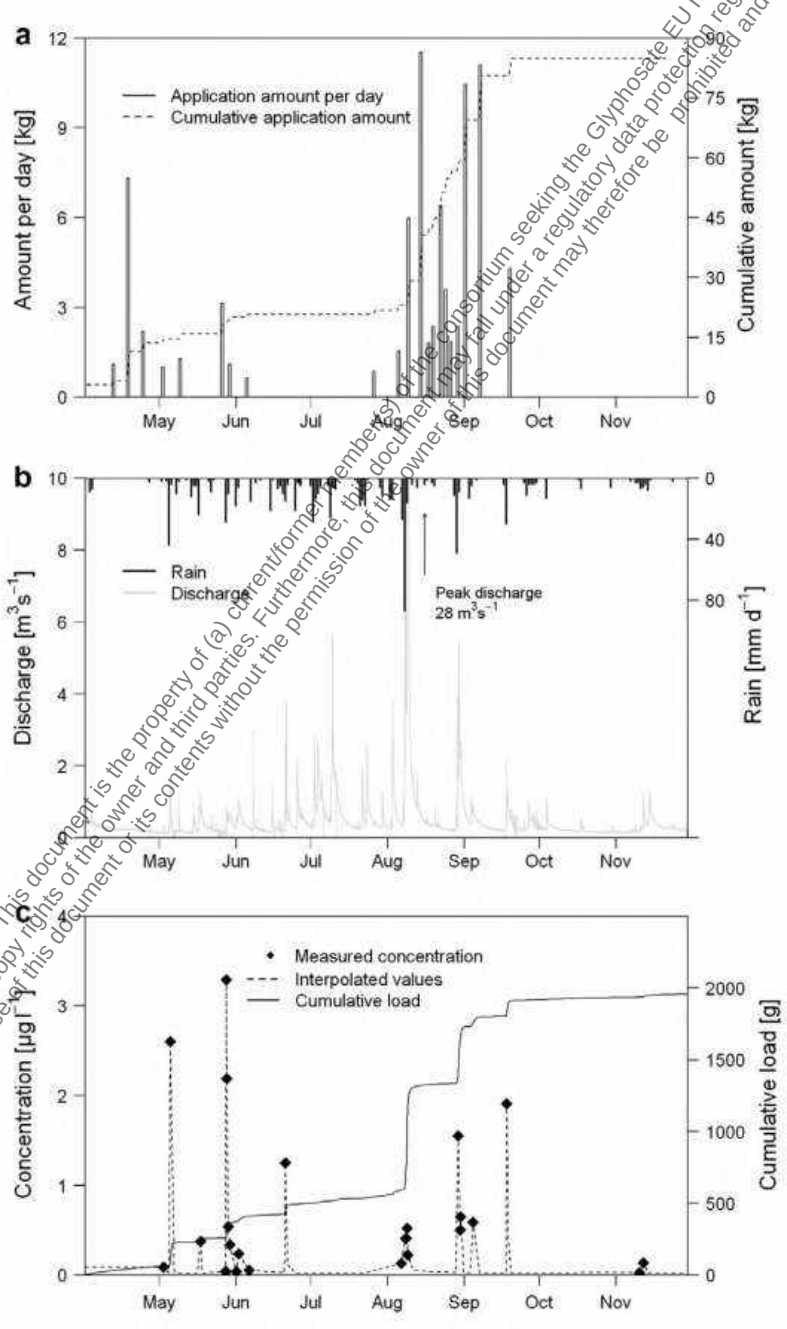
The occurrence of glyphosate in the creek at the outlet of the whole catchment (site 1, Figure 7.5-176b) was studied for ten rain events throughout the entire study period. As expected, peak discharge concentrations were much higher than the concentrations during base flow (Figure 7.5-177c). Between April and October, peak concentrations up to 3.30 µg/L were measured, whereas base flow concentrations were between 0.024 µg/L and 0.13 µg/L. The base flow concentrations fluctuated irregularly during the year and no clear trend could be observed. Peak concentrations were higher in spring than in late summer and they were significantly above those of other measured herbicides, although these had comparable application volumes and were supposed to be more mobile. Even during the flood situation in August, while the discharge reached 28 m^3/s, glyphosate was detected with a peak concentration of 0.52 µg/L. In November, glyphosate peak concentrations were still above 0.10 µg/L, although the last agricultural application was carried out 2 months before.

Minimum total load

We calculated a minimum load for the whole catchment based on the known concentrations measured at the outlet of the catchment. For this purpose, the measured peak concentrations were interpolated considering the discharge dynamics. For rain events without glyphosate measurements and for base flow periods, the lowest base flow concentration of 0.024 µg/L was assumed (see Figure 7.5-177c). In total, a load of 1.9 kg glyphosate was found in surface water from end of April to end of November (Figure 7.5-177c). If the whole load was assigned to agricultural use and compared to the agricultural application amount of 88 kg, the resulting agricultural loss rate would be 2.2 %. This value is considerably higher than the calculated agricultural loss rates of atrazine (0.8 %) or isoproturon (<0.5 %) in the catchment in the investigated period. Atrazine is known as an herbicide with a relatively high mobility (K_d of 0.2–18 L/kg, Field DT_{50} of 16–77 d). In contrast, glyphosate is not supposed to be mobile in soil due to its sorption to

soil particles (K_d of 13.2–427 L/kg) and its degradation to AMPA (Field DT_{50} of 7–63 d). Annual agricultural loss rates found in field studies were in the range of 0.1 %. Furthermore, the agricultural application amount of atrazine until the end of May was three times higher than the one of glyphosate. However, the atrazine load in surface water was less than half the load of glyphosate. Even though minimum assumptions were used, the overall glyphosate load in surface water strongly indicated that diffuse agricultural inputs are not the only source.

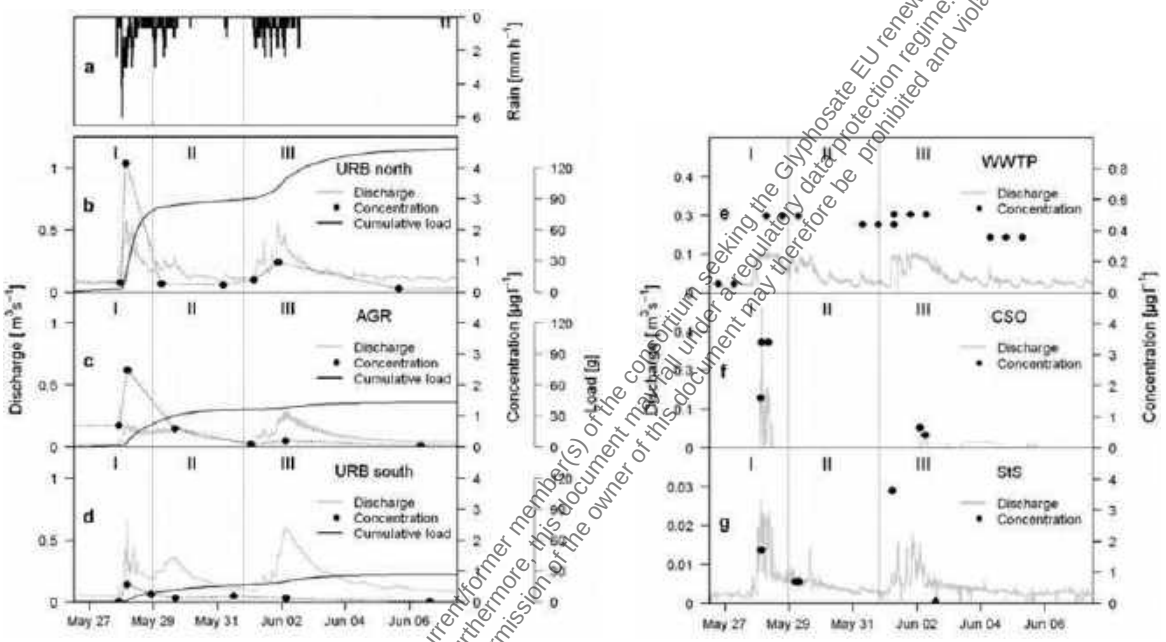
Figure 7.5-177: **(a) Agricultural application amount (per day and cumulative), (b) precipitation and discharge at the outlet of the catchment (site 1), (c) glyphosate concentration dynamics (site 1) and cumulative load of the total catchment from March to November 2007**



Event dynamics

Thanks to the partitioning of the study catchment into four sub-catchments with various land use, the contribution of the agricultural and urban sources could be assigned more distinctively. In addition to the seasonal dynamics at the outlet of the total catchment event-based investigations on the sub-catchment scale were carried out.

Figure 7.5-178: May/June event: rain intensity (a), discharge, concentration and cumulative load dynamics in surface water (b–d): URB_{north} (b), AGR (c), URB_{south} (d), discharge and concentration dynamics in the urban drainage system (e–g): wastewater treatment plant (e), combined sewer overflow (f), storm sewer (g)



Characteristics of the selected rain event

One rain event at the end of May and beginning of June 2007 (hereafter called ‘‘May/June event”) was examined in detail. The event was chosen due to elevated glyphosate concentration at the outlet of the catchment. Furthermore, in previous studies the hydrographs during this event had been studied in more detail to investigate the hydrological response of the sub-catchments and the urban drainage system and to determine the origin of the water. These observations were supplemented with knowledge concerning the concentration dynamics of two other herbicides (atrazine and mecoprop). The event was divided into three main intervals (Figure 7.5-178); the first rainfall was short and heavy (interval I) followed by two intervals of moderate rainfall (intervals II and III). During interval I, the discharge increased rapidly (URB_{north} and URB_{south}), as a result of run-off from hard surfaces. Furthermore, high concentrations of urban wastewater tracers such as caffeine were observed (up to 6 µg/L) and the concentrations of the purely agricultural herbicide atrazine were generally low (up to 0.2 µg/L), which indicates that diffuse agricultural inputs were less significant. Additionally, the overflow in sub-catchment URB_{south} was mainly active during interval I. These observations led to the conclusion that during interval I the discharge was mainly composed of water from urban areas. In intervals II and III, atrazine concentrations increased which indicates that the input from agricultural areas gained in importance.

Glyphosate concentrations at the outlets of the sub-catchments

Based on the source and the transport behavior of a compound its input dynamics may be predicted. Compounds applied in urban areas often show high concentrations during first flush and a subsequent fast concentration decrease as seen for mecoprop. Agricultural use leads to concentrations correlating with the discharge (except during first flush) comparable to the behavior of atrazine. High glyphosate concentrations

were measured at every site during the selected event (Figure 7.5-178); only in catchment URB_{south} the concentrations were significantly lower (Figure 7.5-178d). For all sub-catchments, the maximum glyphosate concentration was detected during interval I with the highest value of 4.2 µg/L in URB_{north} (Figure 7.5-178b). During the recession part of the hydrograph in interval II, the concentrations decreased to base flow levels and thus followed the discharge pattern. In interval III, the concentrations increased again; however to lower values than during the discharge peak in interval I. In spite of no known agricultural application in sub-catchment AGR, glyphosate concentrations at the outlet were high (Figure 7.5-178c). In interval I, the concentration of glyphosate increased rapidly and earlier than the concentration of mecoprop and atrazine. Mecoprop concentration pattern showed a small increase at peak discharge but not as pronounced as glyphosate. Probably, fast runoff from roads was an important input pathway of glyphosate. In summary, the concentration dynamics of glyphosate at the outlet of the sub-catchments was dominated by first flush peaks from sealed areas followed by lower concentration peaks from diffuse sources.

Glyphosate concentrations in the urban sewer system

The concentrations in the urban drainage system were in the same range as those found in the surface water samples. The concentrations in the WWTP rose with the first discharge peak and slightly decreased after the second with concentrations ranging from 0.06 to 0.51 µg/L (Figure 7.5-178e). The CSO was only active during short time periods in intervals I and III with main contributions in interval I and concentrations ranging from 0.43 to 3.4 µg/L (Figure 7.5-178f). The peak concentrations of URB_{south} can be explained by this overflow activity. The concentrations in the StS were higher during interval III than during interval I (Figure 7.5-178g). Due to the high concentrations in the surface water during interval I and in the urban drainage system, we concluded that during this particular event urban sources were important for the occurrence in surface water.

AMPA concentrations in the surface water and urban sewer system

The main transformation product of glyphosate in soil and in water is aminomethylphosphonic acid (AMPA). However, since AMPA is also a transformation product of phosphonates, it is not a specific metabolite of glyphosate. Phosphonates are used as chelating agents in various industrial applications. Furthermore, they are ingredients of domestic laundry and cleaning products. In an urban sewer system, the main input of AMPA may result of the degradation of phosphonates used as detergents. At the outlet of the total catchment the concentrations of AMPA were between 0.12 and 0.55 µg/L and varied less than those of glyphosate. During the May/June event the concentrations in the sub-catchments were between 0.04 and 1.11 µg/L and showed concentration dynamics similar to glyphosate (Figure 7.5-179a) with the exception of sub-catchment URB_{north}, where the AMPA concentration rose again during intervals II and III. This was probably due to a continuous input from the WWTP (Figure 7.5-179b).

Event loads

In order to confirm the importance of urban inputs for glyphosate, we calculated the load of the different parts of the urban drainage system (WWTP, CSO, and StS) by extrapolating the measured data. These loads were compared to the load in surface water. We only considered the three sub-catchments URB_{north}, AGR, and URB_{south}. DRAI was not included, since the load from this sub-catchment could only be determined indirectly by subtracting the load of URB_{north}, AGR, and URB_{south} from the load found at the outlet of the catchment. The loads of the StS and the CSO were extrapolated according to the catchment area of the separate and combined sewer system. In total, 120 ha of the urban area (without buildings) were drained by the combined and 46 ha by the separate sewer system (Figure 7.5-176c). The calculated loads amounted to 29 g for the WWTP, 54 g for the CSO and 42 g for the StS. Compared to the sum of the load found in surface water at the outlets of the sub-catchments (209 g), the contributions of the three urban input ways were all in the same range (WWTP 14 %, combined 26 % and separate system 20 %) and correspond in total to three fifths of the load in surface water.

Wastewater treatment plant – WWTP

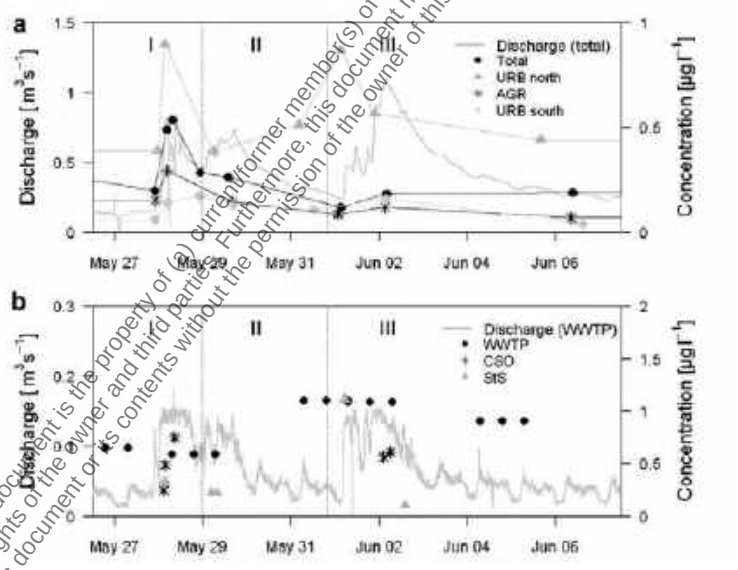
The concentrations in the WWTP effluent (up to 0.51 µg/L) were somewhat lower compared to values of 2 µg/L measured in the US or 1.5–1.9 µg/L in Austria. In WWTPs, glyphosate partially dissipates due to sorption and degradation; however, until now only few studies have investigated the removal of glyphosate in WWTPs. In a pilot plant a removal rate of 90–95 % was found for concentrations of 500 mg/L.

Nowack *et al.* (2002) investigated phosphonates in WWTPs and found removal rates in the same range (85–93 %). As glyphosate contains a phosphonate group, similar behavior in WWTP was assumed. Using an average removal rate of 90 %, the resulting amount in the WWTP inflow during the May/June event would be approximately 300 g, which equals to 2 % of the estimated total yearly amount (18 kg) used in the urban areas of the catchment.

Combined sewer system – CSO
Although the investigated overflow of the combined sewer system was only active during short periods of time (mostly at the beginning of a rain event), the input was significant. During the first interval of the May/June event, the load from URB_{south} was composed only of the load of the overflow. The activity of the CSO was thus crucial for the load dynamics of glyphosate.

Separate sewer system – StS
In contrast to the CSO where most of the input through over-flows of the combined system was covered by our sampling site and to the WWTP where the entire input was considered, the input through the separate sewer system was not optimally represented. Since there were several additional storm sewers present in the study catchment, the uncertainty of the extrapolation was high. Furthermore, the use data in the catchment area of the considered storm sewer was based on a comparatively small sample size. However, the main conclusion that urban sources had a wide influence was not affected by the uncertainty concerning the input from the separate sewer system.

Figure 7.5-179: Concentration dynamics of AMPA during the May/June event in surface water (a) and in the urban drainage system (b)



Event loads based on agricultural loss rates
To validate the loads, the input from agriculture was assessed by agricultural loss rates and the applied amount. The residual load was then assigned to urban inputs. The agricultural loss rate to surface waters was defined as the total amount reaching the surface waters divided by the amount applied on the fields in the catchment during one year. Although only one rain event was considered, we used overall loss rates, conscious that the input from agriculture was therefore overestimated. We assumed a loss rate of 0.1 % to represent diffuse losses and a rate of 1.0 % to consider improper handling or disposal. The loads based on these loss rates accounted for 3.9–39 % of the overall load from the three sub-catchments, which means that less than two fifth of the overall load can be explained by agriculture. These approaches thus indicate that the application of glyphosate in urban areas has considerable effects on the total load of glyphosate in surface water.

Conclusion
Monitoring (over a period of 9 months) was conducted in four sub-catchments with differing land uses: agricultural and urban use was surveyed allowing for a detailed interpretation of the monitoring results. The peak discharge concentrations of glyphosate during storm events were 4.15 µg/L. Fast runoff from hard surfaces led to a fast increase in glyphosate concentrations, which did not coincide with the concentration peak normally observed from agricultural fields. The load from the wastewater treatment plant, the combined sewer system, and the separate sewer system were all in the same range. It was clear that the majority of the total glyphosate load originated from urban areas. To evaluate the representativeness of the loss rate found for the study season (2.2 %) at the investigated study site (25 km^2) the loss rate for the river Rhine was estimated using national use data and measured loads from the Rhine monitoring station at Basel. River Rhine is the most important Swiss stream and drains two third of the country (28 000 km^2). The calculated loss of approximately 2 % for the river Rhine is in the same range as for the study site located in the North-East of Switzerland and thus confirms that the findings seem to be representative also for a larger scale.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes a monitoring experiment in Switzerland covering a catchment with urban and agricultural land use. Glyphosate and its metabolite AMPA were analyzed. A comparison between the contribution of agricultural use and urban use to the overall load was conducted. Due to a specific definition of sub-catchment areas and their evaluation, a specific conclusion for the agricultural area can be given. It was clear that the majority of the total glyphosate load originated from urban areas. Analytical approaches were sufficiently described.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/070
Report author	Botta, F. *et al.*
Report year	2009
Report title	Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems
Document No	Chemosphere (2009), doi:10.1016/j.chemosphere.2009.05.008
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

A study of glyphosate and aminomethyl phosphonic acid (AMPA) transfer in the Orge watershed (France) was carried out during 2007 and 2008. Water samples were collected in surface water, wastewater sewer, storm sewer and wastewater treatment plant (WWTP). These two molecules appeared to be the most

frequently detected ones in the rivers and usually exceeded the European quality standard concentrations of 0.1 µg/L for drinking water. The annual glyphosate estimated load was 1.9 kg/year upstream (agricultural zone) and 179.5 kg/year at the catchment outlet (urban zone). This result suggests that the contamination of this basin by glyphosate is essentially from urban origin (road and railway applications). Glyphosate reached surface water prevalently through storm sewers during rainfall events. Maximum concentrations were detected in storm sewers just after a rainfall event (75–90 µg/L). High concentrations of glyphosate in surface water during rainfall events reflected urban runoff impact. AMPA was always detected in the sewerage system. This molecule reached surface water mainly via WWTP effluent and also through storm sewers. Variations in concentrations of AMPA during hydrological episodes were minor compared to glyphosate variations. Our study highlights that AMPA and glyphosate origins in urban areas are different. During dry periods, detergent degradation seemed to be the major AMPA source in wastewater.

Methods

Study area

The research sites are situated in the Orge River catchment (956 km^2) in the North of France. The catchment is situated 30 km in the southern part of Paris metropolitan area and the Orge River is a tributary of the Seine River. This catchment shows an urbanization gradient from prevalently agricultural areas and partially forested upstream to more densely urbanized areas nearer the connection with the Seine River resulting in two zones of pesticides contribution. Glyphosate was one of the main molecules applied on roadsides and railways.

Sample campaigns were organized to gather data according four different levels: the first one at the basin scale to calculate the budget of glyphosate load in the Orge River, the second one at the urban area scale to verify the impact of the sewage network on the river contamination, the third part at the network scale to study the transfer of glyphosate and its degradate by runoff in urban areas and the last part at the waste water treatment plant scale to verify the potential impact of urban wastes on surface waters.

Basin area:
From January 2007 until December 2007, a bi-weekly sampling and analysis of glyphosate and AMPA were conducted in three locations in the Orge basin. Epinay-sur-Orge is situated downstream of the Yvette River, whereas Sermaise and Athis-Mons are situated, respectively, upstream and downstream of the Orge River. The Yvette River is the most important tributary of the Orge River (about 30 % of the total surface) and represents a highly urbanized part of the watershed.

Urban area:
Sampling was carried out in three different seasons of the year (autumn, winter and spring) in order to determine if urban applications are responsible for surface water contamination by glyphosate. Three points were sampled one in the Orge river and the other two in a small urban tributary, the Boële river (upstream and downstream). To evaluate the sewer contribution to the surface water contamination, samples were collected in the outfalls of two storm sewers discharging directly to the Boële River, between the up- and downstream points in different weather conditions.

Urban sewerage system:
This urban catchment has two big sewers that are running parallel. One is the main wastewater sewer and the other one is a storm sewer called Ru de Fleury drains a surface of 4.4 km^2. The area is located downstream of the Orge River in a residential zone. Sampling was carried out according to glyphosate application by public services. Samples were collected continuously with automated samplers during the sample campaigns.

Wastewater treatment plant effluent:
In order to know the wastewater treatment plant effluent contribution to surface water contamination, a small stream catchment was studied. The Predecelle River is a small tributary of the Orge River located in

the centre of the Orge basin. Five sites were sampled on the Predecelle River and one in the WWTP effluent on September 25, 2007 during dry weather conditions.

Analytical conditions

Glyphosate and AMPA were analysed by HPLC with a fluorescence detector following use of a derivatization agent. The quantification limit for glyphosate and AMPA in water was 0.1 µg/L.

Results

Basin area:
In the Orge River, annual glyphosate fluxes increased from upstream to downstream. For glyphosate the estimated annual flux was 1.9 kg year^{-1} in the upstream point while the same compound had an annual flux of 179.0 kg year^{-1} in the downstream point of Orge catchment. AMPA had an annual flux of 156.8 kg year^{-1} in the Orge downstream point and 1.7 kg year^{-1} in the upstream point. For glyphosate, the downstream point loads were 100 times bigger compared to the loads in the Orge upstream. This difference is also detectable for AMPA. In the downstream point of the Orge River, the glyphosate load is more than 20 kg year^{-1} higher than the AMPA load. For the Yvette River outlet, the estimated annual flux was 92.3 kg year^{-1} for the glyphosate and 52.8 kg year^{-1} for the AMPA. Yvette fluxes represented 50 % of glyphosate and 30 % of AMPA of total fluxes of the Orge river.

The results of this study and the pesticide use inquiries indicate that urban applications of pesticides are responsible for Orge catchment contamination, particularly glyphosate. In the urban parts of the Orge watershed (downstream Orge and Yvette), glyphosate load was higher than AMPA. This is not the case in the agricultural area, where treatments are applied to soil.

Urban area:
In order to assess the contribution of the urban applications, analyses were performed upstream and downstream of an Orge tributary in an urbanized sector (Boële River). Except for the sampling on December 10, 2007, glyphosate was always detected in the Boële River, as shown in the table below. Concentrations in the downstream point of the tributary were usually higher than in the one upstream and the concentrations registered in the Orge point were always lower. As the Orge River receives less urban rainfall via sewers than the Boële River, the increase in glyphosate concentration can be explained by the urban applications of glyphosate.

The concentrations of AMPA in the Boële River tributary points were always higher than concentrations registered in the Orge River. Values ranged from 0.3 to 1.0 µg/L in the tributary, whereas they ranged from 0.2 to 0.8 µg/L in the Orge River. Comparison of the observed concentrations upstream and downstream of the Boële River indicated that glyphosate and AMPA were essentially of urban origin. The impact of urban application was related to direct runoff from impervious surfaces towards the stream. AMPA occurrence during dry weather conditions in urban areas indicated that it might originate from detergent degradation especially out of the pesticide application period. A general increase in AMPA concentration after the beginning of treatment (campaigns of May, June and July) can be explained due to phosphonate and glyphosate degradation.

Evidence was given for a glyphosate concentration increase in the Boële River water flowing through an urbanized zone. Storm sewer outfalls were identified as a potential pinpoint pollution source. On the whole, glyphosate and its metabolite concentrations during the four samplings between June and July varied according to weather conditions. Glyphosate was always detected after rainfall events, ranging from 0.3 to 1.7 µg/L. After dry weather periods, glyphosate concentrations were detected in three out of four occasions at concentrations lower than 0.25 µg/L. AMPA was always detected in the outfalls during this period at up to 0.9 µg/L.

Table 7.5-207: Concentrations of glyphosate and AMPA in the Boële River, in the Orge River and in two outfalls

	October 22, 2007		December 10, 2007		April 3, 2008		April 15, 2008		May 16, 2008		June 13, 2008		June 19, 2008		June 25, 2008		July 4, 2008	
Weather condition	Dry weather		After rainfall		Dry weather		Dry weather		After rainfall		After rainfall		After rainfall		Dry weather		After rainfall	
µg L^{-1}	Gly	AMPA	Gly	AMPA	Gly	AMPA	Gly	AMPA	Gly	AMPA	Gly	AMPA	Gly	AMPA	Gly	AMPA	Gly	AMPA
Boele upstream	0.233	1.026	<0.1	0.355	0.125	0.475	<0.1	0.231	0.753	0.751	0.749	0.762	0.82	1.193	0.156	1.067	0.233	1.423
Boele downstream	0.439	0.733	<0.1	0.343	0.207	0.465	0.182	0.469	0.831	0.681	1.082	1.186	0.701	1.068	0.138	1.131	0.335	1.925
Outfall A	ND	ND	ND	ND	0,119	0.316	ND	ND	ND	ND	0.662	0.43	0.369	0.311	<0.1	0.707	0.264	0.579
Outfall B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.372	0.15	1.681	0.947	0.129	0.21	0.159	0.222
Orge river	0.196	0.786	<0.1	0.233	0.139	0.372	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Urban sewerage system:
AMPA was found in the storm sewer during dry weather conditions outside of the glyphosate application period and resulted from detergent use. The glyphosate found in storm water (up to 90 µg/L) was linked to glyphosate application by the local authorities 2 days beforehand. Glyphosate was found also in the wastewater sewer after the beginning of rainfall due to water transfer from the storm sewer to the wastewater sewer.

Wastewater treatment plant effluent:
Samples taken from points in the Predecelle River indicate that the main input of glyphosate is from urban applications and WWTP effluent resulting in concentrations of 1.5 and 1.62 µg/L, respectively, which decrease downstream where no input of glyphosate occurred. For AMPA, concentrations of 0.51 and 3.54 µg/L were found at the urban and WWTP effluent sampling points, respectively. Under dry weather conditions, detergent degradation seemed to be the source of AMPA in surface water receiving treated wastewater.

Conclusion
Investigation in the Orge Basin showed that non-agricultural application of glyphosate has a significant contribution to the glyphosate annual load. Urban runoff is responsible for glyphosate peaks in the Orge River in accordance with literature and glyphosate is more sensitive to rainfall compared to AMPA.

Glyphosate was not found in the storm sewer under dry weather conditions and outside of application periods. However, it was detected during application periods and rainfall events in storm sewers and in wastewater sewers. This means that in a separate sewerage system, during rainfall events, glyphosate may be transferred to surface waters directly via storm sewers and also indirectly *via* WWTP discharge.

AMPA was always detected in all samples (waste, storm and surface waters). Highest concentrations were measured in wastewater samples. It was also found in storm sewers during dry weather conditions and outside of glyphosate application periods. The results show the domestic origin of AMPA in sewer systems. This AMPA can be a metabolite formed from some detergents.

The result of this study confirms AMPA inputs through WWTP discharge and underlines that glyphosate used in urban areas reaches streams mainly by storm sewers.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article investigates urban sources of glyphosate and AMPA in surface water in the Orge River catchment in the North of France over two years. The methods and results are sufficiently described. In surface water, glyphosate was found up to 1.7 µg/L and AMPA up to 1.93 µg/L.
The article is considered reliable with restriction.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/071
Report author	Ghanem, A. *et al.*
Report year	2007
Report title	Concentrations and specific loads of glyphosate, diuron, atrazine, nonylphenol and metabolites thereof in French urban sewage sludge
Document No	Chemosphere (2007), doi:10.1016/j.chemosphere.2007.05.022
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Indirect soil pollution by heavy metals and organics may occur when sewage sludge is used as fertilizer. The nature and amounts of pollutants contained in sewage sludge need to be defined in order to assess the environmental risk. Results were obtained for the surfactant nonylphenol and herbicides; glyphosate, diuron and atrazine and their major degradates in sewage sludge sampled from three wastewater treatment plants and one composting unit in the vicinity of Versailles, France for one year. The presence of glyphosate and aminomethylphosphonic acid was demonstrated at the mg/kg (dry matter) level in all samples.

Methods

Sewage sludge was sampled monthly from July 2004 to June 2005 in three urban wastewater treatment plants and one composting unit in the vicinity of Versailles (France). In all cases, the treatment process included screening, grit removal, primary sedimentation with use of chemical coagulants (except the plant of Saint-Cyr), phosphorus and nitrogen elimination and conventional activated sludge treatment.

The plants of Plaisir and Elancourt were each connected to a separate sewer system and an urban catchment area with moderate industrial activity. The plant of Saint-Cyr has a similar catchment area, but it is connected to a combined sewer system. The WWTP of Plaisir provided dried (pelleted) sludge, whereas sludge treatment was obtained by liming in Elancourt and Saint-Cyr. In the unit of Gazeran, sludge was composted with wood chips as a bulking material. Sludge also originated from several WWTPs, located in a rural area with a mixture of agricultural (cattle breeding) and industrial activities. Wastewaters were collected by several sewer systems, mainly of the combined type.

Centrifuged samples were used for all analyses, as these contained the highest extraction yields. Other samples were then collected after drying, composting or liming to show an effect of sludge treatment on chemical content. Unfortunately, some technical problems in the composting plant of Gazeran prevented a complete campaign of sampling. Sludge samples (1 kg wet weight) were collected, frozen within 1 h after sampling and stored at -20 °C until analysis.

The concentrations of glyphosate and AMPA in sludge samples were determined in alkaline extractions purified on a strong anion-exchanger resin before FMOC-Cl derivatization on the same solid support. Samples were concentrated by reversed-phase SPE before analysis by LC-ESI-MS/MS in the MRM (Multiple Reaction Monitoring) mode.

The method for glyphosate and AMPA analysis showed mean recoveries of 70 % (RSD < 9 %) for glyphosate and 63 % (RSD < 5 %) for AMPA, using centrifuged sludge samples collected before liming, composting or drying. Limits of quantifications (LOQs, S/N of 5) were 35 and 50 µg/kg d.m. (dry matter) for glyphosate and AMPA, respectively.

Results and discussion
Glyphosate and AMPA were quantified in all the samples. The highest mean values for glyphosate were detected in the samples from Plaisir and Elancourt (1.1 and 1.4 mg/kg d m.), whereas sludge from Saint-Cyr was less contaminated (0.4 mg mg/kg d.m.). Accordingly, mean values of 20.3 (Plaisir), 11.5 (Elancourt) and 2.8 (Saint-Cyr) mg/kg d.m. were calculated in the sludge for AMPA. The concentrations of AMPA should be attributed to glyphosate degradation. Nevertheless, aminophosphonates (EDTMP and DTPMP) contained in household cleaning products can be converted to AMPA in wastewaters and WWTPs. This urban source could explain the high amounts of AMPA measured. Samples from Saint-Cyr were the most contaminated (46.6 µg/kg d m.) as compared to those of Plaisir and Elancourt (11.2 and 20.0 µg/kg d.m.).

Despite incomplete sampling, the data for Gazeran revealed a contamination level similar to that observed for Plaisir and Elancourt for glyphosate and AMPA. Although these compounds are widely used in urban areas as herbicides, it was difficult to define a clear relationship between sludge contamination and periods of weed treatment.

Conclusion
Substantial amounts of herbicides (glyphosate and its metabolite AMPA) were detected in sewage sludge originating from urban areas in France. It can be concluded that an important part of the herbicides detected comes from domestic households.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article investigates indirect soil pollution by heavy metals and organics when sewage sludge is used as a fertilizer from three urban wastewater treatment plants and one composting unit in the vicinity of Versailles (France) over one year. The authors concluded that an important part of the glyphosate and AMPA detected came from domestic households.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/072
Report author	Peschka, M. *et al.*
Report year	2006
Report title	Trends in pesticide transport into the River Rhine
Document No	Hdb Env Chem Vol. 5, Part L (2006): 155–175 DOI 10.1007/698_5_016
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The occurrence of relevant pesticides in the River Rhine and two of its tributaries is presented over a period of ten years. Trace determinations of 66 target pesticides and their metabolites in water from the River Main and the River Nidda were performed on continuously sampled wastewater and surface water utilizing different solid phase extraction protocols and detection by gas chromatography mass spectrometry, directly or after derivatization. The transport rates of pesticides in municipal wastewater treatment plant (WWTP) effluents and surface waters were determined from data obtained in 1994, and these show that WWTPs contribute significantly to the pesticide pollution in the surface water. A trial education program providing improved methodology, spraying equipment and support to farmers living close to a single WWTP lead to a drastic reduction (more than 90 %) in the total pesticide transport caused by this WWTP.

During two extensive sampling campaigns in 1999 and 2000, mixed samples from a total of 106 (for 1999) and 35 (for 2000) WWTPs in agricultural used areas from Hesse (Germany) were investigated for selected priority pesticides and metabolites. In this case, the mitigation measures mentioned above were found to be unsuccessful overall, which is most likely attributable to less interaction with the pesticide users as compared to projects in small villages with high public attention.

Methods

A total of 62 pesticides were selected including glyphosate and its metabolite AMPA.

Sampling:
Receiving streams (the Main and the Nidda) and WWTP located in agricultural areas were chosen for study in Hesse, Germany. Mainly grain and maize, but also rape and sugar beet are grown over the catchment area of the River Nidda. No companies discharging industrial waste containing pesticides were located on the river. At Frankfurt-Nied, the Nidda joins with the River Main, which subsequently joins with the River Rhine close to Bischofsheim. The Main receives discharges from many chemical industries, including those producing pesticides. The period from April to May was selected for sampling, as this time frame reflects the peak period for pesticide application.

River samples were taken twice a week from the Rhine during a period of ten years (1993–2003). In the period from 6th April to 17th May 17 1999, a total of 106 WWTP effluent samples were collected twice as three-week mixed samples. The sites found to be most polluted in 1999 were then sampled again over the same period in 2000. During the same time period, mixed samples from the WWTP at Woelfersheim, Hesse, Germany were also taken daily from 1994 to 1998. Mixed weekly surface water

samples were collected automatically from the Main during pesticide application time (April to June), and collected as two-week mixed samples for the rest of the year.

Analysis:
Rhine samples were filtered if necessary and then enriched over C-18 cartridges. Main, Nidda and WWTP samples were passed through glass fiber filters, prewashed with methanol and Milli-Q water before solid phase extraction (SPE) was performed. Analysis was by GC/MS.

Results and discussion
Glyphosate was present in the river Main from April to September at a concentration of up to 0.1 µg/L. In the Nidda it was present over the whole year at a maximum concentration of 0.4 µg/L, which is due to the higher amount of waste water in the Nidda. The concentration of the metabolite AMPA exceeded the glyphosate concentration by several times.

The results from bank filtration experiments showed that glyphosate was removed after a distance of about 200 m, whereas AMPA needed about 300 to 500 m to be completely eliminated. The experiments were carried out at the waterside of the Main.

Glyphosate and AMPA were not detectable in groundwater, even though they had been applied in massive amounts around rail tracks since 1991.

Water treatment at the WWTP included several steps, namely floc filtration, gravel filtration, and activated carbon filtration. In order to evaluate the efficiencies of those steps, samples were taken before and after each step so that the glyphosate and AMPA could be quantified. The first step, flocculation with activated silicic acid and addition of potassium permanganate and aluminum salts, gave an elimination rate of 39 ± 14 % for glyphosate and 22 ± 15 % for AMPA. Gravel filtration reduced both by less than 10 %. Activated carbon filtration also reduced glyphosate by < 10 %, and AMPA by 21 ± 9 %. These results showed that glyphosate and its metabolite were not completely removed in a raw water treatment facility.

Conclusion
Sampling from sewage drains leading to WWTPs showed that farms connected to sewage drains are the most important source of pollution. Analysis of puddles on roads and paths also showed pesticide contamination, which will also be a source of pesticide entry into sewage drains through rainfall wash-off. It can be assumed, however, that the main sources of pollution are the cleaning of spraying tools in farmyards and the pesticide lost from spraying machines traveling by road.

The diffuse pollution problem is a difficult one to tackle. Mitigation measures to circumvent diffuse pollution, even those resulting from many small point pollutions (such as those that were partially successful in this study) depend strongly on the motivation of the pesticide users and the level of interaction with them achieved, since a measurable result will only be obtained through the responsible application and use of pesticides by the farmers.

The removal rates for glyphosate and AMPA for some low-chemical processes were reported: flocculation with activated silicic acid and addition of potassium permanganate and aluminium salts, removal rate of 39±14 % for glyphosate and 22±15 % for AMPA; for gravel filtration removal rate of <10 % for both compounds; and for activated carbon removal rates of <10 % for glyphosate and 21±9 % for AMPA.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article investigates urban sources of glyphosate in surface water in the Main and Nidda Rivers in Germany. The methods and results are briefly described. The removal rates for glyphosate and AMPA for some low-chemical processes were reported: flocculation with activated silicic acid and addition of potassium permanganate and aluminium salts, removal rate of 39±14 % for glyphosate and 22±15 % for AMPA; for gravel filtration removal rate of <10 % for both compounds; and for activated carbon removal rates of <10 % for glyphosate and 21±9 % for AMPA.
Glyphosate was found in surface water at a concentration of up to 0.4 µg/L.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/073
Report author	Augustin, B.
Report year	2003
Report title	Urban areas - source of pesticide-contamination of surface water?
Document No	Mitt. Biol. Bundesanst. Land- Forstwirtsch. 394, 2003;
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

In Rhineland Palatinate in Germany, numerous (14-day-mix) samples of surface water (Mosel, Nahe, Selz) were repeatedly monitored for pesticide pollution between 1997 and 1999. Investigations focused on 35 different active ingredients including glyphosate. Glyphosate results were presented for the Selz river in 1997 and indicated detections in water sources in periods during the year. An additional investigation of a sewage disposal plant ("Hahnheim"), which drains into the Selz river, clearly showed that waste water contained glyphosate at a concentration about ten times as high as in the river water.

Up to the present there are no indications for the presence of glyphosate in drain water from agricultural areas. The author speculated that as glyphosate was detectable during the entire year, it is unlikely that it derived from application of farmland, vineyards or orchards. The fact that larger quantities are used on urban areas indicated that there might also be runoff from sealed areas.

Methods
14-day-mix samples from the Selz river at lngelheim in the period 3 March to 8 December 1997 and a wastewater treatment plant at Hahnheim (which drains into the Selz river) in the period 17 March to 9 July 1997 were analysed for glyphosate. Glyphosate was also analysed in runoff water from a concrete surface.

Results
In 14-day-mix samples from the Selz river at lngelheim, concentrations of glyphosate up to approximately 1.8 µg/L were measured with maximum concentrations occurring in April to June 1997. In 14-day-mix samples from a wastewater treatment plant at Hahnheim, concentrations of glyphosate up to approximately 9 µg/L were measured in the period April to July 1997.

Glyphosate analysed in runoff water from a concrete surface was found at much higher concentrations of up to 17.9 mg/L after 2 mm rain over 1 hour. The concentration decreased in runoff after longer periods.

Conclusion
Glyphosate was detected in the Selz river in periods during the year. An additional investigation of a sewage disposal plant ("Hahnheim"), which drains into the Selz river, clearly showed that waste water contained glyphosate at a concentration about ten times as high as in the river water. Glyphosate was found in runoff water from a concrete surface at much higher concentrations than in waste water and the river.

Up to the publication date (2003) there were no indications for the presence of glyphosate in drain water from agricultural areas. The author speculated that since glyphosate was detectable during the entire year, it was unlikely that it derived from application of farmland, vineyards or orchards. The fact that larger quantities were being used on urban areas indicated that there might be runoff from sealed areas.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article investigates urban sources of glyphosate in surface water in the Selz River in Germany over one year. The methods and results are only briefly described. The author speculated that as glyphosate was detectable throughout the year it was unlikely that it derived from application to farmland, vineyards or orchards; that larger quantities were used in urban areas (due to increased residential building) and probably originated from runoff from hard surfaces.
A maximum concentration of glyphosate up to 1.8 µg/L was reported in surface water.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

B. Water

B.2b Transitional water

Concentrations of glyphosate (GLY), AMPA and HMPA in transitional water arising from public monitoring datasets have been collected from regional/national environment agencies as well as published peer reviewed publications from literature searches and rated as potentially relevant/reliable are reported in this section.

There are two new applicant studies presented on transitional waters. [redacted] (2020, CA 7.5/001) describes the collection of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA. [redacted] (2020, CA 7.5/002) assesses the data collected by [redacted] (2020, CA 7.5/001). These two recent studies were designed to be the more comprehensive than previous studies by considering additional metabolites, compartments and time periods. [redacted] (2020, CA 7.5/002) covers a range of environmental compartments, however, the study summary below only includes the results relevant to transitional waters.

The maximum measured concentrations in transitional waters for GLY were 0.18 µg/L (Germany) and 1.2 µg/L (UK) and for AMPA was 0.9 µg/L (Germany), which were all well below the surface water RACs and EQS thresholds.

The available data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in the transitional water compartment.

Applicant studies

New studies/assessments

1. Information on the study

Data point:	CA 7.5/001
Report author	[redacted]
Report year	2020
Report title	Collection of public monitoring data for European countries for the compartments soil, water, sediment and air for Glyphosate, AMPA and HMPA
Document No	110057-1
Guidelines followed in study	Methodology is based on the Groundwater Monitoring guideline document (Gimsing *et al.*, 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations') Minimum quality criteria of monitoring data described by the FOCUS Ground Water Work Group chapter 9.5 (European Commission, 2014)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The report provides information about the outcome of a search for readily accessible and available monitoring data in European countries at a regional/national level for the time period 1995-2019. The main focus was on the time period 2012-2019 while earlier years are already covered by existing data. The search included raw data, requested from regional/national authorities or downloadable from their websites, as well as aggregated data extracted from reports compiled by authorities.

Data from 14 European countries were considered: Austria, Belgium, Denmark, France, Germany, Hungary, Ireland, Italy, The Netherlands, Poland, Romania, Spain, Sweden and the United Kingdom. The countries represent the major markets of products containing glyphosate sold in the EU. The data compilation included the active substance glyphosate and its metabolites AMPA and HMPA, in the soil, groundwater, surface water, tidal water, drinking water, sediment and air environmental compartments.

As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities and other bodies of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were not actually included in any of the monitoring programs.

Tidal Water Compartment Conclusion

Raw datasets for transitional water bodies were only provided by Germany and England. No aggregated datasets were identified or provided by any countries.

I. MATERIALS AND METHODS

The general methodology of data collection of public monitoring data and minimum quality criteria is based on existing guideline documents for groundwater monitoring programs. The underlying principles have been applied to all environmental compartments, especially where no specific guidance is at hand. Data search, acquisition and processing approaches are described below. The same approach was applied for each country, compartment and substance. Country specific adaptations to the general procedure were made in order to generate a harmonized database. The data collected for this report refers to third party organization data regarding all environmental compartments (SOIL, GW, SW, TD, DW, SD, AIR) and was further differentiated into the two different data types, i.e. raw data and aggregated data. Aggregated data refers to information provided in publicly available reports, e.g. from environmental agencies or research institutes. Such reports might hold only summary information on substance findings over space and time and may intersect with the raw data. Raw data refers to mid to long term time series of data that are provided on request by e-mail or by database from governmental authorities and are therefore recognized as official monitoring data. These datasets hold the information of sampling values, quality information (sampling, treatment, limit of detection - LOD, limit of quantification - LOQ) as well as information of location and time of sampling.

The following data source types were investigated in order to collect monitoring data:

- E-mail requests: a general e-mail was sent to the national responsible authorities with regard to the required information.
- Governmental webpages: the official webpages of the national responsible authorities were searched for information regarding available reports and datasets.
- Public online databases: available data from online databases were downloaded as provided by the webpages of governmental authorities and other institutions.

The data search resulted in a very heterogeneous collection of tabular data and reports in different formats and structure. Data were processed into a harmonized tabular format by selecting relevant information and adapting data organisation. In general, the complete datasets were included in the final harmonized database as provided by the authorities, but obvious duplicates were deleted. In general, all entries for the digital database were checked for consistency and plausibility. For the raw data it was assumed that information was already subjected to critical scrutiny by the respective organization. For the aggregated data the same assumption was made with quality assurance of the data (mostly summaries) being the responsibility of the authors of the respective reports.

II. RESULTS AND DISCUSSION

The final data collection of raw data and aggregated data is summarised for each compartment and each country in Table 7.5-208.

Tidal Water

- Germany (DE)
  - The regional authority in Mecklenburg-Vorpommern provided raw data on tidal waters.
  - No aggregated data were provided.
- Poland (PL)
  - The responsible authorities for monitoring data in Poland are the Polish Geological Institute and the Chief Inspectorate Of Environmental Protection. The latter authority confirmed by e-mail that in Poland there is currently no public monitoring of glyphosate or its metabolites in surface water.
- Romania (RO)
  - The responsible authority for monitoring data is the Ministry of Water and Forests. The Water Resources Management Directorate confirmed on behalf of the Ministry of Water and Forests that no public monitoring of glyphosate or its metabolites is carried out in any water compartment in Romania.
- United Kingdom (UK)
  - For tidal waters, raw data were available for England from the EA webpage.
  - No other country in the UK provided raw data for tidal waters,
  - No aggregated monitoring data from reports were provided and included in this report.

Table 7.5-208: Overview of public monitoring data availability of raw data (R) and aggregated data (A)

Country	Soil	Water				Sediment	Air
		Ground	Surface	Tidal	Drinking		
Austria	-	R, A	R, A	-	A	-	-
Belgium	-	R	R	-	A (Flanders)	-	-
Denmark	-	R, A	A	-	A	-	-
France	-	R	R	-	A	R	-
Germany	R (Brandenburg)	R, A	R, A	R	R (Schleswig-Holstein), A	-	-
Hungary	-	A (one research article)	A (one research article)	-	-	-	-
Ireland	-	R, A	R, A	-	R, A	-	-
Italy	-	R (Lombardia), A	R, A	-	-	-	-
The Netherlands	-	R, A	R, A	-	R	-	-

Table 7.5-208: Overview of public monitoring data availability of raw data (R) and aggregated data (A)

Country	Soil	Water				Sediment	Air
		Ground	Surface	Tidal	Drinking		
Poland	confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Romania	confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Spain	-	R, A	R, A	-	A	-	-
Sweden	-	R, A	R	-	R, A	R	-
UK England	-	R	R	R	A	-	-
UK Northern Ireland	-	R	-	-	-	-	-
UK Scotland	-	-	R	-	-	-	-
UK Wales	-	-	R	-	A	-	-

R raw data available; A aggregated data from reports available; - no raw or aggregated data available

III. CONCLUSIONS

The present collection of public monitoring data for glyphosate, AMPA and HMPA in soil, groundwater, surface water, drinking water, tide water, sediment and air resulted in a comprehensive database of 'raw monitoring data from national authorities' and 'aggregated monitoring data from reports published by national authorities'. As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were actually not included in any of the monitoring programs.

Raw datasets for transitional water bodies were only provided by Germany and England. No aggregated datasets were identified or provided by any countries.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes the collection process of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA
The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Document No	-
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al.*, 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The report provides information about the outcome of an analysis of public monitoring data comprising environmental concentrations of glyphosate (GLY) and its primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA) collated from readily available public monitoring databases held by national/regional environment agencies. This data collection and analysis was designed to expand previous reviews to include other compartments and supplement them for surface water, groundwater and drinking water. Public monitoring data from the following Member States (MS) were assessed for the water, sediment and soil compartments: Austria (AT), Belgium (BE), Denmark (DK), France (FR), Germany (DE), Ireland (IE), Italy (IT), Netherlands (NL), Spain (ES), Sweden (SE) and the United Kingdom (UK). Three MS, namely Poland (PL), Hungary (HU), and Romania (RO) confirmed that they do not conduct analyses for GLY, AMPA and HMPA in any environmental compartment. No data for HMPA was identified for any MS or compartment. Note that at the time the study was started the UK was a Member State and is referred to as a Member State throughout the report.

Analyses of the large spatial and temporal dataset of measured concentrations occurring in several environmental compartments, namely surface water, groundwater, drinking water, tidal water, sediment and soil, were conducted to assess their state. This analysis not only sought to assess the state of the environmental compartment but also to consider the potential impacts this might have on biota, ecosystems and human health by using regulatory endpoints and thresholds from a range of European (EU) Directives. These included the Water Framework Directive (Directive 2000/60/EC) and associated Groundwater (2006/118/EC), Drinking Water (1998/83/EC) and Priority Substances (2008/105/EC28) Directives in addition to the Plant Protection Products Directive (1107/2009/EC).

<u>Transitional Waters</u>

A small number (~800 samples from 22 sites) of GLY and AMPA analyses from brackish transitional/tidal environments were analysed. These were from two MS, namely DE and UK, from an individual region in

each MS, Mecklenburg-Vorpommern and England, respectively. The data were assessed against the surface water RAC of 400 µg/L for GLY and 1200 µg/L for AMPA, given these are brackish waters. Additional ecosystem impact assessments were conducted using SW EQS-MAC and EQS-AA where these were defined for these MS. No information on HMPA was available.

Compliance was 100 % with no exceedances of the RAC, EQS-MAC or EQS-AA indicated by the data for both GLY and AMPA. The maximum measured concentrations of 0.18 µg/L (DE) and 1.2 µg/L (UK) for GLY, as well as 0.9 µg/L (DE) for AMPA, were well below the RAC and EQS thresholds.

Transitional Waters Compartment Conclusion

While limited in number, spatial and temporal scope the available transitional water data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in this environmental compartment.

I. MATERIAL AND METHODS

The dataset analysed comprised individual groundwater analysis records as well as existing aggregated analyses extracted from reports sourced from regional/national environment agencies (see [redacted], 2020, CA 7.5/001). The approach taken for the data processing was precautionary in that it preserved samples in the analysis where there was any doubt regarding their reliability. No records were excluded from the analysis. Similarly, no attempt to remove outliers was undertaken. Analysis and assessment of the data against thresholds was undertaken in statistical software R (R Core Team, 2019) and graphs produced in Excel. The same endpoints as that used for surface water (SW) bodies were utilised. This is consistent with these being predominantly brackish waters rather than marine waters. The UK endpoints for such waters are reported as being the same as for SW:

- Ecotoxicological endpoint: Regulatory acceptable concentration (RAC) of 400 µg/L for GLY and 1200 µg/L for AMPA.
- Ecosystem endpoint: Environmental quality standards (EQS) where these were proposed/available (see Table 7.5-209) at a Member State level, comprising annual average EQS (EQS-AA) and maximum allowable concentration (EQS-MAC); No values for these endpoints are available at a European level.

Table 7.5-209: Summary of Environmental Quality Standards (EQS), average annual (AA) and Maximum allowable concentration (MAC) utilised for the different Member States

Member State	GLY		AMPA	
	EQS-AA	EQS-MAC	EQS-AA	EQS-MAC
	µg/L			
DE - Germany	28	NA	96	NA
UK – United Kingdom	196[1]	398[1]	NA	NA
EU Combined Dataset	NA	NA	NA	NA

NA – Not available as not defined

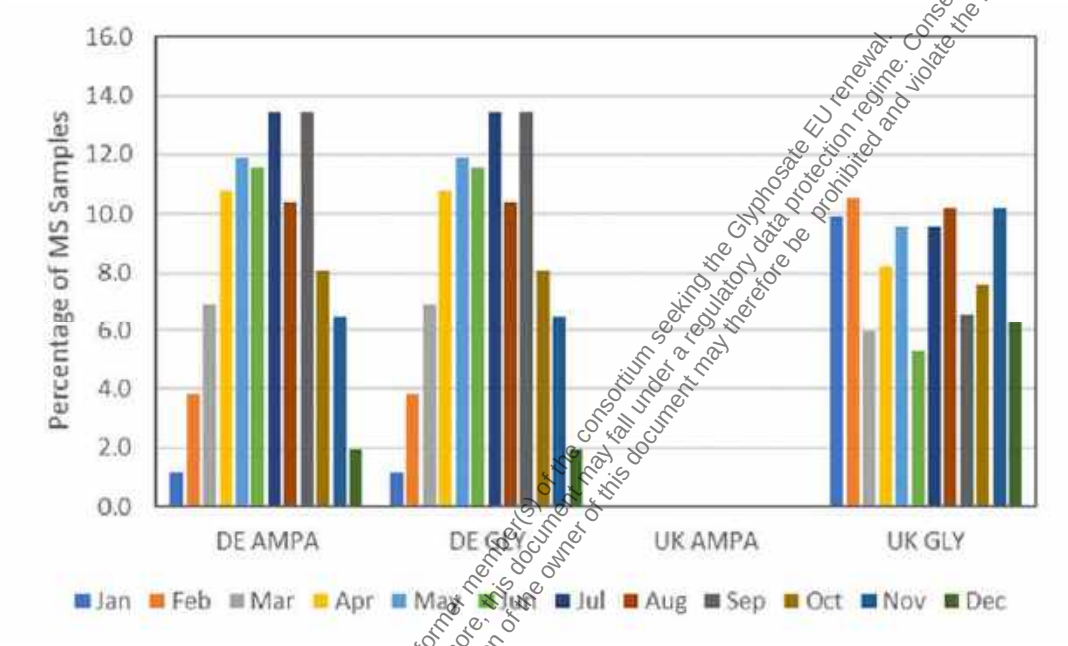
[1] - These are 90th percentiles, applied as maximum values in the first instance in a precautionary approach

II. RESULTS AND DISCUSSION

The amount of data supplied and analysed for this study is very limited (~800 samples from 22 sites) and as such is biased both spatially and temporally. These include a variety of tidal water bodies including estuaries, lagoons and near shore brackish areas. The bulk of the data (~46 % for GLY and 100 % for AMPA) came from the DE dataset which comprises 15 sites located along the Baltic Sea coastline of Germany in the Bundesland of Mecklenburg-Vorpommern. This dataset covered 9 years spanning the

period 2009 – 2018 (see Table 7.5-210). Monthly sampling effort for both GLY and AMPA appeared to be unimodal with lower sampling intensities in the winter (see Figure 7.5-180). The dataset from the UK comprised 8 sites distributed unevenly along the east coast of England. It covered 9 years spanning the period 2000 to 2009. Monthly sampling effort appeared to be variable throughout the year. There was insufficient data to create a combined European dataset and as such only individual MS data were presented.

Figure 7.5-180: Bar chart of tidal water monthly glyphosate (GLY) and AMPA sampling effort within each Member State



Analysis of the GLY tidal water dataset indicated that GLY was quantified in 6.9 % (DE) to 8.9 % (UK) of samples (see Table 7.5-210), albeit the number of samples was quite limited (260 in DE; 303 in UK). Compliance was 100 % given no analyses exceeded the RAC of 100 µg/L or came close to doing so with the maximum measured concentrations being 0.18 µg/L (DE) and 1.2 µg/L (UK). As such, compliance with the UK EQS-MAC was 100 % given none of the UK samples exceed the national EQS-MAC of 398 µg/L. There was insufficient data in the DE dataset to calculate average annual concentrations, however, three sites in the UK do have sufficient data to do so (see Table 7.5-211). At these three sites 100 % compliance with the UK EQS-AA of 196 µg/L was demonstrated as no exceedance of the EQS-AA was indicated. Intuitively, given the median and maximum concentrations (see Table 7.5-210) exceedance of the EQS-AA for either MS seems extremely unlikely at any sites.

All of the AMPA data came from the DE dataset and comprises 260 samples taken at 15 sites. Compliance of 100 % with the RAC of 1200 µg/L was indicated given there were no exceedances of the RAC. The maximum measured concentration was low, being 0.9 µg/L. While no EQS-MAC was set for DE, the data available does not suggest such an EQS would be exceeded where it is available. There was insufficient data in the DE dataset to calculate average annual concentrations, however, given the median and maximum concentrations (see Table 7.5-210) exceedance of the EQS-AA of 96 µg/L seems extremely unlikely.

III. CONCLUSIONS

Compliance with transitional water regulatory endpoints and thresholds was 100 % with no exceedances of the RAC, EQS-MAC or EQS-AA indicated by the data for both GLY and AMPA. The maximum measured concentrations of 0.18 µg/L (DE) and 1.2 µg/L (UK) for GLY, as well as 0.9 µg/L (DE) for AMPA, were well below the RAC and EQS thresholds. While limited in number, spatial and temporal scope the available transitional water data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in this environmental compartment.

Table 7.5-210: Summary results of glyphosate (GLY) and AMPA analyses in tidal water bodies

MS	Substance	Number of Sites	Number of Samples	Years	LOQ (µg/L)	Samples with LOQ≤0.1 µg/L		Detected >LOQ		Detected >RACS			Detected > EQS-MAC			Measured Concentration (µg/L)
					Mean (min - max)	Sites	Samples	Samples	%	Sites	Samples	% Samples	Sites	Samples	% Samples	Median[1] (min - max)
DE	AMPA	15	260	2009 - 2018	0.03 (0.02 - 0.03)	15	260	86	33.1	0.0	0	0.0	ND	ND	ND	0.03 (0.014 - 0.9)
	GLY	15	260	2009 - 2018	0.02 (0.01 - 0.03)	15	260	18	6.9	0.0	0	0.0	ND	ND	ND	0.02 (0.010 - 0.18)
UK	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	8	303	2000 - 2008	0.10 (0.10 - 0.10)	8	303	27	8.9	0	0	0.0	0	0	0.0	0.10 (0.100 - 1.2)

ND – not determined as data not available or EQS-MAC not defined
[1] Values <LOQ and <LOD are treated as equal to LOQ and LOD as a precautionary estimate of the median

Table 7.5-211: Summary of Annual Average (AA) Environmental Quality Standard (EQS) statistics for those Member States (MS) where such a threshold is available

MS	Substance	Number of Sites	Number of Years	Number of Sites with 12 Samples per Year	Number of Years with 12 Samples per Year	Number of Sites > AA-EQS	Percent of Sites > AA-EQS	Number of Years > AA-EQS	Percent of Years > AA-EQS
DE	AMPA	15	47	0[1]	0[1]	ND	ND	ND	ND
	GLY	15	47	0[1]	0[1]	ND	ND	ND	ND
UK	AMPA	NA	NA	NA	NA	NA	NA	NA	NA
	GLY	8	43	3	27	0	0.0	0	0.0

[1] Insufficient data available with <12 samples per year being taken at each site; ND – not determined as insufficient data; NA – no data available

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the analysis of public monitoring data for key European countries for the compartment transitional/tidal waters for glyphosate and AMPA. Monitoring data for two countries were available. The maximum measured concentrations in transitional waters of 0.18 µg/L (DE) and 1.2 µg/L (UK) for GLY, as well as 0.9 µg/L (DE) for AMPA, were well below the RAC and EQS thresholds. The available data do not indicate any risk to biota or ecosystems from measured GLY and AMPA concentrations in the transitional water compartment.
The study is considered valid.

Assessment and conclusion by RMS:

Existing studies/assessments

There are no existing applicant monitoring data or studies covering transitional waters.

Relevant literature articles

There are no existing relevant literature articles covering transitional waters.

B. Water

B.3 Drinking water

Concentrations from public monitoring datasets of glyphosate (GLY), AMPA and HMPA in drinking water have been collected from regional/national environment agencies as well as published peer reviewed publications from literature searches and those rated as potentially relevant/reliable are reported in this section.

There are three new applicant studies presented for drinking water. [redacted] (2020, CA 7.5/001) describes the collection of public monitoring data for European countries for the compartment soil, water, sediment and air for glyphosate, AMPA and HMPA. [redacted] (2020, CA 7.5/002) assesses the data collected by [redacted] (2020, CA 7.5/001). These two recent studies were designed to be the more comprehensive than previous studies by considering additional metabolites, compartments and time periods. [redacted] (2020, CA 7.5/002) covers a range of environmental compartments, the study summary below only includes the results relevant to the drinking water environmental compartment. [redacted] (2015, CA 7.5/074) updates a previous investigation period described by an existing study of [redacted] (2008, CA 7.5/075).

The existing applicant studies by [redacted] (2008, CA 7.5/075) and [redacted] (1997, CA 7.5/076) are presented for completeness.

Two publications are also presented outlining concentrations found in drinking water:

- Malaguerra *et al*. (2012, CA 7.5/077) considered drinking water data for Zealand, Denmark.
- Bruchet *et al*. (2011, CA 7.5/027) reported drinking water concentrations following bank filtration of water from the Seine, Paris, France.

A summary of maximum concentrations of glyphosate (GLY) and AMPA in drinking water reported by these studies and publications is presented in Table 7.5-212 while the maximum reported rates of exceedance of various thresholds by these datasets are summarised in Table 7.5-213. No data for HMPA was identified.

Maximum measured concentrations of GLY up to 0.92 µg/L are reported. GLY compliance with the regulatory drinking water threshold of 0.1 µg/L is very high (>99.84 % of samples) as exceedances are exceedingly rare (<0.16 % of samples) and when they do occur, they are well below the lifetime health-based Acceptable Daily Intake (ADI) concentration of 1500 µg/L used for consumer risk assessment.

Maximum measured concentrations of AMPA up to 3.0 µg/L are reported. AMPA compliance with the arbitrarily defined regulatory threshold of 10 µg/L for non-relevant metabolites is absolute as exceedances do not occur. Concentrations above the precautionary threshold of 0.1 µg/L are exceedingly rare (<0.22 % of samples) and when they do occur, they are well below the lifetime health-based ADI concentration of 3960 µg/L used for consumer risk assessment.

Malaguerra *et al*. (2012, CA 7.5/077) constructed a statistical model to assess factors influencing GLY concentrations in drinking water abstracted from local groundwater sources and concluded that distance from surface water was a driving factor. They postulated that infiltration from SW sources or slower degradation in riparian areas were possible reasons for this observation. Some exceedances reported in the datasets and reports assessed by the applicant studies arise from apparently untreated water sources, e.g. household groundwater wells. Exceedances within the Swedish dataset assessed in [redacted] (2020, CA 7.5/002) were old (≤2007) with later periods in the dataset reflecting the significant strides that have been made since the introduction of the water protection regulations in 2004 through delineation of water protection zones. Bruchet *et al.* (2011, CA 7.5/027) demonstrate that bank filtration, the lateral movement of groundwater through the phreatic aquifer, removed GLY and AMPA very effectively resulting in no

detectable residues in drinking water. Groundwater case studies in [redacted] (2020, CA 7.5/002) investigating situations where public monitoring suggested elevated rates of detection demonstrate that local factors like open hand dug wells may influence detections of GLY and AMPA and that localised investigations to understand the situation better with a view to adapting local practice through targeted stewardship programs or defining drinking water protection zones around wells is the most appropriate means of addressing these situations where they arise.

Similarly, [redacted] (2020, CA 7.5/002) demonstrated that existing water treatment removal efficiencies (95 % for AMPA, and 99 % for GLY) used in the production of drinking water will address typical measured concentrations in surface water abstracted for drinking water. More so when considered in conjunction with abstraction (selective abstraction into bank side storage, bank abstraction) and source management (blending of sources) used by water companies within their water supply chain.

The available measured environmental concentrations available suggest neither GLY nor AMPA pose a risk to human health *via* drinking water. Safe use with respect to drinking water is demonstrated for the vast majority of use environments in Europe.

Table 7.5-212: Summary of reported maximum concentrations of glyphosate (GLY) and AMPA in drinking water

Reference	Context	Maximum Concentration (µg/L)	
		GLY	AMPA
[redacted] 2020, CA 7.5/002	EU Summary	0.61	0.85
		0.92[1]	3.0[1]
[redacted] 2015, CA 7.5/074	EU Summary	NR	NR
[redacted] 2008, CA 7.5/075	EU Summary	NR	NR
Malaguerra, F. *et al.,* 2012, CA 7.5/077	Zealand, DK	NR	NR
Bruchet, A. *et al.,* 2011, CA 7.5/027	FR bank filtration	<0.1	<0.1

[1] Aggregated report values

Table 7.5-213: Summary of reported rates of concentrations of various thresholds for measured concentrations of glyphosate (GLY) and AMPA in drinking water

Reference	Context	Exceedance threshold and rate		
		Threshold (µg/L)	GLY (%)	AMPA (%)
[redacted] 2020, CA 7.5/002	EU Summary	0.1	0.10	0.13
		10	NA	0.00
		0.1[1]	0.16[1]	0.05[1]
[redacted] CA 7.5/074	EU Summary	0.1	0.09	0.22
[redacted] 2008, CA 7.5/075	EU Summary	0.1	NR	NR
Malaguerra, F. *et al.,* 2012, CA 7.5/077	Zealand, DK	0.01	9.3	8.4
		0.1	NR	NR
Malaguerra, F. *et al.,* 2012, CA 7.5/077	FR following bank filtration	0.1	0.00	0.00

[1] Aggregated report values

Applicant studies

New studies/assessments

1. Information on the study

Data point:	CA 7.5/001
Report author	[redacted]
Report year	2020
Report title	Collection of public monitoring data for European countries for the compartments soil, water, sediment and air for Glyphosate, AMPA and HMPA
Document No	110057-1
Guidelines followed in study	Methodology is based on the Groundwater Monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations') Minimum quality criteria of monitoring data described by the FOCUS Ground Water Work Group chapter 9.5 (European Commission, 2014)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
The report provides information about the outcome of a search for readily accessible and available public monitoring data in European countries at a regional/national level for the time period 1995-2019. The main focus was on the time period 2012-2019 while earlier years are already covered by existing data. The search included raw data, requested from regional/national authorities or downloadable from their websites, as well as aggregated data extracted from reports compiled by authorities.

Data from 14 European countries were considered: Austria, Belgium, Denmark, France, Germany, Hungary, Ireland, Italy, The Netherlands, Poland, Romania, Spain, Sweden and the United Kingdom. The countries represent the major markets of products containing glyphosate sold in the EU. The data compilation included the active substance glyphosate and its metabolites AMPA and HMPA, in the soil, groundwater, surface water, tidal water, drinking water, sediment and air environmental compartments.

As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities and other bodies of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were not actually included in any of the monitoring programs.

Drinking Water Compartment Conclusion
Public monitoring data for glyphosate or its metabolites in drinking water were available for 10 countries (AT, BE, DE, DK, ES, FR, IE, NL, SE and UK). In most cases information was only accessible as

aggregated monitoring data. Raw data were rarely available for reasons of national security in the case of public wells or due to data protection in cases where data were owned by private companies. Raw data was provided by the German federal state Schleswig-Holstein, Ireland and Sweden.

I. MATERIALS AND METHODS

The general methodology of data collection of public monitoring data and minimum quality criteria is based on existing guideline documents for groundwater monitoring programs. The underlying principles have been applied to all environmental compartments, especially where no specific guidance is at hand. Data search, acquisition and processing approaches are described below. The same approach was applied for each country, compartment and substance. Country specific adaptations to the general procedure were made in order to generate a harmonized database. The data collected for this report refers to third party organization data regarding all environmental compartments (SOIL, GW, SW, TD, DW, SD, AIR) and was further differentiated into the two different data types, i.e. raw data and aggregated data. Aggregated data refers to information provided in publicly available reports, e.g. from environmental agencies or research institutes. Such reports might hold only summary information on substance findings over space and time and may intersect with the raw data. Raw data refers to mid to long term time series of data that are provided on request by e-mail or by database from governmental authorities and are therefore recognized as official monitoring data. These datasets hold the information of sampling values, quality information (sampling, treatment, limit of detection - LOD, limit of quantification - LOQ) as well as information of location and time of sampling.

The following data source types were investigated in order to collect monitoring data:

- E-mail requests: a general e-mail was sent to the national responsible authorities with regard to the required information.
- Governmental webpages: the official webpages of the national responsible authorities were searched for information regarding available reports and datasets.
- Public online databases: available data from online databases were downloaded as provided by the webpages of governmental authorities and other institutions.

The data search resulted in a very heterogeneous collection of tabular data and reports in different formats and structure. Data were processed into a harmonized tabular format by selecting relevant information and adapting data organisation. In general, the complete datasets were included in the final harmonized database as provided by the authorities, but obvious duplicates were deleted. In general, all entries for the digital database were checked for consistency and plausibility. For the raw data it was assumed that information was already subjected to critical scrutiny by the respective organization. For the aggregated data the same assumption was made with quality assurance of the data (mostly summaries) being the responsibility of the authors of the respective reports.

II. RESULTS AND discussion

The final data collection of raw data and aggregated data is summarised for each compartment and each country in Table 7.5-214.

Drinking water

- Austria (AT)
  - No raw monitoring data from national authorities for drinking water in Austria were identified.
  - Aggregated monitoring data from annual reports on drinking water quality were downloaded from the Federal Ministry of Labour, Social Affairs, Health and Consumer Protection.
- Belgium (BE)
  - No raw monitoring data from national authorities for drinking water in Belgium were identified.

- Aggregated monitoring data from reports published by national authorities in Belgium for drinking water were obtained from the Flemish EPA.

- Germany (DE)
  - Raw monitoring data from national authorities for drinking water were provided by the state of Schleswig-Holstein.
  - Aggregated monitoring data from reports published by national authorities for drinking water were downloaded from the German EPA.
- Denmark (DK)
  - No raw monitoring data from national authorities for drinking water in Denmark were identified.
  - Aggregated monitoring data from reports published by national authorities for drinking water were downloaded from the Danish EPA.
- Spain (ES)
  - No raw monitoring data from national authorities for drinking water in Spain were identified.
  - Aggregated monitoring data from reports published by national authorities for drinking water were obtained from the Ministry of Health, Consumption and Social Welfare in form of annual reports.
- Europe (EU)
  - No aggregated monitoring data from reports published by EU institutions or international organizations for drinking water at EU level were identified for glyphosate or its metabolites.
- France (FR)
  - No raw monitoring data from national authorities for drinking water in France were identified.
  - Aggregated monitoring data from reports published by national authorities for drinking water were obtained from the Ministry of Solidarity and Health.
- Hungary (HU)
  - The Ministry of Interior confirmed that no monitoring programs were in place that included glyphosate or metabolites.
- Ireland (IE)
  - Raw monitoring data from national authorities for drinking water were downloaded from the SAFER portal of the Irish EPA.
  - Aggregated monitoring data from reports published by national authorities for drinking water were downloaded from the Irish EPA and from the governmental page on the Water Framework Directive.
- Italy (IT)
  - No drinking water monitoring data for glyphosate or its metabolites were identified for Italy.
- The Netherlands (NL)
  - No raw monitoring data from national authorities for drinking water in the Netherlands were identified.
  - Aggregated monitoring data from reports published by national authorities for drinking water were downloaded from RIVM, the Inspection of Environment and Transport and the E-depot of Wageningen University & Research.
- Poland (PL)
  - The responsible authorities for monitoring data in Poland are the Polish Geological Institute and the Chief Inspectorate of Environmental Protection. The latter authority confirmed by e-mail that in Poland there is currently no public monitoring of glyphosate or its metabolites.
- Romania (RO)
  - The responsible authority for monitoring data is the Ministry of Water and Forests. The Water Resources Management Directorate confirmed on behalf of the Ministry of Water and Forests that no public monitoring of glyphosate or its metabolites is carried out in any water compartment in Romania.
- Sweden (SE)
  - The national monitoring data sent to us by SLU do not comprise drinking water. However, SLU also provided another in-official database containing raw data for drinking water issued from other sources than national monitoring.
  - Aggregated monitoring data from reports published by national authorities in tabular form for drinking water were downloaded from the SLU homepage.

- United Kingdom (UK)
  - Aggregated monitoring data from reports published by national authorities for drinking water in England and Wales were downloaded from the Drinking Water Inspectorate.

Table 7.5-214: Overview of public monitoring data availability of raw data (R) and aggregated data (A)

Country	Soil	Water				Sediment	Air
		Ground	Surface	Tidal	Drinking		
Austria	-	R, A	R, A	-	A	-	-
Belgium	-	R	R	-	A (Flanders)	-	-
Denmark	-	R, A	A	-	A	-	-
France	-	R	R	-	A	R	-
Germany	R (Brandenburg)	R, A	R, A	R	R (Schleswig-Holstein), A	-	-
Hungary	-	A (one research article)	A (one research article)	-	-	-	-
Ireland	-	R, A	R, A	-	R, A	-	-
Italy	-	R (Lombardia), A	R, A	-	-	-	-
The Netherlands	-	R, A	R, A	-	A	-	-
Poland	confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Romania	confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Spain	-	R, A	R, A	-	A	-	-
Sweden	-	R, A	R	-	R, A	R	-
UK England	-	R	R	R	A	-	-
UK Northern Ireland	-	R	-	-	-	-	-
UK Scotland	-	-	R	-	-	-	-
UK Wales	-	-	R	-	A	-	-

R raw data available; A aggregated data from reports available; - no raw or aggregated data available

III. CONCLUSIONS

The present collection of public monitoring data for glyphosate, AMPA and HMPA in soil, groundwater, surface water, drinking water, tide water, sediment and air resulted in a comprehensive database of 'raw monitoring data from national authorities' and 'aggregated monitoring data from reports published by national authorities'. As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were actually not included in any of the monitoring programs.

Public monitoring data for glyphosate or its metabolites in drinking water were available for 10 countries (AT, BE, DE, DK, ES, FR, IE, NL, SE and UK). In most cases information was only accessible as

aggregated monitoring data. Raw data were rarely available for reasons of national security in the case of public wells or due to data protection in cases where data were owned by private companies. Raw data was provided by the German federal state Schleswig-Holstein, Ireland and Sweden.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the collection process of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA
The report is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Document No	-
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al.*, 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
The report provides information about the outcome of an analysis of public monitoring data comprising environmental concentrations of glyphosate (GLY) and its primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA) collated from readily available public monitoring databases held by national/regional environment agencies. This data collection and analysis was designed to expand previous reviews to include other compartments and supplement them for surface water, groundwater and drinking water. Public monitoring data from the following Member States (MS) were assessed for the water, sediment and soil compartments: Austria (AT), Belgium (BE), Denmark (DK), France (FR), Germany (DE), Ireland (IE), Italy (IT), Netherlands (NL), Spain (ES), Sweden (SE) and the

United Kingdom (UK). Three MS, namely Poland (PL), Hungary (HU), and Romania (RO) confirmed that they do not conduct analyses for GLY, AMPA and HMPA in any environmental compartment. No data for HMPA was identified for any MS or compartment. Note that at the time the study was started the UK was a Member State and is referred to as a Member State throughout the report.

Analyses of the large spatial and temporal dataset of measured concentrations occurring in several environmental compartments, namely surface water, groundwater, drinking water, tidal water, sediment and soil, were conducted to assess their state. This analysis not only sought to assess the state of the environmental compartment but also to consider the potential impacts this might have on biota, ecosystems and human health by using regulatory endpoints and thresholds from a range of European (EU) Directives. These included the Water Framework Directive (Directive 2000/60/EC) and associated Groundwater (2006/118/EC), Drinking Water (1998/83/EC) and Priority Substances (2008/105/EC28) Directives in addition to the Plant Protection Products Directive (1107/2009/EC).

Drinking water

Drinking water monitoring data were identified and evaluated for DE (German federal state Schleswig-Holstein), IE (GLY only) and SE. These data comprise analyses from both treated and untreated sources likely taken at the tap of the consumer. In addition, data analysis of SW data was undertaken assuming these were used as raw water for drinking water considering water treatment removal efficiencies when treating these waters. Case study investigations of raw drinking water sources in the Meuse river and around Berlin (DE) were conducted to investigate elevated frequencies of detection highlighted by regulators in NL and DE.

Glyphosate

The GLY public monitoring dataset was comparatively small (~8 000 samples collected from ~3 100 sampling sites). Compliance with the DrW threshold of 0.1 µg/L is very high (99.90 % of samples) with detections ≥ 0.1 µg/L being rare (~0.10 % of analyses). All 5 samples in SE that are ≥ 0.1 µg/L come from apparently untreated sources. All exceedances are old (≤2007) and significant strides have been made in SE since the introduction of the water protection regulations in 2004 through delineation of water protection zones. Where exceedances do occasionally occur the maximum concentration of 0.61 µg/L (recorded in DE) is well below the lifetime health-based ADI concentration of 1500 µg/L. These findings are consistent with aggregated report values of ~0.16 % sample exceedance and maximum concentrations up to 0.92 µg/L (recorded in ES). Likewise, this maximum value is well below the lifetime health-based ADI concentration. These values compare favourably with the ~0.09 % of samples ≥ 0.1 µg/L in the previous data collection.

Case studies of GLY concentrations in SW, conducted for the river Meuse and the Spree/Havel river system in the Berlin area, conclude that the glyphosate sources from agriculture and urban and railway hard surface uses cannot clearly be distinguished. However, the data does suggest that baseline concentrations likely derive from agricultural uses and that urban and railway uses are key drivers of peak concentrations and in turn exceedance of the 0.1 µg/L water quality threshold of raw surface waters.

AMPA

The AMPA public monitoring dataset was similarly small (~7 000 samples collected from ~2 300 sampling sites). Compliance with the regulatory threshold of 10 µg/L is absolute at 100 %. Compliance with the DrW threshold of 0.1 µg/L is very high (99.87 % of samples) with exceedances being rare (~0.13 % of analyses). All 7 samples in SE that are ≥ 0.1 µg/L come from apparently untreated sources. All exceedances are old (≤2007) and significant strides have been made in SE since the introduction of the water protection regulations in 2004 through delineation of water protection zones. Where exceedances do occasionally occur the maximum concentration of 0.85 µg/L is well below the lifetime health-based ADI concentration of 3960 µg/L. This is consistent with aggregated report values of ~0.05 % exceedance and maximum concentrations of up to 3.0 µg/L (recorded in NL). Likewise, this maximum value is well below the lifetime health-based ADI concentration. These values compare favourably with the ~0.22 % of samples ≥ 0.1 µg/L in the previous data collection. It should be borne in mind that AMPA may originate from sources other than GLY, for example detergents.

HMPA

No monitoring data were available for HMPA.

Surface Water as a Raw Drinking Water Source

For surface water destined to be drinking water, there are almost always water treatment processes applied to remove bacteria and viruses and other organic micro-pollutants. Undertaking a simplistic data analysis where raw SW concentrations are factored with the known optimal treatment removal efficiencies (95 % for AMPA, and 99 % for GLY) does not alter the conclusions of no risk to human health from the assessment of drinking water datasets, especially when considered within the broader context of abstraction (selective abstraction into bank side storage, bank abstraction) and source management (blending of sources) within the water supply chain.

Drinking Water Compartment Conclusion

No information on HMPA was available. The analysis of the dataset available for drinking water for GLY and AMPA indicates that compliance is very high given detections above 0.1 µg/L are very rare and when they do sporadically occur, they occur at low concentrations that are well below human health thresholds. The measured environmental concentrations available suggest neither GLY nor AMPA pose a risk to human health *via* drinking water.

I. MATERIAL AND METHODS

The dataset analysed comprised individual surface water analysis records as well as existing aggregated analyses extracted from reports sourced from regional/national environment agencies (see [redacted] 2020, CA 7.5/001). The approach taken for the data processing was precautionary in that it preserved samples in the analysis where there was any doubt regarding their reliability. As such no records were excluded from the analysis. Similarly, no attempt to remove outliers prior to the analysis or calculation of statistics was undertaken. Analysis and assessment of the data against thresholds was undertaken using the statistical software R. For drinking water the monitoring data was evaluated against the following thresholds and endpoints:

- Drinking water endpoint: Standard drinking water threshold of 0.1 µg/L for parent compounds (GLY) and relevant metabolites;
- Drinking water threshold: Regulatory drinking water threshold of 10 µg/L for non-relevant metabolites (AMPA);
- Regulatory toxicology endpoints: Drinking water concentrations (see Table 7.5-215) based on 10 % of the lifetime Acceptable Daily Intake (ADI) values for a 60 kg person consuming 2 L of water per day (EFSA, 2010; WHO, 1993; 2011). This is more precautionary than the current WHO guidelines (2011) which use 20 % of ADI:

  - o GLY – 1500 µg/L based on an ADI of 0.5 mg/kg bw/day derived from the NOAEL using a safety factor of 100 (EFSA, 2012)
  - o AMPA – 3960 µg/L based on 1.32 mg/kg bw/day for AMPA derived from the NOAEL using a safety factor of 200 (EFSA, 2012)

In addition, the raw SW datasets were analysed further against the threshold of 0.1 µg/L following implementation of the following treatment effectiveness factors to the dataset:

- GLY – 60 to 99 % removal
- AMPA – 25 to 95 % removal

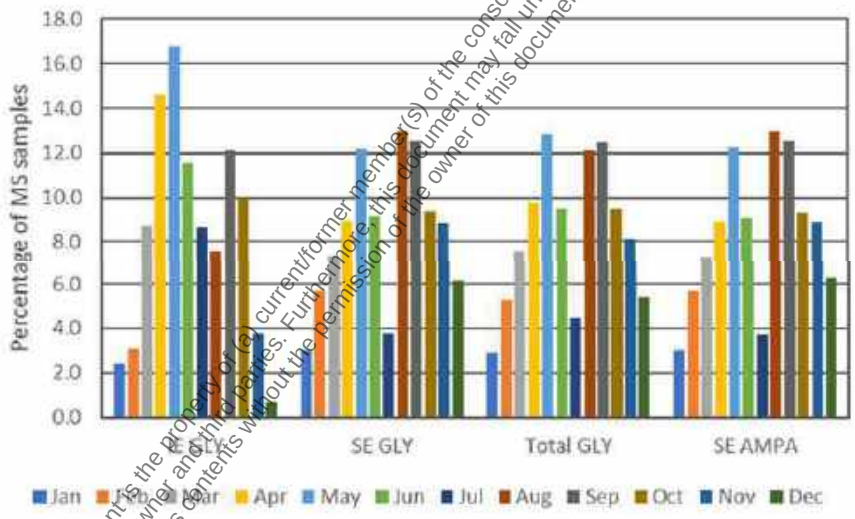
The combined European surface water dataset for GLY and AMPA was factored by the lower and upper removal efficiencies for each compound and then reanalysed using the same approach as was undertaken for surface water.

II. RESULTS AND DISCUSSION

Monitoring Data Assessment

Very little unaggregated drinking water data was available for analysis. This is largely because it is considered confidential by either the agency holding it or the organisation that supplied it to the agency, often on the grounds of consumer/national security. The data supplied and analysed was biased both spatially and temporally. The bulk of the data (~86 % for GLY and 99 % for AMPA) came from the SE dataset and while this dataset comprises >2 000 sites the coordinates for these sites were unavailable and as such the spatial distribution of these could not be assessed further. Similarly, none of the 767 sites in the IE dataset were supplied with coordinates. The small unrepresentative dataset from Germany is limited to the federal state of Schleswig-Holstein. The SE data comprises records from 1998 to 2014, the DE data covers 2012 to 2018 while that from IE are from 2017 only. Both the IE and SE datasets displayed a bimodal distribution of monthly sampling effort (see Figure 7.5-181), with peaks in the spring/summer (April/May/June) and autumn (August/September/October). There was insufficient data to create a combined European dataset and as such only individual MS data were presented. There was insufficient data to plot the DE data.

Figure 7.5-181: Bar chart of drinking water monthly glyphosate (GLY) and AMPA sampling effort within each Member State



Glyphosate

Across all MS the GLY public monitoring dataset compiled comprised >8 300 samples collected from >3 100 sampling sites (see Table 7.5-216). Given the limited size of the dataset and the limited number of MS from which it was sourced, a combined European dataset was not created.

Compliance with the drinking water threshold of 0.1 µg/L was high (99.90 %) given few exceedances (~0.10 %). All 5 samples in SE that are ≥ 0.1 µg/L came from 5 apparently untreated sources (2 drilled wells, 2 dug wells, 1 unspecified GW source). Only 1 site had more than a single sample to assess if exceedance was systematic and for that dug well a further sample 7 weeks later was <LOD. All exceedances were old (≤2007) and significant strides have been made in SE since the introduction of the water protection regulations in 2004. Maximum concentrations were 0.61 µg/L in DE, 0.074 µg/L in IE and 0.17 µg/L in SE. These were well below the life-time ADI based concentration of 1500 µg/L (see Table 7.5-215). In addition, GLY exceedances extracted from aggregated data in official reports (see Table 7.5-217) ranged between 0.00 % in AT and 0.29 % in ES with an average of ~0.16 % of samples ≥ 0.1 µg/L. Maximum

concentrations were up to 0.92 µg/L in ES. This value was well below the life-time ADI based concentration. These values compared well with the previous data collection ([redacted] 2008, CA 7.5/075; [redacted] 2015, CA 7.5/074) where ~0.09 % of samples analysed for GLY were found to equal or exceed 0.1 µg/L (see Table 7.5-218).

Across all MS the AMPA public monitoring dataset compiled comprised >7 000 samples collected from >2 300 sampling sites (see Table 7.5-216). Compliance with the regulatory drinking water threshold of 10.0 µg/L for non-relevant metabolites was 100 %. Compliance with the more precautionary threshold of 0.1 µg/L was high (99.87 % of samples) given the small number of exceedances (0.13 % of samples). Five of the SE samples that were ≥ 0.1 µg/L came from 5 apparently untreated sources (2 drilled wells, 3 dug wells) while 2 further samples came from "treated surface water from groundwater" from a single site. Only 1 site had more than a single analysis to assess the temporal nature of these exceedances and for this site the 4 samples of treated surface water from groundwater the concentrations suggested an exceedance event of <12 months in duration. In all 4 samples the GLY concentration was <LOQ which suggests this AMPA was from SW from other sources than GLY. All exceedances were old (<2007) and significant strides have been made in SE since the introduction of the water protection regulations in 2004. The maximum concentration was 0.85 µg/L (DE). This is well below the life-time based ADI concentration of 3960 µg/L (see Table 7.5-215). In addition, AMPA exceedance rates extracted from aggregated data in official reports (see Table 7.5-217) range between 0.00 % in DK and 0.05 % of samples in DE with an average of ~0.05 % for all MS combined. Maximum concentrations were up to 3.0 µg/L in NL. This value is well below the life-time based ADI concentration. These values compared well with the previous data collection (Horth and Gendebien, 2008, CA 7.5/075; Horth, 2015, CA 7.5/074) where ~0.22 % of samples analysed for AMPA were found to equal or exceed 0.1 µg/L (see Table 7.5-218). It should be borne in mind that AMPA may originate from sources other than GLY, for example detergents.

Table 7.5-215: Summary of acceptable daily intake (ADI) concentrations used in the assessment (*after* EFSA, 2010; WHO, 1993; 2011)

Compound	Intake in % of ADI	ADI derived from NOAEL and safety factor (mg/kg bw/day)	Maximum daily intake (mg/kg person/day)	Maximum daily intake 60 kg person consuming 2 L water /day (mg/day)	Acceptable intake based on 10 % ADI (µg/L)
GLY	10	0.5	0.05	3	1500
AMPA	10	1.32	0.132	7.92	3960

Raw Surface Water as a Source of Drinking Water

For completeness, an assessment of raw surface water bodies against the threshold of 0.1 µg/L was undertaken on the assumption that they might be used as raw water sources for drinking water production. The effects of factoring the raw surface water data to account for typical treatment on the exceedance rate were calculated (Table 7.5-90). For GLY the compliance rate improved with water treatment as the exceedance rate of 0.1 µg/L of ~23 % in the baseline scenario was reduced to ~12 % considering 60 % reduction during water treatment and reduced even further to ~0.1 % for treatment reduction rates near 99 %. Similarly, for AMPA the compliance rate improved with water treatment as the exceedance rate of 0.1 µg/L of ~48 % in the baseline scenario was reduced to ~42 % considering 25 % reduction during water treatment and reduced even further to ~5 % for treatment reduction rates near 95 %. If one considers a threshold of 10 µg/L for the non-relevant metabolite AMPA then the compliance was high (99.9 %, 99.8 % and 100 % respectively) in the baseline, the 25 % reduction and 95 % reduction scenarios. Water treatment is geared to the quality of the raw waters, for better quality source waters lower water treatment removal rates would ensure compliance with the drinking water threshold. For source waters of lower quality the higher removal rates suggest compliance. These basic analyses suggest that the water treatment process would likely account for any environmental concentrations of GLY and AMPA in raw SW abstracted to produce potable water for human consumption, especially when compared against the life-time based ADI

concentrations.

Limitations of the simplistic analysis were highlighted e.g. it was unable to take account of the fact that water companies would construct and optimise water treatment processes in order to achieve compliance. It should also be borne in mind that a significant portion of the AMPA in raw SW arises from other parent compounds like detergents. As such, this simplistic analysis indicates that raw SW abstracted and treated for human consumption would meet the required quality as the actual data on measured concentrations at the consumers tap demonstrates.

An evaluation of Member State specific thresholds is also outlined to illustrate the nuance specific in these systems and their drinking water supply with a view illustrating how these might be considered alongside public monitoring datasets. These are not summarised further here.

Table 7.5-216: Summary of the unaggregated drinking water (DrW) data for glyphosate (GLY) and AMPA sourced from Ireland and Sweden

Member State	DE					IE		SE				
Substance	GLY		AMPA			GLY		GLY		AMPA		
Threshold	DrW: 0.1 µg/L	LTHAC: 1500 µg/L	Threshold: 0.1 µg/L	DrW: 10.0 µg/L	LTHAC: 3960 µg/L	DrW: 0.1 µg/L	LTHAC: 1500 µg/L	DrW: 0.1 µg/L	LTHAC: 1500 µg/L	Threshold: 0.1 µg/L	DrW: 10.0 µg/L	LTHAC: 3960 µg/L
Number of sites	16	16	14	14	14	767	767	2335	2369	2321	2356	2356
Number of samples	18	18	15	15	15	1211	1211	6917	7135	6848	7054	7058
Number of samples > threshold	3	0	1	0	0	0	0	5	0	7	0	0
% of samples > threshold	16.7	0.00	13.3	0.00	0.00	0.00	0.00	0.072	0.0	0.10	0.00	0.0
Number of sites > threshold	3	0	1	0	0	0	0	5	0	6	0	0
% of sites > threshold	18.8	0.0	14.3	0.0	0.0	0.00	0.0	0.21	0.0	0.26	0.00	0.0
Number of consecutive samples > threshold	0	0	0	0	0	0	0	0	0	2	0	0
% of samples that are consecutive samples > threshold	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.0	0.029	0.00	0.0
Max number of samples > threshold at a single site	1	0	1	0	0	0	0	1	0	5	0	0
Max number of consecutive samples > threshold at a single site	1	0	1	0	0	0	0	1	0	5	0	0

NA – No data available
LTHAC - lifetime health-based ADI concentration

Table 7.5-217: Summary of drinking water (DrW) monitoring data aggregated in reports for glyphosate (GLY) and AMPA

MS	Substance	Number of reports identified	Reports with data relating to threshold						Maximum value (µg/L)
			Number of reports	Date range	Number of samples	Threshold (µg/L)	Samples above threshold	% samples above threshold	
AT	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	2	2	2011 - 2017	2020	0.1	0	0.00	NS
BE	AMPA	1	1	2016	1169	0.1	1	0.09	0.087
	GLY	1	1	2016	1157	0.1	2	0.2	0.051
DE	AMPA	3	3	2011-2016	9525	0.1	5	0.05	NS
	GLY	3	3	2011-2014	4531	0.1	9	0.20	NS
DK	AMPA	1	1	2014-2016	1336	0.1	0	0.00	NS
	GLY	1	1	2014-2016	1337	0.1	1	0.07	NS
ES	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	10	9	2008-2018	>5313	0.1	>10	0.22/0.29[1]	0.92
EU Trans	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
FR	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
IE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	1	0	NA	NA	NA	NA	NA	NS
IT	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
NL	AMPA	11	0	NA	NA	NA	NA	NA	3.0
	GLY	11	0	NA	NA	NA	NA	NA	0.3
SE	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	ND	ND	ND	ND	ND	ND	ND	ND
UK	AMPA	ND	ND	ND	ND	ND	ND	ND	ND
	GLY	9	0	NA	NA	NA	NA	NA	NS

[1] Report data includes sample counts and % values – The first value is the average using count data only while the second is the average of report averages
ND – No data identified; NS – Not specified; > as missing values to calculate total

Table 7.5-218: Summary of glyphosate (GLY) and AMPA monitoring data in drinking water across Europe, 2008-2015 (*after* [redacted] 2015, CA 7.5/074)

Country	Years	Monitoring number of sites or samples		Detection number		Concentration ≥ 0.1 µg/L	
		GLY	AMPA	GLY	AMPA	GLY	AMPA
Austria	2011-13	751	15	?	?	0	0
Belgium - Flanders	2013	17	17	0	2	0	0
Czechia	2014-15	64	67	2	3	0	0
Denmark	2011-13	882	-	?	?	0	0
France	2008-12	2624	589	10	13	10	13
Germany	2009-13	2484	2952	3	1	3	1
Ireland	2012-13	0	-	0	-	0	-
Portugal	2013-14	-	-	0	-	0	-
Spain	2009-13	2038	-	7	-	7	-
Sweden	2009-14	2848	2825	3	0	0	0
Switzerland	2014	2	-	0		0	-
Netherlands	2010-13	4	-	?		0	0
UK	2008-14	13487	-	4	-	4	-
Total	**2008-15**	**25201**	**6465**	**29**	**25**	**24**	**14**
%				**0.11**	**0.39**	**0.09**	**0.22**

Table 7.5-219: Summary of the exceedance rate of the baseline and treatment mitigated scenarios for glyphosate (GLY) and AMPA

Metric	GLY		AMPA		
	DrW: 0.1 µg/L	LTHAC: 1500 µg/L	Threshold: 0.1 µg/L	DrW: 10.0 µg/L	LTHAC: 3960 µg/L
	%	%	%	%	%
Baseline	33.0	0.0034	47.5	0.4	0.0011
Lower limit – 25 %/60 % Reduction	22.0	0.0024	42.3	0.2	0.0007
Upper limit – 95 %/99 % Reduction	0.1	0.00	4.8	0.0	0.0004

LTHAC - lifetime health based ADI concentration

III. CONCLUSIONS

No information on HMPA was available. The analysis of the dataset available for drinking water for GLY and AMPA indicates that compliance is very high given detections above 0.1 µg/L are very rare and when they do sporadically occur, they occur at low concentrations that are well below human health thresholds. The measured environmental concentrations available suggest neither GLY nor AMPA pose a risk to human health *via* drinking water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the analysis of public monitoring data for key European countries for the compartments soil, water and sediment for Glyphosate and AMPA.
The available data do not indicate any risk to human health from measured GLY and AMPA concentrations in the drinking water compartment.
The report was seen to be valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/074
Report author	[redacted]
Report year	2015
Report title	Survey of glyphosate and AMPA in drinking water supplies in Europe - 2015 update report
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
The report represents a review of glyphosate and AMPA monitoring results for drinking water across Europe. This review is based on an earlier review carried out in 2008, which has been updated to include the latest available information with respect to glyphosate and AMPA in drinking water. For this update, information was sought for all 28 Member States of the European Union plus Norway and Switzerland. For 19 countries, no monitoring data was available. Where available, drinking water quality reports issued by the national or regional responsible authorities were assessed. For Sweden a pesticide database, which included drinking water monitoring results, was available. Other information was obtained from web and literature searches, the EU synthesis report and from professional contacts.

Glyphosate and AMPA were present in water intakes in Belgium, Czech Republic, France, Germany, Spain, Sweden and in England & Wales. With the exception of France (23 samples in the period of 2008-2012), Germany (4 samples in the period of 2009-2013), Spain (7 samples in the period of 2009-2013) and England & Wales (4 samples in the period of 2008-2014) the measurements did not exceed the individual pesticide standard for drinking water of 0.1 µg/L. Glyphosate has not been found at concentrations at or above 0.1 µg/L in Austria, Belgium, the Czech Republic, Denmark, Sweden, Switzerland and the Netherlands.

All exceedances were isolated cases (different years and locations) not indicating any consistent contamination. Whilst there is much more monitoring data available than in 2008, there is clearly no evidence of an increasing number of glyphosate detections over the period of 2008-2015.

In France, the exceedances mainly occur in small supplies, which are much more vulnerable to contaminations, as they are often wells situated in farms where pesticides are handled. Where separate information is available, it is clear that the highest proportion of detections or exceedances is found in small supplies. There were no reported exceedances for AMPA in most countries, with exceptions in France (13 samples) and Germany (one sample).

I. MATERIAL AND METHODS

The report represents a review of glyphosate and AMPA monitoring results for drinking water across Europe. This review is based on an earlier review carried out in 2008, which has been updated to include the latest available information with respect to glyphosate and AMPA in drinking water. For this update, information was sought from Austria, Belgium, Czech Republic, Denmark, France, Germany, Ireland, Italy, Norway, Poland, Portugal, Slovak Republic, Spain, Sweden, Switzerland, the Netherlands and the UK. Information was obtained from web and literature searches, the EU synthesis report and from professional contacts. Where available, drinking water quality reports issued by the national or regional responsible authorities were assessed. For Sweden, data was extracted from a national pesticides database, which includes drinking water monitoring results. No relevant information was available for Bulgaria, Croatia, Cyprus, Estonia, Finland, Greece, Hungary, Latvia, Lithuania, Luxembourg, Malta, Romania and Slovenia.

II. RESULTS AND DISCUSSION

Table 7.5-220: Summary of glyphosate and AMPA monitoring and detection in drinking water in 13 EU countries, 2008 – 2015

Country	Year(s)	Monitoring		Detection (number)		Concentration ≥ 0.1 µg/L (number)		Reliability of results
		Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA	
Austria	2011-13	751	15	?	?	0	0	good
Belgium-Flanders [1]	2013	17 [2]	17 [2]	0	2	0	0	good
Czech Republic [1]	2014-15	64	67	2	3	0	0	x
Denmark	2011-13	882	-	?	?	0	0	good
France [1]	2008-12	≥2624	589 [2)]	≥10	≥13	10	13	x
Germany	2009-13	2484	2952	≥3	≥1	3	1	x
Ireland	2012-13	?	-	0	-	0	-	+
Portugal	2013-14	?	-	0	-	0	-	+
Spain [1]	2009-13	>2038	-	≥7	-	7	-	x
Sweden [1]	2009-14	2848	2825	3 [3]	6 [3]	0	0	x
Switzerland	2014	2 [2]	-	0	-	0	-	x
The Netherlands	2010-13	>4	-	?	?	0	0	x
UK								
-England & Wales	2008-14	13487	-	≥4	-	4	-	good
-Northern Ireland	2012-13	?	-	?	-	0	-	+
-Scotland [1]	2012-13	?	-	?	-	0	-	+
Total	***2008-15***	***≥25 201***	***≥6 465***	***≥29***	***≥25***	***24***	***14***	
%				***0.11***	***0.39***	***0.09***	***0.22***	

- not relevant
? no information
x insufficient information to judge reliability of results
+ based on risk assessment
[1] may include small supplies
[2] sites or water supply zones (WSZ)
[3] no Glyphosate detection after 2009, no AMPA detection after 2012

Glyphosate has not been found at concentrations at or above 0.1 µg/L in Austria, Belgium, the Czech Republic, Denmark, Sweden, Switzerland and the Netherlands. A small number of sporadic results >0.1 µg/L has been reported from France (23 samples in the period of 2008-2012), Germany (4 samples in the period of 2009-2013), Spain (7 samples in the period of 2009-2013) and England & Wales (4 samples in the period of 2008-2014). All exceedances were isolated cases (different years and locations) not indicating any consistent contamination. Whilst there is much more monitoring data available than in 2008, there is clearly no evidence of an increasing number of glyphosate detections over the period of 2008-2015.

III. CONCLUSION

A considerable amount of glyphosate monitoring has been carried out in drinking water in recent years in several European countries. There are only a small number of isolated detections or exceedances of the drinking water standard. It is clear that glyphosate detections are more frequent in small supplies (e.g. private wells on farms where pesticides are handled). These isolated glyphosate detections cannot be considered significant in terms of a risk of non-compliance with the drinking water standard. Despite its widespread usage, there is no evidence of any increase in glyphosate detections in drinking water over the period 2000-2015.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study provides an overview on monitoring data for drinking water from 13 European countries. No specific guideline is applicable to this data point.
The study was considered valid.

Assessment and conclusion by RMS:

Existing studies/assessments

1. Information on the study

Data point:	CA 7.5/075
Report author	[redacted]
Report year	2008
Report title	Review of glyphosate and AMPA in drinking water in selected European countries
Report No	UCC7729.04
Document No	BVL No. 2310278
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (no experimental work performed)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

Drinking water quality reports issued by the responsible national authorities in Belgium, Denmark, France, Germany, Ireland, The Netherlands and the UK, together with some additional information, were assessed with respect to glyphosate and AMPA in drinking water for public supplies (private supplies also for Denmark and Northern Ireland), and in some cases for raw water intakes (Germany and The Netherlands). For Sweden a pesticides database which included drinking water results was available.

Glyphosate and AMPA were present in water intakes in Belgium, Germany and The Netherlands, but did not exceed the individual pesticide standard for drinking water of 0.1 µg/L. A small number of sporadic results > 0.1 µg/L in finished water have been reported from France (25 samples in the period 2001-03), The Netherlands (two each in 2005 and 2006) and the UK (four in England & Wales in 2004, three in Northern Ireland in 2004 and one in 2005). All were isolated detections and none were considered significant, i.e. no reports of improvement measures being needed because of the presence of glyphosate in drinking water. Three of the four exceedances in England & Wales were attributed to probable problems with the analysis, due to the generally spurious occurrences; similar explanations may well apply to exceedances reported from elsewhere. There were no reported exceedances for glyphosate (or AMPA) in large public supplies in Denmark, there were however some detections and exceedances in small private supplies. Special investigations revealed that all wells affected were abstracting shallow groundwater (probably supplied untreated) in conditions where there was rapid infiltration of surface water from nearby fields or run-off from treated court yards in the vicinity. A similar situation may be the case in Sweden, where a small number of glyphosate and AMPA detections and exceedances were found in drinking water; these seemed to be mainly derived from groundwater, but no further sample details were available.

I. MATERIAL AND METHODS

Reporting by EU Member States to the Commission under the Drinking Water Directive 98/83/EC (1998) will be incorporated into WISE (Water Information System for Europe) in the near future. However, at present, there are no clear indications of the details of reporting, and data are available in various forms for some Member States.

Available information was sought for Belgium, Denmark, France, Germany, Ireland, Spain, Sweden, The Netherlands, Czech Republic, Greece, Italy and the UK. Information was obtained from websearches and professional contacts.

Drinking water quality reports issued by the responsible national authorities were reviewed for Belgium, Denmark, France, Germany, Ireland, Spain, The Netherlands and the UK. For Sweden data were accessed from a database.

No relevant information was obtained from Greece and Italy. The Czech Republic confirmed that glyphosate was not among the substances monitored.

II. RESULTS AND DISCUSSION

The results are summarized in the table below.

Table 7.5-221: Summary of glyphosate and AMPA monitoring and detection in drinking water in eight EU countries

Country	Year(s)	Monitoring		Detection (number)		Concentration ≥ 0.1 µg/L (number)	
		Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
Belgium	2002-04	not known	not known	?	?	0	0
Denmark	2002-04 [1]	probably	probably	?	?	0	0
	2001-05 [2]	yes	yes	54 [3]		21 [3]	
France	1993-98	not known	not known	?	?	0	0
	2001-03	yes	yes	26	22	18	15
	2004-06	probably	probably	?	?	0	0
Germany	2002-04	probably	probably	?	?	0	0
	2005	yes	yes	0	0	0	0
Ireland	2005-06	not known	not known	?	?	0	0
Sweden	2000-07	yes	yes	7	14	4	≥ 4
Spain	2002-04	nm	nm	nr	nr	nr	nr
The Netherlands	2000-06	yes	probably	14	?	2	?
UK							
- England	2000-06	yes	not known	?	?	4	?
- Northern Ireland	2002-06	yes	not known	?	?	6 [4]	?
- Scotland	2005	not known	not known	?	?	≤ 2 [5]	?

1 large public supplies
2 small/private wells of shallow groundwater, probably untreated
3 glyphosate and AMPA presented as combined amounts
4 2 of these in private supplies
5 only 2 exceedances of the pesticide standard but substance(s) not specified
nm = not monitored, nr = not relevant, ? = no information

III. CONCLUSION

No glyphosate exceedances of the individual pesticide standard for drinking water of 0.1 µg/L were reported from Belgium, Germany and Ireland. A small number of sporadic results > 0.1 µg/L have been reported from France (25 samples in the period 2001-03), The Netherlands (two each in 2005 and 2006) and the UK (four in England & Wales in 2004, three in Northern Ireland in 2004 and one in 2005). All were isolated detections and none were considered significant, i.e. no reports of improvement measures being needed because of the presence of glyphosate in drinking water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study compiles drinking water quality data for glyphosate and AMPA from national authorities in Europe. The methods and results are sufficiently described.
Therefore, the study was considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/076
Report author	[redacted]
Report year	1997
Report title	Glyphosate in drinking water/ letter from Harison, F. (PSD York)
Report No	-
Document No	-
Guidelines followed in study	None
GLP	No
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	*Summary was compiled from information available in the glyphosate Monograph (2000).* In the United Kingdom the Drinking Water Inspectorate of the Department of Environment collates information and publishes reports on the quality of drinking water. Data for 1991-1994 are taken from the report "Nitrate, Pesticides and Lead 1991 to 1994". Data for 1995 and 1996 are from the individual years reports "Drinking Water 1995" and "Drinking Water 1996". Data from "Drinking Water" for the years 1995 and 1996 are given for each individual company. In this period the number of water companies in existence was 31 in 1995 and 29 in 1996. However, only three companies monitored glyphosate.
Short description of results:	Glyphosate monitoring data from "Nitrate, Pesticides and Lead 1991 to 1994":

	1991	**1992**	**1993**	**1994**
Total number of determinations	61	138	1217	1347
Number of determinations >0.1 µg/L	0	0	3	3
Max. concentration in drinking water (µg/L)	-	-	0.35	0.37

Glyphosate monitoring data from "Drinking Water 1995" and "Drinking Water 1996":

Water company	**Dwr Cymru Cyfyngedig**	**Mid Southern Water plc**	**South East Water Ltd.**
Determinations in 1995			
Total	904	84	386
Number exceeding 0.1 µg/L	1	2	0
% exceeding 0.1 µg/L	0.1	2.4	0
Determinations in 1996			
Total	829	66	274
Number exceeding 0.1 µg/L	1	0	0
% exceeding 0.1 µg/L	0.1	0	0

Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monitoring data from 1991 to 1996 are considered not representative for current use conditions of glyphosate. Data are superseded by new monitoring data collection.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4b

Relevant literature articles

1. Information on the study

Data point:	CA 7.5/077
Report author	Malaguerra, F., *et al.*
Report year	2012
Report title	Pesticides in water supply wells in Zealand, Denmark: A statistical analysis
Document No	Science of the Total Environment 414 (2012) 433–444
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Data from the Danish National Borehole Database are used to predict drinking water well vulnerability to contamination by pesticides, and to identify the dominant mechanisms leading to well pollution in Zealand, Denmark. The frequency of detection and concentrations of 4 herbicides and 3 herbicide metabolites are related to factors accounting for geology (thicknesses of sand, clay and chalk layers), geographical location (distance to surface water and distance to contaminated sites), redox conditions and well depth using logistic regression, the binomial test and Spearman correlation techniques. Results show that drinking water wells located in urban areas are more vulnerable to BAM and phenoxy acids contamination, while non-urban area wells are more subject to bentazone contamination. Parameters accounting for the hydraulic connection between the well and the surface (well depth and thickness of the clay confining layer) are often strongly related to well vulnerability. Results also show that wells close to surface water are more vulnerable to contamination, and that sandy layers provide better protection against the leaching of oxidizable pesticides than clay aquitards, because they are more likely to be aerobic. 4-CPP is observed more often at greater well depth, perhaps because of anaerobic dechlorination of dichlorprop. The field data are used to create a set of probabilistic models to predict well vulnerability to contamination by pesticides.

Materials & Methods

Study area

The island of Zealand, Denmark, includes the city of Copenhagen and was selected as a study area for this study. Geologically, the island is mainly composed of a succession of clayey and sandy tills deposited during the Last Glaciation over a chalk bedrock. This geological setting is characteristic of high latitudes and can be found in many parts of the world, like Canada and the northern United States. Zealand is a good case for statistical analysis of drinking water well data because it is highly populated but still contains large agricultural areas, wells contaminated by pesticides or other compounds are common, and rigorous water well sampling data is available over a long period. Moreover, Zealand has a relatively uniform geology, and so the processes relevant to pesticide transport can be assumed to be similar for the whole island. In order to examine the importance of the geological setting, a statistical analysis was also performed on data from wells placed in the west part of Denmark's Jutland peninsula. The area lies west of the limit of the last glacier front and is mainly composed of thick sandy layers originating from glacial erosion of tertiary or glaciofluvial deposits. The region is less populated than Zealand but includes a larger number of drinking water wells.

Pesticides considered

Seven compounds were considered in the study: 2,6-dichlorobenzamide (BAM), MCPP (mecoprop), dichlorprop, 4-chlorophenoxypropanoic acid (4-CPP), bentazone, glyphosate and aminomethylphosphonic acid (AMPA). These compounds are among the most frequent pesticides and pesticide byproducts observed in Danish drinking water wells. BAM is the degradation metabolite of dichlobenil, an herbicide mostly used in urban areas such as paths, roads, courtyards and sports grounds, and which has been banned in Denmark since 1997. Unlike its mother compound, dichlobenil, which is strongly sorbed in topsoils, BAM is leachable. Thus, the stock of dichlobenil sorbed onto soil organic matter is slowly degrading and BAM is being continuously released into groundwater. Even though BAM degradation has been observed, it is widely believed to be very persistent in aquifers. In 2009, BAM was detected in 17.1 % of the groundwater wells investigated in the Danish groundwater monitoring program and the MAC was exceeded in 5.2 % of the wells. BAM findings are the most important cause of drinking water well closure in Denmark.

MCPP and dichlorprop are phenoxy acids employed as selective, hormone-type herbicides and are widely used for agricultural, horticultural and domestic purposes. In Denmark these pesticides were partially banned in 1997 and are now used only for limited purposes. MCPP and dichlorprop do not sorb significantly onto aquifer sediments and are only weakly degraded under anaerobic conditions. 4-CPP is often found in con- junction with MCPP and dichlorprop since it is an impurity of the production process, but some studies suggests that 4-CPP may originate from the anaerobic dechlorination of dichlorprop. In 2009, these three phenoxy acids have been found in 8 % of active water supply wells, and the MAC was exceeded in 1 % of the cases: after BAM, they were the most frequently found compounds. Bentazone is a selective herbicide mainly used in cultivated areas. It is very mobile and leachable. Bentazone can be quickly degraded in the upper soil layer, but there is evidence of its persistence in aquifers. It has been found in 3.9 % of Danish monitoring wells and herbicide concentrations were higher than the MAC in 0.9 % of sampled wells. Glyphosate is a broad-spectrum non-selective herbicide, and is mostly commercialized under the trade name of Roundup and is the most sold chemical for weed control in agricultural, silvicultural and urban environments (both worldwide and in Denmark). Microbial degradation of glyphosate produces AMPA as a primary degradation product. Glyphosate and AMPA sorb strongly onto aquifer sediments, especially to clay minerals, and they are degradable under both aerobic and anaerobic conditions. Little monitoring data are available for glyphosate and AMPA, because their sampling is not recommended by Danish regulations and because they are difficult to analyze. However, glyphosate and AMPA have been recently found in 4.4 % and 3.8 % of the GRUMO monitoring wells respectively, and the MAC was exceeded in 1.4 % (glyphosate) and 1.1 % (AMPA) of the wells. The frequency of detection of glyphosate and AMPA in Danish wells has been increasing in recent years. MCPP, bentazone, glyphosate and AMPA are all included in the list of substances being considered for addition to the list of priority substances in the European Union (European Union directive 2008/105/EC).

Data

In Denmark, water is provided by a large number of clustered drinking water wells where water quality is regularly monitored. Over the last few decades, a unique comprehensive well database has been assembled recording the specifications of each well, and the results of regular chemical analyses. The full database for the Zealand Island was obtained from the Geological Survey of Denmark and Greenland (GEUS). Active drinking water wells were selected from the full database; both wells belonging to waterworks and private wells were included in the analysis. The number of wells sampled and the number of analyses for the compounds considered in this study are presented in Table 7.5-222. Well depths (*D*) were extracted from the database and defined to be the distance from the surface to the bottom of the well. The data- base describing borehole geology used a classification scheme containing 205 different categories. This categorization is too detailed for the purpose of this study, and so the geological information was grouped into 3 main geology types: sand, clay and chalk. For each well, the sum of the layer thicknesses of every group was calculated and provided the parameters *Ds* (total sand layer thickness), *Dcl* (total clay layer thickness) and *Dch* (total chalk thickness). It has to be noted that information on layer discontinuity was lost in this procedure. The data on pesticide concentrations are very heterogeneous because the wells were monitored at different times with different frequencies. The value *C* was chosen to be the maximum value of pesticide concentration recorded at a given well. The distance between the drinking water wells and the closest stream (*dSW*), and the minimum distance between the drinking water wells and contaminated sites (*dCS*) were calculated using a Geographical Information System; stream coordinates were provided by the Danish National Environmental Research Institute, and the locations of contaminated sites were provided by the Danish Environmental Protection Agency. The CORINE 2006 database was used to determine whether wells were located in urban areas or not: the binary variable *LU* had a value of 1 if the well was included in the category "artificial surface" and 0 otherwise. The selection criterion was based on the well location and not on the well catchment. The predominant redox conditions were determined at each well from records of oxygen, nitrate, ferrous iron and sulfate concentrations. If several measurements were available at the well, the classification was made using the mean value of the selected compound.

Table 7.5-222: Number of samples analyzed for pesticides and number of sampled wells in Zealand and Jutland

Compound	Zealand		Jutland	
	Wells sampled	Number of samples	Wells sampled	Number of samples
BAM	2269	9207	697	2324
MCPP	2276	8433	711	2619
Dichlorprop	2276	8445	711	2619
4-CPPP	516	1193	82	145
Bentazone	2233	7084	691	2208
Glyphosate	289	708	44	108
AMPA	286	691	44	108

Results

Well characteristics

General statistics for the well characteristics were calculated for 2605 wells in Zealand and 2156 in western Jutland, and results are presented in Table 7.5-223. Jutland wells are generally deeper, are placed in thicker sand layers and are overlain by more variable clay layer thicknesses. Despite the depth of the wells in Jutland, almost none are as deep as the chalk bedrock. As expected, the depth of the well and the thickness of the clay and sand layers influenced the redox conditions, due to their effect on the water travel time. The thickness of the clay layer was the most significant parameter: almost no wells were found to pump oxic water when clay layers were thicker than 30 m, while about 20 % of the wells overlain by a clay layer thinner than 8.5 m had an oxic redox chemistry. In Zealand, well redox conditions were insensitive to the distance from streams, while in Jutland, less reduced conditions were observed as the distance from surface water increased. More oxic wells were found in Jutland, where about 30 % of the shallow wells (less than 35 m deep) were oxic, compared to only 20 % in Zealand.

Redox dependence
Glyphosate and AMPA were analyzed in a limited number of wells, and mainly low concentrations were detected. Nevertheless, results show that the occurrence of glyphosate was slightly higher in anoxic water. The percentage of wells contaminated with AMPA was not dependent on the redox conditions, but higher concentrations were found in oxic and anoxic waters.

Table 7.5-223: Characteristics of Danish drinking water wells

Parameter	Symbol	Unit	Min	Max	Mean	Median	σ
Zealand							
Well depth	D	m	2.8	150.5	52.6	48.8	23.0
Thickness of sand layer	D_s	m	0	103.6	14.1	9.6	14.8
Thickness of clay layer	D_{cl}	m	0	119.0	20.1	16.4	16.4
Thickness of chalk layer	D_{ch}	m	0	107.3	15.9	12	17.6
Distance to streams	d_{SW}	m	0.1	9527	680	495	748
Distance to contaminated sites	d_{CS}	m	0	6435	760	610	644
Jutland							
Well depth	D	m	6.8	304.0	89.8	80.0	52.9
Thickness of sand layer	D_s	m	0	218.0	62.9	57.5	39.5
Thickness of clay layer	D_{cl}	m	0	198.8	25.4	19	27.4
Thickness of chalk layer	D_{ch}	m	0	57.0	6.02	0	0.2
Distance to streams	d_{SW}	m	2.7	4029	471	453	485

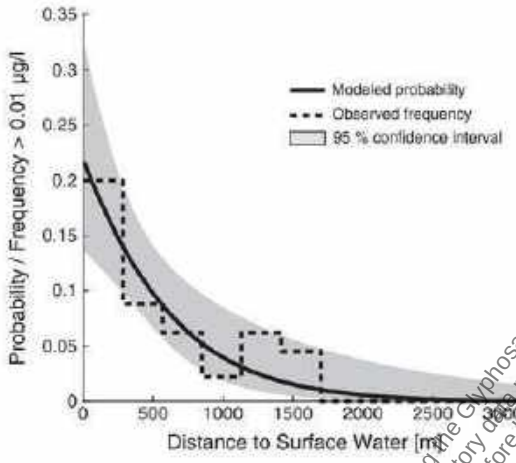
Logistic regression
Pesticide occurrences above two concentration thresholds were used to perform logistic regression: 0.01 µg/L, which is the usual detection limit and 0.1 µg/L, which is the maximum allowable concentration according to the EU Groundwater Directive. For dichlorprop, 4-CPP, bentazone, glyphosate and AMPA, the logistic regression did not produce any significant results for occurrences above 0.1 µg/L.

Ordinary logistic regression coefficients were used to predict the probability of pesticide occurrence, while standardized coefficients provided information on the relative importance of each parameter. The thickness of the clay layer and the distance between pumping wells and streams were significant for most of the compounds, and suggested that thicker clay layers and a greater distance to surface water will lead to a smaller probability of well contamination. The occurrence of phenoxy acids and bentazone were negatively correlated to the thickness of the clay layer. Logistic regression confirmed the dependence of BAM, MCPP and bentazone occurrence on land use. In fact, the coefficients linked to land use were well determined for all three compounds, and the sign of the regression coefficients was positive for BAM and MCPP and negative for bentazone. The occurrence of glyphosate and AMPA could only be linked to the distance to streams.

Standardized logistic regression coefficients indicated that the thickness of the clay layer was the most important parameter influencing the occurrence of BAM, dichlorprop and low MCPP concentrations, while the thickness of the sand layer controlled findings of bentazone, 4-CPP and high MCPP concentrations.

Results from the logistic regression were used to build logistic models for predictions of well contamination. Figure 7.5-182 shows the predicted probability of well contamination by glyphosate (>0.01 µg/L), depending on the distance between the well and the closest stream. Predicted probabilities, 95 % confidence intervals for predictions and the observed frequency of detection are plotted in the same graph. The model fits the observed frequencies well and observations are always included in the 95 % confidence intervals. It should be noted that these probabilities should not be interpreted as a probable frequency of detection, but rather the probability of finding the compound at least once.

Figure 7.5-182: Observed frequency of detection of glyphosate and the associated logistic model



Less data on drinking water well contamination were available for west Jutland drinking water wells, both because fewer wells have been sampled (Table 7.5-222), and because only a few wells have recorded pesticide concentrations above the detection limit. Thus, the p-values of the results were often above 0.05, and significant results were obtained only for BAM.

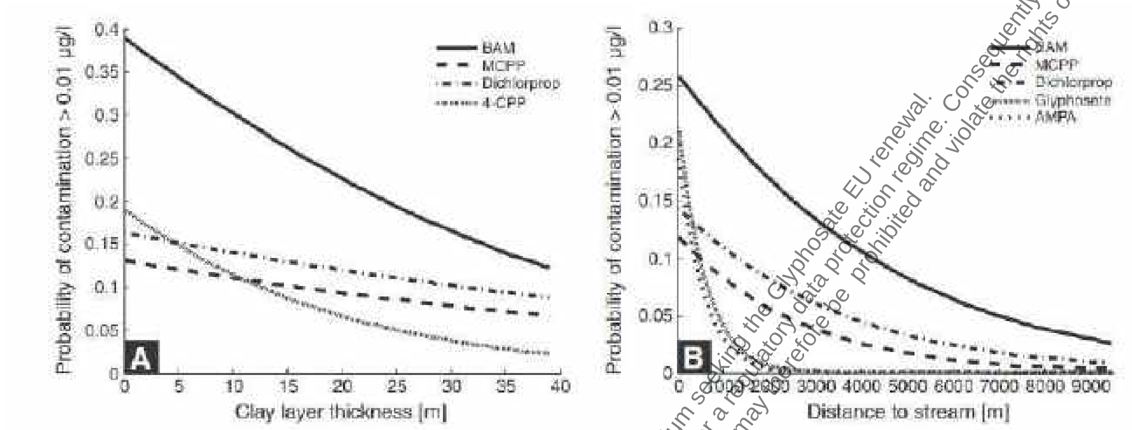
As in Zealand, BAM was found more frequently in urban area wells. The frequency of BAM findings above 0.1 and 0.01 µg/L were inversely related to the thicknesses of the clay and sand layers, and were positively correlated to the distance to streams.

Discussion

Glyphosate and AMPA

The low number of glyphosate and AMPA samples increases uncertainty in the determination of correlation coefficients and logistic regression parameters, and data interpretation becomes difficult. Nevertheless, the concentration and the occurrence of these compounds seem to decrease with the distance from streams. Moreover, logistic regression shows that the dependence between distance to streams and pesticide occurrence is much stronger for glyphosate and AMPA than for the other pesticides (Figure 7.5-183B). Previous studies show that glyphosate transport to surface water in agricultural areas is mainly due to surface runoff and that glyphosate is usually not transported in subsurface drainflow. A run- off transport mechanism is suggested by the fact that glyphosate and its metabolite AMPA are the most common compounds found in Danish streams at concentrations over 0.1 µg/L, with 26.7 % and 38.2 % respectively of samples in Danish streams recording such high concentrations. The greater occurrence of these compounds close to streams may be due to the infiltration of runoff water containing high glyphosate concentrations in riparian zones or because of slower degradation rates due to the prevalence of anaerobic conditions close to surface water.

Figure 7.5-183: Influence of the thickness of the clay layer (A) and the distance to streams (B) on well contamination. The plots show the modeled probabilities of well contamination by selected pesticides, and were obtained considering a hypothetical well 53 m-deep with a 14 m-thick sand layer. In (A) the distance to the stream was kept fixed at 680 m, in (B) the thickness of the clay layer was assumed to be 20 m



Conclusion

This study has shown that in Denmark, the land use affects the contamination of drinking water wells by pesticides: wells in urban areas are more contaminated by BAM and phenoxy acids, while wells in non-urban areas are more contaminated by bentazone. Logistic regression and correlation analysis suggests that the thickness of the clay layer overlying the wells is the most important parameter affecting contamination by persistent pesticides and that thicker sand layers are promoting degradation of aerobically degradable contaminants. In Zealand, well contamination was higher in the wells close to streams, suggesting that groundwater–surface water processes can play a major role in drinking water contamination by pesticides, even when pumping from confined aquifers. This study also suggest that 4-CPP in aquifers may originate from the dechlorination of dichlorprop in anaerobic environments, and that contaminated sites can be a major source of dichlorprop. Comparison of well pollution between Zealand and Jutland suggested that sandy layers can provide a better protection against the leaching of aerobically degradable pesticides than clay aquitards, since they are more likely to host aerobic conditions and therefore promote pollutant oxidation. Finally, we provided probability estimates of drinking water well pollution by BAM, MCPP, dichlorprop, 4-CPP, glyphosate and AMPA, which can be used for risk assessment purposes.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the statistical correlation of the occurrence of some pesticides, incl. glyphosate in groundwater wells with different characteristics of the wells (e.g. geology, geographic information, depth etc.). No measured values are reported.

Glyphosate and its metabolite AMPA are the most common compounds found in Danish streams at concentrations over 0.1 µg/L, with 26.7 % and 38.2 % respectively of samples in Danish streams recording such high concentrations (despite the lower number of samples for these two substances). Infiltration of surface runoff proposed.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/027
Report author	Bruchet, A. *et al.*
Report year	2011
Report title	Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge
Document No	European Journal of Water Quality 42 (2011) 123-133
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at an officially recognised testing facility
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

C. Sediment

Concentrations of glyphosate (GLY), AMPA and HMPA in sediment arising from public monitoring datasets have been collected from regional/national environment agencies as well as published peer reviewed publications from literature searches and rated as potentially relevant/reliable are reported in this section.

There are two new applicant studies on sediment. [redacted] (2020, CA 7.5/001) describes the collection process of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA. [redacted] (2020, CA 7.5/002) assesses the data collected by [redacted] (2020, CA 7.5/001). These two recent studies were designed to be the more comprehensive than previous studies by considering additional metabolites, compartments and time periods. [redacted] (2020, CA 7.5/002) covers a range of environmental compartments, however, the study summary below only includes the results relevant to this environmental compartment.

Existing data by [redacted] (1972, CA 7.5/035) was presented for completeness.

Several publications Lerch *et al.* (2017, CA 7.5/041), Napoli *et al.* (2016, CA 7.5/005), Maillard and Imfeld (2014, CA 7.5/051), Sabatier *et al.* (2014, CA 7.5/078), Imfeld *et al.* (2013, CA 7.7/55), Zgheib *et al.* (2012, CA 7.5/060) and Maillard *et al.* (2011, CA 7.5/064) report sediment concentrations that are not directly comparable with the sediment compartment that is typically risk assessed as part of the approval process, e.g. sediments in runoff water prior to entering a surface water body or entering/retained by artificial wetlands. Others report the concentrations in units that make it difficult to interpret the results e.g. as loads in mg or as concentrations ng/cm^2/yr.

The overall monitoring data presented in this section are summarised in Table 7.5-224. Not all of these are directly suitable for use in assessing the state of the sediment environmental compartment.

Table 7.5-224: Summary of reported maximum concentrations of glyphosate (GLY) and AMPA in sediment

Reference	Context	Maximum Concentration (mg/kg or µg/L)	
		GLY	AMPA
[redacted] 2020, CA 7.5/002	Predominantly riverine	2.84 mg/kg <4.0 µg/L	9.56 mg/kg <4.0 µg/L
Lerch, R.N., 2017, CA 7.5/041	Sediment in field runoff attenuated by a buffer strip	Expressed as input normalised loads in %	NA
Napoli, M. *et al.*, 2016, CA 7.5/005	Sediment in field runoff (before entering SW)	0.68 mg/kg	0.71 mg/kg
Maillard, E., Imfeld, G., 2014, CA 7.5/051	Suspended sediment entering/exiting artificial wetland	All sediment data expressed as loads e.g. mg	All sediment data expressed as loads e.g. mg
Sabatier, P *et al.*, 2014, CA 7.5/078	Lake sediment	NA	Concentrations given as ng/cm^2/yr
Imfeld G. *et al.*, 2013, CA 7.5/055	Artificial wetland sediment	<LOD (LOQ stated as 10 µg/kg)	<LOD (LOQ stated as 10 µg/kg)
Zgheib, S. *et al.*, 2012, CA 7.5/060	Suspended sediment in urban storm runoff before entering SW	8.3 mg/kg	4 mg/kg
Maillard, E. *et al.*, 2011, CA 7.5/064	Suspended sediment entering artificial wetland	0.045 mg/kg	0.021 mg/kg

NA – Not applicable

Applicant studies

New studies/assessments

1. Information on the study

Data point:	CA 7.5/001
Report author	[redacted]
Report year	2020
Report title	Collection of public monitoring data for European countries for the compartments soil, water, sediment and air for Glyphosate, AMPA and HMPA
Document No	110057-1
Guidelines followed in study	Methodology is based on the Groundwater Monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations') Minimum quality criteria of monitoring data described by the FOCUS Ground Water Work Group chapter 9.5 (European Commission, 2014)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The report provides information about the outcome of a search for readily accessible and available monitoring data in European countries at a regional/national level for the time period 1995-2019. The main focus was on the time period 2012-2019 while earlier years are already covered by existing data. The search included raw data, requested from regional/national authorities or downloadable from their websites, as well as aggregated data extracted from reports compiled by authorities.

Data from 14 European countries were considered: Austria, Belgium, Denmark, France, Germany, Hungary, Ireland, Italy, The Netherlands, Poland, Romania, Spain, Sweden and the United Kingdom. The countries represent the major markets of products containing glyphosate sold in the EU. The data compilation included the active substance glyphosate and its metabolites AMPA and HMPA, in the soil, groundwater, surface water, tidal water, drinking water, sediment and air environmental compartments.

As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities and other bodies of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were actually not included in any of the monitoring programs.

Sediment Compartment Conclusion

There were hardly any official programs in place targeting monitoring of glyphosate or its metabolites residues in sediment. Raw data for glyphosate and AMPA were available for France and Sweden.

I. MATERIAL AND METHODS

The general methodology of data collection of public monitoring data and minimum quality criteria is based on existing guideline documents for groundwater monitoring programs. The underlying principles have been applied to all environmental compartments, especially where no specific guidance is at hand. Data search, acquisition and processing approaches are described below. The same approach was applied for each country, compartment and substance. Country specific adaptations to the general procedure were made in order to generate a harmonized database. The data collected for this report refers to third party organization data regarding all environmental compartments (SOIL, GW, SW, TD, DW, SD, AIR) and was further differentiated into the two different data types, i.e. raw data and aggregated data. Aggregated data refers to information provided in publicly available reports, e.g. from environmental agencies or research institutes. Such reports might hold only summary information on substance findings over space and time and may intersect with the raw data. Raw data refers to mid to long term time series of data that are provided on request by e-mail or by database from governmental authorities and are therefore recognized as official monitoring data. These datasets hold the information of sampling values, quality information (sampling, treatment, limit of detection - LOD, limit of quantification - LOQ) as well as information of location and time of sampling.

The following data source types were taken into account in order to collect monitoring data:

- E-mail requests: a general e-mail was sent to the national responsible authorities with regard to the required information.
- Governmental webpages: the official webpages of the national responsible authorities were searched for information regarding available reports and datasets.
- Public online databases: available data from online databases were downloaded as provided by the webpages of governmental authorities and other institutions.
- Professional contacts: information indicated by experts in frequent professional contact to governmental authorities and other institutions were considered in order to complement data sources and datasets.

The data search resulted in a very heterogeneous collection of tabular data and reports in different formats and structure. Data were processed into a harmonized tabular format by selecting relevant information and adapting data organisation. In general, the complete datasets were included in the final harmonized database as provided by the authorities, but obvious duplicates were deleted. In general, all entries for the digital database were checked for consistency and plausibility. For the raw data it was assumed that information was already subjected to critical scrutiny by the respective organization. For the aggregated data the same assumption was made with quality assurance of the data (mostly summaries) being the responsibility of the authors of the respective reports.

II. RESULTS AND discussion

The final data collection of raw data and aggregated data is summarised for each compartment and each country in Table 7.5-225.

Sediment

- France (FR)
  - Raw monitoring data for sediment were downloaded from NAIADES.
- Sweden (SE)
  - Raw monitoring data from national authorities for sediment were provided by SLU per e-mail. Furthermore, raw monitoring data for sediment for Sweden was directly downloaded from the SLU homepage.

Table 7.5-225: Overview of public monitoring data availability of raw data (R) and aggregated data (A)

Country	Soil	Water				Sediment	Air
		Ground	Surface	Tidal	Drinking		
Austria	-	R, A	R, A	-	A	-	-
Belgium	-	R	R	-	A (Flanders)	-	-
Denmark	-	R, A	A	-	A	-	-
France	-	R	R	-	A	R	-
Germany	R (Brandenburg)	R, A	R, A	R	R (Schleswig-Holstein), A	-	-
Hungary	-	A (one research article)	A (one research article)	-	-	-	-
Ireland	-	R, A	R, A	-	R, A	-	-
Italy	-	R (Lombardia), A	R, A	-	-	-	-
The Netherlands	-	R, A	R, A	-	R	-	-
Poland	confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Romania	confirmation by corresponding authorities that no monitoring programs were in place that included glyphosate or metabolites						
Spain	-	R, A	R, A	-	A	-	-
Sweden	-	R, A	R	-	R, A	R	-
UK England	-	R	R	R	A	-	-
UK Northern Ireland	-	R	?	-	-	-	-
UK Scotland	-	-	R	-	-	-	-
UK Wales	-	-	R	-	A	-	-

R raw data available; A aggregated data from reports available; - no raw or aggregated data available

III. CONCLUSIONS

The collection of public monitoring data for glyphosate, AMPA and HMPA in soil, groundwater, surface water, drinking water, tide water, sediment and air resulted in a comprehensive database of 'raw monitoring data from national authorities' and 'aggregated monitoring data from reports published by national authorities'. As a result of the search, the corresponding authorities of the three countries Hungary, Poland and Romania confirmed that neither glyphosate nor its metabolites were included as analytical targets in official monitoring programs. Authorities of all other countries provided raw data or aggregated data for at least one compartment and compound. Moreover, the metabolite HMPA and the compartment air were actually not included in any of the monitoring programs.

There were hardly any official programs in place targeting monitoring of glyphosate or its metabolites residues in sediment. Raw data for glyphosate and AMPA were available for France and Sweden.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the collection process of public monitoring data for European countries for the compartment soil, water, sediment and air for Glyphosate, AMPA and HMPA.
The report is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Document No	-
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
The report provides information about the outcome of an analysis of public monitoring data comprising environmental concentrations of glyphosate (GLY) and its primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA) collated from readily available public monitoring databases held by national/regional environment agencies. This data collection and analysis was designed to expand previous reviews to include other compartments and supplement them for surface water, groundwater and drinking water. Public monitoring data from the following Member States (MS) were assessed for the water, sediment and soil compartments: Austria (AT), Belgium (BE), Denmark (DK), France (FR), Germany (DE), Ireland (IE), Italy (IT), Netherlands (NL), Spain (ES), Sweden (SE) and the United Kingdom (UK). Three MS, namely Poland (PL), Hungary (HU), and Romania (RO) confirmed that they do not conduct analyses for GLY, AMPA and HMPA in any environmental compartment. No data for HMPA was identified for any MS or compartment. Note that at the time the study was started the UK was a Member State and is referred to as a Member State throughout the report.

Analyses of the large spatial and temporal dataset of measured concentrations occurring in several environmental compartments, namely surface water, groundwater, drinking water, tidal water, sediment and soil, were conducted to assess their state. This analysis not only sought to assess the state of the environmental compartment but also to consider the potential impacts this might have on biota, ecosystems and human health by using regulatory endpoints and thresholds from a range of European (EU) Directives. These included the Water Framework Directive (Directive 2000/60/EC) and associated Groundwater (2006/118/EC), Drinking Water (1998/83/EC) and Priority Substances (2008/105/EC28) Directives in addition to the Plant Protection Products Directive (1107/2009/EC).

Sediment

A small number (~2 700 analyses from ~550 sampling sites) of GLY and AMPA analyses from riverine sediment were collected and analysed. These were from two MS, FR and SE. No information on HMPA was available. No GLY or AMPA RACs were available for the sediment compartment as such studies are not triggered because of low toxicity.

The maximum measured concentrations were 2.84 mg/kg (FR), <4.0 µg/L (FR) and 0.05 mg/kg (SE) for GLY and 2.84 mg/kg (FR)/<4.0 µg/L (FR) for GLY and 9.56 mg/kg (FR)/<4.0 µg/L (FR) for AMPA.

Sediment compartment conclusions

Limited sediment monitoring data, in number, spatial and temporal scope, are available.

I. MATERIAL AND METHODS

The dataset analysed comprised individual sediment analysis records as well as existing aggregated analyses extracted from reports sourced from regional/national environment agencies (see [redacted] 2020, CA 7.5/001). The approach taken for the data processing encompassed a precautionary approach that preserved samples in the analysis where there was any doubt regarding their reliability. As such the number of records excluded from the analysis were small, especially relative to the total number of samples prior to removal. Similarly, no attempt to remove outliers was undertaken. Analysis and assessment of the data against thresholds was undertaken in Excel. The monitoring data was not evaluated against thresholds or endpoints as these are not available:

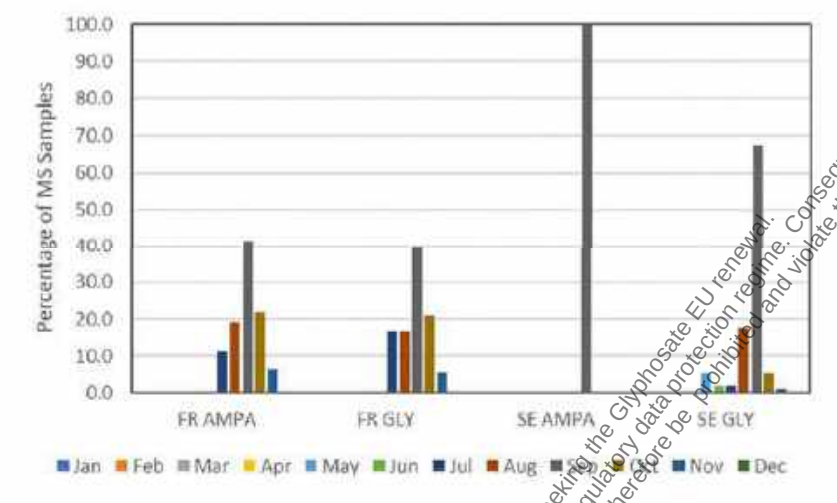
- Ecotoxicological endpoint: No ecotoxicological endpoints in this compartment are available for GLY and AMPA because sediment studies are not triggered.
- Ecosystem endpoint: Environmental quality standards (EQS) are not available at a Member State or at a European level.

II. RESULTS AND DISCUSSION

The data is limited (~2 700 analyses from ~550 sampling sites) and as such is biased both spatially and temporally. While it is not stated which kinds of waterbody were sampled, visual assessment of monitoring locations in GIS suggests that the samples are predominantly riverine. The bulk of the data (~91 % for GLY and ~99 % for AMPA) comes from the FR dataset which comprises ~541 sites, primarily in the north of France from a subset of departments. This dataset covers 13 years spanning the period 2005 – 2017. Monthly sampling effort for both GLY and AMPA is limited to the months of May through December and appears to be unimodal with lower sampling intensities in the early/latter months (see Figure 7.5-184).

The dataset from SE comprises ~12 sites distributed around the country targeting research catchments and locations. The GLY dataset covers 10 years spanning the period 2003 to 2012 while the AMPA data is restricted to 2006. Monthly sampling effort appears to be inconsistent and targets predominantly September. There was insufficient data to create a combined European dataset and as such only individual MS data were presented.

Figure 7.5-184: Bar chart of sediment monthly glyphosate (GLY) and AMPA sampling effort within each Member State



Analysis of the GLY sediment dataset indicates that GLY is quantified in ~5.6 % (FR) to ~48.2 % (SE) of samples (see Table 7.5-226), albeit the number of samples is quite limited (66 samples in µg/L and 1051 in mg/kg for FR; 114 in mg/kg for SE). The maximum measured concentrations were 2.84 mg/kg (FR), <4.0 µg/L (FR) and 0.05 mg/kg (SE).

Analysis of the AMPA sediment dataset indicates that AMPA is quantified in ~20.0 % (SE) to ~48.2 % (FR) of samples (see Table 7.5-226), albeit the number of samples is quite limited (66 samples in µg/L and 1088 in mg/kg for FR; 114 in mg/kg for SE). The maximum measured concentrations were 9.56 mg/kg, <4.0 µg/L (FR) and 0.15 mg/kg (SE).

III. CONCLUSIONS

There are limited sediment data available. The maximum measured concentrations were 2.84 mg/kg (FR)/<4.0 µg/L (FR) for GLY and 9.56 mg/kg (FR)/<4.0 µg/L (FR).

Table 7.5-226: Summary results of glyphosate (GLY) and AMPA analyses in sediment

Member State	Substance	Number of Sites	Number of Samples	Years	LOQ	Detected >LOQ		Detected >RAC			Measured Concentration
					Mean (min - max)	Samples	%	Sites	Samples	% Samples	Median[1] (min - max)
FR	GLY	503	1051	2007 - 2017	0.1 mg/kg (0.01 - 1.0)	59	5.6	NA	NA	NA	0.1 mg/kg (0.01 - 2.84)
	GLY	48	66	2005 - 2017	0.2 µg/L (0.02 – 4.0)	17	25.8	NA	NA	NA	0.2 µg/L (0.02 – <4.0)
	AMPA	505	1088	2007 - 2017	0.1 mg/kg (0.01 - 1.0)	281	25.8	NA	NA	NA	0.1 mg/kg (0.0014 - 9.56)
	AMPA	48	66	2005 - 2017	0.1 µg/L (0.01 - 4.0)	31	47.0	NA	NA	NA	0.2 µg/L (0.02 – <4.0)
SE	GLY	12	114	2003 - 2012	0.04 mg/kg (0.004 - 0.1)	55	48.2	NA	NA	NA	0.06 mg/kg (0.0 - 0.9)
	AMPA	10	10	2006	0.2 mg/kg (0.2 - 0.2)	2	20.0	NA	NA	NA	0.0 mg/kg (0.0 - 0.15)

[1] Values <LOQ and <LOD are treated as equal to LOQ and LOD as a precautionary estimate of the median NA – Not applicable

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the analysis of public monitoring data for key European countries for the compartments soil, water and sediment for Glyphosate and AMPA. The maximum measured sediment concentrations were 2.84 mg/kg (FR)/<4.0 µg/L (FR) for GLY and 9.56 mg/kg (FR)/<4.0 µg/L (FR) for AMPA.
The report is considered valid.

Assessment and conclusion by RMS:

Existing studies/assessments

1. Information on the study

Data point:	CA 7.5/035
Report author	[redacted]
Report year	1972
Report title	Run-off of MON-0573 from Inclined Soil Beds
Report No	AgRR 275
Document No	
Guidelines followed in study	US EPA Guidelines for Registering Pesticides, 2nd draft, 5172, part XI
GLP	No
Previous evaluation	Not accepted in RAR (2015)
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The summary is provided in the surface water monitoring subchapter of this document.

Relevant literature articles

1. Information on the study

Data point:	CA 7.5/041
Report author	Lerch, R.N. *et al.*
Report year	2017
Report title	Vegetative Buffer Strips for Reducing Herbicide Transport in Runoff: Effects of Buffer Width, Vegetation, and Season
Document No	Journal of the American Water Resources Association (JAWRA) 53(3):667-683.
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/005
Report author	Napoli, M. *et al.*
Report year	2016
Report title	Transport of Glyphosate and Aminomethylphosphonic Acid under Two Soil Management Practices in an Italian Vineyard
Document No	Journal of Environmental Quality 45:1713-1721 (2016)
Guidelines followed in study	None
Deviations from current test guideline	N Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the soil monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/051
Report author	Maillard, E., Imfeld, G.
Report year	2014
Report title	Pesticide Mass Budget in a Stormwater Wetland
Document No	Environmental Science & Technology 2014, 48, 8603−8611
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/078
Report author	Sabatier, P. *et al.*
Report year	2014
Report title	Long-term relationships among pesticide applications, mobility, and soil erosion in a vineyard watershed
Document No	PNAS vol. 111 no. 44
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (CARSO-Laboratoire Santé Environement laboratory, Lyon, France)
Acceptability/Reliability:	Reliable

2. Full summary

In this article, a retro-observation approach is presented, based on lake sediment records to monitor micropollutants and to evaluate the long-term succession and diffuse transfer of herbicides, fungicides, and insecticide treatments in a vineyard catchment in France. The sediment allows for a reliable reconstruction of past pesticide use through time, validated by the historical introduction, use, and banning of these organic and inorganic pesticides in local vineyards. The results also revealed how changes in these practices affect storage conditions and, consequently, the pesticides' transfer dynamics. For example, the use of post-emergence herbicides (glyphosate), which induce an increase in soil erosion, led to a release of a banned remnant pesticide (dichlorodiphenyltrichloroethane, DDT), which had been previously stored in vineyard soil, back into the environment. Management strategies of ecotoxicological risk would be well

served by recognition of the diversity of compounds stored in various environmental sinks, such as agriculture soil, and their capability to become sources when environmental conditions change.

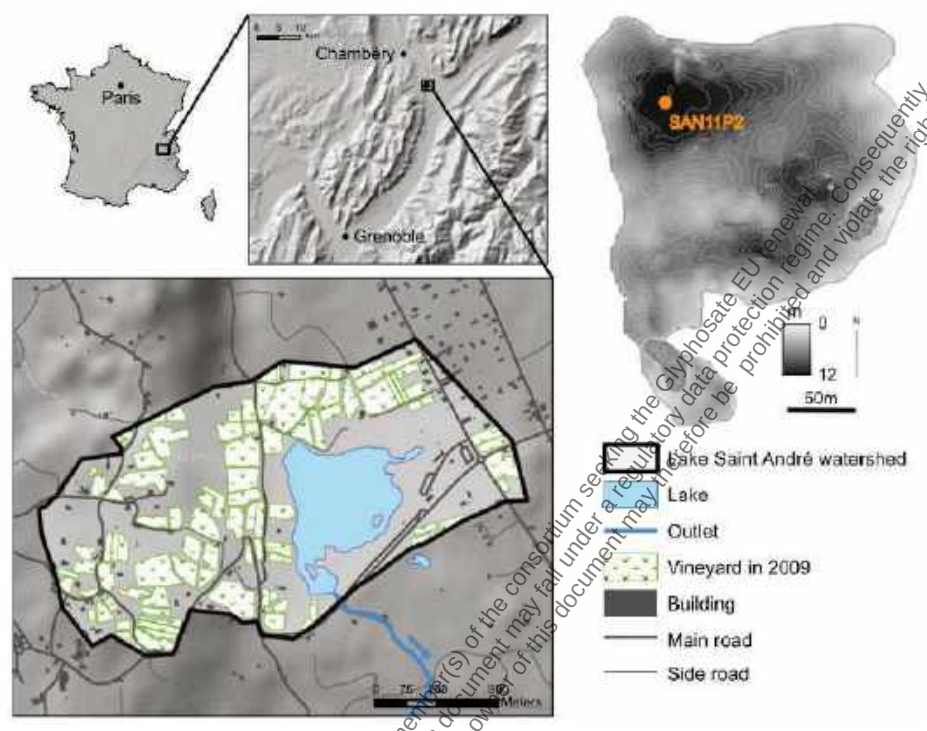
Materials and Methods

The study focused on Lake Saint André, which is located in eastern France at an elevation of 295 m above sea level. Vineyards make up 36 % of the watershed and drain only this landslide deposit.

Logging

Three 1-m-long cores [registered in the International Geo Sample Number (IGSN)/System for Earth Sample Registration Database (www.geosamples.org) as SAN11P1 (IGSN: EDYSAN004), SAN11P2 (IGSN: EDYSAN001), and SAN11P3 (IGSN: EDYSAN007)] were collected from Lake Saint André in December 2011 (Figure 7.5-185), using an Uwitec gravity corer (Environnement, Dynamique et Territoires de Montagne). In the laboratory, the cores were split, photographed, and logged in detail, noting all physical sedimentary structures and the vertical succession of facies. The sediment colors were determined, with a spatial resolution of 5 mm, using a Minolta CM 2600d. The grain size distributions of core SAN11P2 were determined using a Malvern Mastersizer S (Environnement, Dynamique et Territoires de Montagne) at a continuous interval of 1 cm. After inserting the bulk sediment into the fluid module of the granulometer, ultrasound was applied to minimize particle flocculation. Core SAN11P2 was also sampled at 1-cm steps and dried at 60°C over the course of 4 d to obtain its dry bulk density, and then the loss on ignition (LOI) of each 1-cm interval was measured using the protocol of Heiri (40). The LOI at 550°C and 950°C corresponds to the organic and carbonate components of the sediment, respectively. The XRF analysis was performed on the surfaces of the split sediment SAN11P3 core at 2-mm intervals, using a nondestructive Avaatech core-scanner (Environnement, Dynamique et Territoires de Montagne, at the Université de Savoie) on the upper 50 cm. The split core surface was first covered with 4-µm-thick Ultralene to avoid contamination of the XRF measurement unit and desiccation of the sediment. The geochemical data were obtained at various tube settings: 10 kV at 1.5 mA for Al, Si, S, K, Ca, Ti, Mn, and Fe; 30 kV at 1 mA for Cu, Zn, Br, Sr, Rb, Zr, and Pb; and 50 kV at 2 mA for Ba. Each individual power spectrum was converted through a deconvolution process into relative components (intensities), expressed in counts per second. The PCA was performed using “R” software.

Figure 7.5-185: The Lake Saint André watershed and the vineyards in 2009 (interpreted from aerial photographs), as well as the bathymetric map with the location of core SAN11P2 retrieved from the deeper part of the lake



Dating

The ^{210}Pb, ^{226}Ra, ^{228}Ra, ^{228}Th, ^{234}Th, ^{241}Am, ^{137}Cs, ^{7}Be, and ^{40}K activities of the samples were analyzed using well-type, germanium detectors placed at the Laboratoire Souterrain de Modane, which is located under 1,700 m of rock. The detector sensitivity allows for the reduction of the sample mass required for a measurement. These improvements allowed for the measurement of both very low radioactivity levels (with background levels of less than 0.6 cpm in the 30-3,000 keV energy range) and small sample weights (1 g). In general, counting times of 24-48 h were required to reach a statistical error of less than 10 % for excess ^{210}Pb in the deepest samples and for the 1963 ^{137}Cs peaks.

Pesticide Analysis

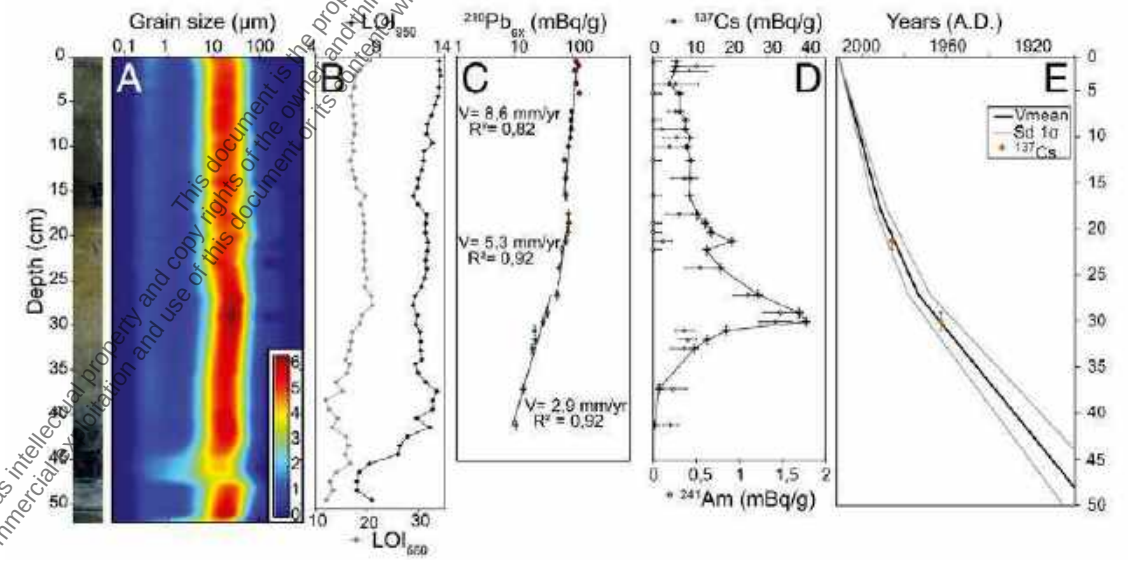
Pesticides were analyzed on cores SAN11P1 and SAN11P2 by the CARSO-Laboratoire Santé Environement laboratory, Lyon, France (www.groupecarso.com), which is COFRAC (Comité français d'accréditation)-accredited (1-1531). Two hundred eighty-two compounds were searched, using three runs: solid dried and sieved sample was extracted with dichloromethane by an accelerated solvent extractor (ASE) system and then concentrated and analyzed by GC/MS in accordance with AFNOR standard XP ×33-012 (205 pesticides searched); solid dried and sieved sample was extracted with dichloromethane by an ASE system and analyzed by high performance liquid chromatography (HPLC) with diode-array detection in accordance with a certified inner standard method (75 pesticides search); and solid dried and sieved sample was extracted with water and evaporated and analyzed by HPLC, using post derivatization in accordance with a certified inner standard method for glyphosate and AMPA.

Results and discussion

Lake Sediment

The lake-bottom sediment cores were characterized in terms of their color, grain size, LOI, and sedimentary structure. The upper 41 cm consists of olive-gray silty clay with constant fractions of carbonate (30 %) and organic content (7.5 %) (Figure 7.5-186B). The grain size distribution of this upper sequence is homogeneous and exhibits two main populations centered at 0.3 µm (carbonate fraction) and 14 µm (Figure 7.5-186A). The levels of major and trace elements were measured using an X-ray fluorescence (XRF) core scanner and were subjected to principal component analysis to constrain sediment end-members. This PCA of the bulk sediment resulted in the identification of four geochemical endmembers: (i) Al, Si, K, Fe, Ti, Rb, Ba, and Zr, which are related to terrigenous input from the watershed (aluminosilicates and heavy minerals present in marls); (ii) Ca and Sr, which are linked to the carbonate productivity in the lake; (iii) S and Mn, which are related to the lake's oxidation state; and (iv) a Cu source that may be correlated with periods of significant vineyard-related activities in the watershed, during which a blend of copper sulfate and calcium hydroxide (Bordeaux mixture) was sprayed as a fungicide. A chronological framework was established via measurements of short-lived radionuclides. A logarithmic plot of ($^{210}Pb_{ex}$) activity (Figure 7.5-186C) shows a general decrease with three distinct linear trends. According to the "constant flux, constant sedimentation rate" (CFCS) model, as applied to each part of the profile, the levels of ^{210}Pb indicate mean accumulation rates of 2.9 ± 0.2 mm/y between depths of 41 and 26.5 cm, 5.2 ± 0.6 mm/y between 26.5 and 17 cm, and 8.7 ± 1.3 mm/y in the upper 17 cm of the core (Figure 7.5-186C). The plot of ^{137}Cs data (Figure 7.5-186D) displays a peak at a depth of 29.5 ± 1 cm, which apparently correlates with the maximum atmospheric production of ^{137}Cs in 1963. This temporal correlation is supported by the ^{241}Am peak at the same depth, which was a result of the decay of ^{241}Pu in fallout from atmospheric nuclear weapons tests. In the upper part of this core, at a depth of 20.5 ± 0.5 cm, a second ^{137}Cs peak corresponds to the time of the Chernobyl accident in 1986 (Figure 7.5-186D). The good agreement between the ages derived from the $^{210}Pb_{ex}$-CFCS model, and the artificial radionuclide peaks provide a well-constrained, continuous age-depth relationship (Figure 7.5-186E) within the sediment sequence, with two primary sedimentation rate changes in ~1973 ± 5 y and 1994 ± 2.5 y.

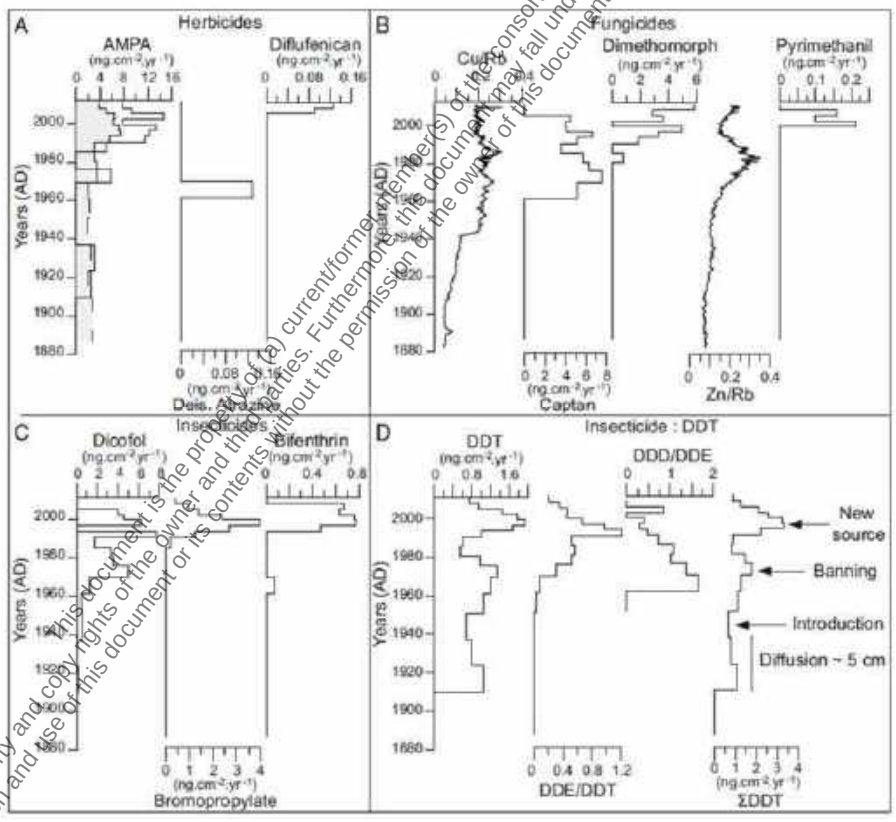
Figure 7.5-186: Data from core SANP2. From left to right: (A) photograph and grain size contour plot with two primary populations centered at 0.3 and 14 µm, (B) LOI at 550°C (organic matter) and 950°C (carbonates), (C) $^{210}Pb_{ex}$ activity, (D) ^{137}Cs activity, and (E) the age model



Sediment Chronology of Pesticides Use

No significant variations in the grain size distribution or the organic content were observed during the last century. Thus, these two parameters could not have affected the absorption/degradation of pesticides in this sediment sequence. Three herbicides (or their metabolites) were identified in the Lake Saint André sediment (Figure 7.5-187A): AMPA [a metabolite of glyphosate]; deisopropyl atrazine (a metabolite of triazine herbicides); and diflufenican [a main ingredient in Buffalo (Bayer)], which is used as a preemergence herbicide. High levels of AMPA were found in the core representing deposition during the previous 20 y, with a primary increase since 1990. AMPA is also present in low but significant concentrations before this period, most likely because of contamination of the deeper part of the core by downward smearing of the very high concentrations found in the upper layers. The metabolite of atrazine, which was used at the end of the 1950s and was banned in 2003, was observed in a sample that dates to the period between 1960 and 1970. Diflufenican, which was introduced at the end of 1990s and is still allowed, was identified in the sediments deposited beginning in 2005.

Figure 7.5-187: Chronological variations in pesticide fluxes. (A) Herbicides: AMPA, deisopropyl atrazine, and diflufenican; (B) fungicides: Bordeaux mixture (Cu/Rb), captan, dimethomorph, mancozeb (Zn/Rb), and pyrimethanil; (C) insecticides: dicofol, bromopropylate, and bifenthrin; (D) DDT and metabolites: DDT, DDE/DDT, DDD/DDE, and ΣDDT. The gray area in the AMPA profile denotes the lower detection limit for this compound

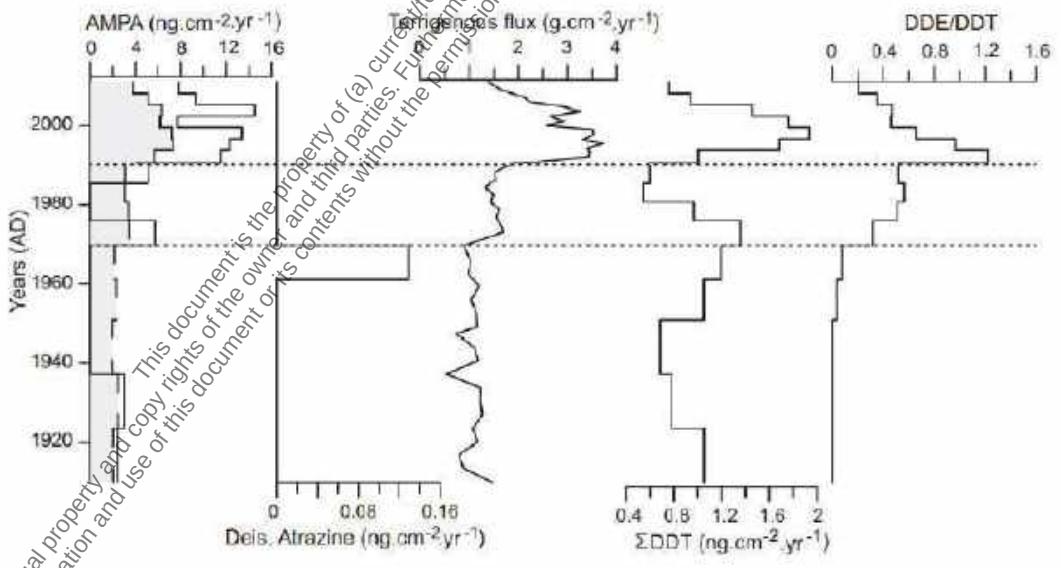


Herbicides and Soil Erosion

We observed three changes in the sedimentation rate (Figure 7.5-186) in this sediment sequence, which display a general increase in the terrigenous flux into the lake going from ~0.9 g cm^{-2}/y (1900–1972) to 1.7 g cm^{-2}/y (1973-1993), and then to as much as 3.4 g cm^{-2}/y (1994-2005). A drastic decrease was then observed during the following years. These variations in the terrigenous sediment supply from the watershed may be directly attributed to soil erosion via vineyard practices. In the early 1970s, the local use

of heavy farm machinery, which is known to contribute to soil erosion and is associated with the first application of preemergence herbicides (Atrazine metabolite) to combat grass between the rows of vines, may have induced the first increase of terrigenous flux into the lake (Figure 7.5-188). In 1990, we observed synchronous increases in AMPA, ΣDDT (with a low DDD/DDE ratio), and terrigenous soil fluxes into the lake (Figure 7.5-188). In the early 1990s, applications of post-emergence herbicides increased widely, including the use of Roundup, as indicated by the high flux of AMPA dating from this period. It has been demonstrated that application of this chemical has a strong effect on soil erosion, as it acts on grass development and leads to permanently bare soil. Moreover, this high flux of sediment supply to the lake is synchronous with the reemergence of banned pesticides, such as DDT and its aerobic metabolites (DDE), which were most likely stored in the vineyard and other agricultural soils in the watershed and subsequently remobilized by the herbicide-triggered rise in soil erosion. In this study, it was demonstrated that the recent widespread use of herbicides (glyphosate) induced an important release and reemergence of contaminants into the environment 20 y after their use was banned. The soils underwent a change in storage conditions, converting from sinks to sources of pesticides. The decrease in pesticide concentrations during the most recent years (Figures 7.5-188 and 7.5-189) may be attributed to French and European regulations controlling the use of micropollutants in agriculture. In summary, our study demonstrates the possibility of reconstructing the use of various pesticides (herbicides, fungicides, insecticides) in an agricultural watershed over the last century, using sedimentary archives. The dates of first use and prohibition of products used to control pests in vineyards and the changes in the soil erosion flux are recorded in the lake sediments. This work demonstrates that this high-resolution analysis of lake sediment allowed the reconstruction of past agricultural practices in this watershed and to precisely determine the 100-y-long dynamics of chemicals (organic and inorganic) used in vineyards. In particular, this study highlights the effects of post-emergence herbicides (glyphosate) on soil erosion and the remobilization of banned remnant pesticides (DDT) stored in vineyard soil.

Figure 7.5-188: Chronological variation in levels of AMPA, deisopropyl atrazine, subaerial flux, and sum of DDT and DDE/DDT. The horizontal dotted lines denote the two primary changes in the sedimentation rate



3. Assessment and conclusion

Assessment and conclusion by applicant:
The article evaluates the long-term relationship among pesticide applications, mobility and soil erosion in a French vineyard watershed. The sediment of an adjacent lake was investigated and compared with available information on historical usage of pesticides. It is postulated, from increasing levels of AMPA in the sediment core post-1990, that the increasing use of glyphosate from the early 1990s led to the remobilization of banned remnant pesticides (e.g. DDT) from vineyard soils.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/055
Report author	Imfeld G. *et al.*
Report year	2013
Report title	Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland
Document No	Chemosphere 90 (2013) 1333–1339
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (Pasteur Institute of Lille (France))
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/060
Report author	Zgheib, S. *et al.*
Report year	2012
Report title	Priority pollutants in urban stormwater: Part 1 – Case of separate storm sewers
Document No	Water research 46 (2012) 6683-6692
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/064
Report author	Maillard, E. *et al.*
Report year	2011
Report title	Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment
Document No	The Science of the total environment (2011), Vol. 409, No. 11, pp. 2317-24
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Pasteur Institute of Lille (France))
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water monitoring subchapter of this document.

D. Air

Concentrations of glyphosate (GLY) and AMPA in air arising from three published peer reviewed literature articles are reported in this section.

A publication by Ravier *et al.* (2019, CA 7.5/079) describes the results of a monitoring exercise of glyphosate and AMPA in the air of four different sites in the southeast of France where glyphosate is applied intensively. AMPA was not found in the samples. The maximum concentration of glyphosate found was 1.04 ng/m^3.

Gasperi *et al*. (2014, CA 7.5/050) reports the results from a qualitative monitoring exercise for micropollutants in total atmospheric fallout and Vialle *et al.* (2013, CA 7.5/056) reports the concentrations of glyphosate and AMPA among some other hundreds of substances in the roof runoff from two experimental sites in France, one in a rural area, the other one in a suburban area.

Glyphosate can be classified as not volatile based on its Henry's law constant and on volatilisation experiments from soil and plants with no significant rates (also see MCA 7.3.1). Due to no significant UV-absorption, direct photolysis in air is not relevant. In case reaching the atmosphere, glyphosate will rapidly be removed by photochemical oxidative degradation (DT_{50} of 1.625 hours).

The findings of the literature articles suggest that drift during spraying operations will be the main atmospheric source of glyphosate as well as wet and dry deposition for glyphosate and AMPA.

Applicant studies

New studies/assessments

No data was identified from requests to and from searches of online data of regional/national environment agencies for the compartment air.

Existing studies/assessments

There is no existing monitoring data on air.

Relevant literature articles

1. Information on the study

Data point:	CA 7.5/079
Report author	Ravier, S. *et al.*
Report year	2019
Report title	Monitoring of Glyphosate, Glufosinate-ammonium, and (Aminomethyl) phosphonic acid in ambient air of Provence-Alpes-Côte-d'Azur Region, France
Document No	Atmospheric Environment 204 (2019) 102-109
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Glyphosate, AMPA, its main metabolite, and glufosinate-ammonium were monitored in ambient air samples collected for two years (2015-2016), at four sampling sites in Provence-Alpes-Côte-d'Azur Region (PACA, France) in areas of different types (i.e. non-agricultural like: city center, zones of 'zero pesticide' policy, industrial areas and agricultural use like: orchards and vineyards). Neither glufosinate-ammonium nor AMPA were detected. The summary focuses on results published for glyphosate and AMPA.

Neither glufosinate-ammonium nor AMPA were detected. Glyphosate was detected at a global frequency of 7 % with frequencies ranging from 0 % (Nice) to 23 % (Cavaillon), according to the sampling site.

Glyphosate concentration reached a maximum level of 1.04 ng/m^3 in the rural site of Cavaillon. This is despite the physicochemical characteristics of glyphosate, which are not favourable to its passage into the atmosphere. The absence of simultaneous detection of glyphosate and AMPA suggests that drift during spraying operation is the main atmospheric source of glyphosate and that resuspension from soil particles is minor.

Materials and methods

Chemicals and reagents

Glyphosate (99 %), glufosinate-ammonium (95 %), and (aminomethyl)phosphonic acid (AMPA, 99 %) reference standards were purchased from Sigma-Aldrich. The main physicochemical properties, the agricultural uses and the legal situation of pesticides studies are summarized in Table 7.5-227.

9-Fluorenmethylcholoroformate (FMOC-Cl, ≥99 %) and stable isotope labeled glyphosate (2-^{13}C, 99 atom% ^{13}C) from Sigma-Aldrich were used as derivatization reagent and internal standard (IS), respectively. HPLC-grade dichloromethane (Sigma-Aldrich), ethylenediaminetetraacetic acid (EDTA), sodium tetraborate decahydrate (Borax), ammonium formate, formic acid, ammonia solution (35 %), LC/MS-grade acetonitrile, and LC/MS-grade methanol (Fisher Scientific) were used for extraction and chromatographic elution. Ultra-High Quality water (UHQ water, 18.2 MΩ/cm at 25°C) was obtained by tap water passed through a Milli-Q water purification system (Direct 8 MilliQ, Merck Millipore). Underivatized standards were dissolved in UHQ water and the stock solutions of each compound at 0.5 g/L for glyphosate and glufosinate-ammonium, and 0.9 g/L for AMPA were stored in a polypropylene bottle (PP) at 4°C.

Sampling and site characterization

Sampling was undertaken at four sampling sites distributed throughout the Provence-Alpes-Côte-d'Azur (PACA) region, France, from January 2015 to December 2016. The description of sampling sites and sampling periods are summarized in Table 7.5-228. The three urban sampling sites (i.e., Avignon, Nice, and Port-de-Bouc) were located in the city centers, whereas the rural site of Cavaillon (hamlet of Les Vignères) was located in an intensive arboriculture area.

Glyphosate and glufosinate-ammonium are expected to exist solely in the particulate-phase. As a result, glyphosate, glufosinate-ammonium, and AMPA concentrations in the atmosphere are assumed to be equal to their particulate-phase concentrations.

Sampling was carried out using a high-volume sampler (Digitel Aerosol Sampler DHA-80) equipped with a PM-10 size selective inlet. Particulate samples (n = 142) were collected on 150 mm diameter ashless quartz microfiber filter (ALBET LabScience). The sampling flow was 30 m^3/h for 24 h. A total of 71 analyses were performed. Each analysis groups two filters, giving a total volume of filtered air around 1400 m^3.

Once collected, samples were stored and protected from light at -18°C until analysis. Moreover, in order to quantify the background contamination from sample handling and storage, field air blanks were done at each site. Typically, they consisted in a brief installation of a filter in the high-volume sampler without air pumping to simulate the sample handling. No contamination was detected, i.e., below the limit of detection.

Table 7.5-227: Physicochemical properties, agricultural uses, and legal situation

Chemical name	CAS number	Molecular weight ($g\ mol^{-1}$)	Vapor pressure (Pa, 25 °C)[a]	Henry's law constant ($Pa\ m^3\ mol^{-1}$, 25 °C)[a]
Glyphosate	1071-83-6	169.1	$1.3{\cdot}10^{-5}$	$2.1{\cdot}10^{-7}$
Glufosinate-ammonium	77182-82-2	198.2	$3.1{\cdot}10^{-5}$	$4.5{\cdot}10^{-9}$
(Aminomethyl) phosphonic acid (AMPA)	1066-51-9	111.0	–	0.16

Chemical name	Solubility in water ($g\ L^{-1}$, 20 °C)[a]	Acceptable Daily Intake ($mg\ kg\ bw^{-1}\ day^{-1}$)[b]	Principal agricultural uses
Glyphosate	10.5	0.3	General treatment, cereals, vegetable crops, orchards, vineyards, non-cropped areas
Glufosinate-ammonium	500	0.02	General treatment, cereals, potatoes, vineyards, non-cropped areas
(Aminomethyl) phosphonic acid (AMPA)	1467	0.3	Transformation product

[a] PPDB: Pesticide Properties DataBase (Lewis *et al*., 2016).
[b] APVMA, 2017.

Table 7.5-228: Description of sampling sites

Sampling site (French department)	Latitude	Longitude	Altitude	Topology	Total analysis number
Avignon (Vaucluse)	43.94976 N	4.80451 E	21 m	Urban	14
Cavaillon (Vaucluse)	43.88128 N	5.00611 E	60 m	Rural	13
Nice (Alpes-Maritimes)	43.70207 N	7.28539 E	4 m	Urban	22
Port-de-Bouc (Bouches-du-Rhône)	43.40195 N	4.98197 E	1 m	Urban	22

Sampling site (French department)	Land use description[a]
Avignon (Vaucluse)	Complex cultivation patterns (33%), Vineyards (30%), Fruit trees and berry plantations (14%) Urban fabric (10%)
Cavaillon (Vaucluse)	Complex cultivation patterns (52%), Fruit trees and berry plantations (18%), Urban fabric (11%)
Nice (Alpes-Maritimes)	Urban fabric (47%), Forests (24%), Scrub and/or herbaceous vegetation associations (16%)
Port-de-Bouc (Bouches-du-Rhône)	Scrub and/or herbaceous vegetation associations (51%), Urban fabric with industrial area (27%), Forests (11%)

[a] Corine Land Cover nomenclature (zone of 10 km radius around the sampling site).

Sample extraction and derivatization

Extraction: Extractions of samples and blanks were carried out using PolyTetraFluoroEthylene (PTFE) or PolyPropylene (PP) vessels to avoid any loss of studied compounds by wall adsorption. In a 70 mL PTFE centrifugation tube, two filters (i.e., one sample) were spiked with 40 µL of IS solution (15.4 mg/L). The sample was then extracted with 20 mL of UHQ water added by 2 mL of Borax (0.05 M) and 0.8 mL of EDTA (0.1 M) solutions using first a mechanical shaker (30 s), then an ultrasonic bath (10 min). Sample was finally centrifuged at 12,000 rpm (12 min). A second extraction was performed with half volume of solutions according to the same procedure. The supernatants of the two successive extractions were collected and filtered together through a polyethersulfone (PES) membrane of 0.45 µm pore size under vacuum.

FMOC (FluorenylMethylOxyCarbonyl) derivatization: The filtrate was derivatized in 10 mL of acetonitrile with 2 mL of FMOC-Cl (50 g/L in acetonitrile). The mixture was stirred, cap closed, for 90 min in the dark at room temperature. After derivatization, acetonitrile was evaporated under nitrogen flow using a concentration workstation (TurboVap II, Biotage) with pressure 1.1 bar and a water bath at 40°C. To remove unwanted by-products and FMOC excess, 6 mL of dichloromethane were added at the residual aqueous solution then removed by settling.

Purification and concentration: Prior to purification and concentration on Solid Phase Extraction (SPE), the pH of the aqueous fraction was adjusted to pH 3 with formic acid 5 % which corresponds to the optimum analyte retention. The extraction cartridge (OASIS HLB cartridge, 6 mL, 150 mg, Waters) was successively conditioned by 2 mL of methanol then 2 mL of formic acid 0.1 %. Impurities were eliminated by a selective washing step constituted by 2 mL of formic acid 0.1 % then 2 mL of UHQ water. Elution was achieved by 4 mL of [methanol/H_2O (70/30) (v/v) + NH_4OH 2 %] solution. The extract was reduced to 1.5 mL by evaporating methanol using a concentration workstation and filtered through a PTFE membrane of 0.2 µm pore size before analysis.

UPLC-MS/MS analysis
Sample extracts were analyzed using an Ultra Performance Liquid Chromatographic (UPLC) system (Acquity, Waters) interfaced with a Quadrupole-Time-of-Flight Mass Spectrometer (Synapt G2 HDMS, Waters) equipped with an electrospray ion source (ESI). The mass spectrometer was used in its resolution mode, up to 18,000 FWHM (Full Width at Half Maximum) at 400 Th and allowed extracted chromatograms with 0.01 Th mass accuracy. The chromatographic separations were carried out on an Acquity UPLC column BEH C18, 1.7 µm particle size, 100 mm × 2.1 mm i.d. (Waters, Milford, MA, USA), at 40°C. The mobile phases consisted in (A) UHQ Water + 5 mM ammonium formate and (B) acetonitrile (Optima®, LC/MS grade, Fisher Scientific). The gradient elution was performed at a flow rate of 0.6 mL/min using 5 %-95 % of (B) within 7.5 min and held at 95 % of (B) for 1.5 min. The injection volume was 10 µL. Analyses were carried out in negative ionization mode and optimum ESI conditions were found using a -0.85 kV capillary voltage, -15 V sampling cone voltage, 450°C desolvation temperature, 120°C source temperature, 20 L/h, and 1200 L/h cone gas and desolvation gas flow rate respectively. Dwell times of 0.25 s/scan were chosen. Data acquisition and mass spectra treatments were provided by the MassLynx software (v.4.1, Waters).

Analytical performance of the method
Method validation was carried out using spiked quartz filter as solid sorbent. The accuracy (including the recoveries) of the analytical method was integrated during calibration (i.e. each concentration levels were spiked on quartz filter and followed by the extraction, derivatization, and analytical protocol). Each concentration level (from 0.04 to 0.63 ng/m^3 for glyphosate, from 0.17 to 2.67 ng/m^3 for glufosinate-ammonium, and from 0.25 to 4.06 ng/m^3 for AMPA, n = 6) are triplicate. Calibration plots showed good linearity with correlation coefficients R^2 ≥0.98 for glyphosate, R^2 ≥0.95 for glufosinate-ammonium, and R^2 ≥0.99 for AMPA. The detection limit (LOD) and quantification limit (LOQ) were determined using the calibration graph residuals for each compound (ICH, 2005). The LOD and LOQ obtained using spiked quartz filter, when air volumes of 1400 m^3 were collected, are equal to 0.05 and 0.14 ng/m^3 for glyphosate, 0.30 and 0.90 ng/m^3 for glufosinate-ammonium, and 0.28 and 0.84 ng/m^3 for AMPA, respectively.

Results

Detection frequency and atmospheric concentrations
Glyphosate was detected at a global frequency of 7 % with frequencies ranging from 0 % (Nice) to 23 % (Cavaillon), according to the sampling site. AMPA, the main glyphosate degradation product, was never detected at any sampling sites. As AMPA is a bio-degradation product formed only in soils, its atmospheric concentrations could be only due to soils aeolian erosion. Since no simultaneous detection of glyphosate and AMPA was observed in the present work, it can be assumed that the aeolian erosion was a pesticide atmospheric source of minor importance and thus, the atmospheric glyphosate concentrations were mainly due to drift during spraying. Glyphosate concentration reached a maximum level of 1.04 ng/m^3 in Cavaillon (Table 7.5-229).

Spatial and temporal detections of glyphosate
According to sampling sites and years, spatial and temporal detection frequencies varied from 0 % (e.g., Nice) to 66 % (i.e., Cavaillon in 2015). With respect to the context of sources (e.g., rural vs. urban), it was not easy to correlate the detections and the environment of the sampling sites.

Spatial distribution

In Nice, sampling was performed in a wooded square in city center, near a cemetery (~550 m South-West), urban parks (~400 m East), and port (~500 m South). Nice was the only site where glyphosate has never been detected (0/22 analysis). The explanation probably lies in the fact that, since 2009, Nice has adopted a 'zero pesticide' policy for the maintenance of green spaces, cemeteries, and roads.

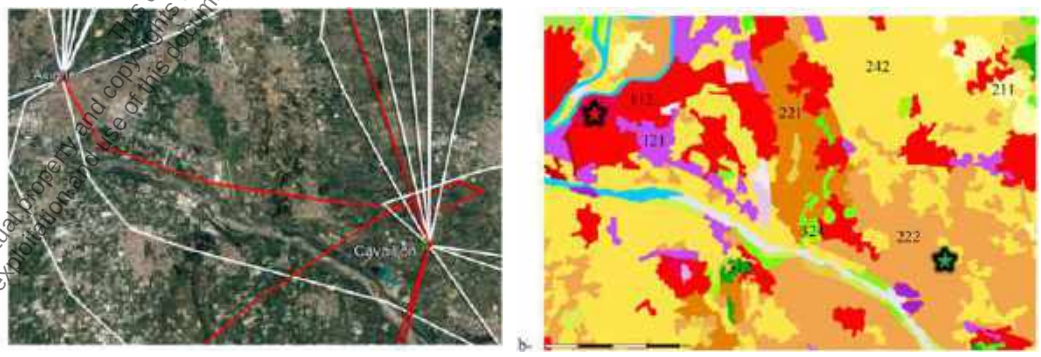
On the other hand, the Cavaillon sampling had a glyphosate detection frequency of 23 % (3/13 analyses). In addition, the highest concentrations, until 1.04 ng/m^3 in April 2015 were measured on this site. Back-trajectories calculated using the NOAA HYSPLYT model (Figure 7.5-189) indicated two regional sources: from North (May 2015) and South-West (April 2015 and June 2016). Samples of Cavaillon were collected in a hamlet named "Les Vignères", a rural site located in an intensive arboriculture area (the nearest orchard is less than 200 m from the sampler). According to the French National Institute for Agricultural Research, mechanical weeding is not always possible in established orchards if it has not been thought upstream, which leads to use of herbicides and especially glyphosate.

The sampling site of Avignon is located in the city center, near a public garden (~200 m North and North-West) and train station (~900 m South). From an agricultural point of view, there is also arable lands (~600 m North), orchards (~2 km North-East), and vineyards (~5 km North-West). Glyphosate was detected only once, in April 2015 (1/14 analysis, 7 %). Back-trajectories (Figure 7.5-189) suggest a South-East source with an air mass passing especially over the orchards surrounding the sampling site of Cavaillon.

The sampling site of Port-de-Bouc is located at the harbor near the train station (~600 m North) and less than 2 km from an industrial complex (refinery, petrochemical facilities). As in Avignon, glyphosate was detected only once in March 2016 (1/22 analysis, 5 %). However, the origin of the air mass coming from the East does not indicate specific areas where glyphosate is intensively used.

These results highlight a higher detection frequency of glyphosate in rural areas than in urban areas, i.e., 87 % (3/13 analysis) against 13 % (2/58 analysis), respectively. If rural and urban sites correspond rather to agricultural and non-agricultural applications, respectively, this is consistent with French sales with non-agricultural applications estimated at 18.6 % in 2015 and 16.1 % in 2016.

Figure 7.5-189: Geographical environment of Avignon and Cavaillon: a- Calculated back-trajectories (NOAA HYSPLIT model - GDAS meteorological data) during sampling (red line: detection of Glyphosate, white line: <LOD). b- Corine Land Cover nomenclature: 112/121-Urban fabric, 211-Arable land, 221-Vineyards, 222-Fruit trees and berry plantations, 242-Heterogeneous agricultural areas, 312-Forests, 324-Scrub and/or herbaceous vegetation associations



Temporal distribution
All detections were made between March and June which is consistent with the main phase of glyphosate applications in late winter and during spring and early summer periods (Table 7.5-229).

It should be noted that of the three sampling sites where glyphosate has been detected (i.e., Avignon, Cavaillon, and Port-de-Bouc), there is no reproducible detection pattern from 2015 to 2016.

Influence of meteorological conditions
The meteorological data collected at the four sampling sites allow the influence of precipitation, temperature, and wind speed on the glyphosate concentrations to be observed. However, it is necessary to be cautious because only 5 out of 71 samples contained glyphosate.

The 5 detections of glyphosate were registered when mean daily temperatures ranged between 9.7 °C (Port-de-Bouc, March 2016) and 21.0°C (Cavaillon, June 2016), which is consistent with the temperatures commonly measured during the application period.

In France, it is forbidden to apply as soon as the wind speed reaches an intensity greater than about 19 km/h. During the days when glyphosate was detected, the wind speed exceeded this value 33 % of the time (hourly measurement), reaching up to a maximum of more than 40 km/h in Port-de-Bouc. These wind speeds can lead to greater resuspension and then long-range transport by aerial drift which will cause injury to nontarget plants. The probability of drift injury occurring increased when winds are gusty or when wind speed will allow spray drift to occur.

Due to its high solubility in water, glyphosate is expected to be removed by rainfall. Only the sampling collected in Port-de-Bouc in March 2016 showed glyphosate detection during a rainy period (precipitation 18.6 mm), suggesting that the measured concentration (0.38 ng/m^3) was potentially higher before the rain event.

Table 7.5-229: Precipitation and atmospheric concentrations of glyphosate, glufosinate-ammonium, and AMPA in all sampling sites

Date		Avignon				Cavaillon			
		Precipitation (mm)	Concentration (ng m^{-3})			Precipitation (mm)	Concentration (ng m^{-3})		
			GLY	GLU	AMPA		GLY	GLU	AMPA
2015	01/21-23								
	02/18-20								
	03/10-12	0	–	–	–	0	–	–	–
	04/20-22	0.2	0.30	–	–	0.2	1.04	–	–
	05/18-20	0	–	–	–	0	0.62	–	–
	06/12-14	12.0	–	–	–				
	07/23-25								
	08/24-26								
	09/15-17								
	10/09-11								
	11/14-16								
	12/04-06								
2016	01/24-26								
	02/25-27	0.2	–	–	–	0.2	–	–	–
	03/15-17	25.3	–	–	–	25.3	–	–	–
	04/25-27	0	–	–	–	0	–	–	–
	05/27-29	0	–	–	–				
	06/14-16	0.4	–	–	–	0.4	0.18	–	–
	07/15-17	0	–	–	–	0	–	–	–
	08/02-04	0	–	–	–	0	–	–	–
	09/04-06	0	–	–	–	0	–	–	–
	10/25-27	2.0	–	–	–	2.0	–	–	–
	11/20-22					24.1	–	–	–
	12/20-22	3.8	–	–	–	3.8	–	–	–

Date		Nice				Port-de-Bouc			
		Precipitation (mm)	Concentration (ng m^{-3})			Precipitation (mm)	Concentration (ng m^{-3})		
			GLY	GLU	AMPA		GLY	GLU	AMPA
2015	01/21-23	20.2	–	–	–	28.8	–	–	–
	02/18-20	0	–	–	–	0	–	–	–
	03/10-12	0	–	–	–	0	–	–	–
	04/20-22	0	–	–	–	0.6	–	–	–
	05/18-20	0	–	–	–	0.2	–	–	–
	06/12-14	0.4	–	–	–	0	–	–	–
	07/23-25	0	–	–	–				
	08/24-26	11.0	–	–	–	5.6	–	–	–
	09/15-17	2.4	–	–	–	0	–	–	–
	10/09-11	0	–	–	–	0	–	–	–
	11/14-16					0	–	–	–
	12/04-06	28.6	–	–	–	2.8	–	–	–
2016	01/24-26	0	–	–	–	0	–	–	–
	02/25-27	0	–	–	–	0.8	–	–	–
	03/15-17	3.6	–	–	–	18.6	0.38	–	–
	04/25-27	0	–	–	–				
	05/27-29					0	–	–	–
	06/14-16	6.6	–	–	–	0	–	–	–
	07/15-17	0	–	–	–	0	–	–	–
	08/02-04	0	–	–	–	0	–	–	–
	09/04-06	0	–	–	–	0	–	–	–
	10/25-27	1.4	–	–	–	1.4	–	–	–
	11/20-22	28.3	–	–	–	20.9	–	–	–
	12/20-22	3.6	–	–	–	5.2	–	–	–

(−) means < Limit of Detection.

Conclusion
Neither glufosinate-ammonium nor AMPA were detected. However, at the same sampling sites, during the same period, detection frequency and maximum concentration of glyphosate were sometimes higher than those found for other pesticides, especially herbicides. This is despite the physicochemical characteristics of glyphosate which are not favorable to its passage into the atmosphere.

The absence of simultaneous detection of glyphosate and AMPA suggests that drift during spraying operation is the main atmospheric source of glyphosate, and that resuspension from soil particles is minor.

However, in the worst-case scenario (1.04 ng/m^3), the expected dose of glyphosate for an average consumer (70 kg body weight) respiring at a rate of 1.5 m^3/h during light exercise is 0.54 ng/(kg day). In these conditions, this value remains well below the chronic reference dose for glyphosate of 1.75 mg/(kg day).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the results of monitoring glyphosate and AMPA in the air of 4 different sites in the southeast of France. Maximum concentration of glyphosate measured at 1.04 ng/m^3
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/050
Report author	Gasperi, J., *et al.*
Report year	2014
Report title	Micropollutants in urban stormwater: occurrence, concentrations, and atmospheric contributions for a wide range of contaminants in three French sites
Document No	Environmental Science and Pollution Research (2014) 21:5267-5281
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions. (no consideration of agricultural areas)

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water monitoring subchapter of this document including the findings for total atmospheric fallout.

1. Information on the study

Data point:	CA 7.5/056
Report author	Vialle, C., *et al.*
Report year	2013
Report title	Pesticides in roof runoff: Study of a rural site and a suburban site
Document No	Journal of Environmental Management 120 (2013) 48 - 54
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water monitoring subchapter of this document.

E. Drinking water treatment

Water treatment for the purpose of generating drinking water

Introduction

Under Article 4 of Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market, it is required that a plant protection product, *"...shall have no immediate or delayed harmful effect on human health, including that of vulnerable groups, or animal health, directly or through drinking water (taking into account substances resulting from water treatment), food, feed or air..."*

An assessment of components potentially formed from drinking water treatment processes is therefore required. The assessment includes potential transformation of the active substance glyphosate and its metabolites AMPA and HMPA into other compounds and the relevance of those components to consumer risk assessment to drinking water.

However, the data requirements listed in Regulations (EU) 283/2013 and (EU) 284/2013 do not stipulate how to address the impact of water treatment processes. No EU agreed guideline or guidance has been adopted yet.

Glyphosate and its metabolite AMPA are considered for the environmental risk assessment of groundwater, and the metabolite HMPA is considered in addition for surface water. However, no data on the presence of HMPA is currently available from public monitoring data sources.

As strongly indicated by data on degradation and adsorption to soil, glyphosate and AMPA are unlikely to be found frequently in groundwater being abstracted as raw drinking water. This is supported by monitoring data available from EU MSs indicating that *ca*. 0.6 % (from *ca*. 3.0 % of sites) of the groundwater samples investigated showed residues of glyphosate at levels ≥ 0.1 µg/L. About 0.002 % (from *ca*. 0.006 % sites) of groundwater samples showed residues of AMPA at levels ≥ 10.0 µg/L.

In contrast, findings of Glyphosate and AMPA were more frequent in surface water monitoring when being referenced to a value of 0.1 µg/L, i.e. in 23 % and 48 % of total samples analysed, respectively, residues

were beyond this threshold. It should be noted that the term 'surface water' is not strictly defined. Though the percentage of findings may appear high, it is not possible to distinguish readily between large scale and smaller scale surface waters and their overall use for drinking water abstraction. Large surface waters like rivers can be a source for raw drinking water by abstraction *via* bank filtration.

As such, an assessment of the likely fate of glyphosate and its metabolites when exposed to water treatment processes has been carried out and is presented below. For pragmatic purposes, differentiation has been made between 'low-chemical' and chemical methods of treatment of raw drinking water.

There are a very wide variety of water treatment processes that may be applied for a given raw drinking water including 'low-chemical' and 'chemical' options. The exact combination depends on the context including characteristics and origin and must be adapted to the source (the 'treatment train').

"Low-chemical" refers to processes with either no involvement of chemicals or, where the treatment is to occur *via* physical processes like complexation and adsorption. It also includes water treatment processes where it is very unlikely that metabolites known to be formed by microbial processes in soil or water/sediment are then transformed under the conditions of that process. For example, the abstracted raw water from most water sources must be cleaned and sieved to remove suspended materials, often achieved by filtration through sand and often followed by concomitant chemical coagulation/flocculation steps.

'Chemical treatment' following low-chemical processes in most 'treatment trains' for drinking water till the consumers tap represents a necessary disinfection step designed to remove hazardous biological material such as bacteria and viruses before it is released. The latter measure is a major water quality objective, achieved, for example, by chlorination.

Chlorination was demonstrated to remove glyphosate residues from water effectively while having the potential to form transformation products. Other chemical treatment like ultra-violet irradiation or ozonation/ozonolysis processes might also result in formation of other potential transformation products. Finally, treatment processes such as activated carbon filtration or reverse osmosis can be excluded as a potential source of transformation products.

The information available in the form of publications or company-sponsored studies to investigate potential transformation routes of glyphosate, AMPA and HMPA under conditions simulating water treatment processes are summarised in the next two sections.

E.1 Low chemical treatment and bank filtration

Applicant studies

[redacted] (2020, CA 7.5/002) covers a range of environmental compartments and subsequent analysis, however, the study summary below only includes the results relevant to water treatment.

Low-chemical water treatment processes are frequently applied to water destined to become drinking water. There are two Monsanto (Bayer) commissioned studies which address the fate of glyphosate and AMPA when subjected to low-chemical water treatment processes. The first of these ([redacted] 2010, CA 7.5/081), contains a review and some original work on removal rates. The same material has also been presented in a peer reviewed publication (Jönsson *et al*., 2013, CA 7.5/084), and the relevant findings summarised below. The second study ([redacted] 2012, CA 7.5/080), is also a review which looks at three low-chemical processes: bank filtration, slow sand filtration and biological activated carbon. The use of bank filtration is relatively limited in Europe, with less than 50 sites specifically designed to utilize this technique. Slow sand filtration is more common in Europe where it has been installed at several hundred treatment works. Biological activated carbon is the most common technique of the three; possibly because it is the easiest to retrofit. The removal rates in this study are also summarised in the peer reviewed publication (Jönsson *et al*., 2013, CA 7.5/084), and the relevant findings will also be summarised below.

Other Information

Of the literature sources available, the following are specifically considered here, with respect to Low: chemical treatment and bank filtration:

In bank filtration, surface water (in a river or lake) filters through the sediment floor or bank, and travels to an extraction well set back from the water body where, following further treatment processes, it is delivered as drinking water. Consequently, the transformation of glyphosate and AMPA when subjected to bank filtration is essentially that which would be expected following aerobic or anaerobic degradation in soil or sediment/water systems: that is, no novel transformation products would be expected as the same microbial and hydrolytic processes take place. As indicated above, in the EU the use of bank filtration is relatively limited, with less than 50 sites specifically designed to utilize this technique. Further, <10 % of raw water for drinking water in the EU involves bank filtration processes (van der Hoek *et al*., 2014, CA 7.5/098) whose main findings are summarised in [redacted] (2020, CA 7.5/002). However, as indicated in (Table 1; Gillefalk *et al*., 2018, CA 7.5/097) and reported in [redacted] (2020, CA 7.5/002), there are several places in the EU where a significant proportion of drinking water involves bank filtration processes (e.g. Paris, Berlin (60 % of drinking water), Düsseldorf (100 % of drinking water)), such that research is available on the fate of glyphosate and AMPA when subjected to bank filtration.

The degradation of ^{14}C-glyphosate in very wet filter sands from three Danish waterworks was investigated at 10°C in the dark for up to 13 days (Hedegaard & Albrechtsen, 2014, CA 7.5/083). The residence time of water *in situ* in rapid sand filters in treatment works was reported as 7.5 – 12 minutes. Under the experimental conditions, glyphosate decreased to 7 – 14 % of initial amounts after 13 days (complete mineralisation); indicating that glyphosate was intrinsically degradable under these conditions (although unlikely to be degraded significantly *in situ*).

Technical scale semi-field investigations (bank filtration and slow sand filter experiments) were carried out with glyphosate and reported in Litz *et al*. (2011, CA 7.5/063). The experimental systems consisted of three enclosures (metal cylinders) of slow sand filter material, with an area of 1 m^2 and a height of 1.85 m (with a filtration length of 1 m) situated within an infiltration pond (area 90 m^2). The flow rate was set at 50 cm/day. Glyphosate was continuously dosed to the enclosures over a 14 day period, and water samples for glyphosate and AMPA analysis were taken for 34 days. These slow sand filter experiments demonstrated that 70 – 80 % reduction in glyphosate concentrations were achieved (for constant inlet concentrations of 0.7, 3.5 and 11.6 µg/L). Modelling (using the VisualCXTFit model) generated a predicted required filtration length of 2.75 – 3.75 m (to give glyphosate concentrations below 0.1 µg/L), and using data from typical Berlin bank filtration sites yielded the same sufficient attenuation within a few days of travel time. Additional experiments on a slow sand filter planted with *Phragmites australis* and an unplanted control demonstrated that the planted slow sand filter enhanced retardation of glyphosate. Overall, the results showed that saturated subsurface passage has the potential to efficiently attenuate glyphosate, with aerobic conditions, long travel times and the presence of riparian boundary buffer strips.

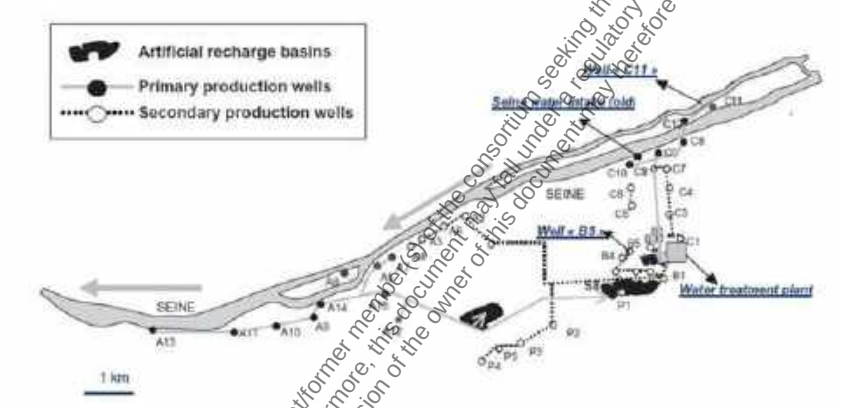
A reactive transport model was developed to evaluate the potential for contamination of drinking water wells by surface water pollution (Malaguerra *et al*., 2013, CA 7.5/085). The model was designed to be applicable to a wide range of aquifers, especially in Denmark. The results of a tracer experiment conducted by other researchers using a river in Switzerland were used to test the model, which was found to adequately model the results of the tracer experiment. Sensitivity analysis showed that the characteristics of the clay aquitard (hydraulic conductivity and thickness) and well depth were the parameters governing the risk of contamination of the wells by pollution in streams. The authors also reported that their results showed that it is unlikely that glyphosate in streams will pose a threat to drinking water wells.

The fate of organic micropollutants during long-term/long-distance river bank filtration, at a temporal scale of several years, was investigated along a row of monitoring wells perpendicular to the Lek River in The Netherlands (Hamann *et al.*, 2016, CA 7.5/082). Analysis for a range of substances (including AMPA) in river and well water was carried out from 1999 – 2013. Models were constructed for transects from the river to three wells, calibrated using tracer experiments. Travel times from the river to the wells were found

to be 1.7 to 3.7 years. Data for AMPA was presented (but not for glyphosate); which was fully removed by bank filtration under these conditions.

A detailed study of the fate of various contaminants (including glyphosate and AMPA) was carried out on a stretch of the Seine downstream of Paris (Bruchet *et al.*, 2011, CA 7.5/027). The investigated area is downstream of urban wastewater plants (Figure 7.5-190), in particular of a plant that treats effluent from 6.5 million people, and comprises 36 primary and secondary wells: the primary wells are located mostly along the river, naturally re-supplied under anoxic conditions through river bank filtration. The primary wells output is pumped and re-infiltrated through a sand-gravel artificial basin (under slightly aerobic conditions) to recharge secondary production wells. Water from the secondary wells is further treated in a drinking water plant that comprises settling with addition of powdered activated carbon, sand filtration, ozonation and final disinfection with chlorine. The plant production is equal to 144000 m^3/day.

Figure 7.5-190: Description of study site showing the four sampling points. Flow of the river is from right to left (from Bruchet *et al.*, 2012)



Grab samples were taken on five occasions during September and October 2008 from the Seine raw water, primary well C11, secondary well B5, and the treated water at the outlet of the drinking water plant. The sampling period covered both low flow conditions (220 m^3/s) and higher flow rates (up to 343 m^3/s). In the river, glyphosate was found at <0.1 – 0.12 µg/L, and AMPA at 0.25 – 0.65 µg/L: but, in both the primary well and the secondary well, concentrations of both substances were <0.1 µg/L, as they were in the drinking water samples. (It is worth noting that "<0.1 µg/L" indicates LOQ, and not an absolute concentration – using it as a basis for determining the removal rate for AMPA would give a removal rate of 85 %, and 17 % for glyphosate; whereas, it is clear from the context that removal is more likely to be 100 %. Indeed, the authors state that "both these compounds are totally removed by bank filtration" in this case.) With respect to glyphosate and AMPA, the study sheds light on the effectiveness of the water treatment train employed for a major surface water to drinking water plant, where the primary treatment process is bank filtration. It seems likely that similar arrangements associated with other major bank filtration complexes have equivalent effectiveness with respect to the removal of glyphosate and AMPA.

It is clear that bank filtration has been shown to be an effective process to reduce or remove glyphosate and AMPA from water destined to be drinking water.

Jönsson *et al.* (2013, CA 7.5/084) reports on some investigations conducted into the fate of glyphosate and AMPA when subjected to UV treatment, in a flow-through pilot reactor. The UV intensity used was significantly higher than typically used in water treatment for disinfection alone; and even then removal of glyphosate was only 36 %, and AMPA was degraded even less.

The publication (Jönsson *et al.*, 2013, CA 7.5/084) also summarises attempts to remove glyphosate and AMPA using activated carbon (often utilized to remove organic micro-pollutants from water) where removal rates were found to be very variable, and reported new investigations using powdered activated carbon – but adsorption of glyphosate and AMPA was low (*ca*. 20 % removal rate). Literature relating to other low-chemical processes (use of coagulants, slow sand filtration, air stripping and membrane filtration) was also summarised; although on some occasions high removal rates for glyphosate and AMPA were reported (e.g. 70 % removal using an iron coagulant), the removal rates were variable. In Peschka *et al.*, (2006, CA 7.5/072), the removal rates for glyphosate and AMPA for some low-chemical processes were reported: flocculation with activated silicic acid and addition of potassium permanganate and aluminum salts, removal rate of 39±14 % for glyphosate and 22±15 % for AMPA; for gravel filtration removal rate of <10 % for both compounds; and for activated carbon removal rates of <10 % for glyphosate and 21±9 % for AMPA. The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30 % , (reported in Ruel *et al.*, 2012, CA 7.5/086). An investigation of the removal rates for glyphosate and AMPA associated with various stages to be found across seven Waste Water Treatment Plants, was reported (Ruel *et al.*, 2011, CA 7.5/087): 30 – 70 % for glyphosate and AMPA for sand filtration, <30 % for AMPA for reverse osmosis and ozone treatment, but >70 % for glyphosate for reverse osmosis and ozone treatment; >70 % for both glyphosate and AMPA for activated carbon filtration. Further information on the efficiency of reverse osmosis followed by activated carbon filtration for removal of organic micropollutants from river bank filtrate is given in Schoonenberg Kegel, F. *et al.* (2010, KCA 7.5/088).

Summary

Removal rates for glyphosate and AMPA when subjected to low-chemical processes are very variable. Table 7.5-230 is summarised from Jönsson *et al.* (2013, CA 7.5/084), and adjusted in the light of the above summarised literature:

Table 7.5-230: Summary of glyphosate and AMPA removal rates following low-chemical treatment processes (based on Jönsson *et al.*, 2013, CA 7.5/084, and adjusted for summarised literature)

Treatment process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 ->95	25 - >95
Aluminium coagulant and clarification	15 - 40	20 - 85
Iron coagulant and clarification	40 - 70	20 – 85
Slow sand filtration	The limited information available suggests that significant removal can be achieved but removal is likely to be highly dependent on conditions	
UV irradiation	Not effective alone at doses used in water treatment	
Activated carbon adsorption	10 – 90	20 – 70

Of these processes, bank filtration, in particular, can be an effective process for removal of glyphosate and AMPA from water, when sufficient residence time within soil/sediment occurs to allow the normal aerobic/anaerobic soil degradation processes to progress to their full extent (total mineralisation; i.e. complete transformation of all the glyphosate/AMPA atoms to CO_2 or equivalent terminal products such as nitrate, phosphate etc.). Further, almost all water passing through bank filtration, and destined for drinking water is also subject to disinfection (see below) which is mostly chlorine-based, which rapidly and effectively removes glyphosate and AMPA.

Applicant studies

New studies/assessments

1. Information on the study

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Document No	-
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The report provides information about the outcome of an analysis of public monitoring data comprising environmental concentrations of glyphosate (GLY) and its primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA) collated from readily available public monitoring databases held by national/regional environment agencies. In addition to this analysis, an assessment of water treatment processes was undertaken through review of published peer reviewed literature.

Removal of Glyphosate and AMPA by Water Treatment Processes

For surface water destined to be drinking water, there are almost always water treatment processes applied to remove bacteria and viruses and other organic micro-pollutants. The vast majority (88 %) of raw water sources for drinking water production are subject to disinfection (Van der Hoek *et al*., 2014, CA 7.5/098). In particular, almost all (99.9 % by volume) the raw water taken from surface water is subject to disinfection; and where surface water is disinfected, chlorine disinfection is applied to a minimum of 62 % of the raw water (Van der Hoek *et al*., 2014, CA 7.5/098). Disinfection and oxidative processes are applied where needed and at predetermined rates for the removal of microbial and organic micro-pollutants, regardless of GLY and AMPA presence. GLY and AMPA are known to be very readily transformed by the most common disinfection methods, ranging from 25 to 95 % for AMPA and 60 to 99 % for GLY (the higher of these values corresponding to chlorination; Jönsson *et al.*, 2013, CA 7.5/084). Transformation products are small molecules, often similar or identical to those found from natural sources. Other chemical treatment processes are also often applied as are low chemical processes (processes with either no involvement of chemicals or where the treatment is to occur via physical processes like complexation and

adsorption) and bank filtration (infiltration of surface water from a river or lake into a groundwater system, induced by water abstraction close to the surface water). Drinking water treatment processes are carefully controlled and the water treatment process train at any given abstraction site optimised to ensure that quality standards are met at the tap of consumers (e.g. GLY < 0.1 µg/L).

I. MATERIALS AND METHODS

An integral part of potentially understanding the patterns of exposure highlighted by the public monitoring data is how raw water sources are treated to produce drinking water. An assessment of water treatment processes was undertaken through review of published peer reviewed literature. This identified treatment processes and the degree to which they are effective at removing glyphosate (GLY) and AMPA during the water treatment process. These can be used to interpret the groundwater and surface water data within the context of drinking water production.

II. RESULTS AND DISCUSSION

Low-chemical Water Treatment and Bank Filtration

Low-chemical water treatment processes are frequently applied to water destined to become drinking water. There are two Monsanto (Bayer) commissioned studies which address the fate of glyphosate and AMPA when subjected to low-chemical water treatment processes. The first of these ([redacted] 2010, CA 7.5/081), contains a review and some original work on removal rates. The same material has also been presented in a peer reviewed publication (Jönsson *et al.*, 2013 CA 7.5/084), and the relevant findings summarised below. The second study ([redacted] 2012, CA 7.5/080), is also a review which looks at three low-chemical processes: bank filtration, slow sand filtration and biological activated carbon. The use of bank filtration is relatively limited in Europe, with less than 50 sites specifically designed to utilize this technique. Slow sand filtration is more common in Europe where it has been installed at several hundred treatment works. Biological activated carbon is the most common technique of the three; possibly because it is the easiest to retrofit. The removal rates in this study are also summarised in the peer reviewed publication (Jönsson *et al.*, 2013, CA 7.5/084), and the relevant findings will also be summarised below.

In bank filtration, surface water (in a river or lake) filters through the sediment floor or bank, and travels to an extraction well set back from the water body where, following further treatment processes, it is delivered as drinking water. Consequently, the transformation of glyphosate and AMPA when subjected to bank filtration is essentially that which would be expected following aerobic or anaerobic degradation in soil or sediment/water systems: that is, no novel transformation products would be expected as the same microbial and hydrolytic processes take place. As indicated above, in the EU the use of bank filtration is relatively limited, with less than 50 sites specifically designed to utilize this technique. Further, <10 % of raw water for drinking water in the EU involves bank filtration processes (van der Hoek *et al.*, 2014, CA 7.5/098). However, as indicated in (see Table 1 in Gillefalk *et al*., 2018, CA 7.5/097), there are several places in the EU where a significant proportion of drinking water involves bank filtration processes [e.g. Paris, Berlin (60 % of drinking water), Düsseldorf (100 % of drinking water)], such that research is available on the fate of glyphosate and AMPA when subjected to bank filtration.

The degradation of ^{14}C-glyphosate in very wet filter sands from three Danish waterworks was investigated at 10°C in the dark for up to 13 days (Hedegaard & Albrechtsen, 2014, CA 7.5/083). The residence time of water *in situ* in rapid sand filters in treatment works was reported as 7.5 – 12 minutes. Under the experimental conditions, glyphosate decreased to 1 – 14 % of initial amounts after 13 days; indicating that glyphosate was intrinsically degradable under these conditions (although unlikely to be degraded significantly *in situ*).

Technical scale semi-field investigations (bank filtration and slow sand filter experiments) were carried out with glyphosate and reported in Litz *et al.* (2011, CA 7.5/063). The experimental systems consisted of three enclosures (metal cylinders) of slow sand filter material, with an area of 1 m^2 and a height of 1.85 m (with a filtration length of 1 m) situated within an infiltration pond (area 90 m^2). The flow rate was set at

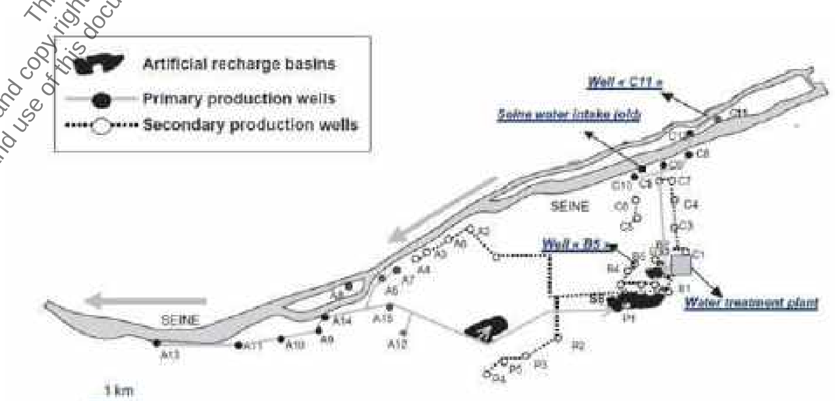
50 cm/day. Glyphosate was continuously dosed to the enclosures over a 14-day period, and water samples for glyphosate and AMPA analysis were taken for 34 days. These slow sand filter experiments demonstrated that 70 – 80 % reduction in glyphosate concentrations were achieved (for constant inlet concentrations of 0.7, 3.5 and 11.6 µg/L). Modelling (using the VisualCXTFit model) generated a predicted required filtration length of 2.75 – 3.75 m (to give glyphosate concentrations below 0.1 µg/L) and using data from typical Berlin bank filtration sites yielded the same sufficient attenuation within a few days of travel time. Additional experiments on a slow sand filter planted with *Phragmites australis* and an unplanted control demonstrated that the planted slow sand filter enhanced retardation of glyphosate. Overall, the results showed that saturated subsurface passage has the potential to efficiently attenuate glyphosate, with aerobic conditions, long travel times and the presence of riparian boundary buffer strips.

A reactive transport model was developed to evaluate the potential for contamination of drinking water wells by surface water pollution (Malaguerra *et al.,* 2013, CA 7.5/085). The model was designed to be applicable to a wide range of aquifers, especially in Denmark. The results of a tracer experiment conducted by other researchers using a river in Switzerland were used to test the model, which was found to adequately model the results of the tracer experiment. Sensitivity analysis showed that the characteristics of the clay aquitard (hydraulic conductivity and thickness) and well depth were the parameters governing the risk of contamination of the wells by pollution in streams. The authors also reported that their results showed that it is unlikely that glyphosate in streams will pose a threat to drinking water wells.

The fate of organic micropollutants during long-term/long-distance river bank filtration, at a temporal scale of several years, was investigated along a row of monitoring wells perpendicular to the Lek River in The Netherlands (Hamann *et al.*, 2016, CA 7.5/082). Analysis for a range of substances (including AMPA) in river and well water was carried out from 1999 – 2013. Models were constructed for transects from the river to three wells, calibrated using tracer experiments. Travel times from the river to the wells were found to be 1.6 to 3.6 years. Data for AMPA was presented (but not for glyphosate); which was fully removed by bank filtration under these conditions.

A detailed study of the fate of various contaminants (including glyphosate and AMPA) was carried out on a stretch of the Seine downstream of Paris (Bruchet *et al.,* 2012). The investigated area is downstream of urban wastewater plants (Figure 7.5-191), in particular of a plant that treats effluent from 6.5 million people, and comprises 36 primary and secondary wells: the primary wells are located mostly along the river, naturally re-supplied under anoxic conditions through river bank filtration. The primary wells output is pumped and re-infiltrated through a sand-gravel artificial basin (under slightly aerobic conditions) to recharge secondary production wells. Water from the secondary wells is further treated in a drinking water plant that comprises settling with addition of powdered activated carbon, sand filtration, ozonation and final disinfection with chlorine. The plant production is equal to 144000 m^3/day.

Figure 7.5-191: Description of study site showing the four sampling points. Flow of the river is from right to left (from Bruchet *et al.*, 2012)



Grab samples were taken on five occasions during September and October 2008 from the Seine raw water, primary well C11, secondary well B5, and the treated water at the outlet of the drinking water plant. The sampling period covered both low flow conditions (220 m^3/s) and higher flow rates (up to 343 m^3/s). In the river, glyphosate was found at <0.1 – 0.12 µg/L, and AMPA at 0.25 – 0.65 µg/L: but, in both the primary well and the secondary well, concentrations of both substances were <0.1 µg/L, as they were in the drinking water samples. (It is worth noting that "<0.1 µg/L" indicates LOQ, and not an absolute concentration – using it as a basis for determining the removal rate for AMPA would give a removal rate of 85 %, and 17 % for glyphosate; whereas, it is clear from the context that removal is more likely to be 100 %. Indeed, the authors state that "both these compounds are totally removed by bank filtration" in this case.) With respect to glyphosate and AMPA, the study sheds light on the effectiveness of the water treatment train employed for a major surface water to drinking water plant, where the primary treatment process is bank filtration. It seems likely that similar arrangements associated with other major bank filtration complexes have equivalent effectiveness with respect to the removal of glyphosate and AMPA. It is clear that bank filtration has been shown to be an effective process to reduce or remove glyphosate and AMPA from water destined to be drinking water. Jönsson *et al.* (2013, CA 7.5/084) reports on some investigations conducted into the fate of glyphosate and AMPA when subjected to UV treatment, in a flow-through pilot reactor. The UV intensity used was significantly higher than typically used in water treatment for disinfection alone; and even then removal of glyphosate was only 36 %, and AMPA was degraded even less.

The publication Jönsson *et al.* (2013, CA 7.5/084) also summarises attempts to remove glyphosate and AMPA using activated carbon (often utilized to remove organic micro-pollutants from water) where removal rates were found to be very variable, and reported new investigations using powdered activated carbon – but adsorption of glyphosate and AMPA was low (*ca.* 20 % removal rate). Literature relating to other low-chemical processes (use of coagulants, slow sand filtration, air stripping and membrane filtration) was also summarised; although on some occasions high removal rates for glyphosate and AMPA were reported (e.g. 70 % removal using an iron coagulant), the removal rates were variable. In Peschka *et al.* (2006), the removal rates for glyphosate and AMPA for some low-chemical processes were reported: flocculation with activated silicic acid and addition of potassium permanganate and aluminum salts, removal rate of 39±14 % for glyphosate and 22±15 % for AMPA; for gravel filtration removal rate of <10 % for both compounds; and for activated carbon removal rates of <10 % for glyphosate and 21±9 % for AMPA. The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30 %, (reported in Ruel *et al.*, 2012, CA 7.5/086). An investigation of the removal rates for glyphosate and AMPA associated with various stages to be found across seven Waste Water Treatment Plants, was reported (Ruel *et al.*, 2011, CA 7.5/087): 30 – 70 % for glyphosate and AMPA for sand filtration, <30 % for AMPA for reverse osmosis and ozone treatment, but >70 % for glyphosate for reverse osmosis and ozone treatment; >70 % for both glyphosate and AMPA for activated carbon filtration.

Low-chemical Water Treatment and Bank Filtration Summary

Removal rates for glyphosate and AMPA when subjected to low-chemical processes are very variable. Table 7.5-231 is summarised from Jönsson *et al*. (2013, CA 7.5/084) and adjusted based on the literature reviewed.

Table 7.5-231: Summary of glyphosate and AMPA removal rates following low-chemical treatment processes (based on Jönsson *et al*., 2013, CA 7.5/084, and adjusted for summarised literature)

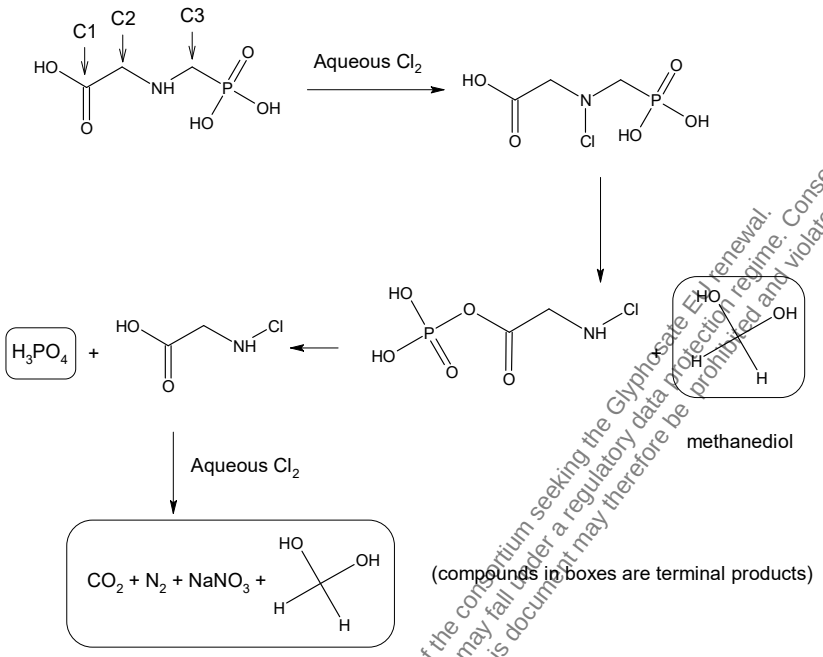
Treatment process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 - >95	25 - >95
Aluminum coagulant and clarification	15 - 40	20 - 85
Iron coagulant and clarification	40 - 70	20 – 85
Slow sand filtration	The limited information available suggests that significant removal can be achieved but removal is likely to be highly dependent on conditions	
UV irradiation	Not effective alone at doses used in water treatment	
Activated carbon adsorption	10 – 90	20 – 70

Of these processes, bank filtration, in particular, can be an effective process for removal of glyphosate and AMPA from water, when sufficient residence time within soil/sediment occurs to allow the normal aerobic/anaerobic soil degradation processes to progress to their full extent (total mineralisation; i.e. complete transformation of all the glyphosate/AMPA atoms to CO_2 or equivalent terminal products such as nitrate, phosphate etc.). Further, almost all water passing through bank filtration, and destined for drinking water is also subject to disinfection (see below) which is mostly chlorine-based, which rapidly and effectively removes glyphosate and AMPA.

Chemical Water Treatment

There is one Monsanto (Bayer) commissioned study ([redacted] 2010, CA 7.5/081) which addresses the fate of glyphosate and AMPA when subjected to water treatment chemical processes. This reviews original work on removal rates when glyphosate and AMPA are subjected to chemical treatment by ozone, chlorine, and chlorine dioxide. The same information has also been presented later in the form of a peer reviewed publication (Jönsson *et al*., 2013, CA 7.5/084), and the relevant findings are summarised below. Neither of these report in detail on the transformation products of glyphosate and AMPA when subjected to water treatment processes. The mechanism of chlorination (when treated with aqueous chlorine) of glyphosate has been investigated exhaustively and reported in two linked publications (Mehrsheikh *et al*., 2006, CA 7.5/095; Brosillon *et al.,* 2006, CA 7.5/094). Using stable isotopes and NMR spectroscopy to identify species generated when glyphosate and glycine are separately treated with aqueous chlorine, it was possible to generate a proposed route of degradation for glyphosate (Figure 7.5-192):

Figure 7.5-192: Proposed mechanism of glyphosate chlorination (compounds drawn in boxes are the terminal products) (from Mehrsheikh *et al.*, 2006, CA 7.5/095)



Glyphosate is totally degraded to small molecules common to the degradation of naturally occurring substances in raw water (e.g. amino acids), and the degradation pathway follows that of glycine. The C1 carboxylic acid carbon of glyphosate/glycine is converted to CO_2; the C2 methylene carbon is converted to CO_2 and methanediol; the nitrogen is transformed into nitrogen gas and nitrate; the C3 phosphonomethylene carbon is converted to methanediol; and the phosphorus moiety produces phosphoric acid. Kinetic models were constructed that allowed the temporal course of the reactions to be simulated; these predicted that under conditions similar to those found in water treatment plants, the chlorination of glyphosate is complete within seconds of contact with chlorine.

The very rapid reaction of glyphosate with aqueous chlorine was confirmed in the investigations reported in Jönsson *et al.* (2013, CA 7.5/084). In this work, incubation was for only 30 minutes, and at 20°C degradation of glyphosate reached 96-100 %; although degradation was less complete at a lower temperature (71 % at 5°C). AMPA degraded faster than glyphosate, >99 % at all temperatures. The investigations indicated that chlorine dioxide is a less effective degrader of glyphosate (17-93 %, 30 minutes, various temperatures/pH values) than aqueous chlorine, and an effective degrader of AMPA (>99 % under all conditions tested).

Another approach to disinfection of drinking water sources is ozonation/ozonolysis, where ozone (O_3) is used to deactivate viruses, bacteria and some parasites. The operation of such processes in the context of treating surface water from three French rivers (Marne, Seine and Oise) to provide drinking water to 4 million people in the Paris region has been reported Boucherie *et al.* (2010, CA 7.5/092). A pilot plant was utilised for the investigations: glyphosate was found to be very rapidly degraded by ozone treatment (>91 %, levels reduced to <0.1 µg/L) and AMPA was rapidly removed (>88 %, levels reduced to <0.1 µg/L); hence, the ozone treatment required to deliver disinfection targets was also effective in removing glyphosate and AMPA to levels below 0.1 µg/L. The use of ozone to degrade glyphosate and AMPA was also investigated in a batch reactor Assalin *et al.* (2010, CA 7.5/091). In these studies, it was clear that the pH of the test solution altered the reactivity of glyphosate and AMPA to ozonation. It was evident that AMPA was produced from glyphosate at all pH's. For glyphosate, at alkaline pH (pH 10) degradation was very rapid and AMPA was also completely degraded (but more slowly); indeed, total carbon content

removal was measured to be 97.5 %, indicating that transformation products were also completely degraded. At acidic pH's (pH 6.5) glyphosate was 80 % removed, with a build-up of AMPA, which didn't appear to be degraded under these conditions.

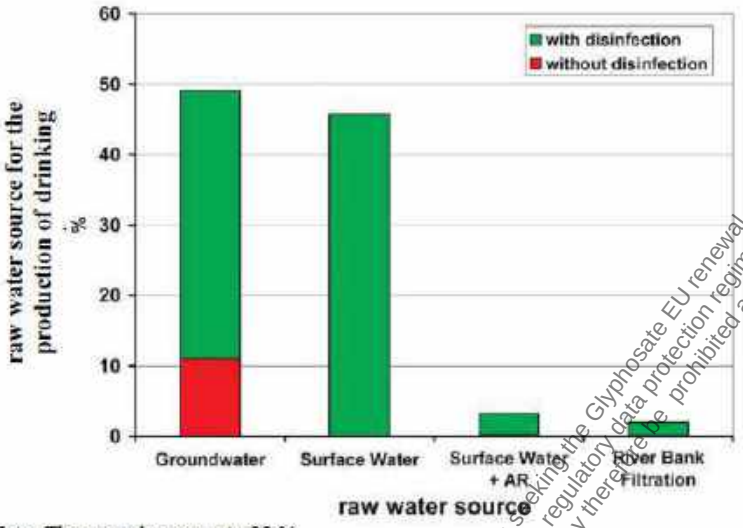
A thorough investigation of the process of ozonation of glyphosate was reported in (Chen, 2011), using batch, semi-continuous tests. It was found that with an initial glyphosate concentration of 5 mg/L, and an ozone concentration of 1.5 mg/L, glyphosate was completely degraded (LOD 0.1 mg/L) within 25 minutes. With an initial pH of 4.9, an initial glyphosate concentration was reduced to <LOD within 25 minutes, and at pH 6.8, was reduced to <LOD within 20 minutes. At a pH of 9.3, the time required to reduce glyphosate to <LOD was 15 minutes. It was demonstrated that as glyphosate was degraded by the oxidation reactions, the amount of AMPA increased, and then AMPA also decreased, and phosphate gradually increased. Indeed, the TOC (total organic carbon) content was degraded by 77.65 % after 30 minutes (when glyphosate had been reduced to <LOD), and further reduced to 93.53 % after 60 minutes of reaction time. Investigation of the presence of intermediates allowed glycolic acid, glycine, phosphoric acid and AMPA to be identified. Under the conditions investigated, it was clear that degradation of glyphosate when subjected to ozonation was rapidly degraded first to a range of intermediates which were in turn subsequently completely degraded.

Partial information on the route of degradation of glyphosate and AMPA, when subjected to ozonation, comes from Klinger *et al.* (2008, CA 7.5/096). The ozonation of a phosphonate complexation agent was investigated, and it was found that this produced glyphosate and AMPA. Consequently, ozonation studies were also conducted on glyphosate and AMPA – at acidic pH (pH 5) it was found that glyphosate was partially degraded to AMPA and orthophosphate; and that AMPA was partially degraded to orthophosphate, under the experimental conditions. An investigation was reported of the removal rates associated with various stages found across seven Waste Water Treatment Plants, including one ozone treatment module (Ruel *et al.*, 2011, CA 7.5/087). For this ozone treatment module, glyphosate was found to have a removal rate of >70 %, whereas for AMPA the removal rate was <30 %. Investigations into the reactivity of glyphosate and AMPA when subjected to ozonation was also carried out at pilot-scale (Jönsson *et al.*, 2013, CA 7.5/084). These studies found that a 15-minute treatment period was enough to result in removal rates of >99 % for both glyphosate and AMPA under the experimental conditions.

Of less importance, from a water treatment perspective (due to rare implementation of the process) is the degradation of glyphosate in water by UV/H_2O_2. One investigation used a high concentration of glyphosate (50 mg/L) to look at the removal of glyphosate from water following the washing out of product containers in Argentina (Manassero *et al.,* 2010, CA 7.5/093). Due to the high concentration of glyphosate used it was possible to identify the compounds formed during the process. It was found that AMPA was not formed from glyphosate under the test conditions, as carbon-phosphate bond cleavage was the first step of the degradation, and after the oxidative removal of one carbon unit, glycine was formed. Glycine is a naturally occurring amino acid, and under the experimental conditions it went on to generate methanediol, formic acid, nitrate anion, ammonium and phosphate anions.

The prevalence across the EU of the treatment processes referred to above, can be inferred from a publication (van der Hoek *et al*., 2014, CA 7.5/098). This paper was the result of a survey carried out amongst the members of the European Federation of National Associations of Water and Wastewater Services. This organisation covered 23 EU MSs and 405 million European citizens, in 2014. Figure 7.5-193 shows that the vast majority of raw water sources for drinking water production (88 %) are subject to disinfection.

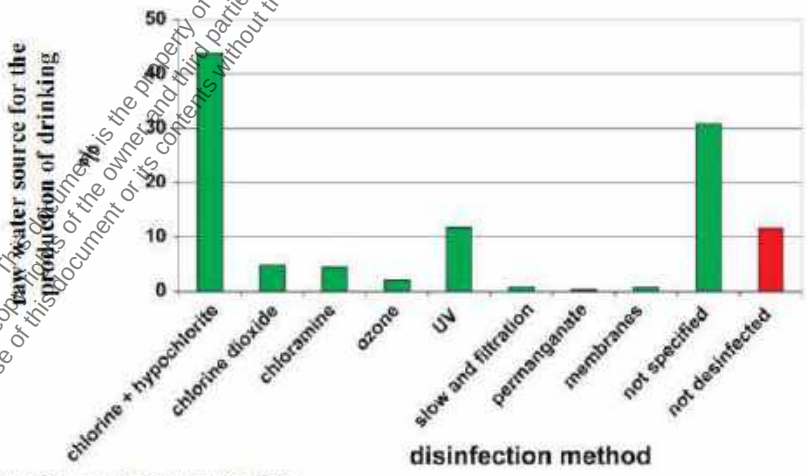
Figure 7.5-193: Raw water sources for drinking water production in Europe (from van der Hoek *et al.*, 2014)



Note: The green bars sum to 88 %

The paper reports that almost all the raw water taken from surface water is subject to disinfection (99.9 %). For bank filtration and artificial recharge (AR), the values are 90.1 % and 92.2 %, respectively. Figure 7.5-194 summarises the disinfection method employed – where surface water is disinfected, the paper reports that chlorine disinfection is applied to 62 % (39 % is 'not specified', but it is very likely that as disinfection by chlorine is by far the most employed method, a significant portion of the 'not specified' is also likely to be chlorine based; hence, 62 % should be considered a conservative minimum value.)

Figure 7.5-194: Raw water sources and treatment scheme (from van der Hoek *et al.*, 2014, CA 7.5/098)



Note: The green bars sum to 88 %

Chemical Water Treatment Summary

Glyphosate and its metabolites (AMPA and HMPA) are most likely to be exposed to chemical water treatment processes via the treatment of surface waters abstracted for the production of drinking water.

Such raw water is very likely to be subjected to a range of treatment processes, and to be subject to disinfection designed to ensure the subsequent drinking water is microbiologically safe to drink. Glyphosate and AMPA are known to be transformed by the most common disinfection methods, transformation products identified are the same as those formed from glycine and other amino acids under the same conditions. Removal rates for glyphosate and AMPA when subjected to disinfection processes are high as summarised in Table 7.5-232.

Table 7.5-232: Summary of removal rates for glyphosate and AMPA following disinfection processes (*after* Jönsson *et al.*, 2013, CA 7.5/084)

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Chlorination	71 - >99	40 - >95
Chlorine dioxide	17 - 93	>99
Ozonation	60 - >99	25 - 95

Furthermore, drinking water treatment processes are carefully controlled, and the characteristics of a specific source raw water needs to be known – as the water treatment process train needs to be optimised to ensure that quality standards are met at the tap of consumers. Consequently, where glyphosate or AMPA are known to be present in the raw water, the drinking water treatment train can be optimised to ensure removal of these substances below the required threshold values.

<u>*Water Treatment Summary*</u>

For drinking water derived from surface water, there is almost always water treatment processes applied to generate the drinking water. The prevalence across the EU of the chemical treatment processes, can be inferred from a publication (van der Hoek *et al*., 2014, CA 7.5/098). This paper was the result of a survey carried out amongst the members of the European Federation of National Associations of Water and Wastewater Services. This organisation covered 23 EU MS's and 405 million European citizens. The report indicates that the vast majority of raw water sources for drinking water production (88 %) are subject to disinfection.

Further, almost all the raw water taken from surface water is subject to disinfection; and where surface water is disinfected, chlorine disinfection is applied to a minimum of 62 % of the raw water. Glyphosate and AMPA are known to be transformed by the most common disinfection methods. Transformation products appear to be small molecules, often similar or identical to those found from natural sources.

Other chemical treatment processes are often applied (either for disinfection or for the explicit removal of micro-pollutants), and low chemical processes are also very frequently applied. Monitoring data is usually only available for raw water, before any water treatment processes have been applied, but for contextualising monitoring data, the effects of these processes should be included. Removal rates for glyphosate and AMPA, for various water treatment processes are summarised in Table 7.5-233.

Table 7.5-233: Summary of removal rates for glyphosate and AMPA following removal processes

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 - >95	25 - >95
Aluminum coagulant and clarification	15 - 40	20 - 85
Iron coagulant and clarification	40 - 70	20 - 85
Activated carbon adsorption	10 - 90	20 - 70
Chlorination	71 - >99	40 - >95
Chlorine dioxide	17 - 93	>99
Ozonation	60 - >99	25 - 95

In addition to disinfection processes, bank filtration can be an effective process for removal of glyphosate and AMPA from water, when sufficient residence time within soil/sediment occurs to allow the normal aerobic/anaerobic soil degradation processes to progress to their full extent (total mineralisation). Generally, drinking water treatment processes are carefully controlled, and the characteristics of a specific source raw water needs to be known – as the water treatment process train needs to be optimised to ensure that quality standards are met at the tap of consumers. Consequently, where glyphosate or AMPA are known to be present in the raw water, the drinking water treatment train can be optimised, where necessary, to ensure removal of these substances below the required threshold values, and therefore, there is a low risk of exceeding the relevant thresholds in drinking water of 0.1 µg/L for glyphosate and 10 µg/L for AMPA, nor for exceeding the life-time health-based ADI concentrations of 1500 µg/L for GLY and 3960 µg/L for AMPA.

III. CONCLUSIONS

For surface water destined to be drinking water, there are almost always water treatment processes applied to remove bacteria and viruses and other organic micro-pollutants. The vast majority (88 %) of raw water sources for drinking water production are subject to disinfection. In particular, almost all (99.9 % by volume) the raw water taken from surface water is subject to disinfection; and where surface water is disinfected, chlorine disinfection is applied to a minimum of 62 % of the raw water. Disinfection and oxidative processes are applied where needed and at predetermined rates for the removal of microbial and organic micro-pollutants, regardless of GLY and AMPA presence. GLY and AMPA are known to be very readily transformed by the most common disinfection methods, ranging from 25 to 95 % for AMPA and 60 to 99 % for GLY. Transformation products are small molecules, often similar or identical to those found from natural sources. Other chemical treatment processes are also often applied as are low chemical processes (processes with either no involvement of chemicals or where the treatment is to occur via physical processes like complexation and adsorption) and bank filtration (infiltration of surface water from a river or lake into a groundwater system, induced by water abstraction close to the surface water). Drinking water treatment processes are carefully controlled and the water treatment process train at any given abstraction site optimised to ensure that quality standards are met at the tap of consumers.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The report describes the analysis of public monitoring data for key European countries for the compartments soil, water and sediment for Glyphosate and AMPA. An assessment of water treatment processes was undertaken through review of published peer reviewed literature. This identified treatment processes and the degree to which they are effective at removing glyphosate (GLY) and AMPA during the water treatment process. These can be used to interpret the groundwater and surface water data within the context of drinking water production.
The report is considered valid.

Assessment and conclusion by RMS:

Existing studies/assessments

1. Information on the study

Data point:	CA 7.5/080
Report author	[redacted]
Report year	2012
Report title	Review of sustainable water treatment
Report No	UC8408v2
Document No	BVL No. 2316001
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

As the European water industry is moving towards 'simple treatments', a review of literature information on the performance of low chemical/energy processes - Bank Filtration (BF), Slow Sand Filtration (SSF) and Biological Activated Carbon (BAC) –for removal of glyphosate and AMPA was conducted. The limited information suggests that BF and SSF can remove glyphosate and AMPA, although the results are inconsistent between studies. No information is available for BAC, but significant removal is not expected through this treatment.

I. MATERIALS AND METHODS

The performance for removal of glyphosate and AMPA by "simple" water treatment process like bank filtration and variations thereof (BF), slow sand filtration (SSF) and biological activated carbon (BAC) was investigated based on literature review. The use of bank filtration is relatively limited in Europe, with less than 50 sites specifically designed to utilize this technique. Slow sand filtration is more common in Europe where it has been installed at several hundred treatment works. Biological activated carbon is the most common technique of the three; possibly because it is the easiest to retrofit.

II. RESULTS AND DISCUSSION

The results of the literature review for removal of glyphosate and AMPA by bank filtration, slow sand filtration and related processes are summarized in the table below. No information was found for biological activated carbon.

Table 7.5-234: Overview on different treatments and results

Compound	Redox conditions	Process	C_0 (µg/L)	Residence time (days)	Removal (%)	Reference
Glyphosate	Anaerobic	BF	0.07	30-300	>30	[redacted] 2000
Glyphosate	Anaerobic	BF	0.12	Unknown	17	[redacted] 2000
Glyphosate	Aerobic & anaerobic	BF and SSF	<0.05 - 0.09	Unknown	~50	[redacted] 2005
Glyphosate	Aerobic	SSF	<0.05 - 0.19	Unknown	>75	[redacted] 2005
Glyphosate	Aerobic	Soil column	10	25	>95	[redacted] 2000
Glyphosate	Aerobic	Batch river water	150000	72	40-72	[redacted] 1993
Glyphosate	Aerobic	Batch soil samples	100 µg/g	50	95	[redacted] 2004
Glyphosate	Initially aerobic	Batch river water	100	56	54 - 89	[redacted] 1994
Glyphosate	Initially aerobic	BF	3.5, 11.6	Half life 7-10 days	80 [1]	[redacted] 2009
AMPA	Anaerobic	BF	0.46	30-300	46 - 87	[redacted] 2000
AMPA	Anaerobic	BF	0.54	450-2000	85 - 94	[redacted] 2004
AMPA	Anaerobic	BF	1.8	Unknown	90	[redacted] 2000
AMPA	Aerobic & anaerobic	BF and SSF	0.23 - 1.1	Unknown	≤95	[redacted] 2005
AMPA	Aerobic	SSF	0.08 - 0.7	Unknown	>89	[redacted] 2005
AMPA	Aerobic	SSF	0.04 - 0.48	Unknown	≤94	[redacted] 1995

BF=Bank Filtration, SSF=Slow Sand Filter, C_0=initial concentration
[1] 80 % removal under test conditions, but removal to <0.1 µg/l identified from modelling for high initial concentrations with half life shown

This table shows that BF and SSF can remove glyphosate and AMPA. The general trend seems to be that the concentration of AMPA is higher than glyphosate but that AMPA is more readily degraded or removed. The degradation of glyphosate seems to benefit from aerobic conditions whereas AMPA is readily degraded both under aerobic and anaerobic conditions.

[redacted] (2009) studied the removal of glyphosate from surface water using a variety of methods; adsorption experiments, degradation experiments, leaching experiments, enclosure experiments, and lysimeter experiments. Overall, the results from the tests carried out confirm that bank filtration should be effective for removal of glyphosate through the range of mechanism investigated.

III. CONCLUSIONS

Glyphosate and AMPA can be removed by sustainable water treatments like BF and SSF. Although no information is available for BAC, this treatment is not expected to effectively remove glyphosate and AMPA from raw water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
A literature review is summarised on removal of glyphosate and AMPA by "simple" water treatment process like bank filtration (BF), slow sand filtration (SSF) and biological activated carbon (BAC). As there is no guideline on assessment of effects of water treatment procedures available, compliance cannot be assessed. Overall, results are sufficiently described.
The report was considered valid to address the data requirement.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/081
Report author	[redacted]
Report year	2010
Report title	Removal of glyphosate and AMPA by water treatment
Report No	UC8154v2
Document No	BVL No. 2316003
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The first part of this study reports the performance of commonly used water treatment processes for the removal of glyphosate and AMPA from raw water during drinking water production. The results show that two of the most common oxidants used in water treatment, ozone and chlorine, can provide a high degree of removal (>95 %) for glyphosate and AMPA under typical conditions used in water treatment. The majority of water treatment works use one (mainly chlorine) or both of these oxidants. The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon (GAC). However, this does not provide an effective barrier to glyphosate or AMPA. Other processes commonly used in water treatment (bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration) would each contribute some removal, but alone would not provide a secure barrier in relation to meeting a 0.1 µg/L standard.

The second part of this study assessed the removal of glyphosate and AMPA by a number of treatment processes in laboratory trials using oxidation and activated charcoal, as well as combinations of ozone, high dose ultraviolet (UV) and hydrogen peroxide in advanced oxidation pilot plant tests. Ozone (O_3) and ozone plus hydrogen peroxide (H_2O_2) are highly efficient in removing glyphosate and AMPA and better than 99 % removal was seen for all conditions tested. Chlorine (Cl_2) was similarly efficient at higher temperature but removal decreased with decreasing temperature to about 70 % at 5°C for glyphosate (but remained >99 % for AMPA). The removal of glyphosate by chlorine dioxide (ClO_2) was not as efficient and more variable, 17-93 % removed, whilst complete removal was achieved for AMPA under these conditions. PAC was the least efficient treatment for glyphosate & AMPA removal, with removals in the range 0-30 %.

Advanced oxidation pilot plant tests with combinations of UV, ozone and hydrogen peroxide confirmed the result of the batch tests with ozone and ozone/peroxide. However, advanced oxidation using UV alone, or UV with peroxide, was less effective for glyphosate removal than ozonation based treatment, particularly with respect to AMPA formation and removal.

I. MATERIALS AND METHODS

The first part of the study was based on a literature review.

In the second part, laboratory batch tests were carried out to investigate the removal of glyphosate and AMPA by oxidation using O_3 alone or in combination with hydrogen peroxide (H_2O_2), Cl_2 and ClO_2, and by adsorption using PAC. In addition, pilot plant tests were conducted on advanced oxidation processes (AOP) to investigate the removal of glyphosate and AMPA by UV radiation and H_2O_2.

The stock solutions of glyphosate and AMPA were prepared by dissolving high purity solids in deionised water. For the AMPA tests using PAC and for all glyphosate tests, a 10 litre sample of Swindon tap water was spiked with 3 µg/L of either glyphosate or AMPA. Samples of the spiked water were taken for analysis to establish the initial concentration of pesticides, and the remainder of the spiked water was used in the tests. This concentration was agreed as the maximum concentration likely to be found in raw waters.

Ozonation alone: A one litre sub-sample of spiked water was ozonated using a pilot-scale O_3 generator and a bubble diffuser stone. Following ozonation for 10 s, the O_3 residual was measured immediately, and at 5 minute intervals, during a 15 minute contact time. At the end of the contact period, the residual ozone was quenched with sodium thiosulphate ($Na_2S_2O_3$).

Ozonation with hydrogen peroxide: A further set of tests were carried out with simultaneous use of O_3 and H_2O_2, at 0.5 and 1.0 mg/L. The ozonation conditions were identical to the test with O_3 alone with the temperature kept constant at 15 ± 0.6 °C. The O_3 residual was measured immediately after ozonation, and then at 5 minute intervals, during a 15 minute contact time. At the end of the contact period, the residual O_3 was quenched with sodium thiosulphate.

Chlorine: One-litre samples of the spiked water were dosed with sodium hypochlorite (NaClO) at 1.5 mg Cl_2/L. The dosed water was left for 30 minutes at the desired temperature. At the end of the contact period, the residual Cl_2 was measured and then quenched with sodium thiosulphate.

Chlorine dioxide: The tests with Cl_2 was repeated but with ClO_2 as the oxidant. The ClO_2 was added as crushed tablets, supplied by Accepta. The initial target concentration of ClO_2 was 1 mg/L.

Powdered Activated Charcoal (PAC): Tests were carried out to investigate the performance of 3 different types of coal based PAC. One litre samples of the spiked water were dosed with the three different PAC at 5, 15, and 25 mg/L. The dosed water was left stirring for 1 hour, to keep the PAC in suspension. The samples were then filtered through GF/C grade filter paper to remove the carbon, prior to analysis.

Advanced oxidation process (AOP) pilot plant test: The AOP pilot rig consisted of in-line hydrogen peroxide dosing, ozone dosing and a UV reactor, which could be used individually or in combination. The retention time in the unit was around 30-60 s, most of which was in the UV reactor. Two tests were performed, each with the same matrix of operating conditions. For the first test, the feed tap water was spiked with glyphosate to the same target concentration as previous tests, 3 µg/L. For the second test, the feed water was spiked with AMPA to a target concentration of 3 µg/L. The matrix of operating conditions was:

UV, dose 740 mJ/cm^2
UV, 1240 mJ/cm^2
UV, 740 mJ/cm^2, + H_2O_2, 5 mg/L
UV, 1240 mJ/cm^2, + H_2O_2, 5 mg/L
O_3, 2 mg/L + H_2O_2, 2 mg/L
O_3, 2 mg/L
O_3, 2 mg/L, with sample left standing for 9 minutes to provide ozone contact time

In the oxidation tests with glyphosate spiking, the treated water samples were also analysed for AMPA, to investigate whether any of the glyphosate was degraded only to AMPA by oxidation.

Workup and analysis:
All samples were analysed for glyphosate and AMPA using the following method. Water samples were treated with fmoc (9-fluorenylmethyl chloroformate) derivatising reagent prior to concentration by solid phase extraction. The extracts are then analysed by high performance liquid chromatography (HPLC) using primary mass spectroscopic (MS) detection in negative ion electrospray with selective ion monitoring. The reported limit of detection (LOD) for the method was 0.006 µg/L for glyphosate and 0.016 µg/L for AMPA.

II. RESULTS AND DISCUSSION

Literature review:
Chlorine, which is one of the most common disinfectants (oxidants) used in water treatment in Europe, can provide a high degree of removal (>95 %) for glyphosate and AMPA under typical conditions used in water treatment. Ozonation, another oxidation process commonly used for pesticide removal, can also provide more than 95 % removal of glyphosate and AMPA. Bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration, commonly used in water treatment, would each contribute some removal, but alone would not provide a secure barrier in relation to meeting a 0.1 µg/L standard. Depending on the treatment processes used, waterworks which include chlorine could deal with between 1 and 4 µg/L (glyphosate + AMPA) in the raw water to maintain less than 0.1 µg/L in the treated water, but if the works also includes ozonation total concentrations of above 30 µg/L could be treated. The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon (GAC). However, this does not provide an effective barrier to glyphosate or AMPA. The results of the literature review are summarized in the table below.

Table 7.5-235: Removal of glyphosate and AMPA by treatment processes

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 to 50	25 to 95
Aluminium coagulant and clarification	15 to 40	20 to 25
	Not a reliable barrier for Glyphosate and AMPA	
Iron coagulant and clarification	40 to 70	20 to 85
	Not a reliable barrier for Glyphosate and AMPA	
Slow sand filtration	Insufficient information but likely to be less effective than bank or dune filtration and therefore of little practical benefit	
Chlorination	74 to >99	40 to >95
	Likely to provide the main barrier to Glyphosate and AMPA at most water treatment works	
Chlorine dioxide	Insufficient information but not expected to be effective	
Ozonation	60 to >99	25 to 95
	Provides an additional barrier at works where already installed for other pesticides and micropollutants	
UV irradiation	No information found. Highly unlikely to be effective alone at doses used in water treatment. May be effective at very high doses not currently used for water treatment.	
UV/hydrogen peroxide	Little direct information available, but indications that a combination of UV with hydrogen peroxide would be effective	
Advanced oxidation	No information found, but would be expected to be effective through free radical mechanisms. Little used for water treatment at the present time.	
Activated carbon adsorption	10 to 90	20 to 70
	Higher removals relate to virgin GAC and are unlikely to be achieved under practical conditions. Not a reliable barrier for Glyphosate and AMPA.	
Membrane filtration	>90 (NF/RO) >50 (UF)* *depending on membrane type	>95 (NF/RO) No information found for UF
	Membrane processes not widely used in water treatment, and unlikely to be installed solely as a barrier to pesticides and other organic micropollutants.	
Air stripping	No information found, not expected to be effective based on chemical characteristics.	

Table 7.5-235: Removal of glyphosate and AMPA by treatment processes

NF = nano filtration
RO = reverse osmosis
UF = ultra filtration

Laboratory batch tests:
Ozone was highly effective in removing both glyphosate and AMPA and virtually complete removal was achieved under all conditions tested. The combination of O_3/H_2O_2 was as effective as O_3 alone in removing glyphosate and complete removal was achieved under all conditions tested. The Cl_2 results indicate that changes in pH had little influence on the removal of glyphosate by chlorine; but that the temperature had a larger influence on the glyphosate removal with 71 % being removed at 5 °C compared to 96 % at 20 °C. The removal of glyphosate by ClO_2 was less effective than that for other oxidants, ranging from 17 % to 93 %. The highest removal was seen for the low pH samples (pH ~6) with high temperature (~22 °C) and high ClO_2 concentrations. However, complete removal of AMPA was seen for all conditions tested, suggesting AMPA is readily removed by ClO_2. Although the results are somewhat scattered, it is clear the investigated PACs would not provide adequate removal of glyphosate and AMPA. The results of the laboratory batch tests are summarized in the table below.

Table 7.5-236: Removal of glyphosate and AMPA during laboratory batch tests

Treatment Process	Glyphosate		AMPA	
	Conditions	Removal (%)	Conditions	Removal (%)
Ozonation	T: ~7, 11, 15 °C Residual O_3: 0.41, 0.76 mg/L Conc.: 2.6 , 2.7 µg/L	>99	T: ~5, 10, 13 °C Residual O_3: 0.5 mg/L Conc.: 3.65 µg/L	>99
Ozonation + hydrogen peroxide	H_2O_2 : 0.5, 1.0 mg/L Residual O_3: 0.09, 0.18, 0.24, 0.46 mg/L Conc.: 2.6, 2.7 µg/L	98 - >99	H_2O_2: 0.5, 1.0 mg/L Residual O_3: 0.16, 0.04 mg/L Conc.: 3.65 µg/L	85 - 97
Chlorine	pH: 6, 7.5, 8.5 T: 5, 10, 20 °C Residual Cl_2: 1.4 mg/L Conc.: 2.17, 3.17 µg/L	71 - >99 (removal decrease with T°)	pH: 6, 7., 8.5 T: 6, 10, 20 °C Residual Cl_2: 1.4 mg/L Conc.: 3.65 µg/L	>99
Chlorine dioxide	pH: 6-8.6 T: 4-23 °C Residual ClO_2: 0.4-1.35 mg/L Conc.: 2.17, 2.47 µg/L	17 - 93 (removal decrease with T°)	pH: 6.2 - 8.4 T: 6, 10, 20 °C Residual Cl_2: 1 - 1.4 mg/L Conc.: 3.65 µg/L	>99
Powdered Activated Charcoal	PAC conc.: 5, 15, 25 mg/L Conc.: 3.13 µg/L	0 - 22	PAC conc.: 5, 15, 25 mg/L Conc.: 3.13 µg/L	0-31

Advanced Oxidation Processes (AOP) pilot plant tests:
UV alone did not remove significant amounts of glyphosate or AMPA even at relatively high doses (1240 mJ/cm^2). UV in conjunction with H_2O_2 showed good removal of glyphosate (approximately 90 %) but significant amounts of AMPA was also generated and AMPA was poorly removed by this treatment (<10 %).

An applied dose of 2 mg/L ozone removed greater than 95 % of the glyphosate, this removal being essentially achieved within 1 minute contact time after the eductor. This indicates a very high rate of reaction with molecular ozone. This is consistent with the previous laboratory tests with ozone, but the earlier laboratory tests showed better removal of AMPA (literature search) by ozone alone. Near complete removal of glyphosate was also seen for the combination of ozone and H_2O_2, >95 % was removed after 1 minute. Again, the removal of AMPA was not as good as in previous tests, but this was probably an effect of the short contact time (1 minute). The results are summarized in the table below.

Table 7.5-237: Removal of glyphosate and AMPA during AOP pilot plant tests

Treatment Process	Glyphosate		AMPA	
	Conditions	Removal (%)	Conditions	Removal (%)
UV (740 mJ/cm²)	1 min contact time Conc.: 1.72 µg/L	25	1 min contact time Conc.: 2.31 µg/L	6
UV (1240 mJ/cm²)	1 min contact time Conc.: 1.72 µg/L	36	1 min contact time Conc.: 2.31 µg/L	32
UV (740 mJ/cm²) H_2O_2 (5 mg/L)	1 min contact time Conc.: 1.72 µg/L Residual H_2O_2: 5.5 mg/L	88	1 min contact time Conc.: 2.31 µg/L Residual H_2O_2: 4.98 mg/L	8
UV (1240 mJ/cm²) H_2O_2 (5 mg/L)	1 min contact time Conc.: 1.72 µg/L Residual H_2O_2: 5.16 mg/L	91	1 min contact time Conc.: 2.31 µg/L Residual H_2O_2: 4.65 mg/L	6
O_3 (2 mg/L) H_2O_2 (2 mg/L)	1 min contact time Conc.: 1.72 µg/L	96 - >99 (duplicates)	1 min contact time Conc.: 2.31 µg/L	35
O_3 (2 mg/L)	1 min contact time Conc.: 1.72 µg/L Residual O_3: 0.83 mg/L	96	1 min contact time Conc.: 2.31 µg/L Residual O_3: 0.90 mg/L	63
O_3 (2 mg/L)	10 min contact time Conc.: 1.72 µg/L Residual O_3: 0.36 mg/L	97	10 min contact time Conc.: 2.31 µg/L Residual O_3:0.52 mg/L	>99

III. CONCLUSIONS

Literature review:

The majority of water treatment works worldwide use chlorine for disinfection, and therefore have an effective barrier for glyphosate and AMPA. Exceptions to this would be works in mainland Europe which use chlorine dioxide for disinfection and protection of the water in distribution, instead of chlorine. In this situation, the removal of glyphosate would be more variable, but complete removal of AMPA (>99 %) could be expected.

The most common water treatment process installed for removal of pesticides worldwide is adsorption using granular activated carbon. This system does not provide an effective barrier to glyphosate and AMPA. However, at many treatment works ozone is also installed for removal of pesticides or other organic micropollutants, and would be highly effective for glyphosate and AMPA removal under the dose and contact time conditions typically used. As expected, UV disinfection processes are not very effective in removing glyphosate and AMPA, but in combination with hydrogen peroxide could provide an efficient barrier for glyphosate (but not AMPA).

Other processes commonly used in water treatment (bankside or dune infiltration, coagulation/ clarification/ filtration and slow sand filtration) would each contribute some removal, but each process in isolation is unlikely to provide a secure barrier in relation to meeting a 0.1 µg/L standard.

Laboratory tests:

Ozone was highly effective in removing both glyphosate and AMPA and virtually complete removal was achieved under all conditions tested. No AMPA was detected in any of the treated samples from the glyphosate tests.

The combination of O_3/H_2O_2 was as effective as O_3 alone in removing glyphosate and complete removal was achieved under all conditions tested. For AMPA breakdown, the hydroxyl radical mechanism is less effective than free ozone.

The results of chlorine treatment indicate that changes in pH had little influence on the removal of glyphosate (96-100 % removal at 20 °C) while the temperature had a larger influence (removal of 71 % at

5°C and 96 % at 20 °C. AMPA concentrations in samples from the glyphosate tests were all non-detectable, confirming the effective degradation of AMPA by chlorine seen in the investigation of the variable concentration of controls.

The removal of glyphosate by ClO_2 was less effective than that for other oxidants, ranging from 17 % to 93 %. The highest removal was seen for the low pH samples (pH ~6) with high temperature (~22 °C) and high ClO_2 concentrations. Low concentrations of AMPA were detected in the glyphosate test samples (1 – 5 % of total glyphosate concentration), suggesting that AMPA was formed as a degradation product when glyphosate was oxidised by ClO_2. However, for AMPA alone, complete removal of AMPA was seen for all conditions tested, suggesting AMPA is readily removed by ClO_2.

PAC was ineffective as a removal treatment for glyphosate, even at the relatively high dose for water treatment of 25 mg/L. No more than 20 % was removed. Removal of AMPA decreases with increasing PAC dose as PAC removes Cl_2 and this stops the degradation of AMPA by Cl_2 present in tap water. Overall, the PACs investigated would not provide adequate removal of glyphosate and AMPA.

Results from AOP tests indicate that advanced oxidation using UV alone, or UV with peroxide, is less effective for glyphosate removal than ozonation-based treatment, particularly with respect to AMPA formation and removal.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In the study, conclusions from a literature review on removal of glyphosate and AMPA by drinking water treatment processes are combined with laboratory experiments on removal efficiency of different treatment procedures. As there is no guideline on assessment of effects of water treatment procedures available, compliance cannot be assessed. Overall, methods and results are sufficiently described. No detailed information is given about the identity and purity of the test items, but this does not have an impact on the results of the study.

The study was considered valid to address the data requirement.

Assessment and conclusion by RMS:

Relevant literature articles

1. Information on the study

Data point:	CA 7.5/082
Report author	Hamann, E. *et al*.
Report year	2016
Report title	The fate of organic micropollutants during long-term/long-distance river bank filtration
Document No	Science of the Total Environment
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

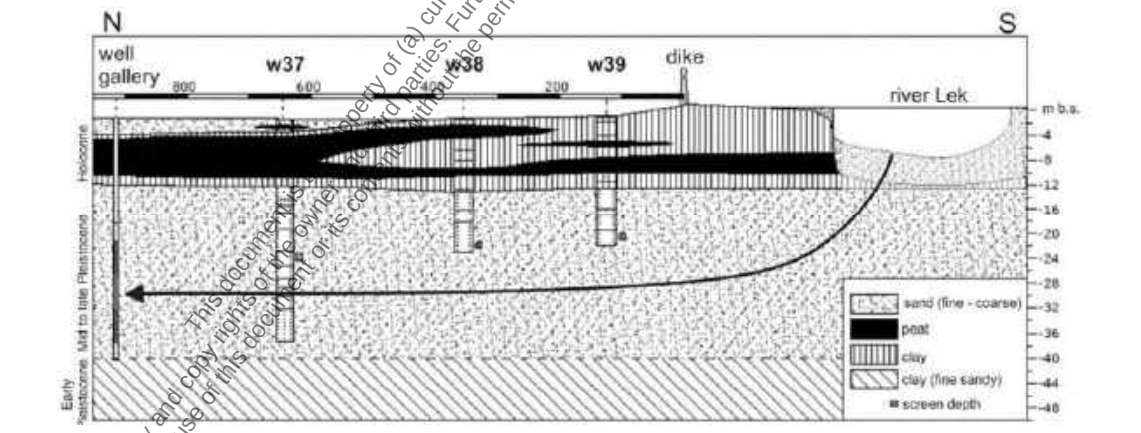
The fate of organic micropollutants during long-term/long-distance river bank filtration (RBF) at a temporal scale of several years was investigated along a row of monitoring wells perpendicular to the Lek River (the Netherlands). Out of 247 compounds, which were irregularly analyzed in the period 1999-2013, including AMPA, only 15 were detected in both the river and river bank observation wells. Out of these, 10 compounds (1,4-dioxan, 1,5-naphthalene disulfonate (1,5-NDS), 2-amino-1,5-NDS, 3-amino-1,5-NDS, AOX, carbamazepine, EDTA, MTBE, toluene and triphenylphosphine oxide) showed fully persistent behavior (showing no concentration decrease at all), even after 3.6 years transit time. The remaining 5 compounds (1,3,5-naphthalene trisulfonate (1,3,5-NTS), 1,3,6-NTS, diglyme, iopamidol, triglyme) were partially removed. Their reactive transport parameters (removal rate constants/half-lives, retardation coefficients) were inferred from numerical modeling. In addition, maximum half-lives for 14 of the fully removed compounds, including AMPA, for which the data availability was sufficient to deduce 100 % removal during sub-surface passage, were approximated based on travel times to the nearest well. The study is one of very few reporting on the long-term field-scale behavior of organic micropollutants. It highlights the efficiency of RBF for water quality improvement as a pre-treatment step for drinking water production. However, it also shows the very persistent behavior of various compounds in groundwater.

Materials and methods

Field Site

The Rodenhuis RBF study site is located on the Lek River, a tributary of the Rhine River in the Netherlands, situated between the cities of Rotterdam and Utrecht. The transect with observation wells w37, w38 and w39 is aligned along the flow direction between the river and the public supply well field at Rodenhuis (Figure 7.5-195), as ascertained by a numerical groundwater flow model (Oasen Drinking Water Company, personal communication).

Figure 7.5-195: Hydrogeological conditions and position of the observation wells along the studied transect (modified from Segers (2006)). The arrow indicates the hypothetical groundwater flow path



Hydrochemical data for the Lek River were taken from the public accessible annual reports of the Rhine River and its tributaries (RIWA, 1999-2013), where monthly analyses of the inorganic and organic compounds are given.

Hydrochemical data from the observation wells was taken from a database provided by the Water Company OASEN. The database includes physico-chemical parameters, major ions, some trace elements and a vast number of organic micropollutants measured from 1999 to 2013. The individual parameters were measured at irregular intervals between 1 and 18 times. Chloride was for example measured 7 times at w37 as compared to toluene which was measured 18 times at the same well.

Altogether, 247 organic substances present above the detection limit in the Lek River were also part of the measurement program at the transect. Out of those, 29 organic micropollutants, including AMPA, were selected for the detailed fate analysis during RBF.

Groundwater flow and reactive transport modeling
Along the transect three 1D models were built, one for each observation well representing the flow path from the river to the well. The use of three separate models was necessary in order to match the measured tracer breakthrough curves during the process of calibration, as insufficient hydrogeological data was available to account for changes in aquifer characteristics over such large distances. The flow and transport simulations were carried out with MODFLOW and MT3DMS, respectively. The extent of the respective models was 370, 606 and 906 m, matching the distance between the river and the observation wells w39, w38 and w37, respectively. The flow conditions were assumed to be steady-state in a homogenous medium. The flow boundaries representing the river and the pumping well were prescribed as 1st and 2nd order boundary conditions, respectively. The latter was adjusted to match the tracer breakthrough curves during calibration. Hydraulic conductivity and effective porosity were chosen according to typical values of medium-grained sands with values of 80 m/d and 0.25, respectively. The resolution of the model grid was 1 m. The temporal discretization was set to monthly time steps according to the availability of measured river data. When data was not available monthly but for longer time intervals, the data was linearly interpolated. The available concentration trends of the conservative tracer chloride in the river and in the observation wells were used to calibrate the flow and non-reactive transport models by adjusting the flow velocities via the production well pumping rates as well as the longitudinal dispersivity.

To simulate the attenuation of the organic micropollutants during RBF, linear adsorption and 1st order degradation were implemented in the models as follows:

$$R\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \lambda C$$

with R [-], retardation coefficient; C [M/L^3], aqueous concentration of a solute; x [L], spatial dimension in flow direction; t [T], time; v [L/T], pore velocity; D [L^2/T], longitudinal dispersion coefficient defined as $D = v\alpha_L$, with α_L [L], longitudinal dipersivity; and λ [T^{-1}], first-order degradation rate constant. The often used degradation half-life is defined as:

$$t_{1/2} = \frac{\ln 2}{\lambda}$$

Retardation by linear adsorption was considered in the model using the conventional linear distribution coefficient K_d [L^3/M]:

$$R = 1 + \rho_b \frac{K_d}{\theta}$$

with ρ_b [M/L^3], bulk density; and θ [-], total porosity. When included, retardation by sorption was assumed to act equally throughout the whole model domain.

First order degradation rate constants (λ's) were likewise uniformly prescribed to the whole model domain, independent of groundwater redox conditions or temperatures. Calibration was carried out by automatically estimating λ for the partially removed and persistent compounds with the model independent parameter estimation tool PEST and manually adjusting K_d to obtain the best fit between measured and modeled data. PEST generated 95 % confidence intervals were provided to inform about the uncertainty of the estimated values of λ.

In cases where the observed concentration time series were not suitable to infer delayed transport by retardation, sorption properties based on the quantitative structure-activity relationship (QSAR) were used for interpretation. For that purpose and due to the fact that some of the investigated compounds will form ions at the prevalent pH conditions at the site, the pH-dependent octanol-water partition coefficient log D_{ow} at pH 7 to 8.5 was used.

Results

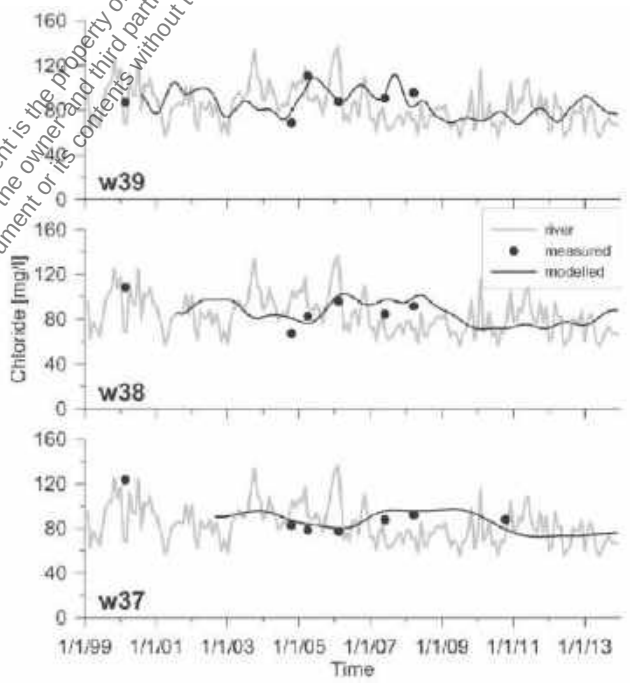
The resulting flow velocities were identical in the models w37 and w38, but slightly lower in model w39 (Table 7.5-238). Increasing dispersivity with increasing distance to the river reflects the scale dependence of dispersion. One limitation for calibration was the frequency of analysis of chloride in the groundwater. While high resolution chloride data was available for the river, chloride was only available for six times in 14 years in groundwater. In our modeling study, more chloride measurements were available for w37, further improving the calibration.

Table 7.5-238: Flow and transport parameters of the calibrated models

Observation well	Distance to river [m]	Flow velocity [m/year]	Travel time [years]	α_L [m]
w39	370	224	1.65	2
w38	606	248	2.44	5
w37	906	248	3.65	10

Following the calibration of the flow and non-reactive transport model, the physical parameters were left unchanged in the reactive simulations. According to their appearance in the observation wells as compared to the river, the compounds were classified into fully removed (no detection in the groundwater observation wells), partially removed (compounds detected in the bank filtrate in decreased concentrations as compared to the river) and fully persistent (no indication for removal at all, even after 3.65 years of sub-surface residence time). Chloride shows conservative behavior as expected (Figure 7.5-196).

Figure 7.5-196: Times series of chloride in the river (measured) and in the observation wells (measured and modelled)



Fully removed compounds

Out of the 29 compounds selected for modeling, 14 substances were non-detectable in the bank filtrate, namely 2-naphthalene sulfonate (2-NS), 2,6-NDS, amidotrizoic acid, AMPA, aniline, bezafibrate, diclofenac, ibuprofen, iohexol, iomeprol, iopromide, ioxitalamic acid, metoprolol and sulfamethoxazol. For these compounds, 1st order degradation rate constants were calculated based on the travel time between river and w39, the mean input concentration at the river and the detection limit at w39 as the maximum possible residual concentration after degradation, assuming that complete degradation takes place somewhere between river and w39. Employing the detection limits and the estimated travel time to w39, the calculated rate constants have to be regarded as minimum values (or rather the half-lives as maximum values). Furthermore, possible retardation was neglected in this calculation. Therefore the degradation rates may be overestimated for retarding substances. To determine the tendency of substances to retard, log D_{ow} values were used. Substances with high log D_{OW}'s are likely to sorb, i.e., aniline, bezafibrate, diclofenac and ibuprofen. Other substances with very low log D_{OW}'s, i.e., amidotrizic acid, AMPA, 2-NS, 2,6-NDS, iohexol, iomeprol, iopromide, ioxatalamic acid, metoprolol and sulfamethoxazol, have more likely been subject to degradation only.

Partially Removed Compounds

The concentrations of triglyme, iopamidol, 1,3,5-naphthalene trisulfonate (1,3,5-NTS) and 1,3,6-naphthalene trisulfonate (1,3,6-NTS) clearly decrease during the RBF. Assuming that no retardation of the mentioned substances takes place based on their low log D_{OW}'s, non-reactive model simulations to a great extent overestimate the measured concentrations at the transect.

Persistent Compounds

Some compounds (MTBE, carbamazepine, AOX, triphenylphosphineoxide (TPPO), toluene, EDTA, 1,5-naphthalene-disulfonate (1,5-NDS), 2-amino-1,5-naphthalene disulfonate (2-amino-1,5-NDS), 3-amino-1,5-naphthalene disulfonate (3-amino-1,5-NDS) and 1,4-dioxan) were present in river and bank filtrate in similar concentrations and the model simulations achieved a best fit when assuming non-reactive and non-sorptive behavior, suggesting persistence over long periods in the sub-surface. Thereby, non-reactive behavior was assumed when the PEST estimated half-life was $t_{1/2}$ >10 years.

Table 7.5-239: Investigated organic micropollutants classified according to their removability at the RBF site in fully removed, partially removed and persistent substances

Fully removed organic micropollutants
(Considered removal processes are degradation and retardation. 1st order degradation rate constants and half-lives of substances with high affinity to adsorb (log D_{OC} > 4) are written in parentheses.)

Substance (CAS nr.)	Application	log $D_{ow}^{(1)}$ at pH 7.0–8.5[2]	$R_{current\_study}$ [−]	$R_{literature}$ [−]	$k_{current\_study}^{(4)}$ [year^{-1}]	$t_{1/2\_current\_study}^{(4)}$ [d]	$t_{1/2\_literature}$ [d] only field studies
2-Naphthalene sulfonate (532-02-5)	Industry	−0.23	1	–	>0.54	<469	Low[w]
2,6-Naphthalene disulfonate (581-75-9)	Industry	−3.43	1	–	>0.37	<683	Low[w]
Amidotrizoic acid (117-96-4)	X-ray contrast agent	−0.62 to −0.64	1	1[p]	>1.95	<130	25–∞[p]
Aminomethyl-phosphonic acid (AMPA) (1066-51-9)	Main metabolite of Glyphosat	−3.16 to −4.33	1	>1[p]	>1.96	<129	37 ± 11[m], 50–300[w]
Aniline (62-53-3)		1.14	1		(>0.33)	(<776)	
Bezafibrate (41859-67-0)	Pharmaceutical	0.97 to 0.49	1		(>0.26)	(<969)	1–2.1[a]
Diclofenac (15307-86-5)	Pharmaceutical	1.37 to 0.77	1	1[(a),(c),(e)], 1.4[h], 4.8[h], 1.4[k], 0.84–1.31[d], >26[f]	(>0.91)	(<280)	1–1.8[a], 36[f], 0.3–∞[a], 3[o]
Ibuprofen (15687-27-1)	Pharmaceutical	1.71 to 0.56	1	1[(a),(c),(e)], 1.1[f], 3[o]	(>0.4)	(<638)	0.7–1.2[a], 1.9[g]
Iohexol 66108-95-0)	X-ray contrast	−1.95	1	1[(a),(e)], 2.6[h]	>1.34	<189	1.4–2.5[a], <7[p]
Iomeprol (48649-41-9)	X-ray contrast	−1.45	1	1[(a),(e)]	>2.16	<117	1.5–2[a], <6[a], 108[ab]
Iopromide (73334-07-3)	X-ray contrast	−0.44 to −0.45	1	1[(a),(e)]	>1.82	<139	1.3–2[a], 0.1[g], 1.5–3[o], <7[p]
Ioxitalamic acid (28179-44-4)	X-ray contrast	−1.47 to −1.49	1	–	>0.72	<353	
Metoprolol (51384-51-1)	Beta blocker	−0.81 to 0.57	1	>9[g], 4.3–10.8[k]	>0.95	<208	
Sulfamethoxa-zol (723-46-6)	Antibiotic	0.14 to −0.14	1	1[(a),(c),(f),(ac)]	>0.58	<433	∞[a], persistent[ba], 22[ff], 30[o], 30–∞[o], 5.3[q]

Partially removed organic micropollutants
(Contemplable removal processes are degradation and retardation)

Substance (CAS nr.)	Application	Log $D_{OW}^{(1)}$ at pH 7.0–8.5[2]	$R_{current\_study}$ [−]	$R_{literature}$ [−]	$k_{current\_study}^{(3)}$ [year^{-1}]	$t_{1/2\_current\_study}^{(3)}$ [d]	$t_{1/2\_literature}$ [d] only field studies
1,1,5-Naphthalene trisulfonate (6654-64-4)	Industry	−6.62	–	–	0.44[3] (0.15–0.54)[5]	569[3] (470–722)[5]	Poorly removable[b][a]
1,3,6-Naphthalene trisulfonate (86-66-8)	Industry	−6.62	–	–	0.24[3] (0.07–0.42)[5]	1044[3] (605–3800)[5]	Removable[c]
Diglyme (111-96-6)	Industrial solvent	0.03	1	Not likely[ae]	0.34[3] (0.26–0.42)[5]	740[3] (598–971)[5]	∞[p]
Iopamidol (60166-93-0)	X-ray contrast agent	−0.74	1	1[(a),(e)]	>1.28[3] (1.15–1.42)[5]	<197[3] (176–221)[5]	1.8–3.5[a], 25–85, 140–∞[a], ≈15[p]
Triglyme (112-49-2)	Industrial solvent	−0.02	1	Not likely[ae]	>0.86[3] (0.69–1.02)[5]	<296[3] (248–367)[5]	Persistent[d]

Persistent organic micropollutants

Substance (CAS nr.)	Application	Log $D_{OW}^{(1)}$ at pH 7.0–8.5[2]	$R_{current\_study}$ [−]	$R_{literature}$ [−]	$k_{current\_study}$ [year^{-1}]	$t_{1/2\_current\_study}$ [d]	$t_{1/2\_literature}$ [d] only field studies
1,4-Dioxan (123-91-1)	Industrial solvent	−1.06	–	1[(b)]	0	∞	Persistent[(b),(aa)]
1,5-Naphthalene disulfonate (81-04-9)	Chemical industry	−3.68	–	1[(f)]	0.004[3] (−0.03–0.07)[5]	63,250[3]	Persistent[(b),(w)], 309[f]
2-Amino-1,5-naphthalene disulfonate (117-62-4)	Industry	−3.26	–	–	0.04[3] (−0.1–0.19)[5]	6325[3]	Persistent[(w)]
3-Amino-1,5-naphthalene disulfonate (131-27-1)	Industry	−4.26	–	–	0	∞	–
AOX			–	1[(f),(ad)]	0.02[3] (−0.03–0.07)[5]	12,050[3]	286[f], <∞[k]
Carbamazepine (298-46-4)	Anticonvulsant	2.77	2.5[g]	≥1[(a),(c),(e),(g),(h),(k),(af),(ag)], 1.84[k], 13[h], 3.8–5.3[v], 1.04–1.16[w], 1.7[f], 2.2[g]	−0.02[5] (−0.08–0.06)[5]	∞[3]	3.5–7[a], persistent[ba], 66[f], 14–∞[k], 35[q], >7300[p]
EDTA (60-00-4)	Chelating agent	−14.2 to −15.51	–	1[(f)]	−0.02[3] (−0.08–0.05)[5]	∞[3]	∞[f],[(g)], ≤∞[k]
MTBE (1634-04-4)	Fuel component, industrial solvent	1.18	1[g]	1[(f),(ah)], 1.05[ai]	−0.02[3] (−0.16–0.04)[5]	∞[3]	82[f]
Toluene (108-88-3)	Industrial feedstock, solvent	2.49	–	2.1[ae]	0.06[3] (−0.11–0.24)[5]	4216[3]	<700[c] (review study)
Triphenylphosphine oxide (791-28-6)	Industrial byproduct	4.76	–	–	0	∞	

Note: footnotes are available in the original article.

Conclusion

In this study, the behavior of a large number of organic micropollutants during river bank filtration was evaluated on the basis of measurements in the period 1999-2013 on a row of monitoring wells with travel times of 1.6-3.6 years. Field studies reporting on such long distance and long-term behavior are rare. Most quantitative information has so far been inferred from laboratory studies or from field-studies with considerably shorter residence times. While the sampling frequency was very high in the river, fewer data were available for the observation wells, somewhat limiting the approach with regard to detailed process understanding. The information whether or not a compound is fully, partially or not at all removed is nevertheless of great value and compounds were classified accordingly.

Overall, only 15 of the 247 compounds detected in the river and analyzed for in the bank filtrate were detected in the bank filtrate. Out of those, 10 were fully persistent (1,4-dioxan, 1,5-NDS, 2-amino-1,5-NDS, 3-amino-1,5-NDS, AOX, carbamazepine, EDTA, MTBE, toluene and TPPO) and 5 only partially removed (1,3,5-NTS, 1,3,6-NTS, diglyme, iopamidol, triglyme).

For compounds detected in the river but not in the observation wells of the transect, including AMPA, at least minimum degradation rate constants were inferred. Many previous studies used the decrease of the concentration of a substance along a flow path time-independently. The long-term time series in this data-set shows how sometimes temporal changes in the river and the time-shift caused by the groundwater travel time can lead to misinterpretations. Instead, numerical model-based interpretations of time-series, which take these variabilities into account are far more suitable to quantify reactive transport parameters such as degradation rate constants.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes a modelling approach to describe long-term/long-distance river bank filtration for 29 compounds including AMPA. There are no new experimental data generated but the modeling approach gives relevant and reliable information on the behavior of AMPA at drinking water abstraction points.

The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/083
Report author	Hedegaard, M., Albrechtsen, H.
Report year	2014
Report title	Microbial pesticide removal in rapid sand filters for drinking water treatment - Potential and kinetics
Document No	Water Research 48 (2014) 71-81
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Filter sand samples, taken from aerobic rapid sand filters used for treating groundwater at three Danish waterworks, were investigated for their pesticide removal potential and to assess the kinetics of the removal process. Microcosms were set up with filter sand, treated water, and the pesticides or metabolites mecoprop (MCPP), bentazone, glyphosate and p-nitrophenol were applied in initial concentrations of 0.03-2.4 µg/L. In all the investigated waterworks the concentration of pesticides in the water decreased – MCPP decreased to 42-85 %, bentazone to 15-35 %, glyphosate to 7-14 % and p-nitrophenol 1-3 % – from the initial concentration over a period of 6-13 days. Mineralisation of three out of four investigated pesticides was observed at Sjælsø waterworks Plant II – up to 43 % of the initial glyphosate was mineralised within six days.

Materials and methods

Degradation potential of filter sand
Filter sand from three Danish waterworks - Islevbro, Sjælsø Plant I and Sjælsø Plant II - was investigated for the removal potential of the pesticides mecoprop (MCPP), bentazone, glyphosate, and the degradation product p-nitrophenol.

The investigations included filter sand from three different groundwater-based waterworks. Selected parameters for water quality can be seen in Table 7.5-240. In order to investigate the potential of filter sand to degrade pesticides, four ^{14}C-labelled pesticides (mecoprop, bentazone, glyphosate and p-nitrophenol) were selected. To investigate pesticide removal at concentrations close to water quality guidelines, pesticides were in general added to an initial concentration of 0.1 µg/L.

Table 7.5-240: Water quality data based on information from the waterworks. The range is given for each parameter for the given time period for wells and the effluent water from the filters. The waterworks monitors for more than 20 pesticides and degradation compounds, but this table only includes detected pesticides

		Islevbro		Sjælsø Plant I		Sjælsø Plant II	
Dry filter sand							
TOC[a]	mg/g	2.42		78.8		0.517	
		Wells 2010–2011	Effluent water 2011–2012	Wells 2011–2012	Effluent water 2012	Wells 2011–2012	Effluent water 2012
Water							
Oxygen	mg/L	0.21–2.2	7.3	0.31–0.62	–	0.25–0.44	–
Nitrate	mg/L	0.018–3.7	2.57	0.043–0.378	–	<0.03–0.061	–
Nitrite	mg/L	0.005–0.017	0.06	<0.0016–0.012	<0.0016–0.0079	<0.0016	<0.0016–0.0077
Ammonium	mg/L	0.25–0.97	0.10	0.38–0.77	<0.004–0.01	0.92–1.26	<0.004–0.016
Manganese	mg/L	0.026–0.1	–	0.005–0.22	<0.001–0.003	0.012–0.054	<0.001
Iron	mg/L	0.01–3.6	0.3	0.2–4.3	<0.01–0.02	0.38–2.6	<0.01–0.05
Sulphate	mg/L	67–160	33.1	9–58	–	<0.5–14	–
Hydrogen sulphide	mg/L	0.012–0.02	–	0.01–0.04	–	0.03–1.19	–
Methane	mg/L	0.01–0.13	–	0.06–0.5	–	1.13–9.2	–
BAM[b]	µg/L	0.014–0.076	–	<0.01–0.018	–	<0.01	–
4-CPP[b]	µg/L	0.016	–	<0.01–0.11	–	<0.01	–
DCPP[b]	µg/L	0.02–0.20	–	<0.01–0.014	–	<0.01	–
2,6-DCPP[b]	µg/L	–	–	<0.01–0.025	–	<0.01	–
MCPP[b]	µg/L	0.04–0.13	–	–	–	–	–
Glyphosate	µg/L	0.022	–	–	–	–	–
Phenol	mg/L	–	–	–	–	<0.05	–
NVOC[a]	mg/L	2.33	2.41	2.2–3.5	1.7–3.9	2.1–3.9	2.7–4.5
Conductivity at 12 °C	mS/m	–	–	55–108	–	66–82	–
Alkalinity	meq/L	5.12–7.18	5.4	–	–	–	–
pH		7–7.9	–	–	–	–	–

– no data available.
[a] TOC = Total organic carbon; NVOC = Non-volatile organic carbon.
[b] BAM = 2,6-Dichlorobenzamide; 4-CPP = (RS)-2-(4-chlorophenoxy)propionic acid; DCPP = (R)-2-(2,4-dichlorphenoxy)propionic acid; 2,6-DCPP = 2-(2,6-dichlorphenoxy)propionic acid; MCPP = (RS)-2-(4-Chloro-2-methylphenoxy)propanoic acid;

Water was collected from the inlet connecting to the clean water tanks. Filter sand was collected from the top 20 cm of the filter bed with a specially designed aluminum bucket on an extendable shaft, which was disinfected with 1 % hypochlorite. The filter sand was transported to the laboratory in an autoclaved plastic bag inside a clean bucket.

Within 2 h of collecting water and filter sand at the waterworks, 250 g wet filter material was transferred with a sterilized spoon to 300 mL serum bottles, which had been acid washed and heated to 555°C for 12 h. A total water volume of 100 mL was added, including volumes of dissolved chemicals.

Abiotic controls were set up with filter sand, which was either autoclaved three times (20 min, 1 bar and 121°C, the microcosms cooled for approx. 30 min - to less than 80 °C - before autoclaving was repeated) or was mixed with sodium azide to a concentration of 2 g/L to inhibit all microorganisms.

Microcosms were closed with Teflon caps and aluminum lids, and they were left at 10 °C in darkness overnight before sampling. Incubation conditions were static. The pH remained at 7 during the experiment and the oxygen concentration was measured before and after the experiment with an HACH HQ40d oxygen electrode.

Sampling was frequent in the initial stages of the experiments and lasted for one to six hours. In the second phase the removal potential of the filter sand was investigated, and sampling was less frequent and lasted for 2-13 days after the experiment started.

The microcosms were spiked with dissolved [^{14}C-]pesticide to a concentration of 0.03-2.4 µg/L (Table 7.5-241). When sampling, 3 mL atmospheric air was added to the microcosms and the 2-3 mL water samples were collected with a syringe through the cap of the microcosms. A 0.25 mm hydrophilic PTFE-filter was used to remove suspended matter from the water sample. The analysis for ^{14}C was based on a double vial system, whereby $^{14}CO_2$ produced in the collected water sample was stripped off and captured by a base trap (1 mL 2M NaOH). Thus, the produced $^{14}CO_2$ and the ^{14}C-activity of the pesticide in the water phase could be quantified.

Table 7.5-241: Initial conditions in the microcosms in the different experiments. Added amount of filter sand and water appear as well as the initial concentration of the added pesticides

Waterworks	Potential of filter sand			Removal kinetics	Effect of oxygen
	Jelevåro	Sjælsø Plant I	Sjælsø Plant II	Sjælsø Plant II	Sjælsø Plant II
Filter sand (g)	250	250	250	250	100
Water (mL)	100	100	100	100	100
Initial concentration					
Mecoprop (µg/L)	0.38	0.04	0.03	–	–
Glyphosate (µg/L)	–	0.05	0.05	–	–
p-Nitrophenol (µg/L)	–	0.16	0.16	–	–
[Carbonyl-^{14}C-]Bentazone (µg/L)	0.1	0.1	0.1	0.1/0.5/2.4	0.6
[Benzene-ring-U-^{14}C]Benzidine (µg/L)	–	–	–	0.16	–

– no data.

Due to frequent sampling in the first 1-6 h, experiments were processed at an ambient temperature (20 °C). After this period, the microcosms were incubated at 10 °C in darkness.

The water content of the filter material was quantified through weight loss after 24 h at 105 °C. The bulk density of the dry filter sand was found by weighing 40 mL, without compressing the filter sand. The amount of total organic carbon (TOC) in the filter sands was measured for the sample. The TOC analysis was carried out by employing a total element carbon analyser (LECO Induction Furnace CS-200) after the removal of carbonates by adding 5 % sulphurous acid (H_2SO_3).

Results

Degradation potential of filter sand

All of the investigated rapid sand filters removed the investigated pesticides partially, either by abiotic or microbial processes (Table 7.5-242), and concentrations in the microcosms decreased during the experiment between 6 and 13 days. MCPP decreased to 42-85 %, bentazone to 15-35 %, glyphosate to 7-14 % and p-nitrophenol to 1-3 % of the initial concentration. Due to the position of the ^{14}C-label in glyphosate only a complete removal of the compound would be detected - partial degradation to the primary metabolite 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA) would not be detected.

Table 7.5-242: Fractionation of ^{14}C-bentazone after incubation with filter material from different filter sands. The fractionation of ^{14}C (or $^{14}CO_2$) of the initial amount of $^{14}C_0$ is shown at two selected times. Data are from microcosms (two replicates) and abiotic controls. The removal of MCPP at Islevbro was tested with both outlet water from filter (OW), and inlet water to filter (IW)

	Fraction of bentazone in water phase ($^{14}C/^{14}C_0$)				$^{14}CO_2$-production from degradation ($^{14}CO_2/^{14}C_0$)			
	Microcosms	Abiotic control	Microcosms	Abiotic control	Microcosms	Abiotic control	Microcosms	Abiotic control
Islevbro	4 hours		13 days		4 hours		13 days	
MCPP OW	60–61%	64%	42–48%	57–61%	–	–		–
MCPP IW	60%	73%	51–57%	73–75%	–	–		–
Bentazone	72–74%	81%	26–33%	74–83%	–	–		–
Sjælsø Plant I	4 hours		6 days		4 hours		6 days	
MCPP	103%	63%	67–74%	67%	–	–		–
Bentazone	62–75%	60%	31–35%	62%	–	–		–
Glyphosate	8–9%	9%	7–8%	4%	–	–		–
4-Nitrophenol	29%	56%	1–3%	22%	–	–		–
Sjælsø Plant II	4 hours		6 days		4 hours		6 days	
MCPP[a]	103%	112%	70–85%	92%	–	–		–
Bentazone	59–71%	101%	15–18%	103%	–	–	8–14%	–
Glyphosate	19–20%	17%	9–14%	8%	31–36%	–	42–43%	–
4-Nitrophenol	28–33%	102%	3%	96%	4%	–	7–10%	–

– No evident tendency in results.
a Low initial concentrations (0.033–0.036 µg/L) – uncertain results.

The mineralisation of pesticides in terms of $^{14}CO_2$ production was observed only at Sjælsø Plant II. After six days, $^{14}CO_2$ production from bentazone reached 8-14 %, glyphosate 42-43 % and p-nitrophenol 7-10 % of the initially added pesticide (mineralisation of MCPP was not detected).

For Islevbro and Sjælsø waterworks Plant I, [^{14}C-]pesticide was removed from the water phase in the abiotic controls, so a part of the pesticide was removed by abiotic processes, such as sorption. For Sjælsø Plant I the removal of MCPP and glyphosate was merely abiotic, since there was no difference between abiotic controls and microcosms. Microbiological removal did not result in immediate mineralisation ($^{14}CO_2$ production), and removal must have been caused by a degradation to a metabolite, which was eliminated from the water phase by sorption or volatilisation, or the compound was taken up by the microorganism. At Sjælsø waterworks Plant II, evident mineralisation was measured for bentazone, glyphosate and p-nitrophenol. Microbiological removal was substantial in this filter, though abiotic processes also had an influence especially on the removal of glyphosate.

Conclusion

The investigations showed a clear removal potential of the pesticides MCPP, bentazone, glyphosate, and p-nitrophenol in rapid sand filters at Danish waterworks. The largest microbial removal was observed with filter material taken from Sjælsø Plant II. At Sjælsø waterworks Plant II bentazone concentration in the water phase decreased as a result of microbial removal to less than 50 % of the initial concentration within 30 min for all tested start concentrations (0.1-2.4 µg/L).

Overall, this study showed that substantial pesticide removal is possible within the contact time of rapid sand filters at Danish waterworks, and that rapid removal is followed by a slower mineralisation of the compound. Hence, there is a potential for microbial removal of pesticides from contaminated groundwater in Danish waterworks. This is of commercial interest due to substantial attention given to the maintenance of today's water treatments.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes experiments on the removal potential of glyphosate in rapid sand filters at Danish waterworks. Under the experimental conditions, glyphosate decreased to 7 – 14 % of initial amounts after 13 days (complete mineralisation); indicating that glyphosate was intrinsically degradable under these conditions (although unlikely to be degraded significantly *in situ*). The experiments are well described. However, no details on analytical methods are given. Further, sampling times and individual results are only reported for bentazone in graphical plots.
The article is considered reliable with restrictions

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/084
Report author	Jönsson, J. *et al.*
Report year	2013
Report title	Removal and degradation of glyphosate in water treatment: a review
Document No	Journal of Water Supply: Research and Technology-AQUA/62.7/2013
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary

Treatment methods such as ozonation and activated carbon are currently used for pesticide degradation and removal. This article provides a review of the reported efficiency in removal and degradation of glyphosate and aminomethylphosphonic acid (AMPA) by some commonly employed treatment options. Additional experiments have been carried out where knowledge gaps were identified. Oxidants used in water treatment, particularly Cl_2 and O_3, are highly effective in degrading glyphosate and AMPA. Removal by coagulation and activated carbon is ineffective as a barrier against contamination in drinking water. UV treatment is also ineffective for glyphosate and AMPA degradation, but the combination of UV/H_2O_2 provided significant degradation of glyphosate, but not AMPA, under the conditions investigated. UV/TiO_2 treatment can degrade significant amounts of glyphosate, but the irradiation time needed is long. Removal or degradation by bank filtration, slow sand filtration, ClO_2 and membranes is variable, but can provide significant removal under the right conditions.

Materials and methods

Batch tests were carried out to investigate the degradation of glyphosate and AMPA by oxidation using Cl_2, ClO_2, O_3, O_3/H_2O_2, and by adsorption using PAC (powdered activated carbon). The stock solutions of glyphosate and AMPA were prepared by dissolving high purity solids in deionized water. Tap water, purged with air to remove residual chlorine, was spiked with stock solutions to achieve a concentration of 3 µg/L of either glyphosate or AMPA. This concentration was chosen to represent a moderately contaminated water. Samples of the spiked water were taken for analysis to establish the initial concentration of glyphosate and AMPA. In the oxidation tests with glyphosate spiking, the treated water samples were also analyzed for AMPA, to investigate whether any of the glyphosate was degraded only to AMPA.

For the ozonation tests, preliminary tests were carried out to find suitable settings to achieve a residual of approximately 0.2-0.4 mg O_3/L after a contact time of 15 min. A 1 L sub-sample of spiked water was ozonated using a pilot-scale O_3 generator (Labo II ozonator from Ozotech Ltd) and a bubble diffuser stone. Following ozonation, the O_3 residual was measured immediately, and at 5 min intervals, during a 15 min contact time. At the end of the contact period, the residual ozone concentration was quenched with sodium thiosulphate. A further set of tests was carried out with simultaneous use of O_3 and H_2O_2, at 0.5 and 1.0 mg/L of H_2O_2. The ozonation conditions were identical to the test with O_3 alone. At the end of the contact period, the residual O_3 and H_2O_2 were quenched with sodium thiosulphate as above.

For the chlorine tests, 1 L samples of the spiked water were dosed with sodium hypochlorite at 1.5 mg Cl_2/L. The dosed water was left for 30 min at the desired temperature. At the end of the contact period, the residual Cl_2 was measured and then quenched with sodium thiosulphate as above. The tests with Cl_2 were repeated, but with ClO_2 as the oxidant. The ClO_2 was added as crushed tablets (Accepta). The initial target concentration of ClO_2 was 1 mg/L.

Tests were carried out to investigate the performance of three different types of coal based PAC; Norit W35, Norit SA Super and Chemviron W. One litre samples of the spiked water were dosed with the three different PAC products at 5, 15, and 25 mg/L. The dosed water was left stirring at room temperature for 1 h to keep the PAC in suspension. The samples were then filtered through GF/C grade filter paper to remove the carbon prior to analysis for glyphosate and AMPA.

The initial results for AMPA showed large variations, even for the spiked untreated control samples. This was found to be caused by a rapid degradation of AMPA by the low concentrations of free chlorine present in the tap water used (<0.2 mg Cl_2/L). Tap water for the subsequent oxidation tests was thoroughly purged with air for 72 h to remove the free chlorine before addition of AMPA. This changed the pH from 7.5 to 8.4. The free chlorine concentration in the purged water was <0.02 mg/L. This rapid degradation of AMPA by chlorine in the control samples was not apparent for glyphosate.

The effects of UV, UV/H_2O_2, O_3, O_3/H_2O_2, and UV/ O_3/H_2O_2 were investigated in a flow through pilot reactor from ITT Wedeco, consisting of in-line H_2O_2 dosing, O_3 dosing and a UV reactor, which could be used individually or in combination. The retention time in the unit was 0.5-1 min, most of which occurred in the UV reactor which has a single low pressure, high output germicidal UV lamp (254 nm, input power to the lamp 330 W). Two tests were performed, each with the same matrix of operating conditions. The feed tank was filled with 2 m^3 of tap water and then left for a minimum of 7 days, during which the free and total chlorine residuals were monitored. Free chlorine residual declined to below the limit of detection (LOD) within 48 h. The feed tank was then spiked with glyphosate or AMPA at a target concentration of 3 µg/L and the water recirculated to ensure the compound was evenly distributed.

The concentrations of O_3, H_2O_2, Cl_2 and ClO_2 were analysed by test kits (Palintest). Samples were treated with 9-fluorenylmethyl chloroformate derivatising reagent prior to concentration by solid phase extraction. The extracts were analysed by high-performance liquid chromatography/mass spectrometry detection in negative ion electrospray with selective ion monitoring. The reported recovery up to 0.3 µg/L was 99 % with a LOD of 0.006 µg/L. The results presented are for single samples.

Results and Discussion

Chlorination

In the tests carried out in this work, the free Cl_2 concentration was relatively stable over the 30 min that the experiments lasted (Table 7.5-243). The results indicate that changes in pH had little influence on the degradation of glyphosate by chlorine; 96-100 % was degraded in the three samples tested at 20 °C. The temperature had a larger influence on the glyphosate degradation with 71 % being degraded at 5 °C compared to 96 % at 20 °C. AMPA concentrations in samples from the glyphosate tests were all non-detectable, confirming the effective degradation of AMPA by chlorine.

Table 7.5-243: Results of chlorination tests in this work

Compound spiked	pH	Temp. (°C)	Free Cl_2 residual (mg L^{-1})		Initial conc. (µg L^{-1})	Final conc.		Removal (%)
			0 min	30 min		Glyph. (µg L^{-1})	AMPA (µg L^{-1})	
Glyphosate	6.00	20.5	1.46	1.46	2.17	0.017	<0.016	99
Glyphosate	7.66	20.5	1.38	1.13	3.17	0.141	<0.064	96
Glyphosate	8.60	20.5	1.46	1.38	2.17	0.083	<0.016	>99
Glyphosate	7.52	4.9	1.38	1.28	3.17	0.915	<0.064	71
Glyphosate	7.52	10.2	1.38	1.24	3.17	0.552	<0.064	83
AMPA	6.25	20.5	1.42	1.28	3.65	N/A	<0.016	>99
AMPA	7.08	20.5	1.46	1.31	3.65	N/A	<0.016	>99
AMPA	8.38	20.5	1.51	1.42	3.65	N/A	<0.016	>99
AMPA	8.38	6.2	1.56	1.46	3.65	N/A	<0.016	>99
AMPA	8.38	9.8	1.56	1.46	3.65	N/A	<0.016	>99

N/A – not analysed.

Chlorine dioxide

The results from the current work with ClO_2 as the oxidant are shown in Table 7.5-244. The degradation of glyphosate by ClO_2 was less effective than that for other oxidants, ranging from 17 to 93 %. The highest degradation was seen for the low pH samples (~pH 6) with high temperature (22 °C) and high ClO_2 concentrations. The increased degradation as pH decreases could be due to changes in the speciation of glyphosate, rather than a direct influence on the oxidative potential of chlorine dioxide. Glyphosate has a second pK_a of 5.44 and the results suggest that the singly deprotonated form of glyphosate ($^{-}OOC\text{-}CH_2\text{-}NH_2^{+}\text{-}PO_3H^{-}$ or H_2L^{-}) could potentially be more readily oxidized by ClO_2 than the doubly deprotonated form ($^{-}OOC\text{-}CH_2\text{-}NH_2^{+}\text{-}PO_3^{2-}$ or HL^{2-}) that dominates between pH 5.44 and 10.13. At pH 6, the concentration of H_2L^{-} is about 30 % of the total concentration of glyphosate, decreasing to about 1 % at pH 7.5 and 0.1 % at pH 8.5.

Low concentrations of AMPA were detected in the glyphosate test samples (1-5 % of total glyphosate concentration), suggesting that AMPA was formed as a degradation product when glyphosate was oxidized by ClO_2. However, for AMPA alone, complete degradation of AMPA was seen for all conditions tested, suggesting AMPA is readily degraded by ClO_2.

Table 7.5-244: Results of chlorine dioxide tests in this work

Compound	pH	Temp. (°C)	ClO_2 residual (mg L^{-1})		Initial conc. (µg L^{-1})	Final conc.		Removal (%)
			0 min	30 min		Glyph. (µg L^{-1})	AMPA (µg L^{-1})	
Glyphosate	6.04	23	0.52	0.39	2.47	0.58	N/A	76
Glyphosate	7.96	23	0.39	0.20	2.47	1.35	N/A	45
Glyphosate	8.60	23	0.39	0.27	2.47	1.42	N/A	43
Glyphosate	8.05	5.2	1.35	1.35	2.47	1.64	N/A	34
Glyphosate	8.05	11.5	1.35	1.16	2.47	1.48	N/A	40
Glyphosate	6.05	21.1	1.23	1.03	2.17	0.16	0.097	93
Glyphosate	7.61	21.1	0.84	0.59	2.17	0.53	0.015	76
Glyphosate	8.56	21.1	1.10	1.03	2.17	0.53	0.043	76
Glyphosate	7.61	4.2	0.39	0.27	2.17	1.79	0.063	17
Glyphosate	7.61	11.6	0.91	0.84	2.17	1.16	0.036	46
AMPA	6.25	20.5	1.35	1.23	3.65	N/A	0.016	>99
AMPA	7.08	20.5	1.03	0.39	3.65	N/A	<0.016	>99
AMPA	8.38	20.5	1.35	1.16	3.65	N/A	<0.016	>99
AMPA	8.38	6.2	1.42	1.10	3.65	N/A	<0.016	>99
AMPA	8.38	10.8	1.35	1.16	3.65	N/A	<0.016	>99

N/A = not analysed.

Ozone, UV and advanced oxidation processes (AOPs)

The ozonation treatment carried out in the current work degraded all of the glyphosate and AMPA to below the LOD after 15 min contact time (Table 7.5-245) and no temperature effect was seen. The initial O_3 concentration was similar between all of the tests and the O_3 demand increased with increasing temperature. Ozone was highly effective in degrading both glyphosate and AMPA and virtually complete degradation was achieved under the conditions tested. No AMPA was detected in any of the treated samples from the glyphosate tests.

Table 7.5-245: Results of ozonation test in this work

Compound spiked	Temp. (°C)	O_3 residual (mg L^{-1})		Initial conc. (µg L^{-1})	Final conc.		Removal (%)
		0 min	15 min		Glyph. (µg L^{-1})	AMPA (µg L^{-1})	
Glyphosate	6.7	0.72	0.48	2.76	< 0.014	N/A	>99
Glyphosate	10.8	0.76	0.44	2.76	<0.014	N/A	>99
Glyphosate	15.2	0.76	0.35	2.76	<0.014	N/A	>99
Glyphosate	6.8	0.42	0.24	2.59	<0.006	<0.016	>99
Glyphosate	11.9	0.41	0.18	2.59	<0.006	<0.016	>99
Glyphosate	15.0	0.41	0.19	2.59	<0.006	<0.016	>99
AMPA	5.1	0.51	0.16	3.65	N/A	<0.016	>99
AMPA	10.5	0.54	0.10	3.65	N/A	<0.016	>99
AMPA	15.4	0.55	0.10	3.65	N/A	<0.016	>99

N/A = not analysed.

A further set of tests was carried out with simultaneous use of O_3 and H_2O_2, at 0.5 and 1.0 mg/L (Table 7.5-246). The ozone concentrations quickly decreased indicating rapid breakdown of the ozone to produce hydroxyl radicals. The initial O_3 concentration was significantly lower in the presence of H_2O_2 due to the reaction between O_3 and H_2O_2 to generate hydroxyl radicals. The combination of O_3/H_2O_2 was as effective as O_3 alone in degrading glyphosate and complete degradation was achieved under the conditions tested. In the sample from the glyphosate tests with the highest H_2O_2 concentration, traces of AMPA were found at <2 % of total glyphosate concentration. With the addition of H_2O_2 the degradation of AMPA seems to decrease with an increasing H_2O_2 dose, although 85 % was still degraded at the highest H_2O_2 concentration.

This is in line with the results from the glyphosate tests, where AMPA was detected at the highest H_2O_2 concentration.

Table 7.5-246: Results of ozonation with hydrogen peroxide at 15°C in this work

Compound spiked	H_2O_2 dose (mg L^{-1})	O_3 residual (mg L^{-1})		Initial conc. (µg L^{-1})	Final conc.		Removal (%)
		0 min	15 min		Glyph. (µg L^{-1})	AMPA (µg L^{-1})	
Glyphosate	0.5	0.46	0.04	2.76	<0.014	N/A	>99
Glyphosate	1.0	0.24	0.04	2.76	<0.014	N/A	>99
Glyphosate	0.5	0.18	0.05	2.59	<0.006	<0.016	>99
Glyphosate	1.0	0.09	0.06	2.59	<0.006	0.042	98
AMPA	0.5	0.16	0.02	3.65	N/A	0.11	97
AMPA	1.0	0.04	0.02	3.65	N/A	0.54	85

N/A = not analysed.

The use of UV, O_3 and AOPs was investigated in this work by the use of a flow through pilot reactor. The tap water used had a temperature of 22 °C, pH between 7 and 7.2, alkalinity between 215 and 219 mg/L $CaCO_3$, and a UV transmittance of 96.7-96.8 %. Measured concentrations of both glyphosate and AMPA were less than the target 3 µg/L (Table 7.5-247) and AMPA was present in the glyphosate stock solution. It has not been determined whether this was a result of decomposition in solution, or AMPA being present in the original glyphosate product. However, it does not impact on the quality of the results, as the test concentrations were high enough to provide reliable data, and were representative of those found in source waters.

The UV dose used in drinking water treatment is typically in the region of 40-100 mJ/cm when used for disinfection alone. Doses >1,000 mJ/cm are usually required for >50 % degradation of organic micropollutants. The doses used in this work were 740 and 1,240 mJ/cm and this resulted in a degradation of 36 % of the spiked glyphosate for the highest dose. The addition of 5 mg/L of H_2O_2 significantly increased the degradation of glyphosate to 88-91 % using the same UV doses, while the AMPA concentration increased. This indicates that AMPA is not readily degraded by UV or UV/H_2O_2 at the conditions used. The ozonation tests were run with 1 min contact time and confirmed the evidence of rapid degradation of glyphosate from previous tests. The AMPA concentration also decreased in the ozonation tests.

Repeating the tests in the flow through system with AMPA it was confirmed that AMPA is poorly degraded by UV and UV/H_2O_2 under the conditions tested; between 6 and 36 % was removed at the doses used. The results from the ozonation tests showed lower degradation of AMPA (35-66 %) than the previous results for 15 min contact time (>99 %). This was due to the shorter contact time of 1 min as the degradation increased to >99 % when the contact time in the flow through pilot plant was increased to 10 min. The results also confirmed the previous finding that the degradation of AMPA in the O_3/H_2O_2 system was reduced compared to the O_3 only system.

Table 7.5-247: Results of UV, O_3 and AOP tests for glyphosate and AMPA removal in this work

Compound spiked	Operating conditions	Initial conc. (µg L^{-1})	Final conc. Glyph. (µg L^{-1})	Final conc. AMPA (µg L^{-1})	Removal (%)
Glyphosate	Feed water	1.72	1.72	0.30	0
Glyphosate	UV 740 mJ cm^{-2}	1.72	1.29	0.34	25
Glyphosate	UV 1,240 mJ cm^{-2}	1.72	1.10	0.42	36
Glyphosate	UV 740 mJ cm^{-2}, H_2O_2 5 mg L^{-1}	1.72	0.21	0.59	88
Glyphosate	UV 1,240 mJ cm^{-2}, H_2O_2 5 mg L^{-1}	1.72	0.15	0.69	91
Glyphosate	O_3 2 mg L^{-1}	1.72	0.068	0.13	96
Glyphosate	O_3 2 mg L^{-1}, H_2O_2 2 mg L^{-1}	1.72	<0.006	0.22	99
AMPA	Feed water	2.31	N/A	2.3	0
AMPA	UV 740 mJ cm^{-2}	2.31	N/A	2.17	6
AMPA	UV 1,240 mJ cm^{-2}	2.31	N/A	1.57	32
AMPA	UV 740 mJ cm^{-2}, H_2O_2 5 mg L^{-1}	2.31	N/A	2.13	8
AMPA	UV 1,240 mJ cm^{-2}, H_2O_2 5 mg L^{-1}	2.31	N/A	1.18	49
AMPA	O_3 2 mg L^{-1} 1 min contact time	2.31	N/A	0.86	63
AMPA	O_3 2 mg L^{-1} 10 min contact time	2.31	N/A	<0.016	>99
	O_3 2 mg L^{-1}, H_2O_2 2 mg L^{-1}	2.31	N/A	1.50	35

N/A = not analysed

Activated carbon

The removal of glyphosate and AMPA by PAC was investigated in this work (Table 7.5-248). Although the results are somewhat scattered, it is clear the PAC was ineffective as a removal treatment for glyphosate, even at the relatively high dose for water treatment of 25 mg/L no more than 20 % was removed. This is not surprising considering the high water solubility (approximately 10 g/L) and low log K_{ow} for glyphosate. No major differences between the different PACs could be seen.

The tap water used for the PAC testing had not been thoroughly de-chlorinated, and the initial concentration of AMPA was therefore lower than expected. However, PAC removes Cl_2 and this stops the degradation of AMPA by Cl_2. This explains why the removal of AMPA seems to increase with decreasing PAC dose. The removal that actually occurs is degradation by Cl_2 and an increased PAC dose removes more Cl_2. A similar, though much less marked, effect is suggested for glyphosate. The conclusion is that the PACs investigated would not provide adequate removal of glyphosate and AMPA.

Table 7.5-248: Results of PAC tests for glyphosate removal in this work

Compound	PAC	PAC conc. (mg L^{-1})	Initial conc. ($\mu g\ L^{-1}$)	Final conc.	Removal (%)
Glyphosate	Norit W35	5.1	3.13	2.51	20
Glyphosate	Norit W35	15.2	3.13	2.76	12
Glyphosate	Norit W35	25.3	3.13	3.14	0
Glyphosate	Norit SA Super	5.2	3.13	2.86	9
Glyphosate	Norit SA Super	15.0	3.13	2.46	22
Glyphosate	Norit SA Super	25.0	3.13	3.03	3
Glyphosate	Chemviron W	5.1	3.13	2.57	18
Glyphosate	Chemviron W	15.1	3.13	2.79	11
Glyphosate	Chemviron W	25.2	3.13	2.72	13
AMPA	Norit W35	5.1	1.57[a]	1.09	31
AMPA	Norit W35	15.2	1.57[a]	1.39	12
AMPA	Norit W35	25.3	1.57[a]	2.12	0
AMPA	Norit SA Super	5.2	1.57[a]	2.92	0
AMPA	Norit SA Super	15.0	1.57[a]	2.28	0
AMPA	Norit SA Super	25.0	1.57[a]	3.25	0
AMPA	Chemviron W	5.1	1.57[a]	1.63	0
AMPA	Chemviron W	15.1	1.57[a]	1.49	5
AMPA	Chemviron W	25.2	1.57[a]	1.92	0

[a]Spiked at 3 $\mu g\ L^{-1}$.

A summary of removal efficiencies for glyphosate and AMPA (based on literature survey and studies reported in the paper) is given in Table 7.5-249.

Table 7.5-249: Summary of removal of glyphosate and AMPA

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 to 50	25 to 95
Aluminium coagulant and clarification	15 to 40	20 to 25
	Not a reliable barrier for Glyphosate and AMPA	
Iron coagulant and clarification	40 to 70	20 to 85
	Not a reliable barrier for Glyphosate and AMPA	
Slow sand filtration	The limited information suggests that significant removal can be achieved but removal is likely to be highly dependent on conditions	
Chlorination	74 to > 99	40 to >95
	Likely to provide the main barrier at most water treatment works	
Chlorine dioxide	17-93	>99
	Removal of glyphosate is variable and works best at lower pH and high temperature. Good removal of AMPA can be expected	
Ozonation	60 to >99	25 to 95
	Provides an additional barrier at works where already installed for other pesticides and micropollutants	
UV irradiation	Not effective alone at doses used in water treatment	
Advanced oxidation	O_3/H_2O_2 provides an additional barrier at works where already installed. UV/H_2O_2 show good removal of glyphosate but not AMPA UV/TiO_2 can degrade significant amounts of both compounds but irradiation times are long	

Table 7.5-249: Summary of removal of glyphosate and AMPA

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Activated carbon adsorption	10 to 90	20 to 70
	Higher removals relate to virgin GAC and are unlikely to be achieved under practical conditions. Not a reliable barrier	
Membrane filtration	>90 (NF/RO) >50 (UF)[1]	>95 (NF/RO) No information found for UF
	Membrane processes not widely used in water treatment, and unlikely to be installed solely as a barrier to pesticides	
Air stripping	Not expected to be effective based on chemical characteristics	

[1]Depending on membrane type

Conclusion

The literature review and laboratory tests showed that glyphosate and AMPA are both readily degraded or removed by a number of common treatment steps at drinking water treatment plants. Biodegradation and adsorption processes can be highly effective in degrading or removing glyphosate and AMPA in bank filtration and SSF. These processes could potentially be of importance in biologically active GAC (granular activated carbon), but the residence time is generally much shorter. Iron-based coagulants are generally more effective than Al-based coagulants in removing glyphosate and AMPA; coagulation is particularly effective if coagulant residuals are removed by filtration. Ozonation and chlorination are highly effective in degrading both glyphosate and AMPA but a decrease in temperature reduces the efficiency. Combining O_3 and H_2O_2 did not improve the degradation compared to O_3 alone; in fact a decrease was observed at high H_2O_2 concentrations. UV doses typically used for disinfection will not degrade significant amounts of either compound. Higher UV doses in combination with H_2O_2 showed good degradation of glyphosate, but not AMPA. Chlorine dioxide is effective for glyphosate and AMPA degradation at around pH 6, but the efficiency decreases with increasing pH and decreasing temperature. UV/TiO_2 treatment can degrade significant amounts of glyphosate, but the irradiation time needed is long. Ultrafiltration (UF), NF (nanofiltration) and RO (reverse osmosis) can also be effective in removing glyphosate and AMPA, but the cut-off for UF needs careful consideration. Activated carbon is not likely to provide a practical removal option for either compound.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes different methods used in drinking water treatment plants with regard to the degradation of glyphosate and AMPA, and presents a useful summary of removal efficiencies for glyphosate and AMPA.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/085
Report author	Malaguerra, F. *et al.*
Report year	2013
Report title	Assessment of the contamination of drinking water supply wells by pesticides from surface water resources using a finite element reactive transport model and global sensitivity analysis techniques
Document No	Journal of Hydrology 476 (2013) 321–331
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

A reactive transport model is employed to evaluate the potential for contamination of drinking water wells by surface water pollution. The model considers various geologic settings, includes sorption and degradation processes and is tested by comparison with data from a tracer experiment where fluorescein dye injected in a river is monitored at nearby drinking water wells. Three compounds were considered: an older pesticide MCPP (Mecoprop) which is mobile and relatively persistent, glyphosate (Roundup), a newer biodegradable and strongly sorbing pesticide, and its degradation product AMPA. Global sensitivity analysis using the Morris method is employed to identify the dominant model parameters. Results show that the characteristics of clay aquitards (degree of fracturing and thickness), pollutant properties and well depths are crucial factors when evaluating the risk of drinking water well contamination from surface water. This study suggests that it is unlikely that glyphosate in streams can pose a threat to drinking water wells, while MCPP in surface water can represent a risk: MCPP concentration at the drinking water well can be up to 7 % of surface water concentration in confined aquifers and up to 10 % in unconfined aquifers. Thus, the presence of confining clay aquitards may not prevent contamination of drinking water wells by persistent compounds in surface water. Results are consistent with data on pesticide occurrence in Denmark where pesticides are found at higher concentrations at shallow depths and close to streams.

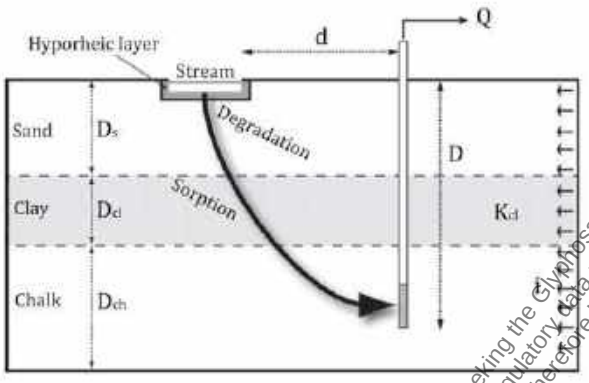
Materials & Methods

Conceptual model

In order to study the link between surface water and a nearby drinking water well, a generic model of contaminant transport from surface water into groundwater is established. The model is designed to quantify the amount of pesticides that can leach from a stream into drinking water during water abstraction in a primary aquifer. The conceptual model is illustrated in Figure 7.5-197. A pumping well is placed at a distance d (m) from a stream and pumps water at a constant pumping rate Q (m^3/d) from a depth D (m). The geology is simplified to be a 3-layer system: a hyporheic layer separates the stream from an underlying sandy aquifer, below which a clay aquitard overlies a chalk aquifer; *Ds*, *Dcl* and *Dch*, respectively, are the thicknesses of the three layers, and *Kcl* is the hydraulic conductivity of the fractured clay till. Clayey glacial tills are very wide- spread in the northern hemisphere, especially at higher latitudes, and represent a frequent aquiclude in countries such as Denmark, Canada or the United States. Thus, the configuration considered in the conceptual model is applicable to a wide range of aquifers. The natural flow in the aquifer is driven by a regional groundwater gradient i (m/m) and to simplify the system, the hydraulic gradient is assumed to be the same in both aquifers. During pumping the well modifies the natural water flow, lowering the water head in the aquifer, so that surface water from the stream can seep into the groundwater and reach

the pumping well. Pollutants in the stream may be retarded by sorption and degraded by microorganisms during their travel to the well. Both the sandy and chalk aquifer are considered to be strictly anaerobic, while the hyporheic zone can be aerobic.

Figure 7.5-197: Conceptual model of the system considered



Model formulation
At steady state, the groundwater flow equation can be written as:

$$\nabla \cdot \mathbf{K} \cdot \nabla H - W = 0 \qquad (1)$$

where K is the hydraulic conductivity tensor, H is the hydraulic head and W is the sink term for water withdrawal. The fate of an aqueous component in groundwater is influenced by advection, mechanical dispersion and diffusion, as well as sources/sinks and geochemical reactions and can be described by the advection– diffusion equation:

$$\left(1+\frac{\rho_b}{n}K_d\right)\frac{\partial C}{\partial t}+\nabla\cdot(\mathbf{v}C)-\nabla\cdot(\mathbf{D}\nabla C)=-kC \qquad (2)$$

Where *qb* is the bulk density, *n* is the soil porosity, *Kd* is the sorption coefficient, v is the pore water velocity, D is the dispersion tensor and *k* is the degradation rate. Degradation kinetics are assumed to follow a first-order rate with half lives for aerobic and for anaerobic conditions. Despite the fact that for many pollutants sorption is often better described by non-linear isotherms, linear sorption isotherms were considered when calculating the retardation factor, because low concentrations are expected and computations are simplified by avoiding sorption related concentration shock fronts and rarefactions. Such a simplification is a common assumption in reactive transport modeling. The model is set up and solved using COMSOL Multiphysics, a finite-element modeling package for solving partial differential equations. The groundwater flow model is solved at steady state, subsequently the transport model is solved transiently, until the concentration of contaminant at the well reach the steady state.

Pesticides considered
Two pesticides and a pesticide metabolite were considered: an older pesticide MCPP (Mecoprop) which is mobile and persistent under anaerobic conditions, glyphosate (Roundup), a newer, readily degradable and strongly sorbing pesticide, and AMPA, which is a more mobile, less degradable glyphosate degradation product, which can therefore accumulate in groundwater. All three compounds have been regularly found in Danish drinking water wells. The three pesticides are known to be more quickly degraded under aerobic conditions than in anaerobic environments. Monitoring in agricultural streams reveals a high occurrence of MCPP in surface waters with detection rates as high as 78 % in some catchments. Glyphosate and AMPA concentrations in streams and groundwater are not always measured because of the cumbersome analytical

procedure. However, glyphosate is the most sold chemical used for weed control in agricultural, silvicultural and urban environments, is likely to be found in surface waters, and despite its high degradability under aerobic conditions, it can pose a threat to groundwater.

Model application to a tracer experiment
In order to test the ability of the model to simulate solute transport from surface water to nearby wells, data from a tracer experiment performed in 2002 on the river Aare, Switzerland (Wanner and Grunner, 2002) were modeled. The experiment investigated the vulnerability of drinking water wells in the riparian zone to river water contamination. A 10 m-thick highly permeable aquifer is pumped by two horizontal wells (ZPW1 and ZPW3) located at around 100 m from the river shore, and one vertical well (VB) located at approximately 50 m from the river shore. In 2002, the two horizontal wells were pumped at 7 and 9.5 m^3/ min respectively while the vertical well had a pumping rate of 2 m^3/min. A pumping test performed in the vertical well revealed a hydraulic conductivity of 5.5e-3 m/s.

A tracer pulse (fluorescein) was injected in the river near the study area, far enough upstream to ensure a good mixing of the tracer in the river close to the sampling area. The maximum river velocity was 1.27 m/s and the duration of the tracer concentration peak was about 2 h. Tracer concentrations at the pumping wells were measured every 2-4 h using in situ fluorometers. No aquitard was present between the river and the drinking water well, and so the geology of the experimental site was different from the geology considered in the conceptual model (Figure 7.5-197). A tracer experiment for the geology setting presented in Figure 7.5-197 would be very difficult to perform, because conventional tracer breakthrough test are difficult to perform for long travel times. Since a confined aquifer tracer test is not available in the literature, the unconfined tracer test is used instead to validate the conceptual model employed in this paper. It includes the main processes simulated by the model, with the exception of the low permeability aquitard. A three-dimensional model of the aquifer was developed: the size of the model domain was 2 by 1 km and the aquifer had a constant thickness of 10 m. The hyporheic zone was modeled as a 2-m thick layer under the stream. Two different isotropic hydraulic conductivities were assigned to the hyporheic zone and the highly permeable aquifer. A fixed head boundary condition was assigned to the river with the head being determined by the topography which had a slope of about 0.1 %. No flow boundary conditions were assigned to the southern boundary since previous studies found water flow streamlines to be mostly in the East–West direction. A fixed hydraulic gradient parallel to the river at the downstream (west) boundary was considered.

The model was calibrated using the tracer breakthrough curves obtained at two pumping wells for five parameters: hydraulic conductivity of the hyporheic zone, hydraulic conductivity of the highly permeable aquifer, longitudinal and transverse dispersivity and hydraulic gradient parallel to the river at the downstream boundary. A Shuffled Complex Evolution Metropolis algorithm (SCEM) was used to determine optimal parameters and confidence intervals.

Sensitivity analysis
The aim of the sensitivity analysis was to determine which parameters most affect the risk of contamination of drinking water wells from pollutants in nearby streams for the conceptual model presented in Section *Conceptual model*. The sensitivity analysis is a generic study of groundwater/surface water interaction and is not restricted to the case study presented in the previous section. Moreover, sensitivity analysis provides information on how parameters influence the seepage of pollutants from the stream to the pumping well. Sensitivity analysis is often considered as a local measure of the effect of a given input on a given output, such as a simple or normalized derivative. Nevertheless local sensitivity relies on point measures, which can be inappropriate to describe the behavior of a model over the whole input parameter space. Here, a global sensitivity analysis (GSA) tool was used to analyze the model over the full extent of the model space.

Many global sensitivity analysis methods are available, most of- ten based on Monte Carlo methods in conjunction with a variety of sampling strategy and sensitivity measures. Since the finite-element solution is computationally expensive, the sensitivity analysis was performed using the Morris method, which belongs to the group of the derivative-based global sensitivity measures (DGSMs) and produces qualitative

results with limited computational effort. The Morris method aims to determine the factors leading to negligible, linear and additive, or non-linear effects, and parameter interactions. The method is based on *elementary effects*, which are attributed to each input. For a model with *k* parameters, the parameter space Ω will be the k-dimensional hypercube with $x_i \in [x_{i_{min}}, x_{i_{max}}]$ for $i \cdots k$, where $x_{i_{min}}$ and $x_{i_{max}}$ are the minimum and maximum values of the a priori distribution for each parameter. In order to observe the model response in several places of the model spaces, a region of experimentation x included in X is constructed as a regular k-dimensional p-level grid, p being a fixed scalar representing the refinement of the grid. Each *xi* may only take on values from $\{x_{i_{min}}, x_{i_{min}} + \Delta, x_{i_{min}} + 2\Delta, \ldots, x_{i_{max}}\}$,
where Δ is a multiple of 1/(1 - *p*). For a given value of x, the elementary effect of the input factor i is defined as:

$$d_i(x) = \frac{[y(x_1, x_2, \ldots, x_{i-1}, x_i + \Delta, x_{i+1}, \ldots, x_k) - y(x)]}{\Delta} \quad (3)$$

The finite distribution of elementary effects associated with the *i*th input factor, named *Fi*, is obtained by randomly sampling different x from Ω. The mean (μ) and the standard deviation (σ) of *Fi* are the most informative sensitivity measures. A high value of μ implies that the factor has a large effect on the output, while a high value of σ means that the elementary effects relative to this factor are significantly different from each other, i.e. the value of an elementary effect is strongly affected by the values taken by the other parameters. Campolongo et al. (2007) proposed that the distribution of the absolute values of the elementary effects, *Gi*, and its mean μ* should also be considered. In fact, if the distribution *Fi* contains negative values some effects may cancel each other when computing the mean, leading to an underestimation of their effect.

The percentage of pollutant in the stream that reached the well at the end of the simulation was chosen as model output considered for sensitivity analysis. Note that the ratio of concentrations at the pumping well to surface water concentrations are independent of initial concentrations since only first order degradation and linear sorption are considered.

Parameter sensitivity was studied on a standard three-dimensional model domain consisting of a 5-m wide stream surrounded by a 1-m thick hyporheic layer, placed in the middle of a 1 km by 1 km area. The abstraction well was modeled as a vertical well with a diameter of 150 mm and a screen length of 6 m (representative of a typical Danish drinking water well). Fixed head boundary conditions were set to vertical boundaries parallel to the stream, while no flux boundary conditions were chosen for the vertical boundaries perpendicular to the stream and for the bottom of the lower horizontal layer.

The effect of the domain size and the distance of boundaries on the stream solute seepage was investigated by performing several simulations with variable geometry of the model domain. Decreasing the distance between the two fixed head boundaries resulted in lower steady-state pollutant concentrations at the pumping well, since more uncontaminated water was coming from the fixed head boundaries. When wells neared the two no-flow boundaries, contaminant concentrations were higher since more water entered the model domain from the stream to compensate for the lower lateral water flow. The 1 km by 1 km domain chosen was the smallest domain ensuring negligible effect of boundaries on contaminant transport from the stream to the pumping well.

An optimal sensitivity analysis should investigate all model parameters, however, due to computational constraints, some parameters were kept fixed to reduce the number of model evaluations needed to obtain results. Values for sand and chalk horizontal saturated hydraulic conductivity were 8.64 and 5 (m/d) respectively, while a lower value (1 (m/d)) was assigned the horizontal hydraulic conductivity of the hyporheic zone. For each layer, the vertical hydraulic conductivity was assigned to be one tenth of the horizontal values. We choose relatively high values for longitudinal and transverse dispersivities (4 m and 0.4 m) since travel distances and water velocity were both high, and to decrease simulation times. Recharge was assumed to be 150 mm/yr, a typical value for a Danish groundwater.

To facilitate the computation of solutions in a reasonable amount of time, sorption coefficients and first-order degradation rates were kept constant. Half lives considered for aerobic conditions were higher than values found in the literature because the model assumed that the hyporheic layer has a thickness of 1 m, while oxygen is usually depleted at depths of 5–40 cm. Thus, using low half lives for aerobic conditions in the whole hyporheic layer would overestimate the pesticide degradation. The fixed thickness of the hyporheic layer of 1 m was necessary to avoid model failures caused by a lack of available memory. The parameter space of the inputs is summarized in Table 7.5-250. Statistics from the Danish National Boreholes Database were used to identify the most representative values for Danish drinking water wells. Empirical cumulative distribution functions of 21,837 wells were employed to obtain intervals representative of 95 % of the drinking wells in Denmark. Clay hydraulic conductivity values are typical for Danish clay tills, and are relatively high in order to consider the permeability increase due to clay till fracturing. Each model run simulated the system for 30 years, which corresponds to the time to remove 99 % of the least degradable compound. When constructing the random model do- main, the pumping well screen was always assumed extracting water below the clay layer.

Table 7.5-250: Parameters intervals used for sensitivity analysis

Parameter	Symbol	Unit	Range
Sand aquifer thickness	D_s	m	1–30
Clay layer thickness	D_{cl}	m	0–30
Chalk aquifer thickness	D_{ch}	m	1–100
Distance from the stream	d	m	3–150
Well depth	D	m	8–100
Aerobic hypoheric zone	O_2		Yes/no
Abstraction rate	Q	m3/h	1–100
Natural hydraulic gradient	i	m/m	−1% to +1%
Clay hydraulic conductivity	K_{cl}	m/s	3e−7–1e−8

Results

Tracer experiment

It was not possible to find parameter values able to fit the breakthrough curves of tracer concentration at the two wells (VB and ZPW1) at the same time. Possibly assuming a uniform, isotropic aquifer is an extreme simplification of the system. However, the purpose of using the tracer experiment data was to test if the model can correctly simulate the transport of solutes from the river to a single pumping well, and not to create a reliable groundwater model of the data. Moreover, the measurements available did not justify a more complex model: the inclusion additional parameters would have led to an over-parametrized model.

Satisfactory results were obtained when considering breakthrough curves of one well at a time. Figure 7.5-198 shows the calibrated breakthrough curve of fluorescein concentrations at the two wells with two optimized parameters sets. Both arrival times and peak concentrations were simulated correctly for the model calibrated with the vertical well (VB) dataset. The model calibrated with the horizontal well (ZPW3) dataset showed a poorer fit to experimental data, but it still could approximate the peak concentration time and the breakthrough mass (difference between observed and simulated breakthrough mass was about 2 %). Parameters values obtained during the two calibrations are presented in Table 7.5-251. The calibration on the vertical well indicated a value of 5.45e-3 m/s for the hydraulic conductivity in the sand layer, which is extremely close to the value of 5.5e-3 m/s measured during the field pumping test.

Figure 7.5-198: Calibrated tracer breakthrough curves at the vertical well VB (A) and at the horizontal well ZPW3 (B)

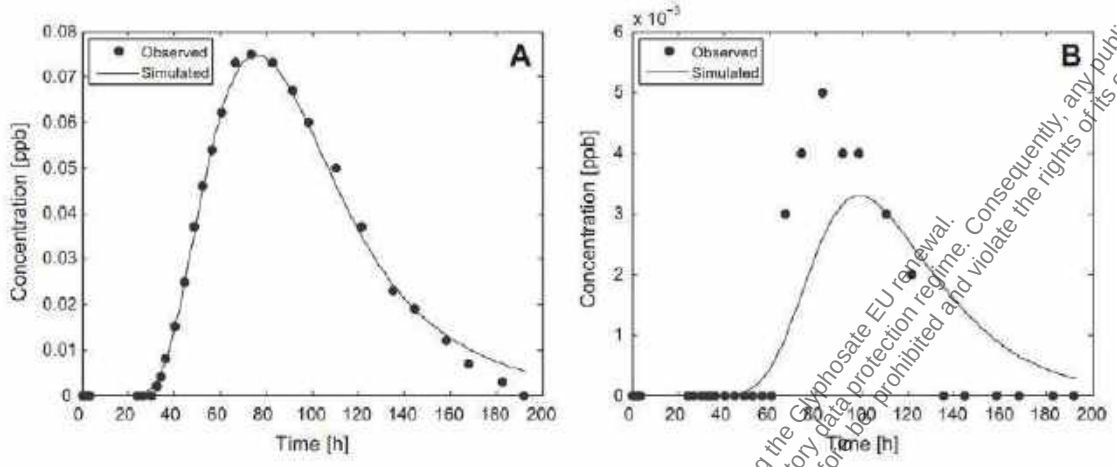


Table 7.5-251: Calibrated parameters for the tracer experiment and 95 % confidence intervals

Parameter	Symbol	Unit	Best fit VB	CI 95%	Best fit ZPW1	CI 95%
Sand hydraulic conductivity	K_s	m/s	5.45e-3	5.29e-3-7.93e-3	7.61e-3	5.99e-3-9.89e-3
Hyporheic zone hydraulic conductivity	K_{hz}	m/s	6.68e-3	6.50e-3-7.78e-3	7.11e-3	5.87e-3-7.91e-3
Longitudinal dispersivity	α_l	m	5.18	5.17-5.32	7.24	6.35-7.89
Transverse dispersivity	α_t	m	0.286	0.285-0.291	0.272	0.253-0.308
Gradient at the downstream boundary	i	-	0.013	0.012-0.051	0.037	0.028-0.043

Sensitivity analysis

The model space was screened through 165 parameter paths (each one consisting in 10 model runs). Results of sensitivity analysis are presented in Table 7.5-252, where the parameters are ranked according to the absolute value of the mean of elementary effects. A more intuitive way to show the sensitivity analysis result is given by σ - μ andn σ - μ* plots as suggested by Morris (1991). For MCPP concentrations at the drinking water well, the hydraulic conductivity of the clay layer (*Kscl*) is the most influential parameter followed by the well depth (*D*), the thickness of clay layer (*dcl*) and the abstraction rate (*Q*). The mean value μ shows whether an increase of a parameter will induce a higher (μ > 0) or lower (μ < 0) contamination to the drinking water well. Results show that a more permeable clay layer (*Kscl* > 4e-8 (m/s)) or an higher abstraction rate (*Q* > 20 (m^3/h)) will lead to higher concentrations at the drinking water well, while deeper wells, longer distance between the stream and the well, and thicker geologic layers will result in lower pollution.

For most of the parameters, values of μ* are very close to the values of |μ|, which means that the parameters are always acting in the same 'direction', i.e. always influencing negatively or always positively the pollutant concentration at the drinking well, and there are no elementary effects that eliminate each other. However, this is not the case for parameter *i*, the regional hydraulic gradient. A given increase or decrease of the regional hydraulic gradient can lead to different consequences depending on the values of other parameters. In order to explain this behavior, we performed random simulations while looking for dependencies between couples of inputs and pesticide concentrations at the drinking water well. Figure 7.5-200 shows the relationship between well depth, regional hydraulic gradient, and MCPP concentrations at the drinking water well: each circle represents a random model simulation and the black dots indicate a simulation for which the MCPP concentration exceeded 0.2 % of the stream contamination. As can be seen, the black dots are located in a well defined triangular zone. Shallow wells can be contaminated with every regional gradient value, while contamination of deep wells only occurs if the regional hydraulic gradient is

close to zero. This is probably due to the fact that for steep hydraulic gradients, the contaminant plume from the stream does not intercept the drinking water well. No interaction between the regional hydraulic gradient and the distance between the well and the stream was found, suggesting that the vertical profile is much more influential for the contaminant transport than the horizontal location of the well. Graphs representing the results for MCPP concentration at the drinking water well are presented in Figure 7.5-199, but similar graphs can be obtained for every model output. Results show that elementary effects for glyphosate and AMPA concentrations at the drinking water wells are very small. Because only very small concentrations of glyphosate and AMPA arrive at the drinking water well (see Figure 7.5-201), absolute changes in pesticide concentrations are very small and consequently Eq. (3) will produce very small elementary effects. Percentages of stream concentrations reaching the drinking water well as a function of the values of most important parameters for the three pesticides considered are shown in Figure 7.5-201. MCPP concentrations seem to be positively correlated to clay hydraulic conductivities values (Spearman $q = 0.14$, $p < 0.001$, Figure 7.5-201A) and negatively correlated to the drinking water well depth (Spearman $q = -0.53$, $p < 0.001$, Figure 7.5-201B). Results also show that MCPP steady state concentrations at the water well can be up to 7 % of stream concentrations, if the well is shallow and the clay hydraulic conductivity is high. The relationship between thickness of the clay aquitard and maximum MCPP concentrations at the drinking well was also investigated. Results also indicate that the well is protected against MCPP leaching from the stream (concentrations in the water wells below 0.01 % of stream water concentration) when clay layer thicknesses are greater than 20 m, for all values of clay hydraulic conductivity and well depth within the considered range.

Maximum concentrations in the drinking water well for glyphosate and AMPA are much lower than for MCPP: only up to 0.025 % of glyphosate stream concentration can be found at the drinking water well (Figure 7.5-201C) and maximum AMPA concentrations are about 0.0024 % of glyphosate stream concentrations (Figure 7.5-201E). Nevertheless, despite very low concentration at the drinking water well, trends between glyphosate and AMPA findings and well depth are found (Spearman q well depth - glyphosate concentrations = -0.44, $p < 0.001$, Spearman q well depth - AMPA concentrations = -0.32, $p < 0.001$) and can be highlighted by plotting concentrations on a logarithmic scale (Figure 7.5-201D and F). Lower glyphosate and AMPA concentrations are found in deep wells.

A separate sensitivity analysis was performed on a model considering only unconfined aquifers. The overall method was identical to the one used for confined pumping wells, but the geometry of the model was changed in order to have contact between the sand layer and the chalk layer. Results of sensitivity analysis indicated the depth of the well D as most influential parameter, followed by the natural hydraulic gradient i, the distance between the stream and the well d, and the thicknesses of the sand and clay layers. Maximum concentrations in the pumping well increased up to 10 % of MCPP and 0.12 % of glyphosate stream concentrations. Up to 0.043 % of stream glyphosate could be found in the well as AMPA. Dependence of MCPP concentrations on well depth is more evident than for confined aquifers (Figure 7.5-202A); moreover, shallow wells are often contaminated with MCPP concentrations above 1 % of stream concentrations. The distance between the well and the stream, which was determined to be an insensitive parameter for confined wells, seems to play a more important role in unconfined aquifers: wells close to the stream are more likely to be contaminated than wells placed far from the stream (Figure 7.5-202B).

Table 7.5-252: Parameter ranking according to the Morris screening. Parameters are ranked according to the mean of elementary effects absolute values μ

Rank	MCPP			Glyphosate			AMPA		
	Parameter	μ^*	σ	Parameter	μ^*	σ	Parameter	μ^*	σ
1	K_{h2}	2.871	7.928	K_{h1}	0.050	0.295	D_s	0.010	0.094
2	D	0.263	0.570	D_s	0.007	0.049	K_d	0.005	0.027
3	D_{s1}	0.185	0.419	D	0.004	0.019	D	0.001	0.004
4	Q	0.178	0.397	Q	0.004	0.021	Q	0	0.004
5	D_{c1}	0.158	0.355	i	0.003	0.025	D_{c1}	0	0.002
6	D_s	0.131	0.255	D_{s1}	0.001	0.005	i	0	0.001
7	i	0.125	0.372	D_{c2}	0.001	0.004	D_2	0	0.001
8	d	0.073	0.195	D_2	0.001	0.005	D_{c2}	0	0
9	D_2	0.001	0.004	d	0.001	0.002	d	0	0

Figure 7.5-199: **Results of sensitivity analysis for MCPP concentrations: the standard deviation (r) of the elementary effects is plotted against their mean l (A) and mean of absolute values µ (B)**

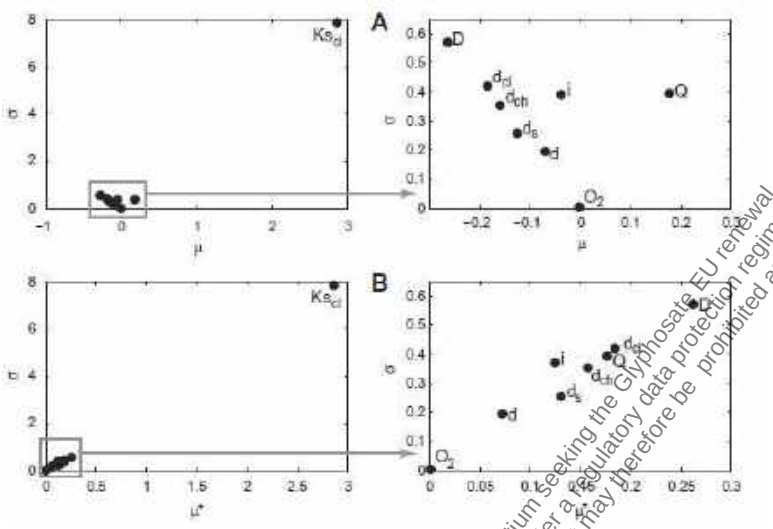
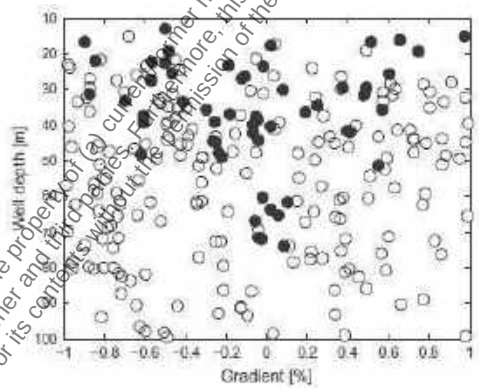


Figure 7.5-200: **Relationship between well depth, natural hydraulic gradient and MCPP well contamination. In White the sampled points, in black, the point for which MCPP concentration at the drinking water well is higher than 0.2 % of the stream concentration**



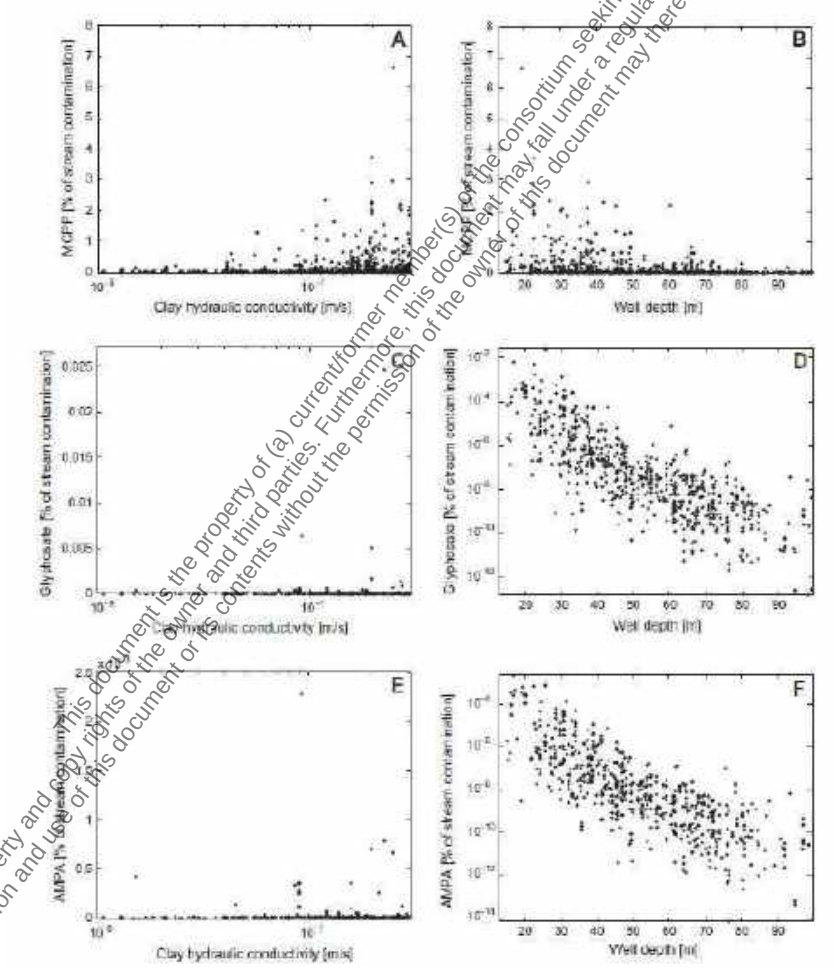
Discussion

Sensitivity analysis
Steady state concentrations at the drinking water wells vary greatly depending on pesticide properties. Highly sorbable, readily degradable compounds like glyphosate and AMPA, reach the wells at very low concentrations due to transformation and dilution processes. The arrival time of such compounds at the drinking water well is delayed because their high sorption coefficients, and so bacteria have more time to degrade the pollutants. In contrast, persistent, mobile pesticides such as MCPP can travel faster from the stream water to the well and higher pollutants concentrations can be found in the drinking water. Results also indicate that the clay aquitard characteristics are the most important parameters controlling infiltration of pollutants from surface water to drinking water wells. If fractures are present in the clay or if the clay layer is thin, pumping wells can be at risk of contamination, independently of the distance between the stream and the pumping well. In the absence of a clay aquitard, contamination from stream pollution is

more likely to occur since sandy sediments are directly in contact with the chalk layer. In this case, the distance between the stream and the pumping well matters, and closer wells are more hydraulically connected to the stream. Sensitivity analysis indicated that deeper wells are less subject to contamination because water travel times are increased and dilution is more effective. However, deep wells are more expensive to drill and require higher operational costs, thus, the majority of drinking water wells (especially those privately owned) are relatively shallow.

The natural hydraulic gradient can also play a major role in contaminant transport from surface water to nearby wells, especially in case of pumping from unconfined aquifers. Depending on the natural hydraulic gradient, the capture zone of the pumping well intersects the stream and surface water can be transported to the drinking water well. The greater the hydraulic gradient; the more elongated the capture zone and so wells have to be shallow to intercept water coming from the stream. On the other hand, if the hydraulic gradient is very low, the capture zone extends vertically and stream water can travel into deep wells.

Figure 7.5-201: Percentages of stream pesticide concentrations leaching into the well plotted against the most sensitive parameters. Note the logarithmic *y*-axis in plots D and F



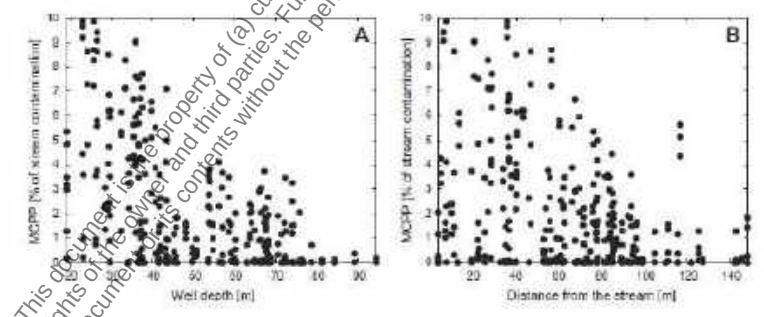
In natural systems, the hydraulic gradient can vary with time and thus increase the contaminant spread. Data on MCPP concentration in drinking water wells from the Danish National Boreholes Database show that both the occurrence and concentrations of MCPP is related to well depth. Thus, a similar trend has been found between simulation results and real data. The comparison also highlights that in both field and

simulated data MCPP concentrations are higher for wells shallower than about 50 m. Sensitivity analysis indicated that the hydraulic conductivity of the clay layer is the most important parameter affecting MCPP leakage into pumping wells (Table 7.5-252), but this relationship could not be verified by the real dataset, because parameters such as the degree of fracturing or the hydraulic conductivity of the clay layer are not included into the Danish borehole database. Real data also shows a relationship between MCPP concentrations measured in drinking water and the distance between the stream and the well. This relationship is not seen in the model results possibly because the streams are placed in valleys, where surface runoff water from cultivated land will flow to riparian zones during rain events, enabling more contaminated water to infiltrate and reach groundwater. Thus, higher pesticide concentration will be found in wells close to surface water. Moreover, near surface water the water table is usually shallower, and the unsaturated aerobic zone is relatively thin or inexistent. Anaerobic conditions will then be predominant and MCPP degradation does not occur.

Implications for drinking water quality

Simulation results indicate that up to 7 % of stream MCPP pollution can reach the drinking water well in confined aquifers. Thus, the European Union drinking water limit for pesticides of 0.1 µg/L can be exceeded for stream MCPP concentrations above 1.5 µg/L. In the case of unconfined aquifers, when up to 10 % of stream MCPP can potentially reach the drinking water well, MCPP stream concentrations of 1 µg/L are enough to threaten drinking water quality. Such concentrations are common in agricultural streams, where non-sorbable, relatively persistent pesticides like MCPP, bentazone and dichlorprop have been regularly found with monthly average concentrations above 2 µg/L. Although pesticides are among the most frequently detected micropollutants in surface waters, emerging contaminants such as pharmaceutical residues and other household residues are gradually becoming a serious issue. Such compounds can affect human health even when present in very small concentrations. Moreover, some of these compounds are mobile and poorly degradable. Studies have shown that some of these substances can reach drinking water wells during bank filtration, when the connection between surface water and pumping well is desired end evident. Our work showed that it is likely that this will occur in confined aquifers too.

Figure 7.5-202: Results of MCPP concentrations plotted versus well depth (A) and distance between the stream and the pumping well (B) in unconfined aquifers



Model limitations

In the model considered here, constant concentrations over a long period of time are considered. In reality, high variability in pesticide stream concentrations are often observed, since pesticides are applied for a specific period of the year, and because most of pesticides fluxes are linked to soil flushing during rain events. In contrast, pesticides originating from landfill leachate plumes are more likely to have a constant effect on surface water if landfills are placed near streams or creeks. The influence of other parameters influencing the fate of pesticides in groundwater such as recharge, dispersion or degradation rates, where not assessed in the sensitivity analysis. Only parameters related to basic well geometry were considered because they are more easily linked to the available information on drinking water wells. Degradation rates values used in this study were close to the lower range of literature values and may lead to an overestimation of contaminant concentrations. On the other hand, the model does not consider the presence of preferential flow paths, which are known to play a major role in contaminant transport and can potentially lead to an

underestimation of pesticides concentrations at the drinking water well. The relative low complexity level of the model considered in this study makes it possible to consider the problem in a general way, neglecting site-specific conditions. More complex models would be physically more accurate, but more computationally expensive and include a higher number of parameters.

Conclusion

Results of the global sensitivity analysis showed that the characteristics of the clay aquitard (hydraulic conductivity and thickness) and well depth are the parameters governing the risk of contamination of drinking water wells by pollution in streams. Results also show that although it is unlikely that glyphosate in streams can pose a threat to drinking water wells, MCPP in surface water can pose a serious risk when pumping in confined and unconfined aquifers. Thus, the presence of confining clay aquitards may not prevent contamination of drinking water wells by persistent compounds in surface water. Comparison between simulation results and pesticides concentration data from the Danish National Boreholes Database showed a similar trend of decreasing MCPP concentrations with well depth. Real data also showed that wells located close to streams are more vulnerable to MCPP contamination, a result not simulated by the model. Some aspects of this study were limited due to computational constraints, therefore efforts should be made in future to enhance the model efficiency. Overall findings suggest that contamination of drinking water wells by pesticides in surface water is possible and may be a serious problem, especially for mobile and persistent compounds.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article reflects a computation model simulation for the contamination of drinking water wells with glyphosate and AMPA via filtration from surface waters. Generalized soil parameters were considered that reflect European agricultural soil characteristics. The derived results represents modelling results, no measured values.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/086
Report author	Ruel, S.M. et al.
Report year	2012
Report title	Occurrence and fate of relevant substances in wastewater treatment plants regarding Water Framework Directive and future legislations
Document No	Water Science & Technology/65.7/2012
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The next challenge of wastewater treatment is to reliably remove micropollutants at the microgram per litre range. During the present work more than 100 substances were analyzed through on-site mass balances over 19 municipal wastewater treatment lines. The most relevant substances according to their occurrence in raw wastewater, in treated wastewater and in sludge were identified, and their fate in wastewater treatment processes was assessed. About half of priority substances of Water Framework Directive (WFD) were found at concentrations higher than 0.1 µg/L in wastewater. For 26 substances, potential non-compliance with Environmental Quality Standard (EQS) of Water Framework Directive has been identified in treated wastewater, depending on river flow. Main concerns are for Cd, DEHP, diuron, alkylphenols, and chloroform. Emerging substances of particular concern are by-products, organic chemicals (e.g. triclosan, benzothiazole) and pharmaceuticals (e.g. ketoprofen, diclofenac, sulfamethoxazole, carbamazepine). About 80 % of the load of micropollutants was removed by conventional activated sludge plants, but about two-thirds of removed substances were mainly transferred to sludge. The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30 %.

Materials and methods

Substances studied and chemical analysis
In total, 117 substances have been selected: 45 substances with EQS and 72 other substances that were chosen according to their potential harmfulness, their reported occurrence and their expected resistance to treatments. The list includes 20 metals, and several organic substances like 4-nonylphenolethoxylates (mono- and di-ethoxylate), 4-nonylphenoxyacetic acid, aminomethylphosphoric acid (AMPA), triclosan, bisphenol A, 33 pharmaceuticals and five hormones.

Wastewater treatment plants selection and sampling
Overall, 19 WWTP treatment lines were studied, chosen as representative of various sizes (100 to 1,000,000 PE) and of various types of treatment processes:

- two primary treatments including primary settling, primary lamellar settling.
- 15 secondary treatments like activated sludge, fixed film processes like biofilter, trickling filter, biodisc, reed bed filter, one membrane bioreactor, one stabilisation pond.
- six tertiary treatment lines including sand filtration, activated carbon filter, ozone oxidation, reverse osmosis.

A total of seven plants were located in rural areas, and eight in urban areas. Half of the plants were equipped with combined sewer, and half with separate sewer. Sampling was performed in the influent and effluent during two or three successive 24 h-periods under dry weather flow conditions, with refrigerated samplers equipped with Teflon pipes and glass containers. Grab samples were collected for treated sludge. Strict procedures of cleaning, sampling, and field blanks were carried out.

Data processing and criteria for relevance determination
The results were described using:

- The frequency of quantification (Fq) and total concentration in influents, effluents and sludges.
- The specific daily average load received at WWTP (g/d/PE), calculated for each substance.
- The removal rate for different processes, with some calculation rules to take into account the variability of concentrations in raw wastewater and the analytical uncertainties associated with low concentrations of substances in complex matrices. If inlet concentration was not higher than 10 times the limit of quantification, removal efficiency was not calculated. Additionally, results were displayed as a removal efficiency range : 0-30 %, 30-70 % or 70-100 %.

The substances with the following criteria were pointed out: Fq>70 % in raw wastewater, removal rate

below 30 %, concentration >1 mg/kg DW (dry weight) in sludge. In treated wastewater the relevance of the substances was determined through the ratio between the effluent concentration (C) and the EQS (noted C/EQS). Three levels of relevance were defined: 'high level' for substances with Fq>70 % and C/EQS>1, 'medium level' for Fq>10 % or C/EQS>1, and 'low level' for Fq<10 % and C/EQS<1.

Results and Discussion

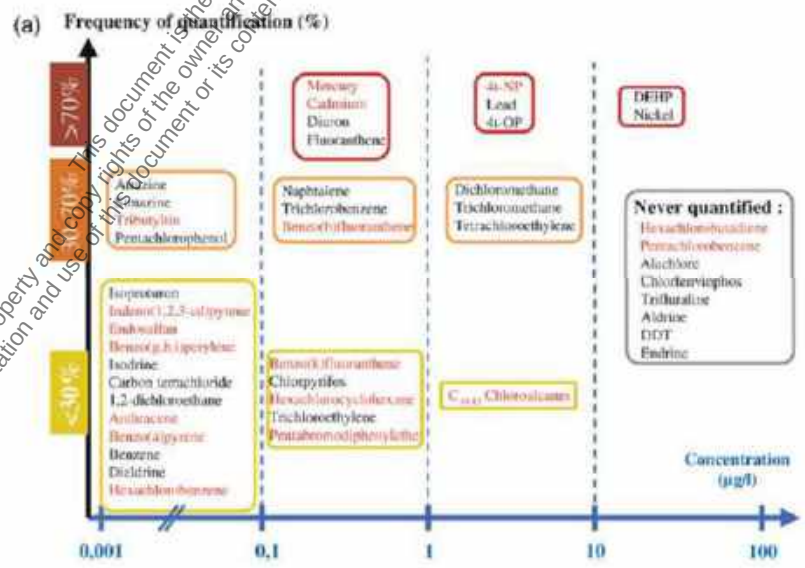
Relevant substances in raw wastewater

In raw wastewater, about half of the 45 substances with EQS and about 80 % of the 72 other studied substances were found at significant concentrations (>0.1 µg/L):

Substances with EQS (Figure 7.5-203):

- The highest Fq was found for DEHP (100 %), which also had the highest mean concentration (67 µg/L). Alkylphenols were also present at high concentration (4.3 µg/L for 4-t-OP; 9.7 µg/L for 4-NP) and very frequently quantified (81 % and 100 % for 4-t-OP and for 4-NP, respectively). Light PAHs (naphthalene and fluoranthene) were frequently found in raw wastewater (59 and 81 %, respectively), with a mean concentration higher than 0.1 µg/L.
- VOCs dichloromethane, trichloromethane and tetrachloroethylene combined a high mean concentration (1.4-2.9 µg/L), with a medium frequency of quantification (30-70 %).
- Among pesticides, diuron was the most frequently quantified (81 %) with a mean concentration of 0.25 µg/L. Atrazine and simazine were found in about half of the samples of wastewater, but their mean concentration was much lower (0.02 µg/L).
- All priority metals were systematically quantified, but with different mean concentrations: 10.6 µg/L for nickel, 5.7 µg/L for lead, 0.36 µg/L for mercury and 0.21 µg/L for cadmium.
- Are also worth mentioning the high mean concentration of C10-C13 chloroalkanes (5.5 µg/L) in the six samples where they were quantified (Fq of 20 %), and the relatively high Fq for trichlorobenzene (47 %) and pentachlorophenol (34 %) with concentrations close to 0.1 µg/L.
- Eight substances with EQS were never quantified, either because their use is now prohibited (pesticides alachlor, aldrine, DDT, endrine, chlorfenvinphos, trifluraline), or because their use is very specialised (e.g., hexachlorobutadiene, pentachlorobenzene).

Figure 7.5-203: Frequency of quantification (%) and mean concentration (µg/L) in domestic raw wastewater (15 WWTP, 32 samples) of substances with EQS (a), other organic substances (b) and metals (c)



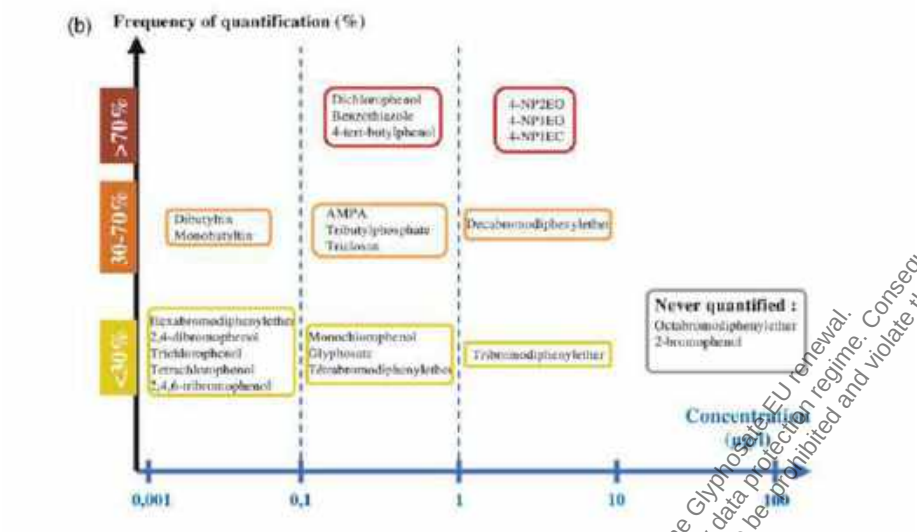
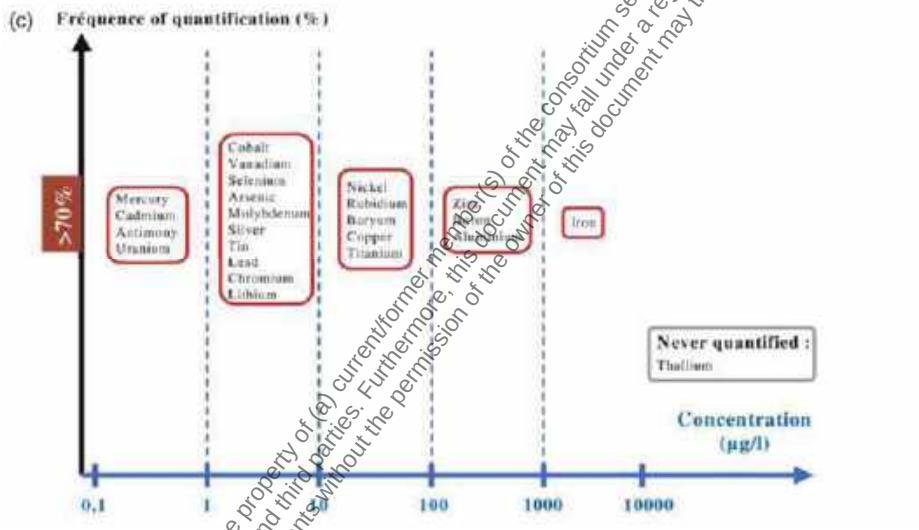


Figure 7.5-203 – continued



Other organic substances (Figure 7.5-203):

- Alkylphenol ethoxylates and carboxylates (4-NP1EO, 4-NP2EO and 4-NP1EC) were systematically quantified at mean concentrations between 2.1 and 6.1 µg/L, which is the same level as priority substances 4-NP and 4-t-OP.
- Benzothiazole (100 %), 4-tert-butylphenol (81 %), dichlorophenol (78 %), tributylphosphate (66 %) and **AMPA (53 %)** were frequently quantified, with mean concentrations between 0.1 and 1 µg/L.
- Very high concentrations of triclosan (up to 49 µg/L) and flame retardants (deca- and tri bromodiphenyl ether: 1.6-2.6 µg/L) were found in some samples (30 %).

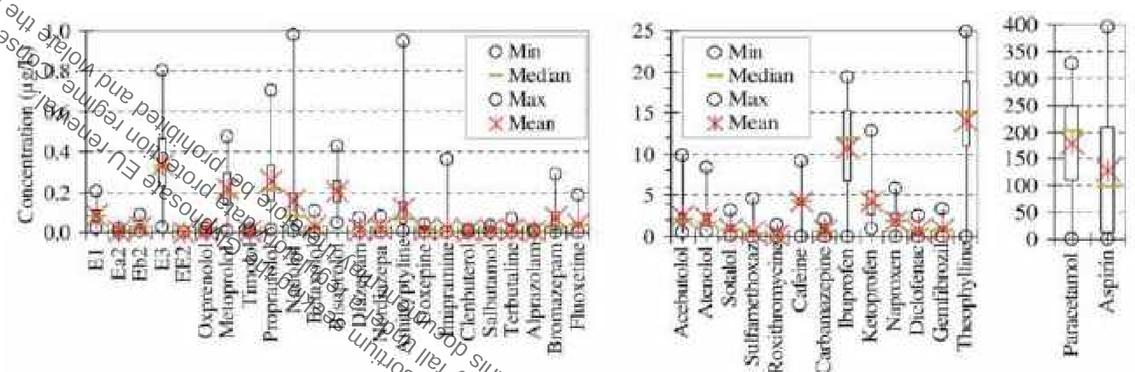
Metals (Figure 7.5-203):

- Metals were systematically quantified, except Ag and Se (occurrence of 70 %).
- Mean concentration for Cd, Hg, Sb and U were between 0.1 and 1 µg/L; mean concentrations of other metals were higher than 1 µg/L, except for Fe, Al, B and Zn for which the mean concentrations were >100 µg/L.

<u>Hormones and pharmaceuticals (Figure 7.5-204):</u>

- Oestrone (E1), 17β-estradiol (Eb2) and estriol (E3) were systematically quantified in raw wastewater. Mean concentrations of E1 and Eb2 were lower than 0.1 µg/L, while mean concentrations of estriol reached 0.34 µg/L.
- A majority of pharmaceuticals were very frequently quantified in raw wastewater (Fq >80 %). Paracetamol and aspirin presented the highest mean concentrations (>100 µg/L). Acebutolol, atenolol, sotalol, sulfamethoxazole, roxithromycine, caffeine theophylline, carbamazepine, ibuprofen, ketoprofen, naproxene, diclofenac and gemfibrozil presented mean concentrations between 0.1 and 10 µg/L. The other pharmaceuticals were never quantified above 1 µg/L.

Figure 7.5-204: Box-plot diagram of concentrations of hormones and pharmaceuticals in domestic raw wastewater (15 WWTP, 32 samples)



Sources of micropollutants can be very variable for each WWTP depending on the period of the year (summer/winter), the location (rural/urban), and the type of activities connected (hospitals, industries). Due to their industrial origin, some substances are quantified at higher concentrations in urban networks (with respect to rural ones): alkylphenols (except 4-NP1EC), VOCs (dichloromethane, trichloromethane trichloroethylene, tetrachloroethylene), chloroalkanes, dichlorophenol, bisphenol A, Ni, Cr and Ag. The concentration of alkylphenol polyethoxylates (adjuvants of detergents in textile industries, additives in paper industries) is two to three times higher in urban WWTPs; these compounds are responsible for the release of alkylphenols (4-NP and 4-t-OP) by biodegradation. **Glyphosate** is more frequently used as a herbicide in urban environments. Comparing the total load of micropollutants in raw wastewater to the load of micropollutants in treated wastewater in WWTPs with conventional activated sludge process, a reduction of about 80 % was observed.

Fate of relevant substances in biological treatments

Three main removal mechanisms in WWTPs need to be considered: biodegradation, adsorption on sludge flocs and stripping to gas phase. The fate of micropollutants in biological treatments will mainly depend on their physicochemical properties. The dissolved phase of the substances in raw wastewater provides a first indication on their propensity to remain in wastewater or to be transferred to sludge. For many substances, the values calculated present a significant variability due to the variability of suspended solids (SS) concentration and to the variability of the volatile suspended solids (VSS) content among the wastewaters tested. Main results are the following:

- Hydrophobic substances (e.g. « heavy » PAH, PBDE, chloroalkanes) were only quantified in particulate phase; DEHP, 4-NP and 4-t-OP, quantified in almost all samples, presented a mean fdiss close to 50 %, meaning that they should be relevant at the wastewater outlet and at the sludge outlet. The transformation product 4- NP1EC was mainly present in dissolved phase (f_{diss} 60 %).
- Most pesticides are hydrophylic (log K_{ow}<3) and were mainly quantified in dissolved phase.
- Metals distribution varied according to their physicochemical properties: f_{diss} >70 % for B, Li, Rb and Mo; f_{diss} <30 % for Zn, Cd, Ag, Ti, Cr, Fe, Pb, Cu, Sn, Al and Hg.

- More than 90 % of hormones were generally found in dissolved phase, in agreement with literature values. Most of the pharmaceuticals were also mainly present in the dissolved phase as most of them are hydrophilic. However, some of them were more evenly distributed, in link with their lower log K_{ow} values. Substances with f_{diss} >70 % (e.g. paracetamol, carbamazepine) have a log K_{ow} ranging between -0.39 and 2.87. At the opposite, f_{diss} of amitriptyline, doxepine and fluoxetine is about 50 % and their log K_{ow} is between 3.99 and 4.95.

Table 7.5-253 provides a classification of the different types of substances addressed, depending on the range of removal efficiencies observed in low load activated sludge process (data from 5 WWTPs). More than 30 % removal efficiency was calculated for about 70 % of the substances quantified in inlet raw water, and more than 70 % removal efficiency for about 50 % of the substances quantified:

- Removal rates were calculated for 23 substances with EQS. More than half of them were removed to more than 70 % due to hydrophobic properties (DEHP, 4-NP, 4-t-OP, heavy PAH, PBDE, chloroalkanes) or volatile properties (chloroform, dichloromethane). Four priority substances (diuron, isoproturon, atrazine, simazine), with hydrophilic properties (log K_{ow}<3) and slow biodegradability (half-life constant >40 days), were found in treated wastewater without significant removal within WWTP.
- Other organic substances were mainly removed from water by adsorption, except for **glyphosate**, **AMPA** and 4-NP1EC that are hydrophilic and not biodegradable. Moreover, alkylphenol carboxylates are produced during biological oxidation of alkylphenol ethoxylates and **AMPA** is a degradation product of glyphosate or detergents, which may increase their concentration at WWTP outlet.
- Metals were distributed among the three ranges of removal rates: 11 metals were efficiently removed, particularly the ones adsorbed onto suspended solids of raw wastewater (Ag, Ti, Cr, Fe, Pb, Cu, Sn, Al, Hg), and also Zn and Cd; seven metals were not removed, in particular B, Li, Rb quantified in the dissolved phase of raw wastewater.
- Hormones were all removed by biotransformation. More than one-third of the studied pharmaceuticals were well removed from water (>70 %) by both adsorption and biotransformation (caffeine, ibuprofen, theophylline, aspirin and paracetamol). One-third is hydrophilic and hardly biodegradable (e.g. carbamazepine, diclofenac), therefore refractory to biological treatments.

Significant differences of removal efficiencies have been measured between different biological treatment processes. Results of Table 7.5-253 should therefore be modulated for each biological process considered.

Table 7.5-253: Fate of substances through low load activated sludge plant (n = 5)

Low load conventional activated sludge	Removal efficiency range <30%	30-70%	>70%
Priority substances with Environmental Quality Standard	Diuron Isoproturon Atrazine Simazine	Nickel Cadmium Naphtalene Chlorpyrifos Trichlorobenzene	Lead Mercury Fluoranthene Benzo(b)fluoranthene Benzo(k)fluoranthene C10-13 Chloroalkanes Pentabromodiphenyl ether DEHP Dichloromethane Chloroform Tetrachloroethylene Trichloroethylene Nonylphenols Octylphenols
Other relevant organic substances	Glyphosate AMPA NP1EC	Mono-, di- chlorophenols Bisphenol A	Triclosan Tri- tetra-, bromodiphenyl ether Hexa- deca-, bromodiphenyl ether Benzothiazole 4-Tertbutylphenol Tributylphosphates NP1EO, NP2EO
Metals	Li, B, V, Co, As, Rb, Sb	Ni, Zn, Se, Cd, Ba, U, Mo	Al, Cr, Fe, Cu, Ag, Sn, Hg, Ti, Pb
Hormones			Estrone (E1) 17α-Estradiol (Ea2) 17β-Estradiol (Eb2) Estriol (E3)
Pharmaceuticals	Carbamazepine Diazepam Nordiazepam Doxepine Oxprenolol Propranolol Sotalol Diclofenac Salbutamol Terbutaline	Sulfamethoxazole Roxithromycine Metoprolol Timolol Atenolol Amitriptyline Fluoxetine	Nadolol Betaxolol Bisoprolol Acebutolol Imipramine Bromazepam Gemfibrozil Bromazepam Ibuprofen Paracetamol Aspirin Ketoprofen Naproxen

Relevant substances in sludge

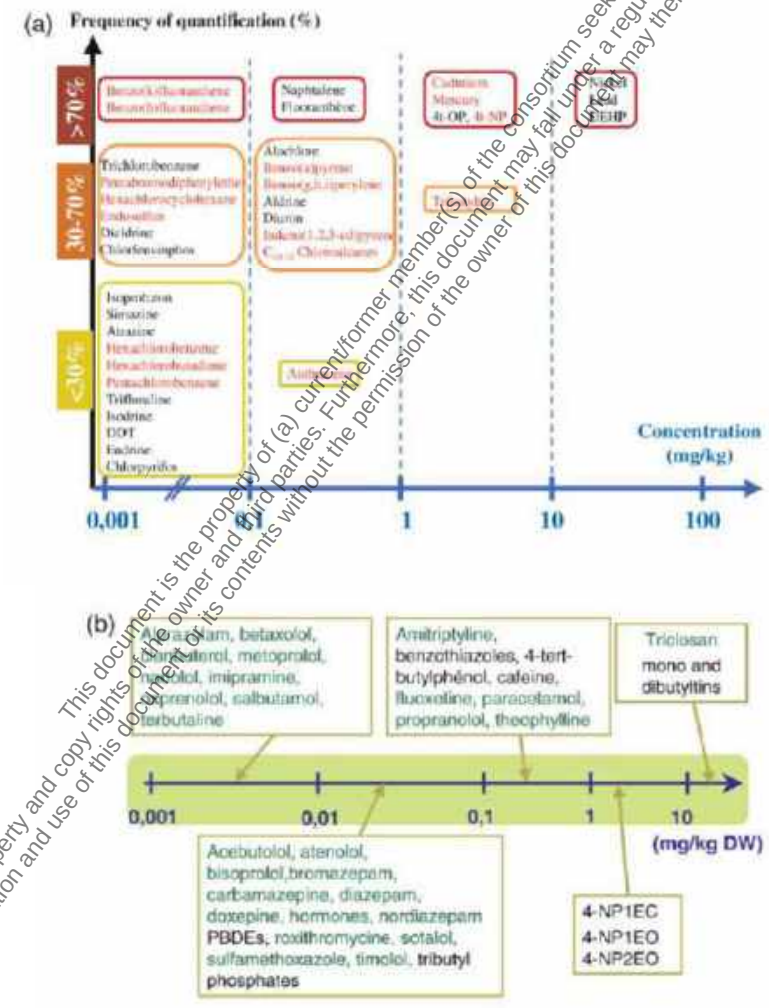
In biological treatment processes, the major removal mechanism was transfer to sludge for about two-thirds of the substances. Twenty-one substances with EQS were frequently measured in sludge, and about 35 % of substances with EQS and 65 % of the others substances were found at concentrations higher than 100 mg/kg DW. All the substances quantified in raw wastewater were also measured in secondary sludge. Nevertheless, the concentrations of PAH (fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene) and metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) were always below the threshold limits for agricultural landspreading in France:

- Some substances with EQS were always quantified in sludge, with concentration levels of <1 mg/kg DW (PAH except anthracene), 1 to 10 mg/kg DW (alkylphenols, Cd, Hg), or >10 mg/kg DW (DEHP, Ni, Pb). Hydrophilic substances (e.g. pesticides) were hardly quantified (<30 %) with low concentrations (<0.1 mg/kg). Several substances with EQS that were never quantified in raw wastewater were sometimes quantified (i.e., hexachlorobutadiene, pentachlorobenzene, chlorfenvinphos, alachlor, DDT).
- Tributyltin (priority substance) was frequently found at a concentration higher than 1 mg/kg, and its degradation products (mono- and di-butyltin) were found at more than 10 mg/kg.
- Other organic hydrophobic substances were often quantified (>70 %) due to high adsorption. Different ranges of concentration were observed: <0.1 mg/kg DW (tributylphosphate),

0.1-1 mg/kg DW (4-tert-butylphenol, benzothiazole), 1-10 mg/kg (4-NP2EO, 4-NP1EO), >10 mg/kg DW (4-NP1EC).

- Metals were quantified in all the samples at concentrations above 1 mg/kg DW, up to 10 or 100 mg/kg DW (Zn,Cu,Ti).
- Two pharmaceuticals (acebutolol, propranolol) were always quantified in sludge, at concentration levels of 0.078 and 0.126 mg/kg DW respectively, due to their high concentration in raw wastewater. Oestrone (E1), carbamazepine and amitriptyline were quantified in 70-90 % of the samples at a concentration of 0.029, 0.075 and 0.195 mg/g DW, respectively. Caffeine, ibuprofen and fluoxetine were quantified in 67 % of the samples at levels of 0.245, 0.245 and 0.104 mg/g DW. Other pharmaceuticals were quantified in less than half of the samples at low concentration levels (<0.1 mg/kg DW), except for aspirin and ketoprofene for which the concentration were 7.9 and 3.8 mg/kg DW respectively, due to high concentrations in raw wastewater (Figure 7.5-205).

Figure 7.5-205: Frequency of quantification (%) and sludge concentration range (mg/kg DW) for substances with EQS (a), and sludge concentration range (mg/kg DW) of other organic relevant substances (b) (17 WWTP, 17 samples)



Relevant substances in treated wastewater

In treated water released by biological treatments, 30 % of substances with EQS and 60 % of other

substances were still quantified at concentrations higher than 0.1 µg/L. Even if a significant decrease of the concentrations was observed through the WWTP, some concentrations higher than 1 µg/L still prevailed for metals (among them Ni had a mean concentration of 5.6 µg/L), DEHP (mean concentration of 4.6 µg/L) and two by-products (4-NP1EC and AMPA with mean concentrations of 2.3 and 3.1 µg/L respectively).

Twenty-six substances with EQS may be a problem regarding the objectives of the WFD (Table 7.5-254):

- Four pesticides (diuron, isoproturon, atrazine, simazine) were classified as medium or high level due to their high Fq and to their poor removal in WWTP. Diuron appeared as the most relevant one as it was frequently quantified at concentrations above the EQS.
- Eight substances were found in almost all samples, sometimes with concentrations above EQS, due to their high concentration in raw wastewater, despite good removal efficiencies in WWTP: four metals, DEHP, two alkylphenols and chloroform.
- Nine substances less frequently found, but with some samples above EQS, due to their high concentration in raw wastewater or to low EQS values: three PAH (fluoranthene, anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene), of four pesticides/biocides (chlorpyrifos, endosulfan, hexachlorocyclohexane, tributyltin), and pentabromodiphenyl ether.
- Five substances were found at low concentrations below EQS, but were quantified in more than 10 % of the samples. A medium risk of reaching EQS was then estimated, as their frequent presence increases the possibility of overcoming EQS. This was the case of naphthalene, pentachlorophenol, trichlorobenzene, dichloromethane and tetrachloroethylene.

It should be noted that for 17 substances (in brackets in Table 7.5-254) current analytical methods do not give reliable results for concentration levels as defined by EQS. According to the QA/QC Directive (2009/90/EC), the criterion for considering a method to be valid for WFD is a limit of quantification equal to or less than one-third of the EQS. Therefore, their emission must be completely eliminated. It should be noted that EQS have been defined for concentration compliance in receiving bodies, not for WWTP effluents.

Indeed, most of the non-regulated substances quantified in raw wastewater were also frequently measured at significant concentrations in treated wastewater. Special concern is related to 4-NP1EC (alkylphenol carboxylate) and AMPA, with higher concentrations at the outlet than at the inlet of WWTP. 4-NP1EC is formed by aerobic degradation of alkylphenols, and AMPA can result from the degradation of glyphosate or from phosphoric acid present in detergent.

Table 7.5-254: Frequency of quantification (Fq) and concentration of substances in treated wastewater released by activated sludge plants (n = 5)

Relevance treated wastewater: frequency & concentration	Priority substances (WFD): Priority substances (to be reduced)	Priority substances (WFD): Hazardous priority substances (to be stopped)	Additional substances with EQS
Metals	Nickel Lead	Cadmium Mercury	
PAH	Fluoranthene Naphtalene	Anthracene (Benzo(a)pyrene) (Benzo(b)fluoranthene) (Benzo(g,h,i)perylene) (Benzo(k)fluoranthene) (Indeno(1,2,3-cd)pyrene)	
Pesticides	Alachlor Chlorfenvinphos Chlorpyrifos Diuron Isoproturon Atrazine (Trifluraline) Simazine Pentachlorophenol	(Hexachlorocyclohexane) (Endosulfan) (Tributyltin)	(Aldrin) DDT (Dieldrin) (Endrin) (Isodrin)
Industry	Benzene Trichlorobenzene DEHP	Hexachlorobutadiene (C10-13 Chloroalkanes) (Pentachlorobenzene) (Hexachlorobenzene) (Pentabromodiphenylether)	
Solvents and surfactants	Dichloroethane Dichloromethane Chloroform Octylphenol	Nonylphenol	Carbon tetrachloride Tetrachloroethylene Trichloroethylene

Dark grey: high frequency >10% and C/EQS>1); light grey: medium frequency >10% or C/EQS>1); no shading: low (frequency <10% and C/EQS<1); (...): analytical limitation: LQ>1/3 x EQS

Conclusion

Relevant substances

- About half of substances with EQS were found at concentrations >0.1 µg/L in wastewater.
- Main loads of micropollutants were identified in raw wastewater: metals >pharmaceuticals >DEHP >alkylphenols > VOCs >other organics.
- For 26 substances with EQS, potential non-compliance with EQS of WFD has been identified in treated wastewater. Main concerns are for Cd, DEHP, diuron, alkylphenols and chloroform.
- Emerging substances of particular concern are by-products (AMPA, NP1EC), other chemicals (triclosan, benzothiazole, chlorophenols, PBDEs) and some pharmaceuticals [analgesics (e.g. ketoprofen, diclofenac), beta-blockers (e.g. sotalol), antibiotics (e.g. sulfamethoxazole), antidepressants (e.g. carbamazepine)].

Fate in WWTPs

- About 80 % of the load of micropollutants are removed by conventional activated sludge plants.
- More than half of substances with EQS were removed to more than 70 % due to hydrophobic or volatile properties. Other organic substances (with no EQS) are mainly removed from water by adsorption. Hormones and more than one third of the studied pharmaceuticals are well removed from water (>70 %) by both adsorption and biotransformation.
- About two-thirds of removed substances were mainly transferred to sludge. All the substances quantified in raw wastewater were also measured in secondary sludge.
- Tertiary treatments may be applied to complete the removal of micropollutants, but this implies additional cost (up to þ 100 % for reverse osmosis) and potential by-products and concentrates (advanced oxidation processes, activated carbon).

- The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30 %.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the occurrence of glyphosate and AMPA among other substances in different wastewater treatment plants in France. The removal rate for glyphosate and AMPA observed in low load activated sludge process (data from five waste water treatment plants) was <30 %.
The analytical methods are poorly described.

The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/027
Report author	Bruchet, A. *et al*.
Report year	2011
Report title	Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge
Document No	European Journal of Water Quality 42 (2011) 123-133
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at an officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the groundwater monitoring subchapter of this document.

1. Information on the study

Data point:	CA 7.5/063
Report author	Litz, N.T. et al.
Report year	2011
Report title	Comparative studies on the retardation and reduction of glyphosate during subsurface passage
Document No	Water research (2011), Vol. 45, No. 10, pp. 3047-54
Guidelines followed in study	None (for filter experiments)
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities (German UBA, German KompetenzZentrum Wasser)
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water subchapter.

1. Information on the study

Data point:	CA 7.5/087
Report author	Ruel, S.M. et al.
Report year	2011
Report title	On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications
Document No	Water Science & Technology 63.11/2011
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted by officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary

The next challenge of wastewater treatment is to reliably remove micro-pollutants at the microgram per litre range in order to meet reuse applications and contribute to reach good status for water bodies. A hundred priority and relevant emerging substances were measured to evaluate at full-scale the removal efficiencies of seven advanced treatment lines (one membrane bioreactor process and six tertiary treatment lines) that were designed for reuse applications. To reliably compare the processes, specific procedures for micro-pollutants were applied for sampling, analysis and calculation of removal efficiencies. The membrane bioreactor process allowed to upgrade the removal efficiencies of about 20 % of the substances measured, especially those that were partially degraded during conventional processes. Conventional tertiary processes like high rate clarification, sand filtration and polishing pond achieved significant removal for some micro-pollutants, especially for adsorbable substances. Advanced tertiary processes, like ozonation, activated carbon and reverse osmosis were all very efficient to complete the removal of polar pesticides and pharmaceuticals; metals and less polar substances were better retained by reverse osmosis. For glyphosate and AMPA, removal rates were reported as being 30 – 70 % for glyphosate and AMPA for

sand filtration, <30 % for AMPA for reverse osmosis and ozone treatment, but >70 % for glyphosate for reverse osmosis and ozone treatment; >70 % for both glyphosate and AMPA for activated carbon filtration.

Table 7.5-255: List of priority and emerging substances studied

	Priority pollutants (WFD) from EC (2008)			
	Priority pollutants	Hazardous priority pollutants	Other dangerous pollutants	Other substances
Metals	Ni, Pb	Cd, Hg		Li, V, Sb, B, Rb, Co, As, Mo, Zn, Ba, Se, U, Ti, Fe, Cu, Cr, Sn, Al, Ag
PAH		Anthracene, benzo(g,h,i) perylene		
	Naphthalene	Fluoranthene		
		Benzo(a)-, indeno(1,2,3-cd)-pyrene		
		Benzo(b)-, benzo(k)-fluoranthene		
Pesticides	Chlorfenvinphos, chlorpyrifos, pentachlorophenol	Hexachlorocyclohexane, endosulfan, tributyltin	Aldrin, DDT, dieldrin, endrin, isodrin	Triclosan mono-, di-, tri-, tetra-chlorophenols
	Diuron			Glyphosate, AMPA
	Alachlor, isoproturon, atrazine, trifluralin, simazine			Bromophenol, mono-, di-butyltin
Industry/solvents/ additives	Di-, tri-chloromethane, DEHP			Tri-, tetra-, hexa-bromodiphenylether
	Dichloroethane, chloroform, benzene		Carbon tetrachloride	Bisphenol A
				Octa-, deca-bromodiphenylether
	4-t-OP (octylphenol)	4-NP (nonylphenol)	Di- tetra-chloroethylene	4-NP1EO and 4-NP2EO (nonylphenol polyethoxylates)
	Trichlorobenzene			4-NP1EC (alkylphenol polyethoxyacids), benzothiazole, 4-terthutylphenol, tributylphosphate
Pharmaceuticals				
Antibiotics				Sulfamethoxazole and Roxitromicin
Hypolipemiants				Gemfibrozil
Bronchodilatants				Clenbuterol, salbutamol, terbutalin
Analgesics				Ibuprofen, paracetamol, aspirin, diclofenac, ketoprofen, naproxen
Antidepressants				Diazepam, nordiazepam, doxepine, alprazolam
				Amitriptyline, imipramine, bromazepam, carbamazepin
Betablockers				Acetobutolol, atenolol, sotalol, metoprolol, propanolol, nadolol, bisoprolol
				Oxprenolol, timolol, betaxolol
Others				Caffeine, theophylline
Hormones				Oestrone, 17a-, 17b-oestradiol, oestriol, ethinylestradiol

Data from (Martin Ruel et al. submitted; Choubert et al. 2011; Gabet-Giraud et al. 2010).
Cinfluent < 0.1 µg/L; light grey shaded 0.1 < Cinfluent < 1 µg/L; dark grey shaded Cinfluent > 1 µg/L.
DDT dichlorodiphenyltrichloroethane; DEHP diethyl hexyl phthalate; PAH, polycyclic aromatic hydrocarbon.

Materials and methods

Micro-pollutants studied

The list of the 33 priority pollutants of the Water Framework Directive (WFD) was considered in this study together with the eight additional pollutants for which an environmental quality standard (EQS) has been defined. Additional substances have been chosen according to their potential harmfulness and their reported occurrence in waters based on French national inventories on dangerous and priority pollutants (see Table 7.5-255). Pharmaceutical compounds (emerging substances) were chosen considering their consumption and their occurrence in wastewater and surface water. A total of 127 micro-pollutants has been selected (including glyphosate and AMPA) but only 100 were quantified at least once in treated wastewaters of activated sludge.

Chemical analysis techniques

Various analytical methods were developed and applied to quantify the selected micro-pollutants (Table 7.5-256). Volatile pollutants were analysed in raw samples. For others, the dissolved phases were analysed due to low suspended solids concentrations (<5 mg/L). Limits of quantification (LoQ) are provided for the dissolved phase. The conventional parameters have also been analysed (total organic carbon, total nitrogen, total phosphorus) to determine if the operating conditions were correct.

Table 7.5-256: Analytical methods applied

Group of micro-pollutants	Extraction	Chromatographic analysis	LoQ in dissolved phase (µg/L)	Reference
Semi-volatile organics (Multiresidue)	Liquid-liquid + florisil	GC-MS-MS	0.010–0.050	Internal protocol
Volatile organic compounds (VOC)	Purge & trap	GC-MS	0.1	ISO 15680 (2004)
Chlorophenols	SPME	GC-MS	0.050–0.150[a]	Internal protocol
Pesticides-antibiotics	SPE	UPLC-MS-MS	0.001–0.002[a]	Internal protocol
Glyphosate/AMPA	SPE	HPLC-MS-MS	0.1	ISO 21458 (2009)
Chloroalkanes	SBSE	TD-GC-ECD	0.5	Internal protocol
PBDEs/bisphenol A	SBSE	TD-GC-MS	0.001–0.1[a]	Internal protocol
Alkylphenols + ethoxylates	SPE	LC-ESI-MS	0.01	Internal protocol
Betablockers, hormones, analgesics, broncho-dilatants, hypolipemiants, antidepressants	SPE	LC-MS-MS UPLC-MS-MS GC-MS	0.0005–0.002	Miège *et al.* 2009b Togola & Budzinski 2008
Mercury		AFS	0.0005	U.S. EPA (2002)
Metals and metalloids	–	ICP-MS	0.01–5	ISO 17294-2 (2005)

AFS, atomic fluorescence spectrometry; ECD, electron capture detector; ESI, electrospray ionisation; GC, gas chromatography; HPLC, high performance liquid chromatography; ICP-MS, inductively coupled plasma – mass spectrometry; LC, liquid chromatography; MS, mass spectrometry; SPE, solid phase extraction; SPME, solid phase microextraction; TD, thermal desorption; UPLC, ultraperformance liquid chromatography.

Wastewater treatment plants (WWTP) selection and sampling

Seven WWTP of various sizes were studied (Table 7.5-257), which included various types of treatment: one full-scale membrane bioreactor (MBR); five full-scale conventional tertiary treatments, including high rate clarification, sand filtration or polishing pond; two advanced tertiary treatments at full-scale (ozonation and micro-filtration (MF) + reverse osmosis (RO)) and two advanced tertiary treatments at pilot-scale (activated carbon filtration and silex filtration + ultrafiltration + RO). The upstream treatment stages achieved both carbon and nitrogen removal to meet regulatory requirements. Influent and effluents of the studied processes were collected under dry weather flow conditions during two successive 24 h or 2 h periods (see Table 7.5-257). Automatic refrigerated samplers, equipped with Teflon pipes and glass containers, were used. Strict procedures of cleaning, sampling and field blanks were carried out. An ISCO bubble flowmeter was used to measure the flow released when a Venturi canal was available at the facility.

Table 7.5-257: Characteristics and operating conditions of the studied process

	Code size (PE)	Upstream treatment (carbon + nitrogen removal)	Process type	Operating conditions	Sampling period of composite sample
MBR	A – 24,000	–	MBR Ultrafor (4 Zenon ZWD500 modules, 10,000 m^2 of membrane surface)	F/M = 0.06 kg BOD_5/kg VSS/d, SRT = 17 d, T = 24 °C, membrane flux = 25 L/h/m^2	2 * 24 h
Tertiary processes	B – 300,000	AS	High rate clarification	Fast settling: 30 mg/L $Al_2(SO_4)_3$, 0.8 mg/L polyelectrolyte, lamellar velocity = 3.2 m/h	2 * 2 h
			+ sand filter	sand filter: velocity = 20 m/h	
	C – 97,000	AS	Sand filter	Sand filter: 3.6 m/h;	2 * 2 h
			+ ozone contact zone	ozone dosage = 10 mg O_3/L, contact time = 40 min	
	D – 25,000	AS	Sand filter	Sand filter: 3.5 m/h	2 * 2 h
			+ MF (84 modules, 1,350 m^2, hollow polypropylene fibres)	MF: 80 L/m^2/h, 1.0 bar	
			+ RO (90 modules filmtec BW30-365 polyamide)	RO: 20 L/m^2/h, 22 bar	
	E – 500 (pilot)	AS	Silex filter	Filter: 7.4 m/h	2 * 2 h
			+ UF (Norit X-flow, membrane 8" SXL-225 FSFC Aquaflex, polyestersulfone)	UF: 50 L/m^2/h, 2 bar	
			+ RO (Trisep, three membranes 8" 8040-X201-TSA, polyamide urea)	RO: 22 L/m^2/h, 8 bar	
	F – 470,000	AS	High rate clarification (full-scale)	Dosage: 70 mg/L $FeCl_3$, 1 mg/L polyelectrolyte, 1 g/L sand dosage, lamellar velocity = 6 m/h;	2 * 2 h
			+ activated carbon filter (pilot) (Filtrasorb-400, height = 1.4 m)	activated carbon: velocity 0.33 m/h, contact time = 2.1 h	
	G – 1,000	Biodisc + reedbed filter	Polishing pond (three tanks, total surface: 2,570 m^2)	Contact time = 15 d	2 * 24 h

AS: Low load activated sludge process (SRT: 15–25 days); MBR: membrane bioreactor; PE: population equivalent; O_3: ozone contact zone; MF: microfiltration; UF: ultrafiltration; RO: reverse osmosis; F/M: loading rate (kg BOD_5/kg VSS/d); VSS: volatile suspended solids; SRT: sludge retention time; T: temperature.

Data processing

Mass balances were performed based on wastewater flow and micro-pollutant concentration data at the inlet and at the outlet of the studied processes. The removal efficiencies (R) were calculated with the following rules to obtain robust information:

– High and low levels of concentration were defined for each substance with respect to the LoQ. Low confidence level was for concentrations between LoQ and 2.5-5 times the LoQ (depending on the substance). High confidence level was for concentrations higher than 2.5-5 times the LoQ, depending on the substance. From analytical practice, at low confidence level, an analytical uncertainty in the range of 50-100 % is a regular value for most substances whereas an analytical uncertainty below 30 % is usual a high confidence level.

– When both inlet and outlet concentrations were lower than the LoQ or within the low level, the removal efficiency value was not calculated.

– When only one concentration, either inlet or outlet concentration, was lower than the LoQ, a value equal to half of the LoQ was adopted and the removal efficiency was calculated.

In addition to these criteria, removal efficiency data was displayed as a removal range (<30 %, 30-70 % and >70 %), since the analytical uncertainty and the variability of the concentrations related to micro-pollutants in wastewater do not allow to certify precise values.

Table 7.5-258: Concentrations (C, µg/L) in the effluent of the conventional AS process, in the effluent of MBR process and in the effluent of each type of tertiary process, and removal efficiency ranges (R, %) for tertiary processes

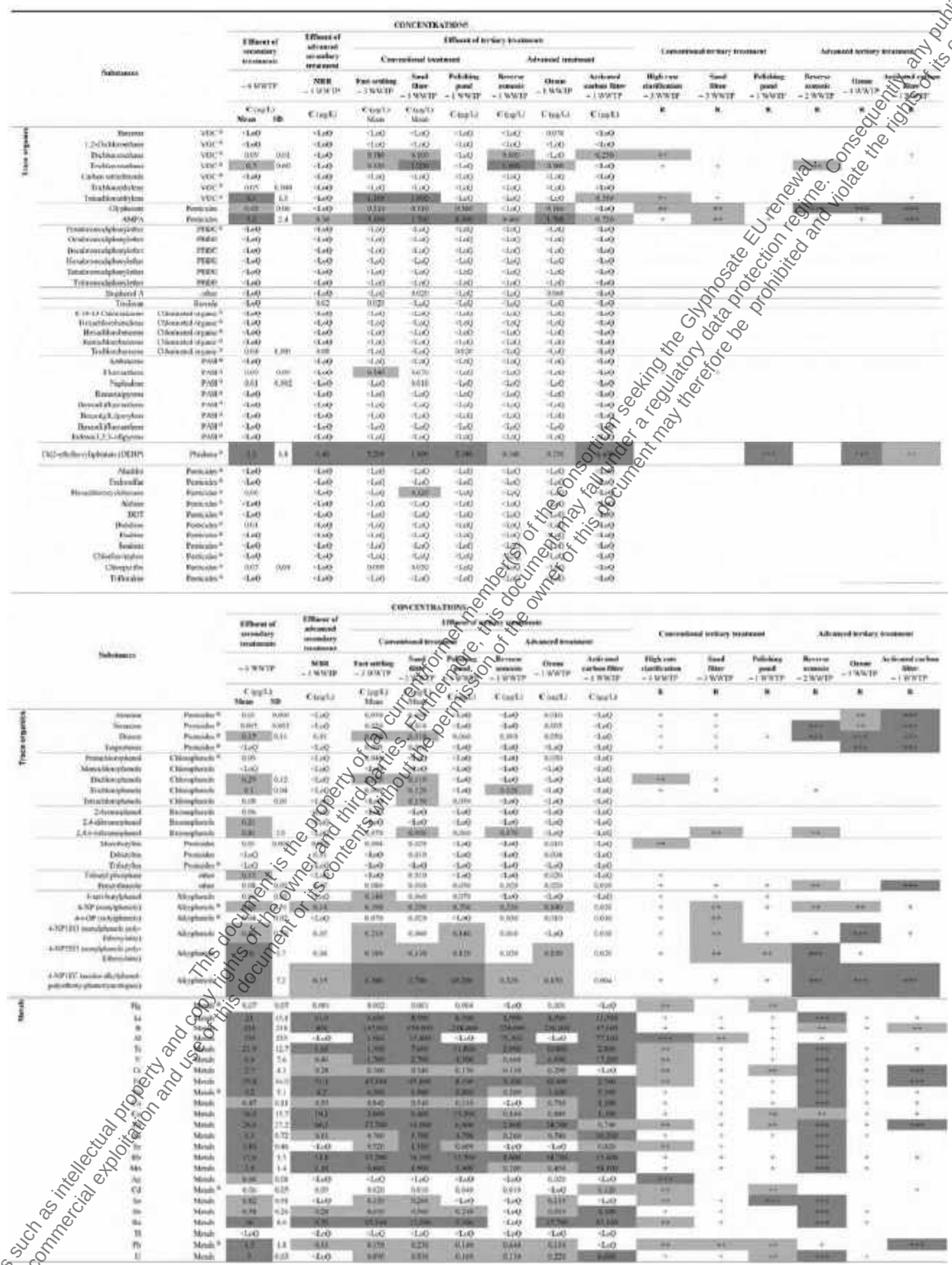


Table 7.5-258 – continued

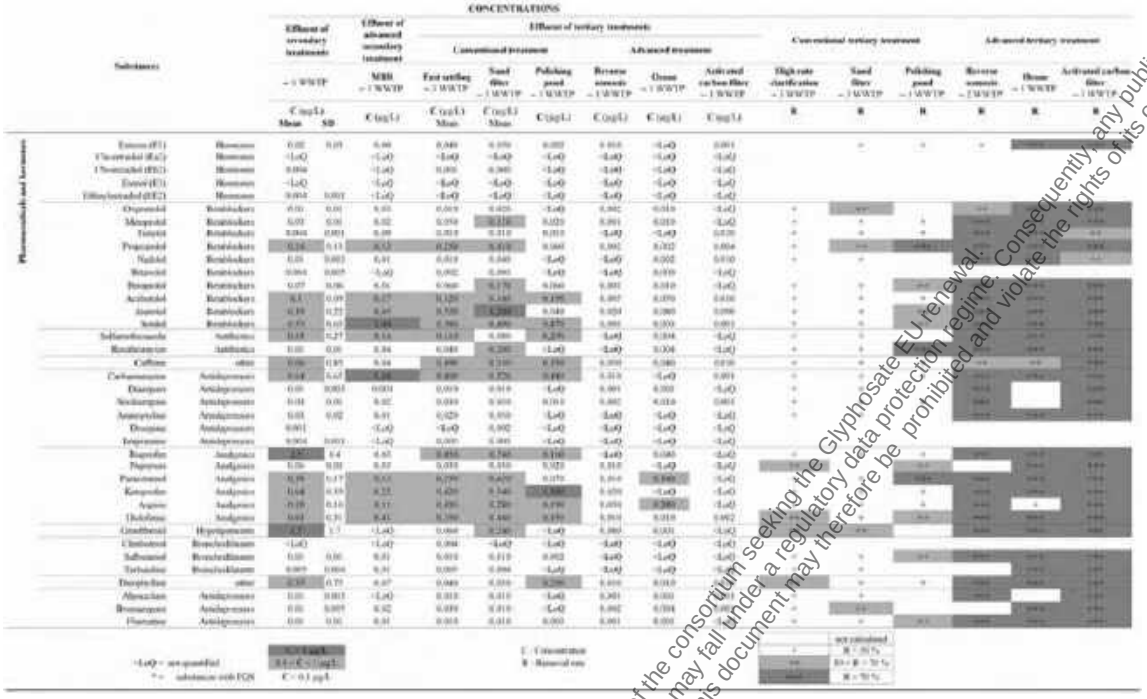


Table 7.5-259: Numbers of substances quantified in effluents of conventional AS process, MBR process, conventional and advanced tertiary processes (calculated from data of Table 7.5-258, total 127 substances)

	Effluent of secondary treatments		Effluent of tertiary treatments						
			Conventional treatment			Advanced treatment			
Parameter	Conventional treatment: AS – six WWTP	Advanced treatment: MBR – one WWTP	Fast settling – three WWTP	Sand filter – three WWTP	Polishing pond – one WWTP	Reverse osmosis – one WWTP	Ozone – one WWTP	Activated carbon filter – one WWTP
Number of quantified substances (C > LoQ)	88	58	81	85	54	51	61	42
Number of non-quantified substances (C < LoQ)	39	69	46	42	73	76	66	85
Number of substances with C > 1 µg/L	23	13	16	15	16	8	10	16
Number of substances with C > 0.1 µg/L	48	30	46	49	35	22	26	22
Total	127	127	127	127	127	127	127	127

Results and Discussion

Table 7.5-258 presents, for each micro-pollutant, the mean concentration (C) in the effluents of the six conventional secondary stages (activated sludge) upstream of the tertiary treatment lines studied, in the effluent of the MBR and in the effluent of each type of tertiary process (conventional and advanced). The removal efficiency ranges (R) of the tertiary processes are also presented. In addition, Table 7.5-259

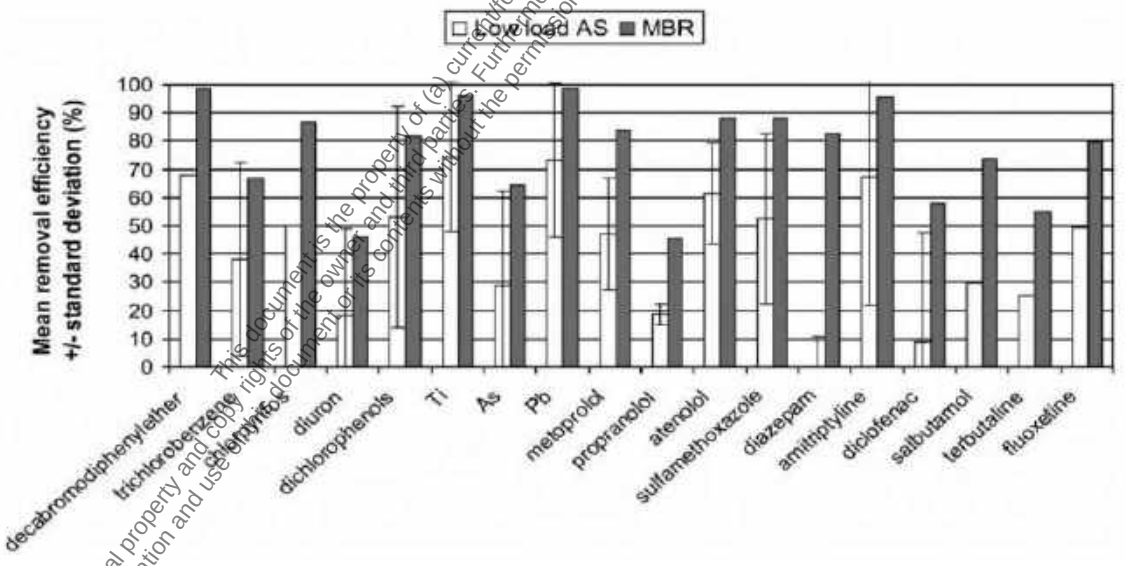
summarises the numbers of substances quantified in effluents of these processes in order to achieve a better comparability.

Efficiency of the MBR process

In the treated water of MBR process, the concentrations were below the LoQ for 69 micro-pollutants (39 were non quantified for low load activated sludge (AS)) as shown in Table 7.5-259; 30 micro-pollutants were measured at concentrations higher than 0.1 µg/L, instead of 48 micro-pollutants for the effluents of AS; and 13 micro-pollutants were measured at concentrations higher than 1 µg/L like DEHP, some metals and two pharmaceuticals sotalol, carbamazepine) (23 for AS). Compared to the effluent of AS, lower concentrations were measured for adsorbable micro-pollutants like decabromodiphenylether, Pb, Hg, 4-NP. These trends suggest a higher level of micro-pollutant retention with MBR compared to AS process. In addition, removal efficiencies at the MBR plant were calculated and compared to the mean removal efficiencies from six low load activated sludge plants, obtained with the same methodology for sampling, analysis and data processing. For 18 substances, removal efficiencies of the MBR were significantly higher than individual values obtained with the AS plants (more than 20 % difference compared to the mean values of AS or above the upper limit of the confidence interval). This suggests a potential improvement of removal efficiency for specific compounds that should be confirmed by other studies. The substances concerned are trichloromethane, naphthalene, chlorpyrifos, AMPA, diuron, sulfamethoxazole, ibuprofen, alprazolam, amitriptyline, and several betablockers (Figure 7.5-206).

Some studies have already shown higher removal for MBR for a limited selection of micro-pollutants referring to the effect of higher sludge retention time. But at almost similar SRT as AS, and with a 20 % higher sludge concentration (5 g mixed liquor total suspended solids/L in MBR instead of 2-4 g/L in AS), the specific bacterial population and the presence of exopolysaccharides in the biological tank of the MBR process may favour adsorption and biodegradation processes.

Figure 7.5-206: Comparison of removal efficiencies of the MBR process with removal efficiencies from low load conventional AS process



Efficiency of conventional tertiary processes

When applying high rate clarification or sand filter to a secondary effluent, the number of quantified micro-pollutants was only slightly reduced from 88 to 81-84 micro-pollutants in the effluents (depending on the plant). The number of quantified micro-pollutants was reduced from 60 to 54 between the inlet and the outlet of the polishing pond (this process was located in a rural area where less micro-pollutants were quantified). Depending on the process, 35 to 49 micro-pollutants are still present at concentration levels

>0.1 µg/L. Between 16 and 19 micro-pollutants were quantified at concentrations higher than 1 µg/L. Three priority pollutants were found at concentrations exceeding the EQS in tertiary treated water (DEHP, 4-NP, chlorpyrifos), which could be a matter of concern when the flow of the receiving body is very low. Differences of removal efficiencies have been measured between the studied conventional tertiary treatment processes. With fast settling tank, removal efficiencies higher than 70 % were measured for two metals (Ag and Al), while 30-70 % removal was calculated for several metals (Zn, Ti, Cr, Pb, Cd and Hg), organic compounds (glyphosate, diclofenac, naproxen, aspirin, gemfibrozil and dichlorophenols) and VOCs (tetrachloroethylene, dichloromethane). Removal efficiencies below 30 % were measured for all other micro-pollutants, in particular for pharmaceuticals and for polar pesticides. For priority pollutants, similar results were recently shown for one fast chemical settler. Through the sand filtration stage, a removal efficiency between 30-70 % was measured for alkylphenols (4-NP, 4-t-OP and ethoxylates), glyphosate/AMPA and some betablockers, whereas high rate clarification had removal efficiencies below 30 % for these substances. With the polishing pond process, removal efficencies lower than 30 % were measured for most micro-pollutants except for some compounds like DEHP, paracetamol, roxithromicin and some betablockers (with removal efficiency higher than 70 %); and bisoprolol, nadolol, sotalol, naproxen, diclofenac, salbutamol and fluoxetine, that were removed with removal efficiencies between 30 and 70 %. In this case, photodegradation and high hydraulic retention time could be the main removal factors.

Efficiency of advanced tertiary processes

The number of quantified micro-pollutants in the effluent of tertiary treatment was reduced from 88 to 42-61 depending on the process. As many as 13 micro-pollutants were never quantified in the effluents of all types of advanced tertiary treatments: chlorobenzene, di-chlorophenols, tetra-chlorophenols, bromophenols, dibromophenols, naphthalene, trichlorobenzene, hexachlorocyclohexane, pesticides (chlorpyrifos, dieldrin), pharmaceuticals (doxepine) and hormones (17β-oestradiol, ethinyl-estradiol). Depending on the process, 22 to 27 micro-pollutants were relevant (concentration levels >0.1 µg/L), that is 50 % less than for AS. Eight to 16 micro-pollutants were quantified with concentrations higher than 1 µg/L. Only DEHP was found at concentrations close to the EQS. Removal efficiencies higher than 70 % were measured for 40-45 micro-pollutants for reverse osmosis and activated carbon filtration. For ozonation, due to low efficiencies of treatment on metals, 31 micro-pollutants were removed at R> 70 %. Ozone oxidation allowed high removal for DEHP (75 %) with double bonds accessible to ozone and hydroxyl radicals, but was not efficient for metals or alkylphenols, confirming previous studies. Reverse osmosis led to the retention of an extended range of micro-pollutants (especially metals and VOCs). DEHP was not retained by reverse osmosis or activated carbon filtration in this study. However, these results should be considered with care since the concentration levels of DEHP in tertiary processes were close to the analytical blanks. Except for metals and VOCs, the activated carbon filtration proved to retain a comparable number of micro-pollutants to reverse osmosis, but with slightly lower removal efficiencies. With the activated carbon filtration AMPA was well removed. For all of these treatments, several pesticides (diuron, simazine, glyphosate) were removed with efficiencies higher than 90 %, and almost 100 % for most pharmaceuticals (including refractory betablockers).

Conclusion

From on-site investigations carried out on seven wastewater treatment plants, the removal efficiencies of conventional and advanced tertiary processes have been assessed for 100 micro-pollutants quantified in secondary effluents.

– Ultrafiltration membrane in biological processes (MBR) could improve removal efficiency for some micro-pollutants in addition to disinfection capacities and suspended solids retention. This is an additional advantage when reuse of wastewater is expected.

– Conventional tertiary processes like fast tertiary settling and sand filtration can already achieve significant (30-70 %) removal for adsorbable micro-pollutants and could therefore be considered as a first complement to the activated sludge process.

– Advanced tertiary processes, like ozone oxidation, activated carbon filtration and reverse osmosis filtration, are efficient to complete the removal of polar pesticides and pharmaceuticals. Reverse osmosis provides a removal of a wider range of micro-pollutants, including metals and less polar organic micro-pollutants, that were not retained by other processes. However, it is also the most expensive technology and the fate of the concentrate should be mastered to get a sustainable process. Ozone oxidation is the less expensive technology but the fate and toxicity of by-products still remains an issue to be investigated. Activated carbon filtration appears as an interesting alternative, but the reliability and the life duration of adsorbing material needs to be further investigated.

– Removal rates for glyphosate and AMPA were: 30 – 70 % for glyphosate and AMPA for sand filtration, <30 % for AMPA for reverse osmosis and ozone treatment, but >70 % for glyphosate for reverse osmosis and ozone treatment; >70 % for both glyphosate and AMPA for activated carbon filtration.

The choice of the most appropriate technology should be made by matching the affordable cost in relation to water quality objectives, either to preserve the receiving water bodies or to secure the reuse of treated wastewater.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the efficiency of different wastewater treatment processes to remove glyphosate and AMPA among other substances from wastewater for reuse application. Different processes are described and their specific efficiency is reported. Removal rates for glyphosate and AMPA were: 30 – 70 % for glyphosate and AMPA for sand filtration, <30 % for AMPA for reverse osmosis and ozone treatment, but >70 % for glyphosate for reverse osmosis and ozone treatment; >70 % for both glyphosate and AMPA for activated carbon filtration.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	KCA 7.5/088
Report author	Schoonenberg Kegel, F. *et al.*
Report year	2010
Report title	Reverse osmosis followed by activated carbon filtration for efficient removal of organic micropollutants from river bank filtrate
Document No	Water science and technology (2010) Vol. 61, No. 10, pp. 2603-10
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

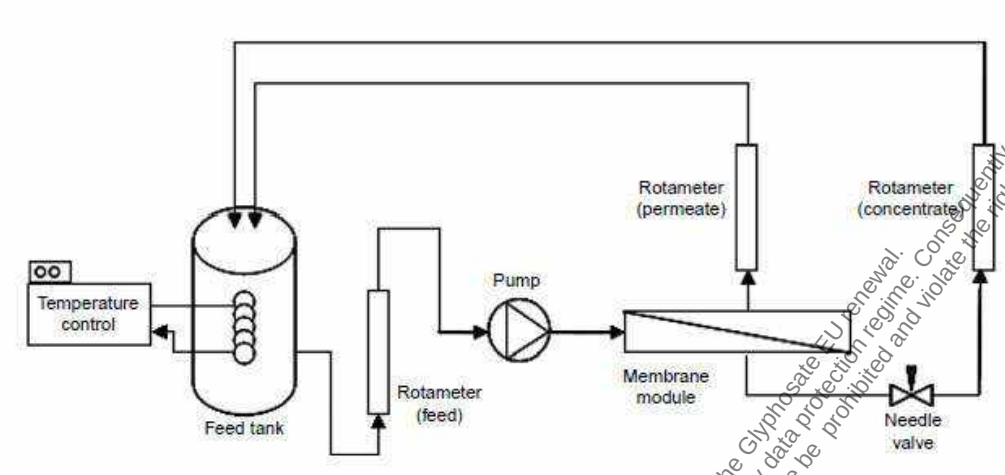
Drinking water utilities in Europe are faced with a growing presence of organic micropollutants in their water sources. The aim of this research was to assess the robustness of a drinking water treatment plant equipped with reverse osmosis and subsequent activated carbon filtration for the removal of these pollutants. The total removal efficiency of 47 organic micropollutants was investigated. Results indicated that removal of most organic micropollutants was high for all membranes tested. Some selected micropollutants were less efficiently removed (e.g. the small and polar NDMA and glyphosate, and the more hydrophobic ethylbenzene and napthalene). Very high removal efficiencies for almost all organic micropollutants by the subsequent activated carbon, fed with the permeate stream of the RO (reverse osmosis) element were observed except for the very small and polar NDMA and 1,4-dioxane. RO and subsequent activated carbon filtration are complementary and their combined application results in the removal of a large part of these emerging organic micropollutants. Based on these experiments it can be concluded that the robustness of a proposed treatment scheme for the drinking water treatment plant Engelse Werk is sufficiently guaranteed.

Materials and methods

Filtration equipment and protocol

The water used for the RO experiments was sampled after the pre-treatment of DWTP (Drinking Water Treatment Plant) Engelse Werk. For the activated carbon experiments, a batch of permeate was used as feed water. The 4-inch, single spiral wound membrane element filtration set-up and protocol for the RO experiments is schematically depicted in Figure 7.5-207. The feed water was fed from a 600 L stainless steel vessel. The feed solution was delivered to a pressure vessel, accommodating a single 4040-membrane element, by a pump. Applied transmembrane pressure was regulated using a needle valve in the concentrate stream, with transmembrane pressure measured with a precision manometer. Adsorption on test unit parts was ruled out, since no significant loss of solutes was observed when the water was recirculated over the installation without a membrane installed. All experiments were performed in a recycle mode with a single batch of water, with both permeate and concentrate recycled back into the feed reservoir. An immersed stainless-steel coil with cooling liquid fed from a cooling system was used to maintain a constant feed water temperature. Membrane filtration experiments were carried out at a constant cross-flow velocity of 0.2 m/s, which corresponds to a feed flow of 1,500 l/h and a concentration polarization factor of 1.07. The recovery is kept constant at approximately 10 %. Permeate flux and temperature were set to approximately 20 l/(m^2 h) and 20 ± 1 °C, respectively. Feed, permeate and concentrate samples were taken after 4 days of filtration and analyzed for organic micropollutants: 4 days was sufficient to reach adsorption equilibrium and ensure that steady-state rejection values were obtained. The granular activated carbon column was 1 m in height and had an inner diameter of 35 mm. The column contained approximately 0.7 L of carbon resulting in a filter bed depth of 0.7 m. The column was fed with a batch of RO permeate from a stainless steel tank. The hydraulic loading was set to 14 L/h resulting in an empty bed contact time of 3 min. Samples of feed and effluent of the column were taken after treatment of 1200 bed volumes to see whether break-through of some micropollutants could already be observed after this time period.

Figure 7.5-207: Membrane filtration set-up for rejection experiments



RO membranes and activated carbon

The membranes used in this study were all commercially available reverse osmosis membranes: Trisep X20 and ACM5 (Trisep Corp., Goleta CA, USA) and Hydranautics ESPA1 and ESPA4 (Nitto-Denko/Hydranautics, Oceanside CA, USA). All membranes are thin film composite membranes with an aromatic polyamide top layer. Before use, all membranes were rinsed with Milli-Q water for two hours in order to remove preservation liquids present in the membranes. Afterwards, the membranes were characterized for pure water permeability with Milli-Q water and for NaCl rejection with a 1,500 ppm NaCl solution in Milli-Q water. Membrane properties are summarized in Table 7.5-260. Membrane contact angles were determined using the sessile drop method. The Zeta potentials were measured in a background solution containing 10 mM NaCl and 1 mM $NaHCO_3$. Membranes with different membrane properties were chosen (e.g. pure water permeabilities), to be able to select the most suitable membrane for the application, based on both energy demand and organic micropollutant removal. The granular activated carbon was supplied by Norit Nederland B.V. (Amersfoort, the Netherlands). The extruded grade Norit Row Supra 0.8 was chosen based on its multi-purpose adsorption characteristics, low hydraulic resistance and high resistance to attrition during regeneration. The bed density of the carbon is 345 kg/m^3 and the raw carbon material is peat. Freshly regenerated activated carbon was used in the experiments, so no pre-loading of natural organic matter (NOM) or other organic pollutants was present on the carbon before the start of the experiments.

Selected organic pollutants and analysis

The spiked organic pollutants were selected for two main reasons. Firstly, some emerging micropollutants, already occurring in Dutch surface- and ground waters were chosen. Examples include glyphosate, carbendazim, bentazon and MTBE. Since the dosing tests were carried out for a drinking water utility, assessment of the removal of problematic substances from the source waters was a necessity. Secondly, other organic solutes were also dosed, and these were mainly selected for their different physico-chemical properties. In a previous publication (Verliefde et al. 2008), it was shown that solute charge, solute hydrophobicity and solute size may all have an influence on solute rejection by NF/RO treatment. Therefore, solutes were divided in different categories of increasing hydrophobicity (expressed as log K_{ow}). Within each category of hydrophobicity, different solutes were chosen with increasing size (expressed as molar mass). Moreover, some charged solutes (positively, as well as negatively charged) were included. All micropollutants were dosed in concentrations that were 200 times higher than the limit of quantitation (LOQ) of the respective analysis method for that pollutant. Thus 99.5 % removal could be quantified. The cocktail of organic micropollutants was prepared as a concentrated stock in 10 L of Milli-Q water. In order to prevent co-solvent effects and possible problems with biological growth in the system, no methanol was used to facilitate dissolution of the pharmaceuticals. For the RO rejection experiments, the desired volume

of this stock solution was then added to the feed tank, containing the Engelse Werk ground water. For the activated carbon experiments, the desired volume of the stock solution was added to a tank containing 750 L of RO permeate (the RO permeate did not contain any residuals of trace organic contaminants). Information on the analytical protocol can be found in (Sacher et al. 2001).

Table 7.5-260: Membrane properties for selected membranes for comparison of organic micropollutant rejection

Membrane	Pure water permeability (m/(s.bar))	Contact angle (°)	% NaCl rejection	Zeta-potential at pH 7 (mV)
Trisep ACM5	1.4×10^{-6}	35	98.5	−20
Trisep X20	9.9×10^{-7}	43	99.5	−10
Hydr. ESPA4	2.1×10^{-6}	70	99.0	n.d.
Hydr. ESPA1	1.5×10^{-6}	25	99.3	−22

Results and Discussion

Reverse osmosis rejection experiments

It was apparent that for almost all solutes rejection values were very high (95 %). However, some solutes showed low rejection. The low rejection of these solutes was consistent for all four membranes. Especially the removal of NDMA was low. This was probably due to the very small size and very compact structure of NDMA. The removal of the hydrophobic solutes ethylbenzene and naphtalene was very low, even though these solutes were larger than, for example, NDMA. This was probably due to hydrophobic interactions of these solutes with the hydrophobic membrane matrices, resulting in an increased partitioning of these solutes into the membranes and thus an increased transport through the membranes. Also the rejection value of glyphosate was lower than expected. Glyphosate is a very polar molecule that has several polar functional groups (positively, as well as negatively charged). At pH 7, there is a high positive charge density in the middle of the molecule, leading to a very high dipole moment (6.7 Debye) in the molecule and charge attraction towards the negatively charged membrane surface. Moreover, since glyphosate is a stretched molecule, steric hindrance is also lower and glyphosate permeates through the membrane quite easily. Both Hydranautics membranes showed lower rejection values for most solutes compared to the Trisep membranes. Comparing the performance of the two Trisep membranes, it was interesting to notice that rejection values were slightly higher for most solutes with the ACM5 membrane than with the X20 membrane, even though the ACM5 has a higher pure water permeability and a lower NaCl rejection (Table 7.5-260). The reason for this difference in organic solute rejection is probably the higher hydrophobicity of the X20 membrane (as shown in the contact angle measurements). The ACM5 membrane is more hydrophilic, which results in an increased transport of water and thus higher fluxes of this element at similar feed pressures, but also results in a decrease of hydrophobic interactions between hydrophobic solutes and the membrane matrix. This results in increased rejection values for hydrophobic solutes. Based on these results, it was decided that the ACM5 membrane will be applied in the new treatment scheme. All experimental rejection data for all solutes on the ACM5 membrane are shown in Table 7.5-261. The experimental rejection values were compared to predicted rejection values, using a QSAR (quantitative structure-activity relationship) model. The modelled rejection values predicted experimental rejection data quite well. As can be seen in Table 7.5-261, the rejection values of some solutes (e.g. dibutylphthalate) could not be determined. This was due to the low feed concentrations of these solutes on the fourth day of the experiments (when rejection is measured), probably due to volatilisation or adsorption of the solutes on the membrane polymer matrix. Adsorption on other test unit parts was ruled out, since almost all test parts were made out of stainless steel.

Based on the rejection values obtained on the single ACM5 membrane element in the laboratory-scale unit, some rough estimations were made for a full-scale installation, operating 80 % recovery. These rough estimations were based on a full-scale rejection model. It was apparent that the ACM5 element performs extremely well in organic micropollutant removal applications: except for NDMA, most problematic organic pollutants (e.g. the pesticides diglyme, triglyme, atrazine, metamitron, bentazon and glyphosate

and the pharmaceuticals phenazon, carbamazepine and ibuprofen) were expected to be removed for more than 90 %. However, 90 % removal is still not complete removal, and a subsequent activated carbon filtration step might still be necessary.

Activated carbon adsorption experiments

The removal of the selected organic pollutants after treatment of 1,200 bed volumes (carbon was freshly regenerated before use) on the ACF column is also summarized in Table 7.5-261. It was apparent that, even with the short contact times used, removal of most micropollutants was high (> 95 %). However, removal of some pollutants, such as NDMA; 1,4-dioxane and 2-methylisoborneol (2-MIB) was more problematic. For NDMA and 1,4-dioxane, this low removal could be expected, due the hydrophilic character of these substances. Moreover, NDMA experienced significant competition from the other organic pollutants because it was dosed in extremely low concentrations (200 ng/l). For 2-MIB, no breakthrough of the solute through the column was expected. 2-MIB is very hydrophobic, and since ACF adsorption mainly occurs through hydrophobic van der Waals interactions, a high removal was expected. Especially the small size of the molecule should make it easy for this molecule to diffuse into the small micropores of the carbon, where it should adsorb readily. Maybe the contact time of 3 min was not enough to allow this pore-diffusion for 2-MIB. Also, log K_{ow} is apparently not always the most suitable parameter to describe adsorption interactions. This is because log K_{ow} measures the differences in interactions of a solute in a water phase and an octanol phase, and octanol does not represent the carbon surface very well. Despite the low removal for NDMA; 1,4-dioxane and 2-MIB, no breakthrough of any other substance through the column was observed. This was partly due to the freshly regenerated carbon, which should have a high adsorption capacity anyway. However, 1,200 bed volumes have already been treated, so the carbon capacity would already be lower than for freshly regenerated carbon. The removal capacity of the carbon was also high, because of the removal of NOM which would normally compete with the organic micropollutants for adsorption sites on the carbon, in the reverse osmosis step. This NOM removal not only diminishes the competition between NOM and the micro-pollutants for adsorption sites on the activated carbon, but also reduces the carbon pore blocking by large NOM molecules. As a consequence short empty bed contact times can be used for the ACF, or the time before regeneration of the column can be extended. This reduces investment costs for the ACF considerably. Compared with full stream RO treatment, split stream RO treatment will result in an increased preloading with NOM of the ACF. Nevertheless split stream RO treatment decrease the NOM preloading of the ACF significant.

Reverse osmosis and subsequent activated carbon filtration

The combined removal efficiency for organic micropollutants of split treatment with the ACM5 membrane and the subsequent activated carbon filter was calculated. The results are also shown in Table 7.5-261. The calculation was based on a RO installation equipped with the ACM 5 membrane, operating at a recovery of 80 % on a by-pass stream of 50 % of the total feed stream. Removal efficiency for all solutes was extremely high, except for the smallest hydrophilic solutes (NDMA and 1,4-dioxane). Fortunately, these two pollutants are absent in the raw water of DWTP Engelse Werk. Moreover, results of ongoing research suggests that small, hydrophilic solutes are preferentially removed by biological degradation in processes such as river bank or dune filtration. The combination river bank filtration RO – ACF would thus be able to remove almost all organic micropollutants. Therefore, we do expect that the proposed treatment scheme can remove these substances if they would be present in the river IJssel.

Table 7.5-261: Solute physico-chemical characteristics, initial feed concentrations, experimental rejection by the ACM5 membrane, experimental removal efficiency by ACF filtration and calculated values for the rejection at 80 % recovery and for the combination of RO (by-pass 50 %) and subsequent ACF

	Properties			Experimental data		Calculated data	
Compound	MW (g/mol)	log K_{ow} (–)	Initial feed concentration (µg/L)	RO Recovery ~ 10%	ACF	RO Recovery ~ 80%	RO (by-pass 50%) subsequent ACF
NDMA	74	−0.57	0.2	74	21	60	45
1,4-dioxane	88	−0.27	2,000	96	18	92	56
NMOR	116	−0.44	0.2	99	99	97	99
Diglyme	134	−0.36	30	99	n.d.	97	n.d.
Glyphosate	169	−4.00	10	90	>99	82	>99
Triglyme	178	−0.76	50	99	n.d.	97	n.d.
Caffeine	194	−0.07	10	99	>99	97	>99
TBA	74	0.35	20,000	99	n.d.	97	n.d.
MTBE	88	0.94	10	>99	>99	>97	>99
Phenazon	188	0.38	2	>99	>99	>97	>99
Metamitron	202	0.83	10	>99	>99	>99	>99
Terbutaline	225	0.90	2	>97	>98	>95	>99
Sulfamethoxazol	253	0.89	2	>99	>99	>97	>99
Sotalol	272	0.24	2	>98	>99	>95	>99
Pentoxifylline	278	0.29	2	>99	>99	>97	>99
ETBE	102	1.92	10	n.d.	>99	n.d.	n.d.
TAME	102	1.92	20	>97	>99	>93	>99
2,4-dinitrophenol	184	1.67	20	99	>95	99	99
Carbendazim	191	1.52	5	99	99	97	99
Monuron	199	1.94	10	99	>99	97	>99
Metribuzin	214	1.70	10	99	>99	97	>99
Metoxuron	229	1.64	10	99	>99	97	>99
Pirimicarb	238	1.70	10	99	>99	97	>99
Bisphenol-S	250	1.65	10	99	>99	97	>99
Metoprolol	267	1.88	2	>99	>99	>99	>99
TCEP	285	1.44	10	n.d.	n.d.	n.d.	n.d.
Benzene	78	2.13	10	88	>99	79	>99
Isoproturon	206	2.87	10	99	>99	97	>99
Chlorotoluron	213	2.51	10	99	>99	97	>99
Atrazine	216	2.61	10	99	>99	97	>99
Diethylphthalate	222	2.42	100	>99	n.d.	>99	n.d.
Diuron	233	2.68	10	n.d.	>99	n.d.	n.d.
Carbamazepine	236	2.45	2	99	>99	97	>99
Bentazon	240	2.34	10	>99	>99	>99	>99
Metobromuron	259	2.38	10	99	>99	97	>99
Dimethenamid	256	2.15	5	99	>99	97	>99
Ethylbenzene	106	3.15	40	18	n.d.	9	n.d.
Naphthalene	128	3.30	50	68	n.d.	53	n.d.

Table 7.5-261 – continued

Compound	Properties MW (g/mol)	log K_{ow} (–)	Initial feed concentration (µg/L)	Experimental data RO Recovery = 10%	ACF	Calculated data RO Recovery = 80%	RO (by-pass 50%) subsequent ACF
2-MIB	168	3.31	50	99	97	97	99
Ibuprofen	206	3.97	2	>99	>99	>97	>99
Mecoprop (MCPP)	215	3.13	10	>99	>99	>97	>99
Bisphenol-A	228	3.32	2	99	>99	100	>99
Linuron	249	3.20	10	>99	>99	>97	>99
Estrone	270	3.13	2	99	>99	100	>99
Dibutylphthalate	278	4.50	100	n.d.	n.d.	n.d.	n.d.
Diclofenac	296	4.51	2	>99	>99	>97	>99
Bezafibrate	361	4.25	2	>99	>99	>97	>99

n.d.: not determined.

Conclusion

The capability of RO and ACF to remove emerging organic pollutants was studied. RO offers very high rejection values for almost all solutes. Lower rejection values are observed for hydrophilic solutes with a negative log K_{ow} value and a low molecular weight, and for relatively low molecular weight hydrophobic solutes. The removal efficiency of the solutes by activated carbon filtration, even with the low contact times used, is extremely high as well. Still, removal of some pollutants, such as NDMA; 1,4-dioxane and 2-methylisoborneol (2-MIB) is more problematic. RO and subsequent activated carbon filtration are complementary and their combination results in removal of a large part of the emerging organic micropollutants, since almost the whole range of solute hydrophobicity is covered. The barrier against micropollutants in the treatment scheme of DWTP Engelse Werk is based on a split stream treatment with reverse osmosis, followed by full-stream activated carbon filtration. The results of this research will be used as input for the analysis program that Vitens performs to monitor the water quality of the river IJssel. The removal capabilities of this treatment scheme are very high for almost all organic micropollutants dosed in this study, except for the smallest hydrophilic solutes (NDMA and 1,4-dioxane). Fortunately, these two pollutants are absent in the raw water of Engelse Werk. The robustness of the treatment scheme is therefore sufficiently guaranteed. Moreover, there is an opportunity to upgrade the scheme by introducing full-stream RO treatment.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes the removal of glyphosate among other substances from drinking water by reverse osmosis followed by activated carbon filtration. The substance properties and analytical methods are insufficiently described. The examined method focus on conservative filtration methods, no degradation products or processes are described.

The study is therefore classified as reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/072
Report author	Peschka, M. *et al.*
Report year	2006
Report title	Trends in pesticide transport into the River Rhine
Document No	Hdb Env Chem Vol. 5, Part L (2006): 155–175 DOI 10.1007/698_5_016
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in the surface water subchapter.

E.2 Chemical water treatment

Applicant studies

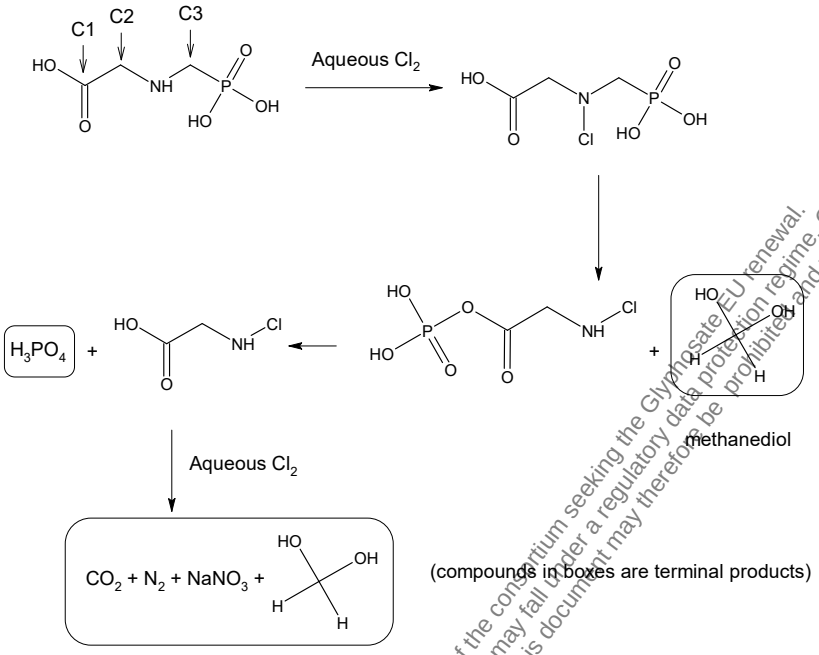
There is one Monsanto (Bayer) commissioned study ([redacted] 2010, CA 7.5/081) which addresses the fate of glyphosate and AMPA when subjected to water treatment chemical processes. This contains a review, and some original work on removal rates when glyphosate and AMPA are subjected to ozone, chlorine, and chlorine dioxide. The same material has also been presented in a peer reviewed publication (Jönsson *et al.*, 2013, CA 7.5/084), and the relevant findings are summarised below. Neither of these report in detail on the transformation products of glyphosate and AMPA when subjected to water treatment processes.

Other Information

Of the literature sources available the following are specifically considered, with respect to Chemical Water Treatment:

The mechanism of chlorination (when treated with aqueous chlorine) of glyphosate has been investigated exhaustively and reported in two linked publications (Mehrsheikh *et al.*, 2006, CA 7.5/095; Brosillon *et al.*, 2006, CA 7.5/094). Using stable isotopes and NMR spectroscopy to identify species generated when glyphosate and glycine are separately treated with aqueous chlorine, it was possible to generate a proposed route of degradation for glyphosate (Figure 7.5-208):

Figure 7.5-208: Route of transformation of glyphosate when subjected to chlorination (from Mehrsheikh *et al.*, 2006, CA 7.5/095)



Glyphosate is totally degraded to small molecules common to the degradation of naturally occurring substances in raw water (e.g. amino acids), and the degradation pathway follows that of glycine. The C1 carboxylic acid carbon of glyphosate/glycine is converted to CO_2; the C2 methylene carbon is converted to CO_2 and methanediol; the nitrogen is transformed into nitrogen gas and nitrate; the C3 phosphonomethylene carbon is converted to methanediol; and the phosphorus moiety produces phosphoric acid. Kinetic models were constructed that allowed the temporal course of the reactions to be simulated; these predicted that under conditions similar to those found in water treatment plants, the chlorination of glyphosate is complete within seconds of contact with chlorine.

The very rapid reaction of glyphosate with aqueous chlorine was confirmed in the investigations reported in (Jönsson *et al.*, 2013, CA 7.5/084). In this work, incubation was for only 30 minutes, and at 20 °C degradation of glyphosate reached 96-10 0 %; although degradation was less complete at a lower temperature (71 % at 5 °C). AMPA degraded faster than glyphosate, >99 % at all temperatures. The investigations indicated that chlorine dioxide is a less effective degrader of glyphosate (17-93 %, 30 minutes, various temperatures/pH values) than aqueous chlorine, and an effective degrader of AMPA (>99 % under all conditions tested).

Another approach to disinfection of drinking water sources is ozonation/ozonolysis, where ozone (O_3) is used to deactivate viruses, bacteria and some parasites. The operation of such processes in the context of treating surface water from three French rivers (Marne, Seine and Oise) to provide drinking water to 4 million people in the Paris region has been reported (Boucherie *et al.*, 2010, CA 7.5/092). A pilot plant was utilised for the investigations: glyphosate was found to be very rapidly degraded by ozone treatment (>91 %, levels reduced to <0.1 µg/L) and AMPA was rapidly removed (>88 %, levels reduced to <0.1 µg/L); hence, the ozone treatment required to deliver disinfection targets was also effective in removing glyphosate and AMPA to levels below 0.1 µg/L. The use of ozone to degrade glyphosate and AMPA was also investigated in a batch reactor (Assalin *et al.*, 2010, CA 7.5/091). In these studies, it was clear that the pH of the test solution altered the reactivity of glyphosate and AMPA to ozonation. It was evident that AMPA was produced from glyphosate at all pH's. For glyphosate, at alkaline pH (pH 10) degradation was very rapid and AMPA was also completely degraded (but more slowly); indeed, total carbon content

removal was measured to be 97.5 %, indicating that transformation products were also completely degraded. At acidic pH's (pH 6.5) glyphosate was 80 % removed, with a build up of AMPA, which didn't appear to be degraded under these conditions.

A thorough investigation of the process of ozonation of glyphosate was reported in Shen *et al.* (2011, CA 7.5/089), using batch, semi-continuous tests. It was found that with an initial glyphosate concentration of 5 mg/L, and an ozone concentration of 1.5 mg/L, glyphosate was completely degraded (LOD 0.1 mg/L) within 25 minutes. With an initial pH of 4.9, an initial glyphosate concentration was reduced to <LOD within 25 minutes, and at pH 6.8, was reduced to <LOD within 20 minutes. At a pH of 9.3, the time required to reduce glyphosate to <LOD was 15 minutes. It was demonstrated that as glyphosate was degraded by the oxidation reactions, the amount of AMPA increased, and then AMPA also decreased, and phosphate gradually increased. Indeed, the TOC (total organic carbon) content was degraded by 77.65 % after 30 minutes (when glyphosate had been reduced to <LOD), and further reduced to 93.53 % after 60 minutes of reaction time. Investigation of the presence of intermediates allowed glycolic acid, glycine, phosphoric acid and AMPA to be identified. Under the conditions investigated, it was clear that degradation of glyphosate when subjected to ozonation was rapidly degraded first to a range of intermediates which were in turn subsequently completely degraded.

Partial information on the route of degradation of glyphosate and AMPA, when subjected to ozonation, comes from (Klinger *et al.*, 2008, CA 7.5/096). The ozonation of a phosponate complexation agent was investigated, and it was found that this produced glyphosate and AMPA. Consequently, ozonation studies were also conducted on glyphosate and AMPA – at acidic pH (pH 5) it was found that glyphosate was partially degraded to AMPA and orthophosphate; and that AMPA was partially degraded to orthophosphate, under the experimental conditions.

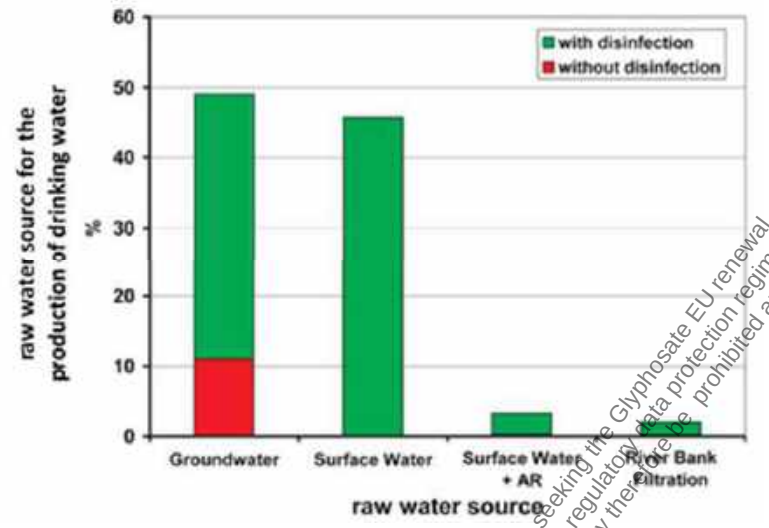
An investigation was reported of the removal rates associated with various stages found across seven Waste Water Treatment Plants, including one ozone treatment module (Ruel *et al.*, 2011, CA 7.5/087). For this ozone treatment module, glyphosate was found to have a removal rate of >70 %, whereas for AMPA the removal rate was <30 %.

Investigations into the reactivity of glyphosate and AMPA when subjected to ozonation was also carried out at pilot-scale (Jönsson *et al.*, 2013, CA 7.5/084). These studies found that a 15 minute treatment period was enough to result in removal rates of >99 % for both glyphosate and AMPA under the experimental conditions.

Of less importance, from a water treatment perspective (due to rare implementation of the process) is the degradation of glyphosate in water by UV/H_2O_2. One investigation used a high concentration of glyphosate (50 mg/L) to look at the removal of glyphosate from water following the washing out of product containers in Argentina (Manassero *et al.*, 2010, CA 7.5/093). Due to the high concentration of glyphosate used it was possible to identify the compounds formed during the process. It was found that AMPA was not formed from glyphosate under the test conditions, as carbon-phosphate bond cleavage was the first step of the degradation, and after the oxidative removal of one carbon unit, glycine was formed. Glycine is a naturally occurring amino acid, and under the experimental conditions it went on to generate methanediol, formic acid, nitrate anion, ammonium and phosphate anions.

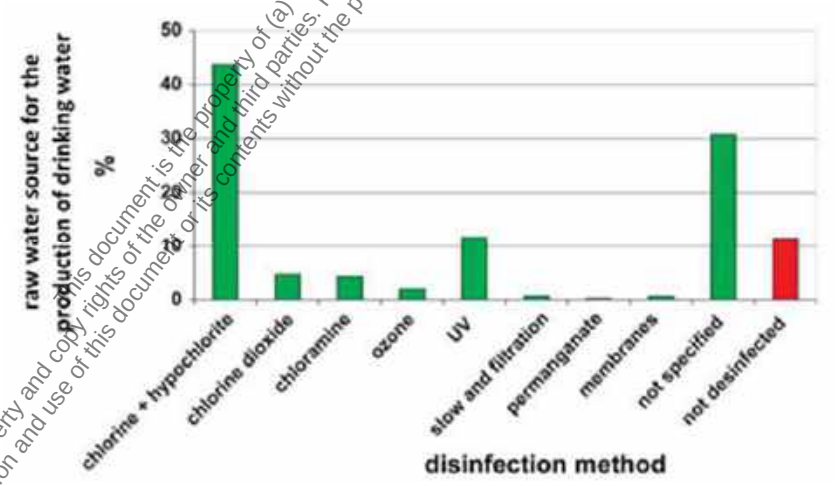
The prevalence across the EU of the treatment processes referred to above, can be inferred from a publication (van der Hoek *et al*., 2014, CA 7.5/098) Drinking water treatment technologies in Europe: state of the art – challenges – research needs) whose main findings are summarised in [redacted] (2020, CA 7.5/002). This paper was the result of a survey carried out amongst the members of the European Federation of National Associations of Water and Wastewater Services. This organisation covered 23 EU MSs and 405 million European citizens, in 2014. Figure 7.5-209 shows that the vast majority of raw water sources for drinking water production (88 %) are subject to disinfection:

Figure 7.5-209: Raw water sources for drinking water production in Europe (from [redacted] 2020, CA 7.5/002: the green columns sum to 88 %)



The paper reports that almost all the raw water taken from surface water is subject to disinfection (99.9 %). For bank filtration and artificial recharge (AR), the values are 90.1 % and 92.2 %, respectively. Figure 7.5-210 summarises the disinfection method employed – where surface water is disinfected, the paper reports that chlorine disinfection is applied to 62 % (30 % is 'not specified', but it is very likely that as disinfection by chlorine is by far the most employed method, a significant portion of the 'not specified' is also likely to be chlorine based; hence, 62 % should be considered a conservative minimum value.)

Figure 7.5-210: Raw water sources and treatment scheme (from [redacted] 2020, CA 7.5/002: the green columns sum to 88 %)



Summary

Glyphosate and its metabolites (AMPA and HMPA) are most likely to be exposed to chemical water treatment processes *via* the treatment of surface waters abstracted for the production of drinking water. Such raw water is very likely to be subjected to a range of treatment processes, and to be subject to disinfection designed to ensure the subsequent drinking water is microbiologically safe to drink. Glyphosate and AMPA are known to be transformed by the most common disinfection methods, transformation products identified are the same as those formed from glycine and other amino acids under the same conditions.

Removal rates for glyphosate and AMPA when subjected to disinfection processes are high as summarised in the table below.

Table 7.5-262: Summary of removal rates for glyphosate and AMPA following disinfection processes (*after* Jönsson *et al.*, 2013, CA 7.5/084)

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Chlorination	71 - >99	40 - >95
Chlorine dioxide	17 - 93	>99
Ozonation	60 - >99	25 - 95

Furthermore, drinking water treatment processes are carefully controlled, and the characteristics of a specific source raw water needs to be known – as the water treatment process train needs to be optimised to ensure that quality standards are met at the tap of consumers. Consequently, where glyphosate or AMPA are known to be present in the raw water, the drinking water treatment train can be optimised to ensure removal of these substances below the required threshold values.

Overall Summary

For drinking water derived from surface water, there is almost always water treatment processes applied to generate the drinking water. The prevalence across the EU of the chemical treatment processes, can be inferred from a publication (van der Hoek *et al.*, 2014, CA 7.5/098) whose main findings are summarised in [redacted] (2020, CA 7.5/002). This paper was the result of a survey carried out amongst the members of the European Federation of National Associations of Water and Wastewater Services. This organisation covered 23 EU MS's and 405 million European citizens. The report indicates that the vast majority of raw water sources for drinking water production (88 %) are subject to disinfection.

Further, almost all the raw water taken from surface water is subject to disinfection; and where surface water is disinfected, chlorine disinfection is applied to a minimum of 62 % of the raw water. Glyphosate and AMPA are known to be transformed by the most common disinfection methods. Transformation products appear to be small molecules, often similar or identical to those found from natural sources.

Other chemical treatment processes are often applied (either for disinfection or for the explicit removal of micro-pollutants), and low chemical processes are also very frequently applied. Monitoring data is usually only available for raw water, before any water treatment processes have been applied, but for contextualising monitoring data, the effects of these processes should be included. Removal rates for glyphosate and AMPA, for various water treatment processes, have been discussed above, and are summarised in Table 7.5-263.

Table 7.5-263: Summary of removal rates for glyphosate and AMPA following removal processes

Treatment Process	Glyphosate removal (%)	AMPA removal (%)
Bank and dune filtration	20 - >95	25 - >95
Aluminium coagulant and clarification	15 - 40	20 - 85
Iron coagulant and clarification	40 - 70	20 - 85
Activated carbon adsorption	10 - 90	20 - 70
Chlorination	71 - >99	40 - >95
Chlorine dioxide	17 - 93	>99
Ozonation	60 - >99	25 - 95

In addition to disinfection processes, bank filtration can be an effective process for removal of glyphosate and AMPA from water, when sufficient residence time within soil/sediment occurs to allow the normal aerobic/anaerobic soil degradation processes to progress to their full extent (total mineralisation). Generally, drinking water treatment processes are carefully controlled, and the characteristics of a specific source raw water needs to be known – as the water treatment process train needs to be optimised to ensure that quality standards are met at the tap of consumers. Consequently, where glyphosate or AMPA are known to be present in the raw water, the drinking water treatment train can be optimised, where necessary, to ensure removal of these substances below the required threshold values, and therefore, there is a low risk of exceeding the relevant thresholds in drinking water of 0.1 µg/L for glyphosate and 10 µg/L for AMPA, nor for exceeding health-based thresholds (glyphosate ADI 0.5 mg/kg bw/day, and AMPA ADI 1.32 mg/kg bw/day).

Applicant studies

New studies/assessments

1. Information on the study

Data point:	CA 7.5/002
Report author	[redacted]
Report year	2020
Report title	Glyphosate (GLY) and the primary metabolites amino methyl phosphonic acid (AMPA) and hydroxy methyl phosphonic acid (HMPA): Public monitoring data assessment and interpretation
Report No	EnSa-20-0322
Document No	-
Guidelines followed in study	Groundwater monitoring guideline document (Gimsing *et al*., 2019) with respect to chapter 7 ('Public monitoring data collected by third party organisations'); Article 5 of Directive 2009/90/EC - Technical specifications for chemical analysis and monitoring of water status.
Deviations from current test guideline	Not relevant
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

The study is relevant for multiple subchapters. The summary is provided in subchapter E.1 (low chemical water treatment and bank filtration) of this document.

Existing studies/assessments

1. Information on the study

Data point:	CA 7.5/081
Report author	[redacted]
Report year	2010
Report title	Removal of glyphosate and AMPA by water treatment
Report No	UC8154v2
Document No	BVL No. 2316003
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The study is relevant for multiple subchapters. The summary is provided in subchapter E.1 (low chemical water treatment and bank filtration) of this document.

Relevant literature articles

1. Information on the study

Data point:	CA 7.5/084
Report author	Jönsson, J. *et al.*
Report year	2013
Report title	Removal and degradation of glyphosate in water treatment: a review
Document No	Journal of Water Supply: Research and Technology-AQUA/62.7/2013
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in subchapter E.1 (low chemical water treatment and bank filtration) of this document.

1. Information on the study

Data point:	CA 7.5/087
Report author	Ruel, S.M. et al.
Report year	2011
Report title	On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications
Document No	Water Science & Technology 63.11/2011
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary

The article was found relevant for multiple subchapters. The summary is provided in subchapter E.1 (low chemical water treatment and bank filtration) of this document.

1. Information on the study

Data point:	CA 7.5/089 CA 7.5/090 (Translation)
Report author	Shen, Y. et al.
Report year	2011
Report title	Ozonation of Herbicide Glyphosate (translated from the original Chinese-language paper)
Document No	Acta Scientiae Circumstantiae,31(8): 1647-1652
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

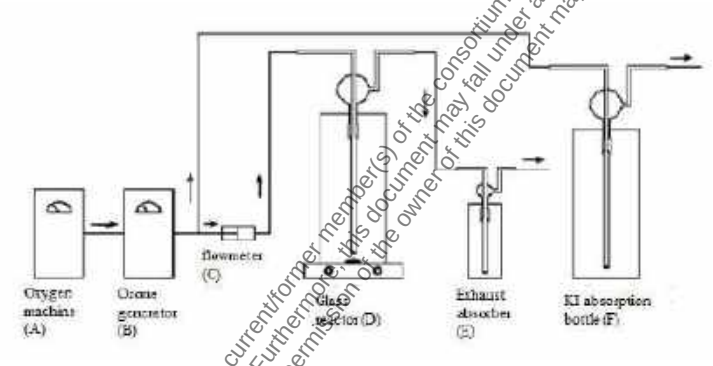
In this work, the influence of pH, ozone dosage and initial concentration of glyphosate on the degradation of glyphosate by ozone was investigated in detail. The pathway for the glyphosate degradation by ozone is also discussed. The results showed that the degradation rate of glyphosate by ozone increased with increasing ozone dosage, and decreased with increasing initial concentration of glyphosate. Under different pH conditions, the removal rate of glyphosate decreased in the following order: basic > neutral > acidic. The degradation of glyphosate by ozone was found to be accomplished by hydroxyl radicals. Intermediates

of glycolic acid, glycine, AMPA, and orthophosphoric acid were identified during the ozonation of glyphosate. AMPA accumulated in the initial reaction time and decreased subsequently. Phosphate ions accumulated as reaction time increased.

Materials and methods

Experimental setup
The test adopted batch and semi-continuous test methods, and the test device is shown in Figure 7.5-211. High-purity oxygen was produced from an air source in medical oxygen machine (A), and ozone gas was produced by discharge of ozone generator (B). After measuring by flowmeter (C), through the bottom of the glass reactor (D), the microporous sand core diffuser ensured that the ozone gas was dissolved in the water. Remaining ozone was absorbed by the potassium iodide absorption bottle (F) after stirring. The reactor was made of quartz with a diameter of 100 mm, a height of 300 mm and a volume of 2.5 L. At the beginning of the test, the valve was first placed into the equilibrium position, the ozone was passed into the KI absorption bottle (F) and stabilized for a few minutes before the valve was transferred to the reaction. The air flow in the equilibrium phase was A-B-F, and the air flow in the test reaction phase was A-B-C-D-E.

Figure 7.5-211: Experimental set-up



Reagents and analytical methods
Glyphosate (purity 99.99 %) and AMPA (aminomethylphosphoric acid) were purchased from Dima; H_3PO_4, NaH_2PO_4, HCl, NaOH were all analytical reagents and purchased from Beijing Chemical Reagent Co.; Water pH was adjusted by 1 mol/L NaOH and determined by 720APLUS Benchtop pH meter (Thermo Orion Co. USA); The UV-vis absorption was determined by U-3010 UV-vis spectrometer (Hiachi Co. Japan); TOC was determined by N/C3000 TOC analyser (Jena, Germany); and the ozone dissolved in water was determined by the indigo method.

The principle of the indigo method for measuring the dissolved ozone concentration in water is to mix the ozone-containing water samples with acidic indigo reagents, and ozone degrades the solution's blue colour. The specific steps are: prepare indigo reagents according to the national standard method, add 9 mL samples to the colorimetric cup with 1 mL indigo reagent, mix and measure with a spectrophotometer.

Glyphosate was determined by HPLC with a pre-column derivatization. The pre-column derivatization was conducted as follow: add 0.5 mL sodium borate buffer solution (0.5 mol/L, pH = 9), 1 mL 4-toluene sulfonyl chloride ($C_7H_7ClO_2S$) acetonitrile solution (1 g/L) to 1.5 mL water sample, mix well and react at room temperature overnight. Then the reaction solution was filtered through 0.45 μm membrane and detected by HPLC. The mobile phase was methanol/50 mmol·L^{-1} NaH_2PO4 solution (pH = 5.5 adjusted by NaOH) (*v*/*v*, 20/80), flow rate was 1 mL min^{-1}, wavelength was 240 nm, injection volume 20 μL, and HYPERSIL GOLD column (250 mm × 4.6 mm × 5 μm, Thermo U.S.) was used. The retention time of glyphosate was 6.303 min, and the limit of detection was 0.1 mg·L^{-1}. The maximum limit of glyphosate in drinking water was

specified as 0.7 mg/L by national standard GB 5749—2006, therefore the established method fully satisfied the requirement of this study.

The simultaneous detection of glyphosate and AMPA was also performed by HPLC with pre-column derivatization. The pre-column derivatization is the same as the above method. The mobile phase was acetonitrile /50 mmol·L^{-1} ammonium acetate aqueous solution (*V*/*V*, 20/80), flow rate was 1 mL·min^{-1}, wavelength was 240 nm, the injection volume was 20 µL, and HYPERSIL GOLD column (250 mm ×4.6 mm, id: 5 µm, Thermo, USA) was used. The retention time of glyphosate was 3.6 min and that of AMPA was 3.2 min. The limit of detection of glyphosate was 0.1 mg/L and that of AMPA was 0.2 mg/L.

Ozone-oxidized glyphosate intermediates were determined by GC-MS. The specific treatment and heating procedures were as follows:

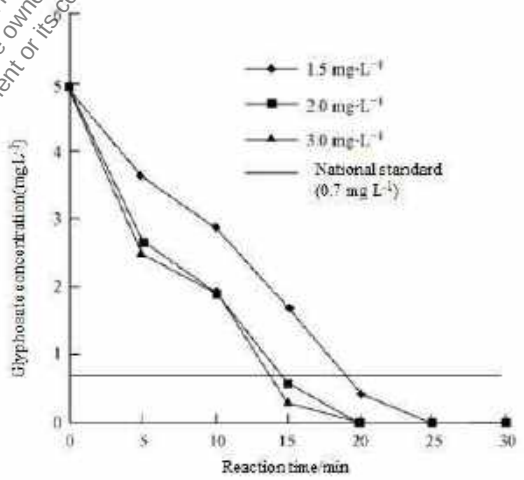
Pre-treatment method: 100 mL water samples at different reaction time was freeze-dried, the obtained solid powder was dissolved in 2.5 mL dichloromethane, and 0.1 mL BSTEA/TMCS silanizing reagents were added for silanization in 60°C water bath for 60 min. Then the anhydrous sodium sulfate calcined at 500°C was used for dehydration. The sample was then filtered with 0.45 µm organic membrane and concentrated to 0.5 mL by nitrogen before injection onto the GC-MS.

Heating procedures: 50°C for 3 min, heated up to 150°C over 5°C ·min^{-1} and for 5 min, then heat up to 250°C by 5°C ·min^{-1} and keep for 20 min. Injector temperature was 280°C, carrier gas was high purity helium, and the gas flow was 1 mL min^{-1}.

Results

Effect of ozone dosage on oxidative removal of glyphosate
In the study, the initial concentration of glyphosate was 5 mg/L, and the dosage of ozone was 1.5, 2.0 and 3.0 mg/L. The reaction was carried out for 30 min, and sampled every 5 min. The residual ozone in the sample was quenched by $NaSO_3$ to study the effect of different ozone dosage on the glyphosate concentration. Figure 7.5-212 shows that glyphosate was almost completely removed after 30 min. The larger the amount of ozone, the shorter the time it took for glyphosate to be completely removed. At 1.5 mg/L of ozone, glyphosate was completely removed at 25 min, while at 2.0 mg/L and 3.0 mg/L of ozone, glyphosate was completely removed at 20 min. There was not much difference for the reaction rates between 2 mg/L and 3 mg/L of the ozone dosage.

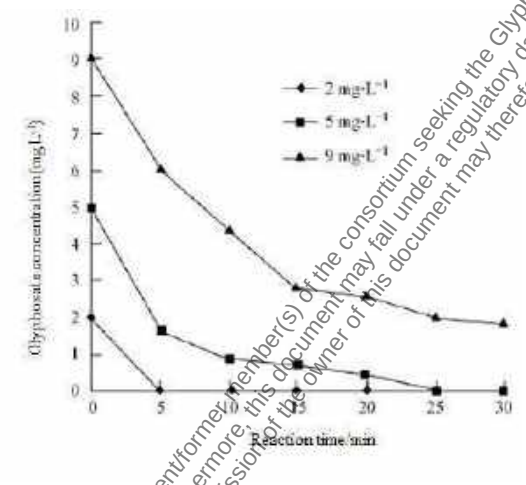
Figure 7.5-212: Degradation of glyphosate with different amounts of ozone



Effect of different initial concentration of glyphosate on the removal of glyphosate by ozone

In order to study the effect of different initial glyphosate concentration on their removal, three initial concentrations of glyphosate 2, 5 and 9 mg/L were applied, and the dosage of ozone was 1.5 mg/L. The reaction was kept for 30 min and the sampling was done every 5 min. The residual ozone in the sample was quenched with Na_2SO_3. It can be seen from Figure 7.5-213 that at the initial concentration of 2 mg/L, glyphosate was completely removed at 5 min, while at the initial concentration of 5 mg/L, glyphosate was completely removed at 25 min, however at the initial concentration of 9 mg/L, glyphosate was not completely removed and its concentration was still 2 mg/L at 30 min. Also, at the beginning of the reaction, the degradation rate was fast, while the degradation rate slowed down gradually as the reaction proceeded. This is because in the beginning the dissolved ozone concentration in the water was relatively high, as the reaction proceeded some of the ozone participated in the reaction and some decayed, then the concentration of ozone in the aqueous solution gradually decreased and the reaction rate slowed down.

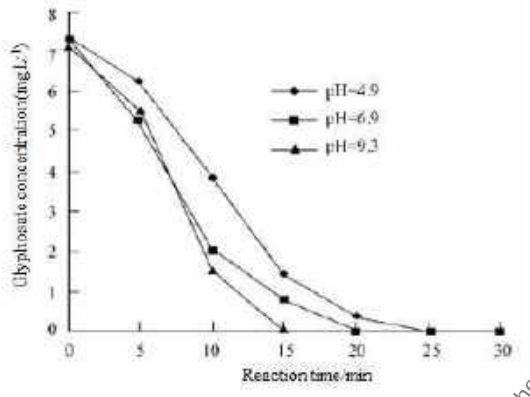
Figure 7.5-213: Degradation of glyphosate at different initial concentrations of glyphosate



Effect of different initial pH on glyphosate oxidation

In the study, three initial pH values were selected, i.e. pH 4.9, pH 6.8, pH 9.3, to investigate the effect of pH on the glyphosate concentration. The initial concentration of glyphosate was 7.2 mg/L, and the ozone dosage was 1.5 mg/L. The reaction was kept for 30 min, sampling was done every 5 min, and the residual ozone was quenched with Na_2SO_3. The results (Figure 7.5-214) showed that glyphosate could always be removed in 30 min even at different initial pH values. The removal was the fastest in the alkaline system, i.e., completely removed at 15 min; it was slower under the neutral condition, i.e., removed completely at 20 min; the removal was the slowest under the acid conditions, i.e., removed completely at 25 min. This is because there are more OH^- in alkaline systems, which can cause the reaction system to produce many hydroxyl radicals. And hydroxyl radicals produce more active radicals by chain reaction, accelerating the rate of oxidation of glyphosate by ozone.

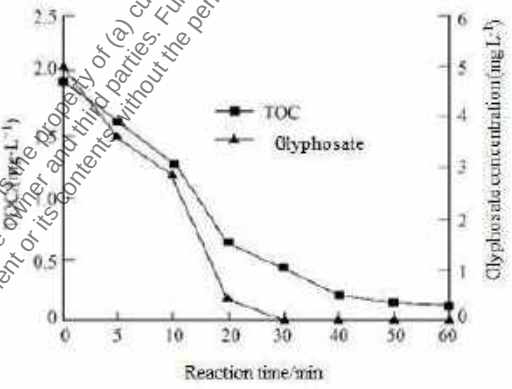
Figure 7.5-214: Degradation of glyphosate at different initial pH



Changes of TOC in the Ozone Oxidation of Glyphosate

The initial concentration of glyphosate was 5 mg/L, ozone dosage was 1.5 mg/L, reaction time was 60 min, sampling time was 0, 5, 10, 20, 30, 40, 50, 60 min, respectively. After sampling, the residual ozone was quenched with Na_2SO_3 to study the change of TOC in the process of glyphosate oxidation. As shown in Figure 7.5-215, the removal of glyphosate by ozone is quite complete, at 60 min the degradation rate of TOC reached 93.52 %. When glyphosate was completely removed at 30 min, the degradation rate of TOC was 77.65 %. This indicates that in the early phase glyphosate is oxidized by ozone to small molecular organics, which are then gradually oxidized until completely mineralized.

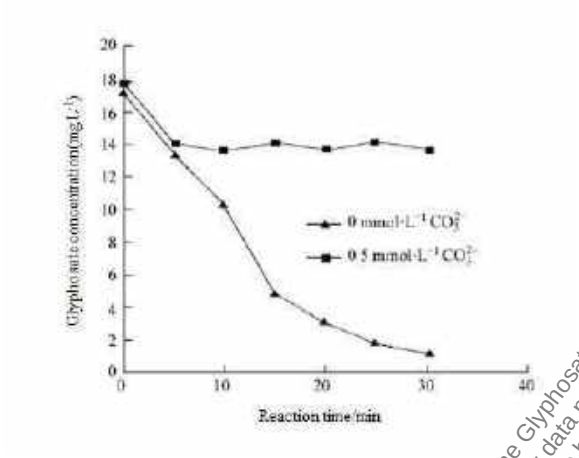
Figure 7.5-215: TOC change during ozonation of glyphosate



Effect of carbonate ions on glyphosate oxidation by ozone

Carbonate ions are typical hydroxyl radical quenchers, which have strong quenching effect on hydroxyl radical. The effect of carbonate ions on ozone oxidation of glyphosate was investigated. Figure 7.5-216 showed that carbonate ions obviously inhibited the rate of glyphosate oxidation by ozone. This indicates that hydroxyl radicals play a major role in glyphosate oxidation by ozone.

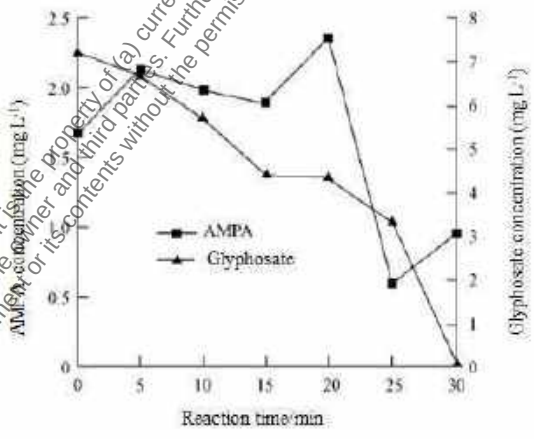
Figure 7.5-216: Effect of carbonate ions on the ozonation of glyphosate



Concentration of product AMPA during glyphosate oxidation

In the study, glyphosate with initial concentration of 7.2 mg/L was selected to investigate the concentration change of product AMPA during ozonation. The reaction time was kept at 30 min, the sampling was conducted every 5 min. The residual ozone was quenched by Na_2SO_3. AMPA was the first product generated from ozonation of glyphosate, as shown in Figure 7.5-217, the concentration of AMPA first increased and then decreased as the concentration of glyphosate decreased. Glyphosate was first oxidized to AMPA, which was then gradually oxidized by ozone to other small molecules.

Figure 7.5-217: Degradation of glyphosate and production of AMPA during ozonation

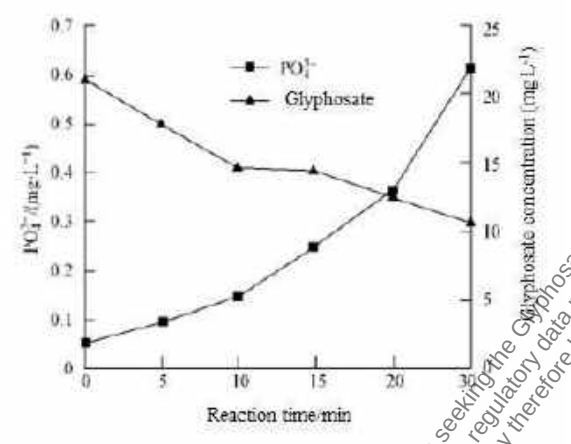


Concentration of PO_4^{3-} during the process of glyphosate oxidation by ozone

20 mg/L was selected as the initial concentration of glyphosate. The reaction time was kept at 30 min, 20 mL was sampled at 0, 5, 10, 15, 20, 30 min, respectively, and the residual ozone was quenched by Na_2SO_3 to investigate the change of concentration of PO_4^{3-} during ozone oxidation of glyphosate. As was shown in Figure 7.5-218, along with oxidation of glyphosate by ozone, the concentration of glyphosate decreased gradually and the concentration of PO_4^{3-} increased gradually. PO_4^{3-} was detected in the initial stage of reaction, indicating the P-C bond was first attacked by ozone molecules and hydroxyl radicals during ozone

oxidation of glyphosate, the phosphorus-containing groups were rapidly oxidized to PO_4^{3-} and the remaining groups continued to be oxidized by ozone molecules and hydroxyl radicals.

Figure 7.5-218: Degradation of glyphosate and production of PO_4^{3-}



GC-MS Analysis of intermediates of glyphosate oxidation by ozone

In order to investigate the intermediate products produced in the process of ozone degradation of glyphosate and then propose a more accurate degradation pathway, a qualitative determination was conducted on the intermediates using GC-MS. The initial concentration of glyphosate for the test was 100 mg/L, ozone was continuously provided, the reaction time was kept at 30 min, sampling was conducted every 5 min, and the residual ozone was quenched by Na_2SO_3. After the pretreatment, the sample was measured with GC-MS, and the total ion flow of ozone oxidation of glyphosate after 60 min is shown in Figure 7.5-219 (Peaks 1, 2, 3, 4 correspond to the products in Table 7.5-264).

Figure 7.5-219: Total ion current during ozonation of glyphosate

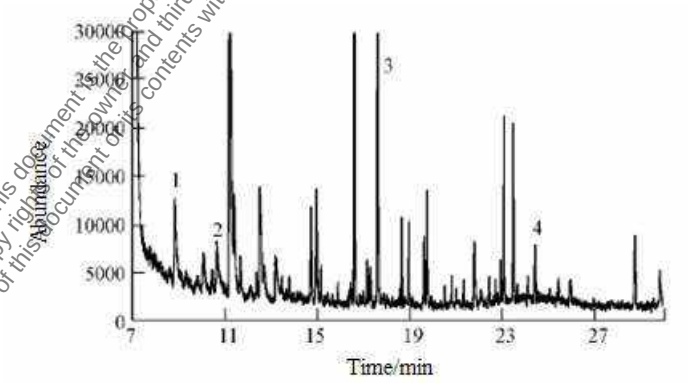


Table 7.5-264 gives the intermediates of reaction at 60 min by GC-MS measurement. The intermediates of glyphosate ozonation included glycolic acid, glycine, phosphoric acid and AMPA. By the analysis of intermediate products, the degradation pathway of glyphosate ozonation was proposed (see Figure 7.5-220). There are four main pathways for the oxidation of glyphosate by ozone, including: cleavage of C-N bonds,

producing glycine and glycolic acid; cleavage of C-P bonds, generating phosphoric acid; cleavage of C-C bonds, forming AMPA.

Table 7.5-264: Intermediate products determined by GC-MS

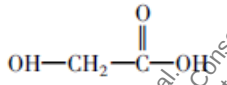
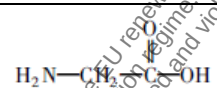
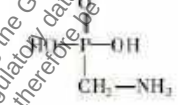
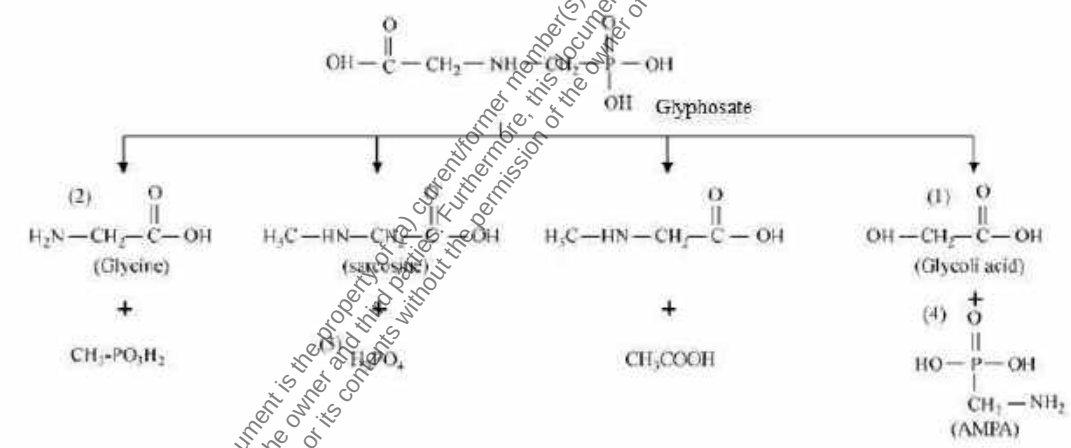
Retention time (min)	Product name (No.)	Structural formula
8.861	Glycolic acid (1)	
10.675	Glycine (2)	
17.627	Phosphoric acid (3)	H_3PO_4
24.367	AMPA(4)	

Figure 7.5-220: Glyphosate degradation pathway



Conclusion

The removal rate of glyphosate by ozone is related to the dosage of ozone, initial concentration of glyphosate and initial pH. The higher the ozone dose, the faster the reaction rate of glyphosate ozonation. The removal rate of glyphosate in a weak alkaline system (pH = 9.3) was faster than that in the medium system (pH = 6.8), with that in the acidic system (pH = 4.9) being the slowest; and the pH of the reaction system changed obviously in the first 20 min, at the later stage of reaction the changes were not apparent.

Ozone oxidation of glyphosate showed a high degree of mineralization, at 30 min the degradation rate of TOC was 77.65 % and at 60 min it was 93.52 %. At the initial stage of the reaction, ozone mainly oxidizes glyphosate to AMPA. After glyphosate is completely removed, most intermediates are completely mineralized to carbon dioxide and water.

The ozone oxidation process follows the reaction mechanism of hydroxyl radical. CO_3^{2-} is a good hydroxyl radical quenching reagent, the rate of ozone oxidation of glyphosate was significantly reduced in the system containing CO_3^{2-} compared to that without the addition of CO_3^{2-}, which indicates that hydroxyl radicals play a major role.

The main intermediates of glyphosate oxidation by ozone were glycolic acid, glycine, AMPA and H_3PO_4, and there were 4 main degradation pathways. The main products of the initial reaction were AMPA and phosphoric acid. AMPA accumulated gradually and then decreased gradually. PO_4^{3-} accumulated gradually from the initial period of reaction.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the degradation of unlabeled glyphosate during ozonation in water with different initial concentrations and different pH values. The degradation products resulting from the ozonation process are described as well.

The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/091
Report author	Assalin M., *et al.*
Report year	2010
Report title	Studies on degradation of glyphosate by several oxidative chemical processes: Ozonation, photolysis and heterogeneous photocatalysis
Document No	Journal of Environmental Science and Health Part B (2010) 45, 89–94
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Several different Advanced Oxidation Processes (AOPs) including ozonation at pH 6.5 and 10, photolysis and heterogeneous photocatalysis using TiO_2 as semiconductor and dissolved oxygen as electron acceptor were applied to study the degradation of glyphosate (N-phosphonomethyl glycine) in water. The degree of glyphosate degradation, the reaction kinetics and the formation of the major metabolite, aminomethyl phosphonic acid (AMPA), were evaluated. Ozonation at pH 10 resulted in the maximum mineralization of glyphosate. It was observed that under the experimental conditions used in this study the degradation of glyphosate followed first-order kinetics. The half-life obtained for glyphosate degradation in the O_3/pH 10 process was 1.8 minutes.

Materials and methods

Chemicals

Glyphosate (purity 99.8 %) and AMPA (purity 99.1 %) were obtained from Monsanto and used without further purification. Analytical grade organic solvents were used for high performance liquid chromatography (HPLC) analysis. Ultra pure distilled-deionized water from a Milli-Q (Millipore Corp.) system was used throughout this study. Commercially available TiO_2 (Degussa P-25) was obtained from Degussa Chemical. All reagents used were of analytical-reagent grade.

Samples

A stock solution containing 1000 mg/L of glyphosate was prepared in deionized water and diluted to the required concentration (42.275 mg/L) for the degradation experiments. The original pH of this solution was about 6.5. The pH was adjusted to 10 by the addition of a NaOH solution for the ozonation experiment.

Ozonation process

Ozone was generated from pure oxygen using an OZO-CAV ZT-2 generator (Inter Ozone Ingenieria Ecologica, Santiago-Chile). The amount of ozone produced was determined spectrophotometrically at 258 nm (ϵ= 3.000 L/mol cm) in the gas phase by passing the mixture of oxygen and ozone through a flow cell. The system reached a steady-state production of ozone in 10 minutes. An ozone concentration of 14 mg/L was applied for 30 minutes in a batch reactor. Samples (42.275 mg/L glyphosate solution, 400 mL) were submitted to ozonation at pH 6.5 and at pH 10 (pH adjusted with a sodium hydroxide solution) at room temperature, using a tubular 500 mL reactor fitted with a sintered glass dispenser that released the gas from the bottom of the reactor. For all experiments, the excess of ozone was passed from the reactor into a glass flask containing a 2 % solution of KI.

Heterogeneous photochemical process

Titanium dioxide (80 % anatase and 20 % rutile, average particle size of 30 nm and BET Method–Brunauer, Emmett and Teller [BET] surface of 50 ± 15 m^2/g) was used without any pretreatment. Aqueous suspensions of 0.1 g of TiO_2/L were used in this experiment. A volume of 200 mL of glyphosate solution (42.275 mg/L, original pH) was placed in the 250 mL cylindrical photoreactor. Illumination was provided by a high-pressure mercury lamp (Philips HPL-N, 125 W; λ>290 nm) with the glass bulb removed. The lamp was fixed in the center of the reactor and cooled by a water jacket, at room temperature. The suspension was bubbled with oxygen (through a sintered glass disk placed in the bottom of the reactor) at a flow rate of about 6 ± 0.2 L/h for 30 minutes. For analytical control, samples were removed and centrifuged at 3500 rpm.

Photolysis process

The same experimental set up, including the passage of oxygen, was used as in the previous section, but without the addition of TiO_2 suspension.

Analytical determinations

Mineralization was followed by measuring the total organic carbon (TOC) through direct injection of filtered samples (pore size of 0.45 µm) into a Shimadzu-5000A TOC analyzer provided with a non-dispersive infrared (NDIR) detector and calibrated with standard solutions of potassium phthalate.

The glyphosate and AMPA concentrations were determined by HPLC, with a Merck-Hitachi HPLC system, model D-7000, with fluorescence detection (excitation at 350 nm and emission at 440 nm). A 300 × 4.6 mm I.D Aminex Glyphosate column and a 100 × 4.6 mm I.D HRLC-Glyphosate guard column (both from Bio Rad) were used.

The flow rate of mobile phase (0.68 g/L KH_2PO_4) was adjusted to 0.7 mL/min. After exiting the column, glyphosate and AMPA were then post column derivatized using 1,2 phthalic dicarboxaldehyde and 2-mercaptoethanol. The retention times for glyphosate and AMPA were 17 and 30 minutes, respectively. The limit of detection (LOD) was established at 0.0075 µg/L, using a signal to noise ratio of 3 for glyphosate and AMPA.

Results

The different treatment processes were applied for the degradation of glyphosate in aqueous solution. The processes studied were photolysis, heterogeneous photocatalysis (TiO_2/UV) and ozonation at two different pH values (6.5 and 10.0). Glyphosate was the only organic compound initially present in the aqueous solutions used in this study.

Ozonation process

In Figure 7.5-221, the variation of the C/C_0 ratio as a function of ozonation treatment time is represented. As can be seen, the ozonation carried out at alkaline pH was more effective for glyphosate degradation. After 17 minutes of treatment the glyphosate was totally removed while, in the ozonation carried out at pH 6.5, after 30 minutes of treatment about 80 % of the glyphosate initially present in solution was removed. Due to the oxidation potential of hydroxyl radicals being much higher than that of the ozone molecule, radical oxidation was faster than direct oxidation and higher glyphosate degradation was therefore observed at pH 10. The HPLC chromatograms of the O_3/pH 10 process at 0, 15 and 30 minutes of treatment time are given in Figure 7.5-222.

Figure 7.5-221: Glyphosate degradation by ozone (pH 6.5) and ozonation based on the hydroxyl radical (pH 10)

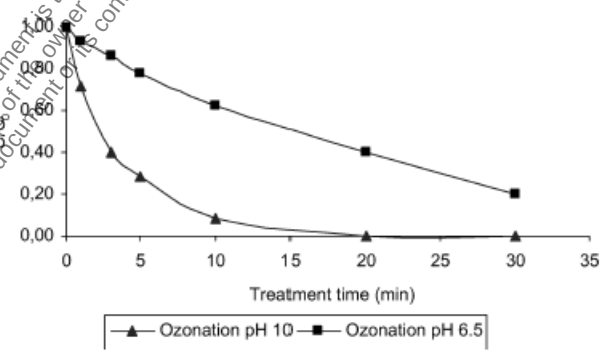


Figure 7.5-222: High performance liquid chromatography (HPLC) chromatograms of samples subjected to O_3/pH 10 at 0, 15 and 30 minutes

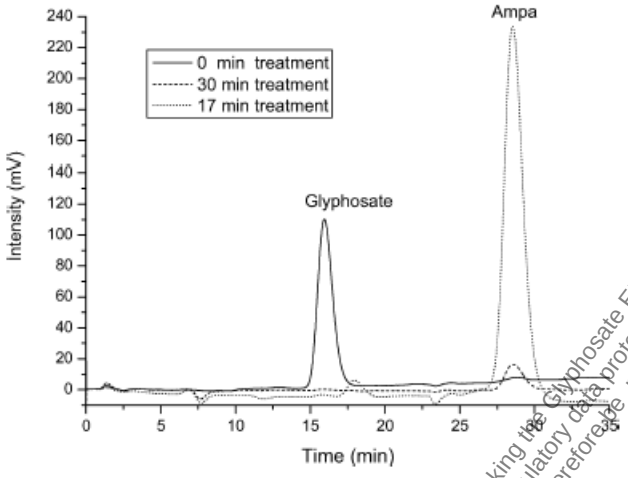
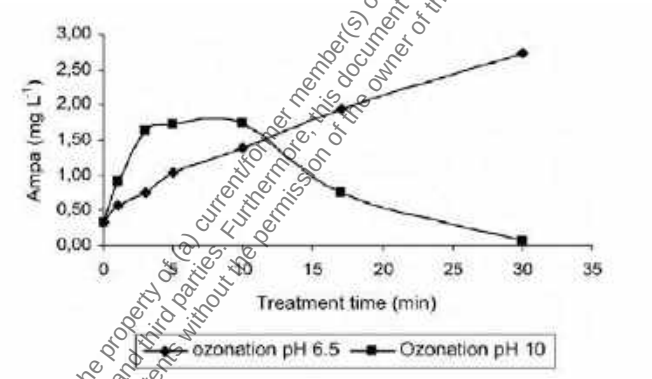


Figure 7.5-223: Aminomethyl phosphonic acid (AMPA) concentration during the ozonation processes



Aminomethyl phosphonic acid (AMPA) is the major metabolite of glyphosate produced by microbial degradation, and is found in plants, water and soil. The results of the present study indicate that the chemical oxidation processes O_3/pH 6.5 and O_3/pH10 produced this metabolite (Figure 7.5-223). Nevertheless, degradation by hydroxyl radicals also removed most of the AMPA produced in 30 minutes of treatment at pH10. For the O_3/pH 6.5 degradation process, this metabolite was continually produced and, apparently, not further degraded.

The degree of pesticide degradation and mineralization can be measured by the reduction of the total organic carbon content of the solution. The results indicate 20 % TOC reduction by application of the O_3/pH 6.5 process. This indicates that other decomposition products, besides AMPA, can be produced during the ozonation process. The ozonation process carried out at pH 10 resulted in 97.5 % TOC removal. These results were very important because they indicate that intermediate compounds (that might be more toxic than the parent compound) were almost totally removed.

Photolytic and photocatalytic degradation

In order to compare the efficiency of the photocatalytic degradation (UV-TiO_2) with direct photolysis (UV), experiments were carried on using the same initial concentration of pesticide, at pH 6.5. The amount of catalyst used was 0.1 g of TiO_2/L.

Figure 7.5-224 shows the disappearance of glyphosate by photocatalysis and photolysis in relation to illumination time. As expected, the direct photolysis was less effective than photocatalysis for glyphosate removal. After 3 minutes of irradiation without TiO_2, only 10.9 % of the initial amount of the compound was degraded while the glyphosate degradation for the same treatment time was 38.7 % for the photocatalytic process. The literature reports that direct photolysis is usually not an option due to the low quantum efficiency for most pesticides. After 30 minutes of UV irradiation in the presence of TiO_2, the residual concentration of glyphosate was 0.06 mg/L (99.9 % efficiency removal). TOC removal for the UV/TiO_2 process achieved 92 % after 30 minutes of treatment time.

Figure 7.5-224: Glyphosate degradation by the ultraviolet (UV) and TiO_2/UV processes

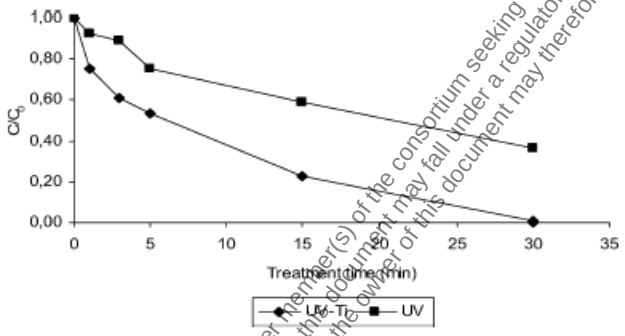
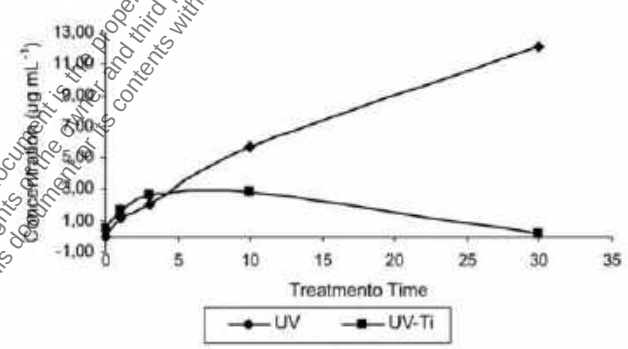


Figure 7.5-225: Aminomethyl phosphonic acid (AMPA) concentration during the ultraviolet (UV) and UV/TiO_2 processes



For both photo-induced processes formation of the AMPA intermediate was also observed (Figure 7.5-225). The amount of AMPA formed during the photocatalytic process was less than the amount formed during the UV process, 2.85 mg/L after 10 minutes of treatment, but this was completely degraded after 30 minutes. For the UV process, the amount of AMPA formed increased during the treatment. At the end of the UV treatment without TiO_2 (30 minutes) the AMPA concentration was 12.1 mg/L indicating that this compound is less easily degraded by UV radiation than glyphosate.

It is believed that the photocatalytic degradation reaction of organic pollutants occurs on the surface of TiO_2 and that O_2 and H_2O are necessary for photocatalytic degradation. Under UV illumination, electron-hole pairs are created on the TiO_2 surface. Oxygen adsorbed on the TiO_2 surface prevents the electron-hole pairs from trapping electrons. Superoxide radical-ions ($^{\bullet}O_2^-$) are thus formed. The $^{\bullet}OH$ radicals are formed from holes reacting with either H_2O or OH^- adsorbed on the TiO_2 surface. $^{\bullet}OH$ and $^{\bullet}O_2^-$ are widely accepted as primary oxidants in heterogeneous photocatalysis. The oxidizing power of the $^{\bullet}OH$ radicals is strong enough to completely oxidize glyphosate adsorbed on the surface of TiO_2.

Comparison between O_3/pH 10 and TiO_2/UV processes

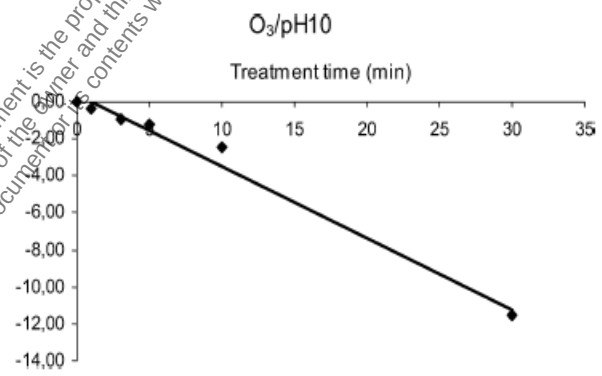
The processes that showed the highest rates for degradation of glyphosate in water were O_3/pH 10 and TiO_2/UV. Both processes were able to efficiently remove glyphosate and also the AMPA generated during the degradation processes. Knowledge of the kinetics and direct comparison of chemical oxidants are required to assess the efficiency of systems engineered for the oxidation of a variety of pollutants. Reliable kinetic studies require obvious substrate decay measurements. Thus, for comparison of the efficiency of these treatment processes, kinetic studies of glyphosate decomposition were carried out.

As several authors have previously reported, the reaction of ozone with organic compounds is second order, first order with respect to each reactant. Therefore, the glyphosate disappearance rate equation can be expressed as:

$$\frac{d[glyphosate]}{dt} = k[O_3][glyphosate]$$

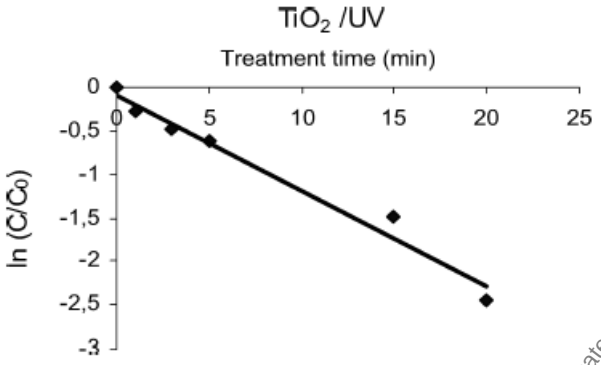
where k is the second order rate constant. In addition, as the initial ozone concentration was in excess with respect to glyphosate, the ozone concentration through each experiment can be considered almost constant. Then, the reaction rate can be reduced to pseudo-first-order kinetics with respect to the ozone concentration. In order to evaluate this pseudo rate constant, the data obtained for glyphosate degradation by O_3/pH 10 were plotted as ln (C/C_0) versus reaction time, and after linear regression analysis ($R^2 = 0.9836$), the slope can be attributed as the apparent first-order rate constant k′ (Figure 7.5-226).

Figure 7.5-226: The pseudo-first-order decay of glyphosate by ozonation at pH 10



Several experimental results have indicated that the photocatalytic degradation rates of pesticides over illuminated TiO_2 follow the Langmuir-Hinshelwood kinetic model. In our investigation, by plotting ln [C/C_0] as a function of time, a straight line was obtained (Figure 7.5-227) that confirms the apparent first-order kinetic law ($R^2 = 0.9743$).

Figure 7.5-227: The pseudo-first-order decay of glyphosate by TiO_2/UV process



The half-life obtained for glyphosate was 1.8 and 6.2 minutes for O_3/pH 10 and TiO_2/UV, respectively. This indicates that for the ozonation carried out at pH 10 a faster rate of glyphosate decomposition was observed under the experimental conditions studied.

Conclusion

The degradation of aqueous solutions containing glyphosate can be realized by oxidative advanced processes. Processes based on the formation of hydroxyl radical, such as Ti/UV and O_3/pH 10, were effective for the degradation of glyphosate and its degradation intermediates, including AMPA, after a short treatment time. Under the experimental conditions used in this study the degradation of glyphosate followed a pseudo first-order kinetic law for both processes studied. The half–lives obtained for glyphosate degradation were 1.8 and 6.2 minutes for O_3/pH 10 and TiO_2/UV, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the removal of glyphosate by ozonation and photocatalysis (Ti/UV) process in water. The results are mainly shown as graphical plots. Thus, insufficient details were reported to evaluate the validity of the rate constants reported.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/092
Report author	Boucherie, C., *et al.*
Report year	2010
Report title	"Ozone" and "GAC filtration" synergy for removal of emerging micropollutants in a drinking water treatment plant?
Document No	Water Science and Technology: Water Supply (2010), Volume 10, Number 5, pp. 860-868
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Veolia Water)
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Ozonation plays an essential role in water disinfection to inactivate viruses, bacteria and some parasites. Ozone treatment rates to attain disinfection goals also result in oxidation reactions of emerging pollutants. Glyphosate, AMPA, amitrole and diuron – the four major pesticides in the Seine, Marne and Oise rivers – are reactive to ozone. Twenty-one pesticides are only partially reactive to ozone and an additional "GAC filtration" is needed to remove them.

Materials and methods
The pilot unit consists of an ozonation-deozonation step linked to a Granular activated carbon (GAC) filtration column. The system is continuously fed by Sand Filtered Water (SFW) from the Neuilly-sur-Marne drinking water plant. Bromide or micropollutants are injected into the feeding line via a static mixer. Moreover the pH can also be automatically controlled by online sodium hydroxide or sulphuric acid injection. The pilot geometry is a rectangular tank with one transfer chamber and three contact chambers and the following characteristics: hydraulic efficiency ratio of 0.70 and hydraulic residence time of 17 minutes for a mean flow rate of 12 m³/h. Gas/Liquid ozone transfer is achieved in the first chamber working as a bubble column with two porous diffusers and counter current ozonated gas and SFW flows. The 300 mm diameter GAC column contains a two meters GAC filter bed. The substrate of this filter bed comes from one full scale GAC filter in Neuillysur-Marne plant with an operating life equivalent to 21,000 bed volumes processed. The column is fed with ozonated-deozonated water from the ozonation unit with 750 L/h mean flow rate and 14 minutes mean contact time between water and filtering medium. The GAC unit is backwashed weekly (air, water and air + water back-wash steps).

The operator of the pilot unit uses a man-machine interface system. This system includes specific automatic regulation loops to control SFW Flow rate, SFW pH, ozone production or ozone residual outlet and ozone quenching upstream GAC filtration. At the beginning of each test, the operator can select a specific test level of ozone or instruct the pilot unit to fix the end-level of ozone. Then spiking of micropollutants is carried out to simulate medium or maximum concentrations found in the Seine, Marne and Oise rivers.

Samples are collected for analysis after a period of two hydraulic residence times, in order to reach a steady state. Some analyses – pH, temperature, alkalinity (AT), UV_{254}, ozone gas and liquid residual – are carried out *in situ*. Ozone concentration measurements in "Air" and "Vent" gases are continuously monitored with sensors "Uvozon" and "BMT 964". Daily controls are also carried out using an iodometric method to assess the ozone concentrations in gases. A sensor "Depolox" continuously monitors the ozone concentration of the water at the pilot outlet. Micropollutants analyses are carried out using High Performance Liquid

Chromatography (HPLC) or Gas Chromatography (GC) methods followed by either fluorescence, or mass spectrometry or UV detection.

Results

Pesticides tests

Six tests were carried out with an ozone treatment level ranging from 0 to 2.3 g/m^3 and with the following experimental conditions: pH = 7.3, 16.9 < T (°C) < 17.7, 0.143 < UV_{254} (cm^{-1}) < 0.184 and A_T = 4.4 meq/L. Table 7.5-265 shows the concentrations of pesticides tested in the spiked SFW. These concentrations remained constant during the test runs which lasted three days.

Table 7.5-265: Average pesticides concentrations in spiked Sand Filtered, ozonated and GAC filtered water matrix

			Water flow rate = 12 m^3/h & pH = 7.3 & 16.9 < T (°C) < 17.7 & 0.143 < UV_{254} (cm^{-1}) < 0.184 & A_T = 4.4 meq/L					
			Ozone treatment rate = 0 g/m^3		Ozone treatment rate: 1.2–2.3 g/m^3 Dissolved ozone residual: 0.13–0.68 mg/L CT: 1.1–8.4 mg/min/L			
Pesticides	Limit of quantification (µg/L)	Average SFW[*] concentration (µg/L) (n = 3)	SCAG1[†] concentration (µg/L) t ~ 5 min	SCAG2[†] concentration (µg/L) t ~ 10 min	SC1[‡] concentration (µg/L) Minimum–Maximum (n = 5)	SC2[§] concentration (µg/L) Minimum–Maximum (n = 5)	SCAG2[†] concentration (µg/L) Minimum–Maximum (n = 5)	Ozone removal (%) Minimum–Maximum
Acetochlore	0.02	0.28	0.05	<0.02	0.14–0.19	0.08–0.14	<0.02	50–71
Alachlore	0.02	0.17	0.03	<0.02	0.09–0.12	0.02–0.11	<0.02	54–88
Amitrole	0.10	1.17	<0.10	<0.10	<0.10	<0.10	<0.10	>91
AMPA[¶]	0.10	0.80	0.70	0.60	0.20–0.70	<0.10	<0.10	>88
Atrazine	0.02	0.16	<0.02	<0.02	0.12–0.15	0.10–0.15	<0.02	19–38
Azoxystrobine	0.02	0.22	0.04	<0.02	<0.02	<0.02	<0.02	>91
Bentazone	0.02	0.26	0.12	0.10	<0.02	<0.02	<0.02	>92
Bromuconazole	0.02	0.20	<0.02	<0.02	0.08–0.16	0.08–0.16	<0.02	21–61
Carbendazime	0.02	0.41	<0.02	<0.02	<0.02	<0.02	<0.02	>95
Carbetamide	0.02	0.35	<0.02	<0.02	0.15–0.21	0.07–0.11	<0.02	69–80
Carbofuran	0.02	0.97	0.29	0.06	0.18–0.54	<0.02–0.07	<0.02	93–> 98
Chloridazone	0.10	1.40	<0.10	<0.10	0.24–0.75	<0.10–0.21	<0.10	85–> 93
Chlortoluron	0.02	0.23	<0.02	<0.02	<0.02	<0.02	<0.02	>91
DCPMU[¶]	0.02	0.09	<0.02	<0.02	<0.02	<0.02	<0.02	>77
DEA[**]	0.02	0.29	<0.02	<0.02	0.27–0.32	0.29–0.54	<0.02	–
DEDIA[††]	0.02	0.27	<0.02	<0.02	0.28–0.33	0.30–0.35	<0.02	–
Deethylterbumeton	0.02	0.26	0.03	<0.02	0.21–0.28	0.22–0.26	<0.02	1–16
DIA[‡‡]	0.02	0.28	<0.02	<0.02	0.23–0.30	0.22–0.26	<0.02	6–20

[*]Spiked sand filtered water.
[†]GAC filtration outlet.
[‡]Transfer chamber outlet.
[§]Contact chambers outlet and.
[¶]Aminomethylphosphonic acid.
[¶]...3,4-dichlorophenyl-methylurea.
[**]Deethylatrazine.
[††]Deethyldeisopropylatrazine.
[‡‡]Deisopropylatrazine.

Table 7.5-265 – continued

			Water flow rate = 12 m^3/h & pH = 7.3 & 16.9 < T (°C) < 17.7 & 0.143 < UV_{254} (cm^{-1}) < 0.156 & A_t = 4.4 meq/L					
			Ozone treatment rate = 0 g/m^3		Ozone treatment rate: 1.2–2.3 g/m^3 Dissolved ozone residual: 0.13–0.68 mg/L CT: 1.1–5.4 mg min/L			
Pesticides	Limit of quantification (µg/L)	Average SFW* concentration (µg/L) (n = 2)	SCAG1† concentration (µg/L) + ~5 min	SCAG2† concentration (µg/L) + ~10 min	SC1‡ concentration (µg/L) Minimum–Maximum (n = 5)	SC4§ concentration (µg/L) Minimum–Maximum (n = 5)	SCAG2† concentration (µg/L) Minimum–Maximum (n = 5)	Ozone removal (%) Minimum–Maximum
Dichloroprop	0.05	0.20	0.05	<0.05	0.14–0.18	0.11–0.13	<0.05	35–45
Difenoconazole	0.04	0.19	<0.04	<0.04	0.04–0.16	<0.04–0.14	<0.04	28– >79
Dimetachlore	0.02	0.22	0.04	<0.02	0.08–0.14	0.06–0.11	<0.02	50–73
Diuron	0.02	0.47	<0.02	<0.02	<0.02–0.13	<0.02	<0.02	>96
Ethofumesate	0.02	0.28	<0.02	<0.02	0.16–0.24	0.10–0.21	<0.02	26–65
Fluquinconazole	0.02	0.14	<0.02	<0.02	<0.02–0.11	<0.02–0.07	<0.02	50– >86
Flusilazole	0.02	0.15	<0.02	<0.02	0.03–0.10	0.04–0.10	<0.02	32–73
Glyphosate	0.10	1.07	0.60	0.50	<0.10	<0.10	<0.10	>91
Hydroxyatrazine	0.02	0.12	<0.02	<0.02	0.11–0.12	0.09–0.10	<0.02	19–27
Imazamethabenz-methyl	0.02	0.21	0.07	0.02	0.15–0.21	0.14–0.19	<0.02	10–33
Isoproturon	0.02	0.45	<0.02	<0.02	<0.02	<0.02	<0.02	>96
MCPA¶	0.05	0.42	0.06	<0.05	0.12–0.19	<0.05–0.06	<0.05	86– >88
Mecoprop	0.05	0.15	0.05	<0.05	<0.05–0.06	<0.05	<0.05	>67
Metazachlore	0.02	0.13	0.03	<0.02	0.03–0.08	0.02–0.07	<0.02	46– >85
Metolachlore	0.02	0.30	0.06	<0.02	0.17–0.20	0.11–0.15	<0.02	50–63
Piclorame	0.05	0.15	<0.05	<0.05	0.10–0.16	0.09–0.14	<0.05	7–40
Prochloraze	0.02	0.14	<0.02	<0.02	<0.02	<0.02	<0.02	>86
Propazine	0.02	0.16	0.03	<0.02	0.13–0.16	0.11–0.16	<0.02	0–30

*Spiked sand filtered water.
†GAC filtration outlet.
‡Transfer chamber outlet.
§Contact chambers outlet end.
¶(4-chloro-2-methylphenoxy)acetic acid.

The presence of glyphosate and AMPA downstream of the GAC filtration unit indicated that neither compound was adsorbed by the column.

The tests with ozone show that three groups of components could be distinguished according to their reactivity to ozone:

- Very reactive molecules are removed as early as transfer chamber outlet: this included glyphosate.
- Less reactive molecules are removed at contact chambers outlet: including AMPA,
- Weak reactive molecules are only partially removed at contact chambers outlet.

Glyphosate and AMPA were not adsorbed in the GAC filter unit, but were reactive or very reactive to ozone.

Conclusion

In the context of a multi-barrier DWTP, ozonation remains an essential disinfection step: its capacity to inactivate viruses is necessary to control health risks. The ozone treatment levels needed to reach disinfection targets can also remove several emerging pollutants by oxidation like pesticides, pharmaceuticals, phthalates, nonylphenols and hormones.

Glyphosate was found to be very rapidly degraded by ozone treatment (>91 %, levels reduced to <0.1 µg/L) and AMPA was rapidly removed (>88 %, levels reduced to <0.1 µg/L).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes the removal of glyphosate and AMPA among other substances from spiked drinking water with a combined ozonation – deozonation - filtration approach. Glyphosate was found to be very rapidly degraded by ozone treatment (>91 %, levels reduced to <0.1 µg/L) and AMPA was rapidly removed (>88 %, levels reduced to <0.1 µg/L); hence, the ozone treatment required to deliver disinfection targets was also effective in removing glyphosate and AMPA to levels below 0.1 µg/L. However, no information about potential break-down products were provided.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/093
Report author	Manassero A. et al.
Report year	2010
Report title	Glyphosate degradation in water employing the H_2O_2/UVC process
Document No	Water research, (2010 Jul) Vol. 44, No. 13, pp. 3875-82
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

Glyphosate is the organophosphate herbicide most widely used in the world. Any form of spill or discharge, even if unintentional, can be transferred to the water due to its high solubility. The combination of hydrogen peroxide and UV radiation could be a suitable option to decrease glyphosate concentration to acceptable limits. In this work, the effects of initial pH, hydrogen peroxide initial concentration, and incident radiation in glyphosate degradation were studied. The experimental device was a cylinder irradiated with two tubular, germicidal lamps. Conversion of glyphosate increases significantly from pH = 3-7. From this value on, the increase becomes much less noticeable. The reaction rate depends on the initial herbicide concentration and has an optimum plateau of a hydrogen peroxide to glyphosate molar concentration ratio between 7 and 19. The expected non-linear dependence on the irradiation rate was observed. The identification of critical reaction intermediaries, and the quantification of the main end products were possible and it led to a proposal of a plausible degradation pathway. The achieved quantification of the extent of mineralization is a positive indicator for the possible application of a rather simple technology for an *in situ* solution for some of the problems derived from the intensive use of glyphosate.

Materials and methods

Chemicals

The following reagents were used: (a) glyphosate (AccuStandard) as standard chromatographic, (b) glyphosate 95 % provided by Red Surcos, (c) hydrogen peroxide (Ciccarelli p.a., >99 %), (d) sarcosine (≥97.5 %, Sigma-Aldrich), (e) glycine (97.3 %, Merck), (f) aminomethylphosphonic acid, AMPA (≥99 %, Sigma-Aldrich), (g) formic acid (98-100 %, Merck), (h) acetic acid (100 %, Merck), (i) glycolic acid (solution 70 % in water, Merck) and (j) catalase from bovine liver, >2000 units/mg (Fluka, 1 unit decomposes 1 µmol H_2O_2 per minute at pH 7.0 and 25°C). Ultra pure water (0.055 µS/cm) was used in all experiments. This water was obtained from an OSMOION purification system made of several filters to eliminate particulate matter, chlorinated compounds, and low molecular weight organic substances. Two reverse osmosis membranes and an ion exchange resin completed the equipment.

Table 7.5-266: Experimental program

Variable	Value
Glyphosate initial concentration (mM)	0.15–0.54
H_2O_2 initial concentration (mM)	0–11.82
Photon fluence rate ($E_{p,0}$) (Einstein cm^{-2} s^{-1}) × 10^9	
Heraeus 40 W lamp (100%)	23.3
Philips 15 W lamp	10.4
Heraeus 40 W lamp (with filter) (16%)	4.2
Reaction time	5 h
Initial pH	3.5–7–10
Temperature	25 °C

Experimental setups and procedures

The photodegradation of glyphosate was carried out in a cylindrical reactor made of Teflon TM, with two parallel, flat windows made of quartz ($V_{Reactor}$ = 110 cm^3). Each window was irradiated with a tubular, germicidal lamp (λ = 253.7 nm) placed at the focal axis of a parabolic reflector made of mirror finished aluminum. The small reactor operated in the loop of a batch recycling system that included a pump, a heat exchanger (for temperature control) and a large volume, well stirred tank with provisions for sampling, temperature control and pH measurements (V_{Total} = 2000 cm^3). Further details on the experimental device can be found elsewhere. Experiments were carried out changing the following variables: (i) initial glyphosate concentrations, (ii) initial hydrogen peroxide concentrations, (iii) initial pH and (iv) incident radiation on the windows of radiation entrance (or, according to IUPAC, the photon fluence rate, $E_{p,0}$) measured with potassium ferrioxalate actinometry (Table 7.5-266). Most of the experiments were done at 0.30 mM of glyphosate initial concentration. Lower and higher concentrations were used to study the behavior of glyphosate degradation at different initial concentrations. Values between 0.30 and 0.45 mM are important from an environmental point of view since they are the average values of glyphosate concentrations found in wastewaters which result from rinsing herbicide containers.

Analytical methods

Glyphosate was analyzed by ion chromatography with a suppressed conductivity detector and employing an Ion Pac AG4A-SC guard column, an AS4A-SC separating column, and an ion self-regenerating suppressor (Alltech DS-Plus) with electrochemical methods. A solution of Na_2CO_3 (9 mM) and NaOH (4 mM) was used as eluent at a flow-rate of 1.5 mL/min. The injection volume was 20 µL. Under this condition the retention time for glyphosate was 4.77 min. The aminomethylphosphonic acid (AMPA) standard could be identified under the same operating conditions. pH was monitored with a HI 98127 Hanna pH meter. Hydrogen peroxide was analyzed using a colorimetric method following techniques reported elsewhere,

and employing a Cary 100 Bio UV visible spectrophotometer. Total organic carbon (TOC) was analyzed in order to compare glyphosate degradation rate with total mineralization rate and also in order to provide more accurate information about possible reaction intermediates. The instrument used was a Shimadzu TOC-5000A. End products were monitored by ion chromatography, and following a procedure similar to the one employed for glyphosate analysis. The identification of glycine, sarcosine and NH_3 was done employing a specific test for free amino acids according to methodology published elsewhere. The presence of formaldehyde was also confirmed using a specific colorimetric method (NIOSH, 1994). Though the possible degradation products monitored were: glycine, sarcosine, AMPA, formaldehyde, acetic acid, formic acid, nitrate anion, ammonium and phosphate anion, only nitrate and phosphate ions were quantified.

Operations

The experimental run was started after every variable of the operating conditions had reached its steady-state and/or uniformity: concentrations, temperature, irradiation rates, etc. The employed equipment permitted the reactor to be isolated from the irradiating system until the starting time was reached. It should be noted that due to the type of equipment used in this work (an irradiated reactor in a recycle that includes a large volume tank) the reaction time plotted in the figures does not represent the irradiation time of the active reaction volume. The real reaction time is the reaction time measured in every experiment and multiplied by the ratio $V_{Reactor}/V_{Total}$ which is a factor <<1.

Results and discussion

Preliminary runs

Two types of previous experiments were carried out in order to investigate the effects of UVC and H_2O_2 separately. The first run was performed employing C^0_{Glyph}: = 0.30 mM, (50 mg/L); C^0_{H2O2} = 2:20 mM; (75 mg/L) and without UV radiation. After 3 h of total time, no noticeable changes in glyphosate concentration were observed. A similar run was performed with C^0_{Glyph}: = 0.30 mM (50 mg/L) and using 40 W Heraeus UVC lamps turned on during 3 h of total time. No signs of direct photolysis were observed, as it had been previously reported elsewhere. This is in agreement with the absorption spectrum of glyphosate, at least in the range from 200 to 400 nm.

Effects of initial pH values

The experiments were carried out at different initial pH: 3.5 (which results from the preparation of the reacting mixture), 7 and 10, and at initial concentrations of glyphosate and hydrogen peroxide of 0.30 mM and 2.20 mM, respectively. pH adjustment was accomplished by the addition of the required amount of 1 N NaOH. The results have shown that the best condition for degradation took place at the highest pH value. However, there are no significant differences between pH 7 and pH 10 (Figure 7.5-228).

Effects of initial H_2O_2 concentration

It is known that there is an optimum concentration of H_2O_2 in the UV/H_2O_2 process. The results, for a total reaction time of 5 h, were analyzed using the final glyphosate conversion under the following operating conditions: C^0_{Glyph}: = 0.30 mM (50 mg/L), pH = 7, two 40 W lamps and H_2O_2 concentration range from 0 to 12.4 mM (Figure 7.5-229a). It is clear that 2.2-5.9 mM (75-200 mg/L) is the range of higher reaction rates. These values are related to the H_2O_2/glyphosate molar ratio between 7.3 and 19.7. Within this plateau, conversion of glyphosate after 5 h was almost 70 %. For a run under the best operating conditions for degradation, Figure 7.5-229b shows the temporal progression of the participating species concentrations. The existence of this optimum is a well-known phenomenon which results from the scavenging effect of the excess of OH radicals on the hydrogen peroxide. The glyphosate decay follows a first-order kinetics with an observed rate constant k = 0.20/h ± 0.01 (3.68/h total process time) with a correlation coefficient of 0.9986. Also, the half-life value was calculated, resulting $t_{1/2}$ = 0.19 h (3.5 h total process time).

Figure 7.5-228: **Experiments made under the following conditions: C^0_{Glyph} = 0.30 mM; (50 mg/L), C^0_{H2O2} = 2.20 mM; (75 mg/L) at different initial pHs and using a UV lamp of 40 W input power: ●, pH 3.5; ■, pH 7 and ▲, pH 10**

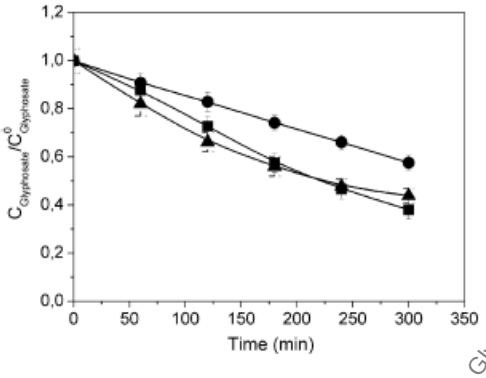
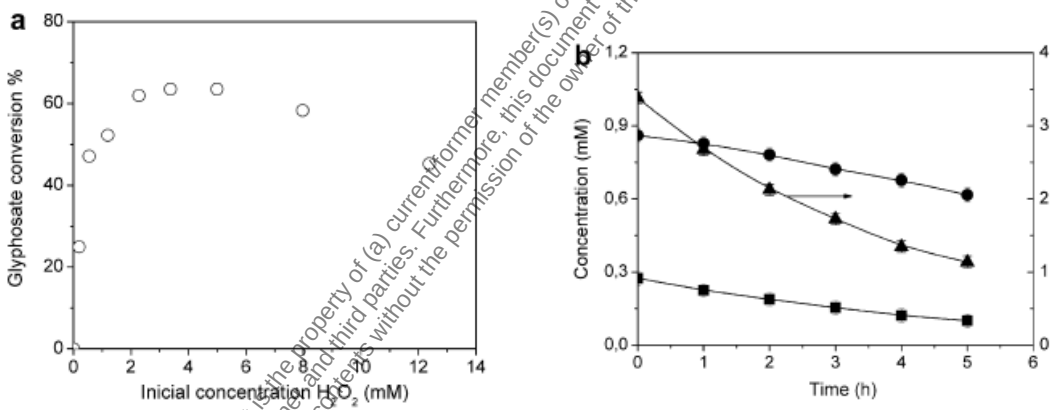


Figure 7.5-229: **(a) Glyphosate conversion, for a fixed reaction time (5 h) vs. initial H_2O_2 concentration. C^0_{Glyph}: 0.30 mM; (50 mg/L), pH = 7 and UV lamp of 40W input power. (b) Glyphosate, H_2O_2 and TOC concentration evolution as a function of time. C^0_{Glyph}:0:27 mM; (46.4 mg/L), C^0_{H2O2} = 3:38 mM; (114.9 mg/L) and UV lamp of 40W input power: ■, glyphosate ▲, :, H_2O_2 and ● C, TOC**



Effects of glyphosate initial concentration

The glyphosate degradation for different initial glyphosate concentrations - between 0.16 and 0.54 mM - and the same hydrogen peroxide initial concentration is shown in Figure 7.5-230. The degradation rate is pseudo-first order with respect to initial concentration.

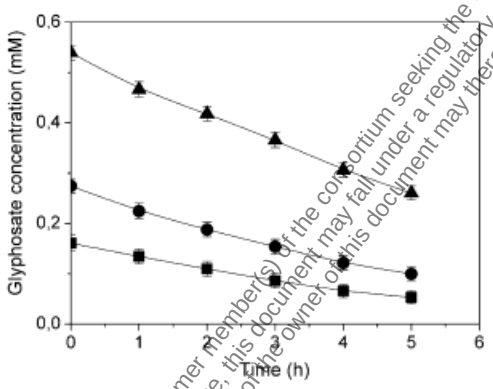
Effect of UV incident radiation intensity

The change on glyphosate concentration under different UV incident radiation rates at the reactor windows, at pH = 7, and for initial glyphosate and hydrogen peroxide concentrations of 0.30 mM and 3.38 mM, respectively, is shown in Figure 7.5-231. For a reaction time of 5 h, with two 40 W lamps (Photon fluence rate, $E_{p,0}$ = 2.3 x 10^{-8} Einstein/(cm^2s), a glyphosate conversion of 63.5 % was reached while conversions with two 15W lamps ($E_{p,0}$ = 10.4 x 10^{-9} Einstein /(cm^2s)) and two 40 W lamps with neutral density filters ($E_{p,0}$ = 4.2 x 10^{-9} Einstein/(cm^2s), were 36.3 % and 20 %, respectively. Please note that this is not a direct indicator of the reaction rate dependence with respect to the absorbed photons because, from the kinetic point of view, the exact information is provided by the average value of the local volumetric rate of photon absorption by H_2O_2 (sometimes called photon absorption rate) and not the fluence rate at the reactor walls.

Total Organic Carbon (TOC) evolution

The total organic carbon (TOC) concentration at every elapsed time is important from two points of view: (i) because it is one of the best indications to conclude that complete mineralization has been achieved. When the TOC concentration is zero, it is certain that the glyphosate and all the reaction byproducts have been entirely degraded. (ii) Because it is always possible to calculate the equivalent theoretical TOC value from the experimentally measured glyphosate concentration at each reaction time. This result can be compared with the above-mentioned experimental TOC. This information is very useful to have an indicator of the existence of stable reaction intermediates; i.e., other organic, carbon-containing compounds, during the progress of the reaction. Figure 7.5-232 depicts the result of a representative run. It proves the existence of different reaction intermediates. It was also observed that, under these experimental conditions, TOC conversion after 5 h was 29 %.

Figure 7.5-230: Glyphosate concentration as a function of time. Initial glyphosate concentration is the parameter: C^0_{Glyph}: 0.54 mM;●, C^0_{Glyph}: 0:27 mM, ■, C^0_{Glyph}: 0.16 mM; UV lamp of 40 W input power and pH = 7



Formation of byproducts

In order to confirm the extent of glyphosate oxidation and to obtain a better understanding of the reaction mechanism involved, a byproduct evaluation is needed. However, given the complex variety of photoproducts that can be produced, an exhaustive identification and quantification of all intermediate products would be very difficult. Hence, this study primarily focused on the major stable byproducts of the reaction. As shown in Figure 7.5-233, the mineralization of glyphosate under a longer run time using UV/H_2O_2 process is evidenced by the evolution of inorganic anions at the highest oxidation states, i.e., phosphate and nitrate. For each mol of glyphosate that is decomposed, one mol of phosphate appears at each reaction time (in the run shown in Figure 7.5-233, after 10 h of total reaction time, the difference between the theoretical and the experimental phosphate concentration was 7 %). However, for nitrate ion the concentration of this end product was below the expected stoichiometric value. In fact, under the operating time shown in Figure 7.5-233, less than 20 % of initial nitrogen is under the nitrate form. In addition to mineral ions, formic acid was detected in the degradation samples. However, other organic acids such as acetic and glycolic acids were not found in this study.

Figure 7.5-231: **Effect of irradiation rates on the reaction rate. Dimensionless glyphosate concentration vs. time. The parameter is the lamp input power for C^0_{Glyph} = 0.30 mM; (50 mg/L), C^0_{H2O2} = 3:38 mM; (115 mg/L) and pH = 7: ●, Heraeus, 40W input power with filter, ▲, Philips, 15W input power, ■ Heraeus, 40W input power**

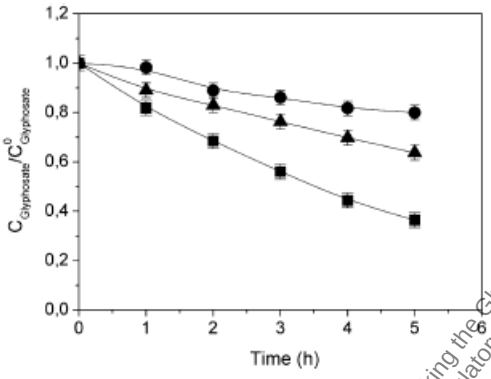


Figure 7.5-232: **Total organic carbon evolution at pH 7 and UV lamps of 40W input power. Conditions: $C^0_{Glyph.}$ = 0:30 mM; (50 mg/L), C^0_{H2O2} = 2:35 mM; (80 mg/L). ○, calculated TOC ●, experimental TOC**

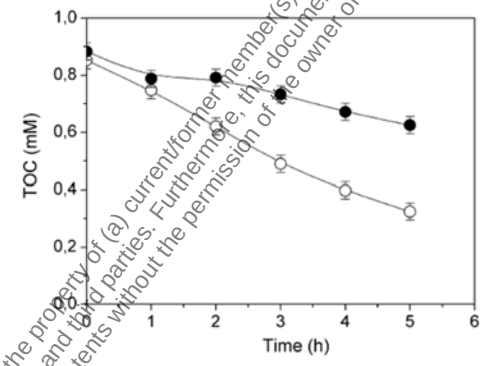
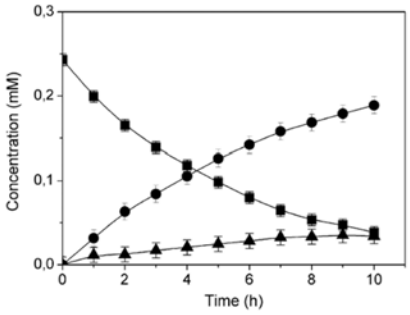


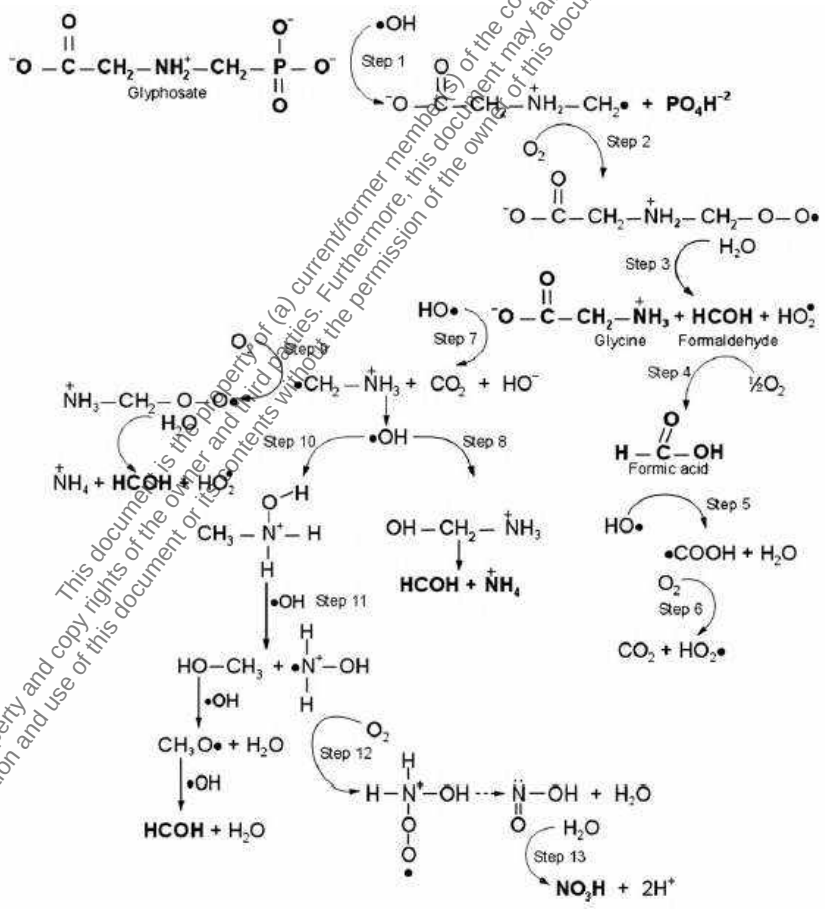
Figure 7.5-233: **Evolution of glyphosate and end products during an extended run made under the best operating conditions for degradation: ■, glyphosate; ●, phosphate and ▲, nitrate. Conditions: $C^0_{Glyph.}$ = 0:24 mM; (41 mg/L), C^0_{H2O2} = 2:4 mM; (83 mg/L)**



A reaction pathway proposal

A plausible reaction pathway of glyphosate decomposition with the H_2O_2/UV system is proposed (Figure 7.5-234). At pH 7 the glyphosate has the three hydroxyl groups ionized and the amino group protonated. The OH formation follows the classical mechanism related to hydrogen peroxide decomposition under illumination. The OH radical attacks glyphosate, which leads to the formation of a carbon centered radical $\bullet CH_2\text{-}NH_2^+\text{-}CH_2\text{-}COO^-$ and phosphate. Since evolution of phosphate occurred during the initial stages of glyphosate decomposition, it may be inferred that C-P cleavage led to formation of phosphate (Step 1). The generated radical can react with molecular oxygen present in the medium at high concentration to give a new radical $COO^-\text{-}CH_2\text{-}NH_2^+\text{-}CH_2\text{-}O\text{-}O$ (Step 2), which reacts directly with water to form glycine, formaldehyde and HO_2 radical (Step 3). The direct formation of glycine without the sarcosine generation was proposed due to verified absence of this compound in the described analytical procedures. The experimental results indicated that when this process was applied, only glycine was present. Furthermore, the absence of AMPA in all samples was confirmed. The generation of formaldehyde was also confirmed as described before. The formaldehyde generated (in all steps) can be directly oxidized to formic acid by the dissolved oxygen under UV light as proposed elsewhere. The steps corresponding to formic acid degradation have been proposed taking into account that the hydroxyl radical formed produces a hydrogen abstraction from the H-C bond to give rise to a •COOH radical. This radical, combined with the existing oxygen in the medium, result in CO_2 and the hydroperoxyl radical HO_2 (Step 5).

Figure 7.5-234: A proposal of a reaction scheme for glyphosate degradation with the UV/H_2O_2 process



For the next oxidation step of glycine in aqueous solution, it is proposed the decarboxylation of the a-amino

acids due to the presence of the •OH radical. It results in CO_2 and $•CH_2NH_3^+$ radical (Step 7). This step is suggested elsewhere to degrade amino acids upon exposure to aqueous titania suspensions and irradiated with UV. The combination of $•CH_2NH_3^+$ and •OH radicals produces formaldehyde and NH_4^+ (Step 8). The generation of NH_3 has been detected as mentioned in the analytical section. There is also another possible step: the addition reaction of molecular oxygen to the $•CH_2NH_3^+$ radical to produce NH_4, formaldehyde and HO_2 radical (Step 9). The nitrate formation would follow an alternative path to the NH_4 formation. The nitrogen radical also reacts with •OH radical in Step 10 to give a protonated hydroxylamine intermediate. This oxidation path is proposed elsewhere as one of various steps during the photodegradation of an amino acid catalyzed by irradiated TiO_2. Afterwards, a possible reaction is that the protonated hydroxylamine reacts with •OH radical to produce methanol and $•NH_2^+$-OH radical (Step 11). This nitrogen radical can react with molecular oxygen to generate $•O\text{-}O\text{-}NH_2^+$-OH nitrogen radical. A similar step is proposed elsewhere in the removal of hydroxylamine by means of processes which generate •OH radicals in aqueous solution. The $•O\text{-}O\text{-}NH_2^+$-OH radical, under reorganization, yields nitrous acid (or nitrite) (Step 12). Then, the nitrous acid, by means of hydrolysis, is transformed into nitric acid (or nitrate) (Step 13). The nitrite and nitrate formation from hydroxylamine is also proposed elsewhere. The nitrate evolution for the longer run time shows that other forms of nitrogen compounds, such as glycine and nitrite, may be present. The methanol formed in Step 11 can be oxidized by •OH radical to generate, first, the $CH_3O•$ radical and then, formaldehyde. This oxidation pathway is described elsewhere as a possible mechanism where methanol is oxidized by the free •OH in solution.

In summary, the glyphosate decomposition stable compounds identified in this study were: glycine, formaldehyde, formic acid, nitrate anion, ammonium and phosphate anion (see compounds in bold face in Figure 7.5-234). The authors note that one of the most interesting outputs of this process is that, at a first glance, it seems that none of the formed byproducts is toxic.

Conclusion

1. As shown in this work, the study of the resulting effects of the most significant operating variables on glyphosate degradation would indicate that the combination of hydrogen peroxide and UV radiation may become a suitable and very simple process to remove glyphosate from water.

2. A proposal for a degradation path based on the observed experimental data has been possible.

3. In a first approach, it seems that glyphosate degradation does not lead to stable toxic end products.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the degradation of glyphosate under H_2O_2/UVC processes and the generation of breakdown products. The experiment is well described. A degradation pathway is proposed.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/094
Report author	Brosillon, S. *et al.*
Report year	2006
Report title	Chlorination kinetics of glyphosate and its by-products: Modeling approach
Document No	Water Research 40 (2006) 2113-2124
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Chlorination reactions of glyphosate, glycine, and sodium cyanate were conducted in well agitated reactors to generate experimental kinetic measurements for the simulation of chlorination kinetics under the conditions of industrial water purification plants. The contribution of different by-products to the overall degradation of glyphosate during chlorination has been identified. The kinetic rate constants for the chlorination of glyphosate and its main degradation products were either obtained by calculation according to experimental data or taken from published literature. The fit of the kinetic constants with experimental data allowed the authors to predict consistently the concentration of the majority of the transitory and terminal chlorination products identified in the course of the glyphosate chlorination process. The simulation results conducted at varying aqueous chlorine/glyphosate molar ratios have shown that glyphosate is expected to degrade in a fraction of a second under industrial aqueous chlorination conditions. Glyphosate chlorination products are not stable under the conditions of drinking water chlorination and are degraded to small molecules common to the degradation of amino acids and other naturally occurring substances in raw water.

Methods

Analytical conditions

Glyphosate and glycine were analyzed by HPLC fluorescence after pre-column FMOC derivatization using diethylether. Aqueous formaldehyde was analysed by HPLC–UV after a pre-column 2,4-DNPH derivatization. For the detection of anions (cyanate, nitrate and phosphate ions), samples were analysed using a Dionex AS9-HC ion chromatography (IonPac) column and suppressed conductivity detection.

Kinetics experiments

For the kinetic measurements, 10-4 M solutions of glyphosate, glycine, or sodium cyanate were chlorinated in a 1000 mL well-agitated reactor using HOCl/substrate molar ratios of approximately 4 and 50. At scheduled times, 5 mL portions of reaction mixture were withdrawn and quenched with an adequate volume of sodium thiosulfate solution, derivatized and analysed.

Results and discussion

Glyphosate chlorination

The dissipation of glyphosate and the formation of its chlorination products after 24 h of reaction conducted in a well-agitated reactor at various chlorine/glyphosate molar ratios at pH 7 indicated glyphosate decay

was complete at chlorine/glyphosate molar ratios close to 2 or higher. The phosphonic acid moiety of glyphosate was converted into phosphoric acid at all ratios of applied chlorine. Nitrate ion was first detected at a chlorine/glyphosate molar ratio of approximately 10 and a maximum concentration was obtained at chlorine levels of 50 M equivalents or higher. In addition to nitrate, nitrogen gas was also a product of glyphosate chlorination. Hydrated formaldehyde (methanediol) and cyanogen chloride (V; CNCl)) were also formed.

The comparison of the products of glyphosate chlorination conducted at pH 7 and 8 showed no significant differences within the pH range relevant to the purification of natural water commonly sourced for drinking water.

To obtain kinetic rate constants, glyphosate chlorination was monitored for 24 h under the reaction conditions in which the chlorine concentration was limited to 4 M equivalents. Glyphosate degradation was complete and very fast, i.e. at the first measurements (10 min), no glyphosate was detected. The concentrations of methanediol and phosphoric acid reached maximums early in the reaction and remained unchanged during the remaining 24 h reaction period. The kinetics of the production of the transitory product cyanate/cyanogen chloride showed two steps: a sharp increase up to 30 min of contact and a slight increase over the remaining 24 h reaction period. Nitrate is not produced in significant amounts under the low excess chlorine chlorination conditions. The chlorination kinetics were carried out in purified water. However, chlorination of glyphosate conducted in typical environmental water samples, from actual water treatment plants, produced identical by-products.

Glycine chlorination

In order to confirm the proposed pathway for glyphosate chlorination, the chlorination of the related amino acid, glycine, was carried out. At low chlorine/glycine molar ratios (2 and 2.5) the cyanogen chloride/cyanate concentration appeared to reach a maximum value after 24 h of reaction, indicating that all of the active chlorine was consumed, hence all the chlorinating reactions were stopped and only hydrolysis could occur. Runs conducted at chlorine/glycine molar ratios of 5–7 mol mol-1 were strikingly different from those conducted at lower ratios. Indeed the amount of cyanogen chloride/cyanate was found to increase to maximum concentrations early in the reaction and then to decline quickly, most likely due to the chlorine-assisted catalytic hydrolysis of cyanogen chloride.

The nitrate concentration reached a plateau for chlorine/ glycine molar ratio of 5–7 mol mol-1 after 2 h. These results confirmed the hypothesis that nitrate is the terminal product of glyphosate/glycine chlorination and cyanogen chloride/cyanate can be considered as transitory intermediates.

Cyanate (VI) chlorination

The chlorination reaction of sodium cyanate(VI) was also investigated for further insight into the kinetic pathway of glyphosate/glycine chlorination. Fast dissipation of cyanate(VI) occurred when chlorination reactions were carried out at a chlorine/cyanate molar ratio of 5 mol mol-1. The cyanate(VI) chlorination reaction solution contained nitrate and inorganic carbons as the only carbon containing product, suggesting carbon dioxide formation under the reaction conditions. It is believed that the aqueous chlorine reaction with cyanate produces carbon dioxide, hydrochloric acid, nitrate, and nitrogen gas.

Chlorination kinetic model

Chlorination kinetics were simulated at the same glyphosate concentration used for the experimental kinetic measurements (10-4 M) to facilitate comparisons. A typical concentration of glyphosate in raw water is expected to be at the 10-8 M level (1 mg/L). Nevertheless, the simulation provided a predictive tool for the estimation of kinetic rate constants and the establishment of overall rate of formation and decline of the short-lived transitory and final products of glyphosate chlorination in an industrial plant environment.

The simulations were computed with a glyphosate concentration of 10-4 M in aqueous chlorine solution at an initial pH 7, for different chlorine/glyphosate molar ratios. The model prediction is consistent with a fast decay of glyphosate as observed in experimental runs. For all simulation runs, the mass balance drawn

around chlorine, carbon, nitrogen, and phosphorous showed excellent conformity with the initial chlorine and glyphosate concentrations, indicating that the resolution process of the proposed model worked well. Nitrate production showed the greatest differences between the observed and the computed concentrations of over 15 % for the higher chlorine/glyphosate molar ratios. The model fits the experimental results quite well as highlighted by the remarkable similarity of the pattern of the evolution of the various glyphosate chlorination products. Therefore, the simulation results support the proposed chemical pathway for glyphosate chlorination and the assumptions made on the reaction rate orders and kinetic rate constants.

Conclusion
It was possible to quantitatively define the decomposition kinetics of glyphosate chlorination and to establish the overall rate of formation and decline of transitory and final chlorination products of glyphosate. The data generated were used to develop mathematical models for predicting glyphosate chlorination and understanding of the nature and lifetime of its transient chlorination products. The simulations of chlorination of glyphosate and model compounds under conditions similar to those used by water treatment plants have shown that chlorination of glyphosate is complete within seconds of contact with chlorine. The initial products of glyphosate chlorination are not stable under the conditions of drinking water chlorination and are degraded to small molecules, such as CO_2, phosphoric acid, nitrate, nitrogen gas, and methanediol, similar to the degradation of amino acids and other naturally occurring substances in raw water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article generated experimental kinetic measurements for the simulation of chlorination kinetics under the conditions of industrial water purification plants.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/095
Report author	Mehrsheikh, A. *et al.*
Report year	2006
Report title	Investigation of the mechanism of chlorination of glyphosate and glycine in water
Document No	WATER RESEARCH 40 (2006) 3003 - 3014
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable

2. Full summary

The chlorination reactions of glyphosate and glycine in water were thoroughly studied. Utilizing isotopically enriched (^{13}C and ^{15}N) samples of glycine and glyphosate and ^{1}H, ^{13}C, ^{31}P, and ^{15}N NMR spectroscopy all significant terminal chlorination products of glycine and glyphosate were identified, and it was shown that glyphosate degradation closely parallels that of glycine. It has been demonstrated that the C1 carboxylic acid carbon of glycine/glyphosate is quantitatively converted to CO_2 upon chlorination. The C2 methylene carbon of glycine/glyphosate is converted to CO_2 and methanediol. The relative abundance of these two products is a function of the pH of the chlorination reactions. Under near neutral to basic reaction conditions (pH 6–9), CO_2 is the predominant product, whereas, under acidic reaction conditions (pH < 6) the formation of methanediol is favoured. The C3 phosphonomethylene carbon of glyphosate is quantitatively converted to methanediol under all conditions tested. The nitrogen atom of glycine/glyphosate is transformed into nitrogen gas and nitrate, and the phosphorus moiety of glyphosate produces phosphoric acid upon chlorination. In addition to these terminal chlorination products, a number of labile intermediates were also identified including N-chloromethanimine, N-chloroaminomethanol, and cyanogen chloride. The chlorination products identified in this study are not unique to glyphosate and are similar to those expected from chlorination of amino acids, proteins, peptides, and many other natural organic matters present in drinking water.

Methods

NMR experiments:
NMR spectra were recorded using a spectrometer. The proton and carbon-13 chemical shift scales were in parts per million downfield from external tetramethylsilane at 0.0 ppm. Phosphorus-31 proton decoupled NMR spectra were referenced to external phosphoric acid in D_2O and ^{15}N spectra were referenced to an external solution of $^{15}NH_4Cl$ in D_2O.

NMR solutions were prepared by dissolving the appropriate amounts of each test material in NMR solvent (neat D_2O or buffered D_2O) in NMR tubes, with concentrations of glycine and glyphosate for the NMR experiments in the range of 0.38–6.25 mg/mL. Chlorination was conducted in un-buffered D_2O at initial pHs of 8, 7 and 5. Additionally, the chlorination reactions were carried out in a 0.48 M borate buffer in D_2O at pH 8 and 9. An appropriate amount of dilute NaOCl solution in D_2O or buffered D_2O was added to the sample in the NMR tube and the sample was sealed, mixed, and analysed immediately by NMR.

High Performance Liquid Chromatography (HPLC) experiments:
Analyses of the radiolabeled experiments were performed using HPLC. A strong cation exchange column was eluted (flow rate: 0.5 mL min^{-1} at 50 °C) with a 0.005 M solution of KH_2PO_4 (adjusted to pH 2.0 with H_3PO_4) containing 4 % methanol for 35 minutes and the HPLC effluent was passed through a radioactive flow detector. Some samples were analyzed by a second HPLC method using an IonPac column, eluting with a 0.009 M sodium carbonate solution at a flow rate of 1.0 mL min^{-1} for 35 min at ambient temperature using either a radioactivity detector or suppressed conductivity detection.

Chlorination products of glycine and glyphosate were monitored by HPLC using the corresponding ^{14}C-labelled test materials in unbuffered water at initial pHs of 9, 8, 7, 6, and 5 with aqueous chlorine at a chlorine to substrate molar ratio of 100:1. Additionally, the chlorination reactions were carried out in a 0.05 M borate buffer at pH 8 and 9 or a 0.05 M phosphate buffer at pH 7, 6, and 5 in separate experiments. For these experiments, appropriate amounts of the dilute aqueous chlorine solution were transferred into a 2-mL amber coloured autosampler vial equipped with a Teflon septum cap. An aliquot of each ^{14}C-stock solution of glyphosate or glycine was added to each autosampler vial containing dilute aqueous chlorine solution (chlorine to substrate molar ratio of 100:1) kept at room temperature in order to achieve a final concentration of 3.51–7.25 µM (0.27–0.55 µg/mL) for [2-^{14}C]glycine; 4.29–13.03 µM (0.73–2.2 µg/mL) for [3-^{14}C]glyphosate, and 3.61–13.25 µM (0.61–2.3 µg/mL) for [2-^{14}C]glyphosate. Aliquots of the reaction mixture were then analysed by HPLC after 2 h and at about 24 h of contact.

Results and discussion

Figure 7.5-96 illustrates the proposed mechanism for the reaction of glycine with aqueous chlorine. N-chloroglycine (I) is formed when one equivalent of aqueous chlorine is reacted with glycine. N-chloroglycine appears to be stable under the reaction conditions in the absence of excess chlorine. When chlorination is conducted with more than 1 equivalent of aqueous chlorine, N,N-dichloroglycine (II) is detected as the predominant product immediately after contact. Decarboxylation and elimination of HCl of the labile N,Ndichloroglycine will provide N-chloromethanimine (III) as a transitory product, which was detected by NMR. From the product distribution reported in this study, it is postulated that a second mole of HCl is eliminated from N-chloromethanimine (III) to possibly form cyanide, which has not been detected in the experiments. The lack of cyanide detection is indicative of its facile chlorination under the reaction conditions to form CNCl (VII) as has been reported. CNCl (VII) undergoes hypochlorite-assisted catalytic hydrolysis to form CO_2. Alternatively, it is postulated that N-chloromethanimine (III) is hydrated to form N-chloroaminomethanol (IV), favoured under acidic reaction conditions. It should be noted that hydration of N-chloromethanimine to form N-chloroaminomethanol is analogous to the widely known hydration of formaldehyde in water to form methanediol. N-chloroaminomethanol (IV) appears to be quantitatively converted to methanediol within 24h of formation as determined by the NMR experiments. Based on the ^{15}N-NMR work it is proposed that the nitrogen atom of glycine, which is initially released as NH_4Cl and chloramines from the hydrolysis of CNCl and/or decomposition of chloroaminomethanol, is eventually converted to N_2 and nitrate due to further reactions with excess aqueous chlorine. The formation of N_2 and $NaNO_3$ from CNCl chlorination with 5.7 excess molar equivalents of aqueous chlorine has been reported previously.

Figure 7.5-236 illustrates the proposed mechanism for the reaction of glyphosate with aqueous chlorine. Analogous to the chlorination of glycine, and based on NMR evidence, N-chloroglyphosate (VIII) is postulated to be the first intermediate in the glyphosate chlorination. Rearrangement of N-chloroglyphosate through a six-membered ring transition state results in transfer of the phosphorus moiety to the carboxylate oxygen and ultimately formation of the acylphosphate intermediate IX and methanediol. Decomposition of intermediate IX by hydrolysis would lead to the formation of phosphoric acid and glycine/N-chloroglycine. Further chlorination of glycine/N-chloroglycine, according to the reaction scheme depicted for glycine would lead to a mixture of methanediol, CO_2, N_2, and nitrate as the final chlorination products.

With the exception of the formation of phosphoric acid, the final chlorination products of glyphosate are identical to those observed for glycine chlorination. The phosphorus-31 NMR study revealed that addition of one or more equivalents of aqueous chlorine to glyphosate in buffered and unbuffered D_2O, and in the pH ranges of 5–9, produced phosphoric acid as the only P-containing terminal chlorination product.

Figure 7.5-235: Proposed mechanism of glycine chlorination (compounds in boxes are terminal products and intermediates are in brackets)

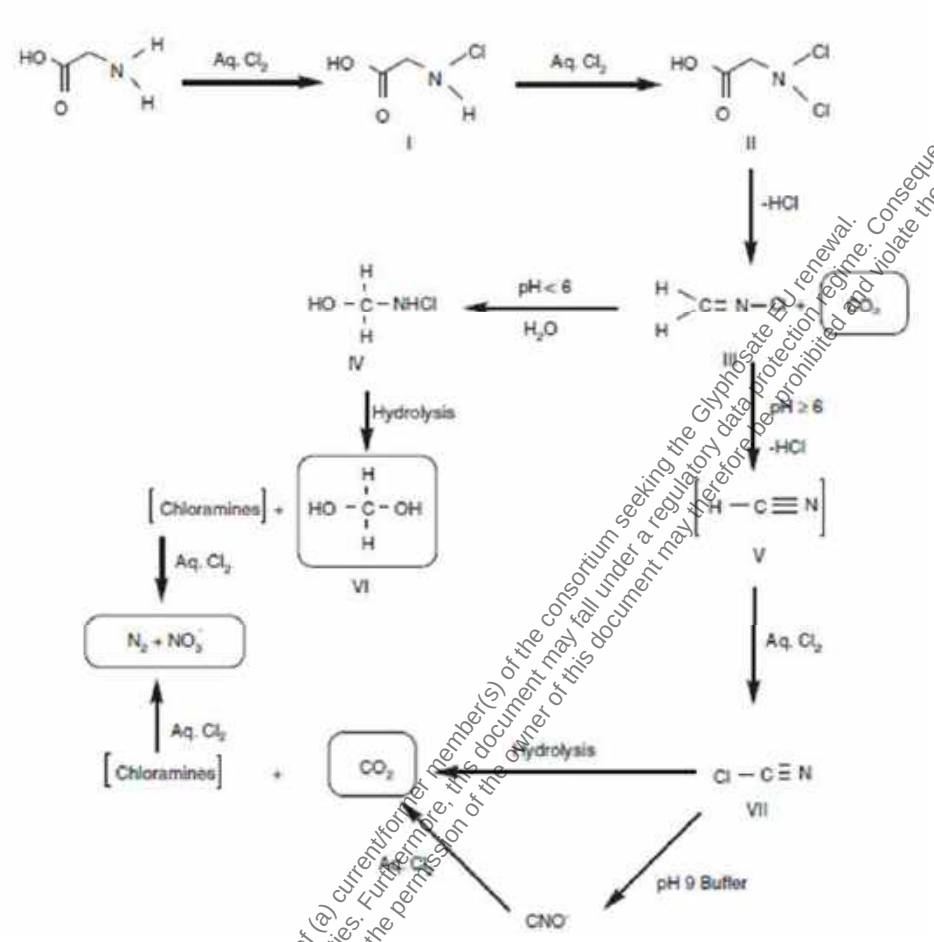
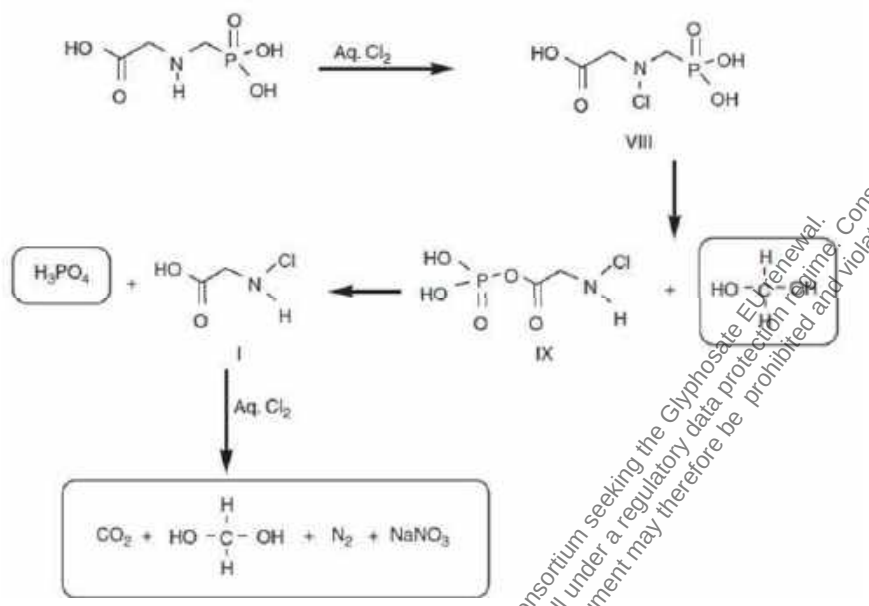


Figure 7.5-236: Proposed mechanism of glyphosate chlorination (compounds in boxes are terminal products)



Conclusion

The results of this study have shown that under aqueous chlorination conditions, glyphosate is totally degraded to small molecules common to the degradation of naturally occurring substances in raw water, and that the degradation pathway follows that of glycine. Utilizing stable isotopes and NMR spectroscopy we were able to identify all significant chlorination products of glycine and glyphosate after total breakdown. It has been demonstrated that upon chlorination the C1 carboxylic acid carbon of glycine/glyphosate is converted to CO_2; the C2 methylene carbon of glycine/glyphosate is converted to CO_2 and methanediol; the nitrogen of glycine/glyphosate is transformed into nitrogen gas and nitrate; the C3 phosphonomethylene carbon of glyphosate is converted to methanediol; and the phosphorus moiety of glyphosate produces phosphoric acid. The terminal glyphosate chlorination products identified in this study (phosphoric acid, CO_2, methanediol, N_2 and nitrate) are not unique to glyphosate and would also be expected as products from chlorination of other natural organic matter present in raw water.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article investigates the mechanism of chlorination of glyphosate and glycine in water. The methods and results are sufficiently described.
The article is considered reliable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 7.5/096
Report author	Klinger, J. *et al.*
Report year	2008
Report title	Formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra (methylenephosphonic acid)
Document No	OZONE SCIENCE & ENGINEERING Vol 20, 99-110
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions

2. Full summary

Because of its widespread use and its low biodegradability, ethylenediaminetetra(methylenephosphonic acid) (EDTMP) might be found in river waters and could even be present in raw waters of drinking water treatment plants. In Europe, average surface water concentrations in the low µg/L range are predicted. Therefore, it is of interest for drinking water supplies whether EDTMP can be eliminated during water treatment processes. Since many water treatment plants have an ozonation step, this paper deals with the behaviour of EDTMP during ozonation. Due to its chemical structure, a reaction scheme for the ozonation of EDTMP similar to the reaction pathway for the ozonation of EDTA was predicted.

The experimental results confirmed the predicted mechanism as well as the formation of glyphosate and AMPA during ozonation of waters containing EDTMP. Ozonation studies were also conducted on glyphosate and AMPA – at acidic pH (pH 5) it was found that glyphosate was partially degraded to AMPA and orthophosphate; and that AMPA was partially degraded to orthophosphate, under the experimental conditions.

Methods
Ozone was produced from high purity oxygen using an ozone generator Ozomat COM 6000. The reactor was a glass bottle with a working volume of 2 L. Ozone concentration in the gas stream was measured at the reactor inlet using an ozone measuring instrument GM 6000. Ozone was transferred into the liquid sample for three minutes with a gas stream of 40 L/h containing about 35 mg/L ozone. While stirring continuously, this time period was long enough to reach equilibrium conditions for dissolving ozone in the aqueous phase. After three minutes, the concentration of dissolved ozone was determined, and the target chemical was added. All reactions were carried out at room temperature in distilled water. Initial concentration of the target chemical was 1 mg/L and initial concentration of dissolved ozone was about 3 mg/L. This ozone dose is close to water works conditions. The pH value after addition of phosphonic acids was constant at pH 5 without adding any buffer solutions. Additionally, experiments in tap water from Karlsruhe were carried out at pH 7. In all cases, total reaction time was 10 minutes. Samples were taken after different reaction times and ozonation was stopped by adding sodium thiosulfate.

EDTMP was preconcentrated by evaporating the sample to dryness, methylated with diazomethane and determined by liquid chromatography and mass spectrometry coupled by a thermospray interface. A Merck LiChrospher 100 Diol (5 um) 125 x 4 mm separation column with a gradient mobile phase containing isopropyl alcohol and n-hexane was used.

Glyphosate and AMPA were determined after extraction on an ion exchange resin by liquid chromatography, post-column derivatization using orthophthaldialdehyde and N,N-dimethyleneaminoethanethiol and fluorescence detection. A strong basic cation exchange separation column with an isocratic aqueous mobile phase containing 0.005 M potassium dihydrogen phosphate and 4 % methanol was used.

Dissolved ozone concentration was determined by the indigo method, which is based on the decolorization of the blue indigo trisulfonate solution by ozone measured spectrophotometrically at 600 nm. Orthophosphate also was determined spectrophotometrically. This method is based on the formation of a molybdenum blue complex which is measured at 880 nm. Phosphonoformic acid was analyzed by ion chromatography with conductivity detection. Standard deviations of the chromatographic methods were about 10 %.

Results and discussion

Within one-minute reaction time, EDTMP is completely eliminated, but orthophosphate is formed up to only 50 % yield. Because orthophosphate formation rises up to 60 % within the entire reaction time of 10 minutes, one-half of the initial EDTMP concentration reacts very fast to orthophosphate and the other half is oxidized to further phosphorus-containing metabolites, such as phosphonoformic acid (PFA) and aminomethylphosphonic acid (AMPA) which may not react or react only very slowly with ozone. This is in accordance with ozone degradation during ozonation of EDTMP. After three minutes reaction time, no further significant ozone consumption takes place. Thus, after 10 minutes reaction time, the concentration of dissolved ozone is still 1.2 mg/L.

Due to the similar chemical structures of EDTMP and ethylenediaminetetraacetic acid (EDTA), an analogous reaction pathway and, consequently the formation of analogous intermediates during ozonation, seems to be quite probable. In order to verify the formation of the predicted ozonation products, not only concentration of orthophosphate but also concentrations of glyphosate, AMPA and phosphonoformic acid were determined during ozonation of EDTMP. Furthermore, the identified oxidation products were treated with ozone in order to check their behaviour during ozonation separately.

Glyphosate, AMPA and phosphonoformic acid could be clearly identified under respective experimental conditions. With an initial concentration of 1 mg/L EDTMP, after 10 minutes reaction time, 1.2 µg/L glyphosate, 100 µg/L AMPA and 63 µg/L phosphonoformic acid were found. As can be seen from the mass balance data given in Table 7.5-267, up to now not all phosphorus-containing oxidation products are identified. This is due to the reaction pathway given in Figure 7.5-237, where other phosphorus-containing intermediates might be formed which are not amenable to analytical methods. Furthermore, the mass balance data in the table shows that no significant reaction takes place after 30 seconds, although dissolved ozone is still present. As already mentioned, this might be due to the low reactivity of the identified phosphorus-containing oxidation products. To verify this assumption, glyphosate, AMPA and PFA were treated with ozone.

Figure 7.5-237: Suggested reaction pathway for the oxidation of EDTMP by ozone

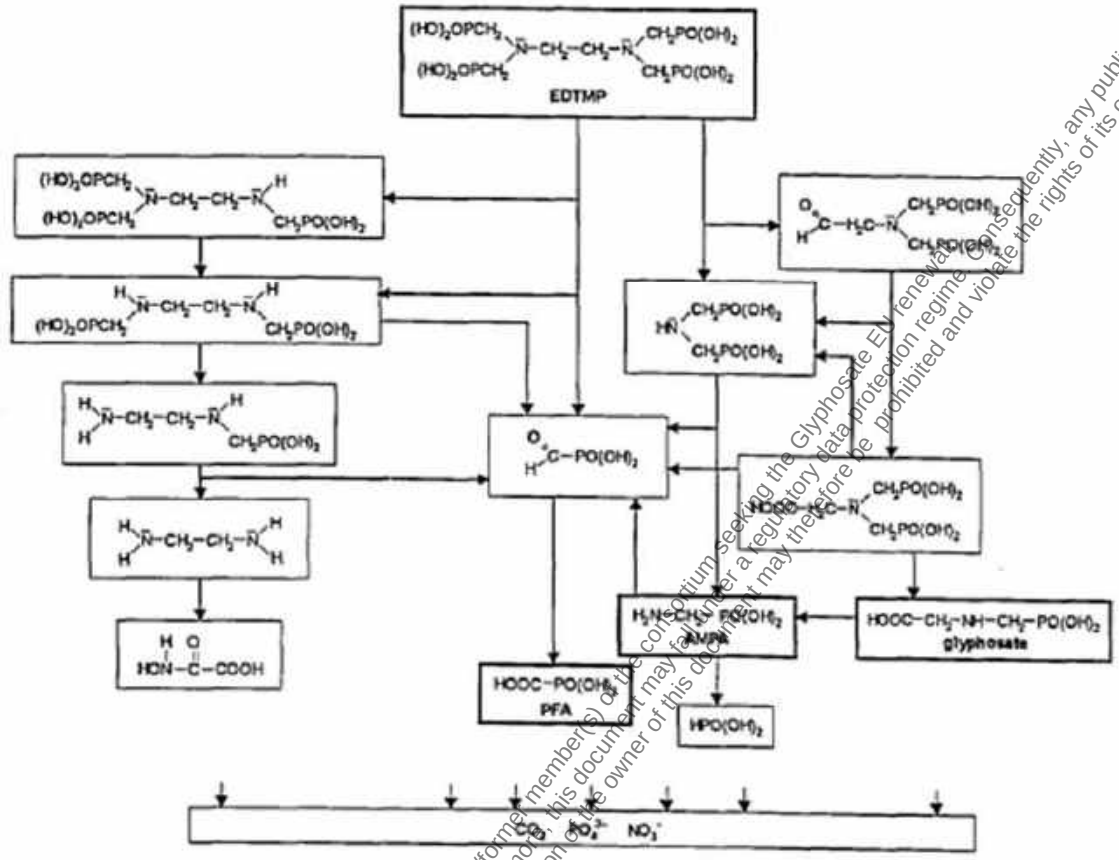


Table 7.5-267: Phosphorus mass balance for the ozonation of EDTMP

reaction time in sec	0	30	300	600
EDTMP in µmol P/L	9.2	0.62	0	0
glyphosate in µmol P/L	0	0.01	0.007	0.007
AMPA in µmol P/L	0	0.84	0.90	0.90
phosphonoformic acid in µmol P/L	0	0.66	0.58	0.55
orthophosphate in µmol P/L	0	4.3	5.4	5.7
sum in µmol P/L	9.2	6.4	6.9	7.2
identified oxidation products in % P	100	70	75	78

In Figure 7.5-98 oxidation of glyphosate and formation of orthophosphate as dependent on ozonation time is shown. It can be seen from this figure, glyphosate is eliminated up to 50 % after ten minutes reaction time. The low reactivity might be due to the amine group of glyphosate which is protonated and therefore inert at pH 5. As orthophosphate concentration is raised up to only 30 %, further phosphorus-containing oxidation products, such as AMPA, phosphonoformaldehyde and PFA must be formed.

In Table 7.5-268 the phosphorus mass balance at different reaction times for the ozonation of glyphosate is listed. Besides orthophosphate, AMPA is clearly identified as an oxidation product, but PFA could not be detected. From this table it can be seen that nearly all phosphorus-containing oxidation products are identified, so the reaction AMPA -phosphonoformaldehyde - phosphonoformic acid might not be a favoured pathway.

In Figure 7.5-239, oxidation of AMPA and formation of orthophosphate is shown. Within a reaction time of ten minutes AMPA is not totally eliminated. Formation of orthophosphate corresponds nearly with elimination of AMPA and PFA is not identified again. This corresponds to the conclusion that the reaction AMPA – phosphonoformaldehyde - phosphonoformic acid is not a favoured pathway.

In Figure 7.5-240, phosphonoformic acid is eliminated only up to 20 % and elimination of PFA corresponds very well with formation of orthophosphate.

As glyphosate, AMPA and PFA are not totally eliminated by ozone under these experimental conditions, they can be identified as oxidation products during ozonation of EDTMP. Thereby the formation of orthophosphate up to only 60 % is explainable. However, PFA was not detected during ozonation of glyphosate and AMPA. This might be due to the high detection limit of 50 µg/L; but this means on the other hand, that formation of PFA, which is clearly identified during ozonation of EDTMP, is also possible by other reaction pathways. This is according to the predicted reaction pathways in Figure 7.5-237. But obviously, the reaction AMPA - phosphonoformaldehyde - phosphonoformic acid is not favoured as 50 % of the EDTMP reacts very fast to orthophosphate, other phosphorus-containing metabolites must be formed, which react very fast and are converted into orthophosphate. Moreover, one can divide the reactions in Figure 7.5-237 into the two main pathways dephosphonomethylation and C-N-cleavage.

Because formation of glyphosate and AMPA during ozonation of EDTMP is of particular importance, additional experiments were carried out in tap water in order to prove whether or not glyphosate and AMPA might be formed under these conditions. In Table 7.5-269 the determined concentrations of glyphosate and AMPA are listed. As can be seen from this table, glyphosate and AMPA are clearly identified also during ozonation of EDTMP in tap water. Comparing Table 7.5-269 with Table 7.5-267, it seems that more glyphosate and less AMPA is formed during ozonation of EDTMP at pH 7 in tap water than at pH 5 in model solutions.

Figure 7.5-238: Ozonation of glyphosate at pH5

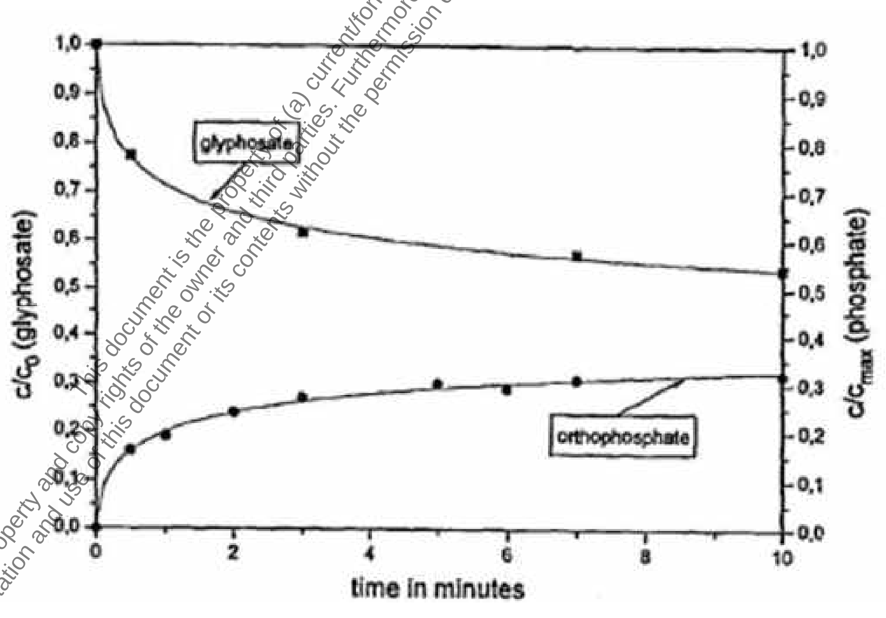


Table 7.5-268: Phosphorus mass balance for the ozonation of glyphosate

reaction time in sec	0	30	180	600
glyphosate in µmol P/L	5.92	4.57	3.65	3.20
AMPA in µmol P/L	0	0.41	0.62	0.69
orthophosphate in µmol P/L	0	0.95	1.61	1.91
sum in µmol P/L	5.92	5.94	5.88	5.80
identified oxidation products in % P	100	100	99	98

Figure 7.5-239: Ozonation of AMPA at pH5

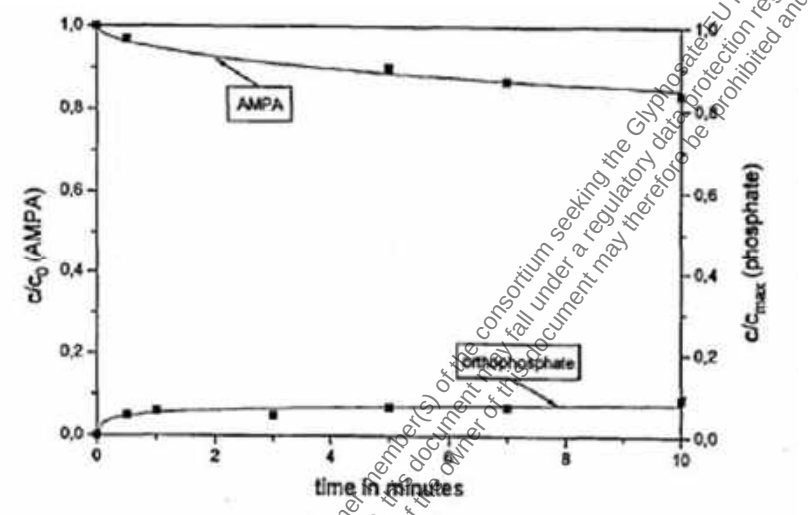


Figure 7.5-240: Ozonation of phosphonoformic acid at pH5

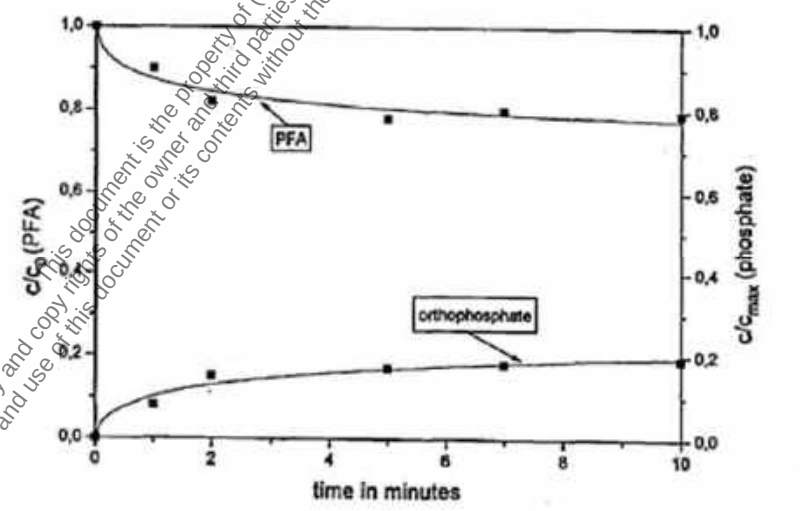


Table 7.5-269: Formation of glyphosate & AMPA during ozonation of EDTMP in tap water (pH 7)

reaction time in sec	30	180	600
glyphosate in µmol/L	0.03	0.05	0.06
AMPA in µmol/L	0.52	0.34	0.36

Conclusion
The experimental results confirmed the predicted mechanism as well as the formation of glyphosate and AMPA during ozonation of waters containing EDTMP.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article investigates the formation of glyphosate and AMPA during ozonation of waters containing ethylenediaminetetra(methylenephosphonic acid). Ozonation studies were also conducted on glyphosate and AMPA – at acidic pH (pH 5) it was found that glyphosate was partially degraded to AMPA and orthophosphate; and that AMPA was partially degraded to orthophosphate, under the experimental conditions.
The methods and results are sufficiently described.
The article is considered reliable with restrictions.

Assessment and conclusion by RMS:

Glyphosate

Annex M-CA 7-01

Annex to the Documen M of the technical section[10]:
FATE AND BEHAVIOUR IN THE ENVIRONMENT

[10] Annex to the Doc ID: 110054-MCA7_GRG_Jun_2020

AIR 5 introduction

During the AIR 2 evaluation process of glyphosate, in the Renewal Assessment Report 2015 version[11], the RMS Germany included public literature articles as part of the RAR Vol 3, Annex B.8 (Appendix). **All articles** included in this version of the RAR Vol 3 2015 have been included in this annex for the sake of completeness, with the aim of providing the EU authorities during the AIR 5 EU process with all information available for glyphosate from previous EU evaluations.

All information presented in this Annex, is an exact copy of the literature information included in the RAR Vol 3 2015 version. When reading the present annex, please note:

- This annex only present articles and not regulatory studies.
- Some references are made to the former Monograph glyphosate 1998.
- If text was strickethrough in the RAR Vol 3 2015, then those sentences were not included in the present annex.
- The numbering of tables in the present annex have not been changed and remain as original presented in the RAR Vol 3 2015 version.
- If text was highlighted in the RAR Vol 3 2015, then those sentences are also highlighted in the present annex.
- If text was given in italic style in the RAR Vol 3 2015, then those sentences are also given in italic in the present annex.

[11] Renewal Assessment Report, Vol 3, Revised 2015

Detailed description of open literature – Soil photolysis

Echavia et al. (2009)

Title: Photocatalytic degradation of organophosphate and phosphonoglycine pesticides using TiO_2 immobilized on silica gel	
Author: Glory Rose Mangat Echavia, Fumiko Matzusawa, Nobuaki Negishi	
Reference: Chemosphere 76: 595-600.	
Year: 2009	
Results and conclusion: Complete (100 %) decomposition of glyphosate was attained within 60 min of irradiation.	
Proposed action: Consider as additional information as no standard test design was followed. Recalculation of endpoints on stability in soil is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Photodegradation of glyphosate using TiO_2 immobilized on silica gel
Protocol	Non-GLP study
Test compound	Glyphosate (99 %) purchased from Wako Pure Chemical Industries, Ltd., Japan (CAS 1071-83-6)
Test system and conditions	The photocatalyst used in the study was the commercial HQC-22 TiO_2 obtained from Shinto V Cerax Company, Japan. The batch photocatalytic reactor or photo reactor consisted of a spiral glass tube packed with 14.0 g of the TiO_2 photocatalyst and wound around a 6Wblack light fluorescent UV lamp. The photo reactor also included a 250 mL glass container that served as a reservoir for the pesticide solutions. The UV lamp emits a wavelength centred mostly at 365 nm with a light intensity of 1.4 $mWcm^{-2}$ (measured 1 cm away from the UV lamp by UV Caremate PRO produced by Fuji Xerox Co.). The external surface of the photo reactor chamber was covered with aluminium sheet to prevent dissipation of UV light.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is low due to the artificial system.
Weight of evidence	
"Positive"/"Negative" evidence	The results of the experiments cannot be compared with standard testing.

Kirmser et al. (2010)

Title: Degradation of the Herbicides Clomazone, Paraquat, and Glyphosate by Thermally Activated Peroxydisulfate
Author: Elena M. Diaz Kirmser, Daniel O. Martire, Monica C. Gonzalez, and Janina A. Rosso
Reference: J. Agric. Food Chem. 2010, 58, 12858–12862
Year: 2010

Results and conclusion: From photochemical activation of peroxydisulfate in flash-photolysis experiments, the bimolecular rate constants for the reaction of sulphate radical with glyphosate ($1.6\ 10^8\ M^{-1}\ s^{-1}$) was obtained. Thermal activation of peroxydisulfate was shown to degrade the herbicide glyphosate. Although the herbicide degradation was observed to take place in less than 1 h, the mineralization of the organic carbon required longer reaction times, because of the formation of stable organic intermediates.	
Proposed action: Consider as additional information as no standard test design was followed. Recalculation of endpoints on stability in soil is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Photodegradation of glyphosate using activation of peroxydisulfate in flash-photolysis experiments
Protocol	Non-GLP study
Test compound	
Test system and conditions	Not relevant
Statistical design	
Relevance	
Environmental relevance	The relevance is low due to the artificial system.
Weight of evidence	
"Positive"/"Negative" evidence	The results of the experiments cannot be compared with standard testing.

Xu et al. (2011)

Title: Degradation of Glyphosate in Soil Photocatalyzed by $Fe_3O_4/SiO_2/TiO_2$ under Solar Light	
Author: Xuan Xu , Fangying Ji, Zihong Fan and Li He	
Reference: Int. J. Environ. Res. Public Health 2011, 8, 1258-1270; doi:10.3390/ijerph8041258	
Year: 2011	
Results and conclusion: Photocatalytic degradation of glyphosate in soil by these photocatalyst under solar irradiation was investigated. Results show that 0.5 % $Fe_3O_4/SiO_2/TiO_2$ has the best photocatalytic activity. The best moisture content of soil is 30 %~50 %. Degradation efficiency of glyphosate reaches 89 % in 2 h when the dosage of photocatalyst is 0.4 g/100 g (soil), and it increased slowly when more photocatalyst was used. Degradation of glyphosate is not obviously affected by sunlight intensity when the intensity is below 6 mW/cm$_2$ or above 10 mW/cm^2, but it is accelerated significantly when the sunlight intensity increases from 6 mW/cm^2 to 10 mW/cm^2.	
Proposed action: Consider as additional information as no standard test design was followed. Recalculation of endpoints on stability in soil is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Degradation of Glyphosate in Soil Photo catalyzed by $Fe_3O_4/SiO_2/TiO_2$ under Solar Light
Protocol	Non-GLP study
Test compound	Non-labelled: Glyphosate (CAS 1071-83-6)
Test system and conditions	Soil for experiments was typical red loam, which was collected from the Banan District, Chongqing, China.
Statistical design	Three replicates
Relevance	
Environmental relevance	There is little relevance for this analysis as the results cannot be compared with reliable studies.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

References

Echavia G.R. *et al.* 2009. Photocatalytic degradation of organophosphate and phosphonoglycine pesticides using TiO_2 immobilized on silica gel. Chemosphere 76: 595-600.

Kirmser E. *et al.* 2010. Degradation of the Herbicides Clomazone, Paraquat, and Glyphosate by Thermally Activated Peroxydisulfate. J. Agric. Food Chem. 2010, 58, 12858–12862

Xu X. *et al.* 2011. Degradation of Glyphosate in Soil Photocatalyzed by $Fe_3O_4/SiO_2/TiO_2$ under Solar Light. Int. J. Environ. Res. Public Health 2011, 8, 1258-1270; doi:10.3390/ijerph8041258

Detailed description of open literature – Rate of degradation in soil laboratory studies

Accinelli et al. (2006)

Title: Influence of Cry1Ac Toxin on Mineralization and Bioavailability of Glyphosate in Soil	
Author: CESARE ACCINELLI, WILLIAM C. KOSKINEN, AND MICHAEL J. SADOWSKY	
Reference: J. Agric. Food Chem. 2006, 54, 164-169	
Year: 2006	
Results and conclusion: Results from laboratory investigations indicate that soil incorporation of purified Cry1Ac toxin in the range of 0.25-1.0 Lg g^{-1}does not influence glyphosate mineralization or its sorption in soil. These results are in contrast to results obtained in previous investigations done using a mixture of Cry toxins at a concentration of 10 µg g^{-1}. The concentration of Cry toxins in soil occurring during the growing season has been estimated not to exceed 1 µg g^{-1}, based on the average concentrations of Cry toxin in crop residues incorporated into the top soil or left at the soil surface. On the basis of these estimates and the results obtained here, the data indicate that concentrations of Cry1Ac comparable to those encountered under field conditions do not have the potential to increase persistence and sorption of glyphosate in soil. Following K_{foc}-values were determined for glyphosate: sandy loam (Italy): 6230 L/kg and 6408 L/kg (US soil).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation or sorption is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	$DegT_{50}$ in soil and sorption in soil
Protocol	Non-GLP standard degradation study modified OECD Guideline 307 and 106
Test compound	CAS 1071-83-6 Unlabelled glyphosate (chemical purity > 98 %) ^{14}C labeled glyphosate (N-phosphonomethyl-2-^{14}C-glycine; radiopurity >99 %, specific activity) 1.18 106 MBq g^{-1})
Test system and conditions	Two soils with different physicochemical properties, taken from areas of the Po Valley (Italy) and of south central Minnesota, were selected for this study. The Italien soil (IT, 0.7 % OC) and the American soil (MN, 0.94 % OC) were both classified as sandy loam. At both locations, the soil was collected from fields that had not received glyphosate applications within the previous 5 years. A portion of the IT and MN soils was mixed with Cry1Ac toxinpowder to obtain a final concentration of 100 µg g^{-1}soil. Aliquots of these two amended soils were mixed with a sufficient mass of IT and MN soils to obtain final soil concentrations of 0.25, 0.5, and 1.0 µg Cry1Ac toxin g^{-1} soil (air-dried basis). The soil moisture in treated soil samples was adjusted to the gravimetric content at -33 kPa using distilled water and incubated in the dark at 25 °C. Isotherms for sorption of glyphosate to IT and MN soils containing different Cry1Ac toxin concentrations were determined using the batch equilibrium method 20 °C for 14 h.
Statistical design	Three replicates were prepared for each soil type and toxin concentration, and controls consisted of soils with no toxin addition.
Relevance	
Environmental relevance	The sorption studies are in principle performed considering the current guidance documents and can be considered for the calculation of sorption parameters. The degradation studies are not documented well enough to be considered further.
Weight of evidence	
"Positive"/"Negative" evidence	The results on degradation and sorption are principally supported by other reliable studies.

Accinelli et al. (2004)

Title: Influence of insecticidal toxins from Bacillus thuringiensis subsp. kurstaki on the degradation of glyphosate and glufosinate-ammonium in soil samples	
Author: Cesare Accinelli, Claudio Screpanti, Alberto Vicari, Pietro Catizone	
Reference: Agriculture, Ecosystems and Environment 103 (2004) 497–507	
Year: 2004	
Results and conclusion: The influence of insecticidal toxins on the persistence of herbicides in soil is analysed. The persistence of GLYP and GLUF was enhanced by the addition of a high rate of Btk. insecticidal crystal toxins extracted and purified from the commercial formulation Dipel 2×. Since no influence of Btk toxins on SMC of the two soils was observed and a rapid decrease of the insecticidal activity of the added Btk toxins was estimated during the 28-day incubation period, the observed increase of GLYP and GLUF persistence was presumably due to the reduction of bio-availability of the two herbicides, modification of the soil nutritive status or other not measured properties, such as soil microbial activity.	
Proposed action: Not be considered for recalculation of endpoints since the study design did not completely follow standard testing (e.g. no radio-labelling)	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP standard degradation study modified OECD Guideline 307
Test compound	Test compounds: CAS 1071-83-6 (GLYP) and 40465-66-5 (GLUF), Glyphosate [N-(phosphonomethyl)glycine] (GLYP) and glufosinate-ammonium [the ammonium salt of dl-homoalanin-4-yl(methyl)phosphinic acid] (GLUF) commercial formulation of Roundup Bioflow (Monsanto Agricoltura Italia S.p.A., Lodi, Italy; isopropylamine salt of GLYP, suspension concentrate, containing 360 g active ingredient, a.i., 1-1 formulation), Basta (Bayer Crop-Science S r.l., Milano, Italy; ammonium salt of GLUF, suspension concentrate, containing 120 g a.i. 1-1 formulation).
Test system and conditions	Surface (0-20 cm) soil samples were taken from two agricultural areas of the Po Valley (Italy), Cadriano and Ozzano. Cadriano soil was classified as a loam (Udic Ustochrepts, fine silty, mixed, mesic) and Ozzano soil as a sandy loam (Udertic Ustochrepts, fine, mixed, mesic). In both the locations, soils were collected from fields with no pesticide application during the last 5 years. Before the beginning of the experiment, soil moisture was adjusted to the gravimetric content at -33 kPa using ultrapure water. Soil samples were kept in the dark in a climatic chamber at 25 °C ± 0.5 for 10 days. The conditioning period of 10 days allowed the soil to establish a steady-state level of microbial activity. Conditioned soil samples were treated with water solutions of the commercial formulation. For herbicide half-life estimation, sampling times were 0, 3, 7, 14, 21 and 28 days after treatment.
Statistical design	Three replicate samples
Relevance	
Environmental relevance	The investigations dealing with the persistence of glyphosate and glufosinateammonium in soil in the presence of insecticidal toxins is not of preliminary interest, but can be considered as additional information.
Weight of evidence	
"Positive"/"Negative" evidence	Though the results did not completely follow the standard procedure the results are in line with results of other reliable studies.

Alexa et al. (2009)

Title: DYNAMIC OF GLYPHOSATE MINERALIZATION IN DIFFERENT SOIL TYPES	
Author: Ersilia Alexa, Mihaela Bragea, Renata Sumalan, Aurel Lazureanu, Monica Negrea, Stancu Iancu	
Reference: ROMANIAN AGRICULTURAL RESEARCH, Number 26/2009, 57-60	
Year: 2009	
Results and conclusion: The experimental results indicate a high microbial degradation of the glyphosate herbicide. The $^{14}CO_2$ quantity accumulated following glyphosate biodegradation under the microorganism action is higher in all 4 analyzed soils, comparing with blind sample (untreated soil). The experimental results show that the rate of glyphosate degradation in time is higher in the firsts 5 days, than the velocity decreases until the curves attained plateaus. The initial rapid phase of degradation was attributed to microbial action on the free glyphosate while the slower phase was due to the subsequent attack on the adsorbed glyphosate.	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Supporting	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP standard degradation study modified OECD Guideline 307
Test compound	Glyphosate-phosphonomethyl-^{14}C-labeled with specific activity 2.2 mCi/mmol (CAS 1071-83-6)
Test system and conditions	Four types of soils were taken under study: black chernozem, vertisol, gleysol and phaeozom with different characteristics. Four types of soils were taken under study: black chernozem, vertisol, gleysol and phaeozom with different characteristics. The soil was onditioned by being moistened to 85 % of the field water capacity. The soils were incubated at 20 °C, for 40 days. The mineralization curves of $^{14}CO_2$ accumulated were compared during 40 days.
Statistical design	Not reported
Relevance	
Environmental relevance	The degradation studies are in principle performed considering the current guidance documents. However, the studies are not documented well enough to be considered further (e.g. time dependent residues not given).
Weight of evidence	
"Positive"/"Negative" evidence	The results are principally supported by other reliable studies.

Alexa et al. (2010)

Title: Studies on the biodegradation capacity of ^{14}C-labelled glyphosate in vine plantation Soils	
Author: Ersilia Alexa, Renata Sumalan, Monica Negrea, Mihaela Bragea, Mariana-Atena Poiana, Isidora Radulov and Aurel Lazureanu	
Reference: Journal of Food, Agriculture & Environment, Vol.8 (3&4)	
Year: 2010	
Results and conclusion: The aim of this paper was to study the biodegradation capacity of glyphosate in soil samples prelevated from vine plantation from Timis county, Romania, belonging to Banat's University of Agricultural Science, Timisoara, in presence of organic and inorganic supplement, at different concentration levels. After addition of glyphosate-phosphonomethyl-^{14}C-labeled, the accumulated $^{14}CO_2$ (as % of total ^{14}C) was monitored during 44 days. Investigated soil shows a high degradation capacity of over 85 % of total radioactivity after 44 days from the treatment application. Addition of inorganic supplement causes a decrease of glyphosate biodegradation capacity to 10.77-12.87 % of total radioactivity, while in presence of straw the accumulated $^{14}CO_2$ (as % of total ^{14}C) during the 44 days ranged between 59.97 and 87.58 %. The amount of $^{14}CO_2$ released reached the highest level in the first 4 days after herbicide application, both in control and experimental variants with organic and inorganic supplement (from 2.61 to 30.27 % of total radioactivity). By glyphosate addition the growth and multiplication of soil microorganisms, whose biomass is digested in the range of 9-12 days of treatment, according to the daily mineralization rate (DMR) values, is stimulated. Our results on the activity of microorganisms showed that glyphosate degradation in soil is mainly performed by micromyces.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP degradation study under laboratory conditions
Test compound	Labelled compound: ^{14}C-glyphosate
Test system and conditions	The soils were incubated at 20 °C, to dark for 44 days. In order to evaluate the biodegradation of ^{14}C-labeled glyphosate during the incubation period, samples were taken every 4 days.
Statistical design	Not reported
Relevance	
Environmental relevance	Low relevance as important environmental parameters influencing the degradations were not reported (e.g. moisture)
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with other studies as important environmental parameters were not determined.

Alexa et al. (2010)

Title: RESEARCH ON THE WEED CONTROL DEGREE AND GLYPHOSATE SOIL BIODEGRADATION IN APPLE PLANTATIONS (PIONEER VARIETY)	
Author: Ersilia ALEXA, Roxana MICU, Monica NEGREA, Renata SUMALAN, Olimpia IORDANESCU	
Reference: Analele Universitatii din Oradea-Fascicula Biologie Tom. XVII/1, 2010, pp. 5-8	
Year: 2010	
Results and conclusion: The experimental results indicate a high microbial degradation of the glyphosate herbicide. Glyphosate mineralization curve reveals two phases of CO_2 release, the first rapid phase, followed by a slow phase, when the mineralization curve reaches a steady plateau. According to the authers initial rapid phase covers a period of approximately 20 days from the beginning of the experiment and is attributed to the action of microorganisms on free glyphosate from soil, while the second phase is attributed to slow action of microorganisms on glyphosate adsorbed on soil components.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation or sorption is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP degradation study under outdoor conditions
Test compound	Labelled compound: Roundup 3 l/ha (glyphosate isopropyl amine salt 360 g/l), C^{14} marked to fosfonometil group with 37kBq (CAS 38641-94-0)
Test system and conditions	A field study was performed determining the mineralisation of glyphosate in soil.
Statistical design	Not reported
Relevance	
Environmental relevance	Low relevance as important environmental parameters influencing the degradations were not reported (moisture, temperature)
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with other studies as important environmental parameters were not determined.

Alexa et al. (2008)

Title: RESEARCHES REGARDING THE MICROORGANISMS INFLUENCE ON GLYPHOSATE BIODEGRADATION	
Author: Alexa Ersilia, Sumalan Renata, Negrea Monica	
Reference: Journal of Agroalimentary Processes and Technologies 14 (2) 2008	
Year: 2008	
Results and conclusion: The degradation capacity is influenced by the micro-biological soils particles and leads to the glyphosate primary metabolite formation, aminomethyl-phosphonic acide (AMPA). For determination of CO_2 content, it shows the microorganisms action over the free glyphosate in soil. The experimental results show microbial bio-degradation of glyphosate and of his metabolite AMPA after 96 hours (4 days) since the treatment application, when the CO_2 quantity release is maximum. The released CO_2 quantity grows until day six, than it reaches a constant level regarding the glyphosate degradation, and the mineralization speed decrease. Regarding the herbicide quantity added, it discovers that the free glyphosate from soil is directly and rapidly degraded by micro-organisms and not affect the microbiological activity, even at the high concentrations applied, double comparing with the quantity used in field.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation or sorption is not necessary	
Type of information (critical, high/low weight, supporting, additionally): Additional	
Reliability	
Endpoint	Principally $DegT_{50}$ in soil (mineralisation), but not explicitly calculated
Protocol	Non-GLP degradation study
Test compound	Non-Labelled glyphosate (CAS 38641-94-0) and AMPA (CAS: 74341-63-2)
Test system and conditions	Four types of soils have been taken under study: Black Chernozem, Vertisol, Gleysol and Phaeozem with different characteristics. The analyzed soils have been taken from horizon A, from a depth of 10 cm. In order to obtain a representative sample, the samples have been taken from different points by splitting the surface in quarters, diagonally and on rows, through the carrots.
Statistical design	Not reported
Relevance	
Environmental relevance	Low relevance as the study was not evaluated according to standard procedures (e.g. FOCUS Deg Kin).
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with other studies as the endpoints was not calculated adequately.

Al-Rajab et al. (2010)

Title: Degradation of ^{14}C-glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils	
Author: Abdul Jabbar Al-Rajab, Michel Schiavon	
Reference: Journal of Environmental Sciences 2010, 22(9) 1374–1380	
Year: 2010	
Results and conclusion: Laboratory degradation studies were performed showing an immediate and high rate of glyphosate degradation after its application on soil. Mineralization of glyphosate after 17 days of incubation reached 32.2 % to 39.7 % of the initial amount applied to the two soils (sandy loam (pH 5.1) or silt clay loam (pH 6.3)). However, the mineralization rate was more rapid and intense for the clay loam soil (pH 7.9) with 48.4 % reached by 12 days of incubation. Thereafter, the mineralization of glyphosate declined gradually for all three soils. The endogenous activity of mineralization was comparable for the three investigated soils. The fast mineralization of glyphosate in clay loam soil appears due exclusively to a bioavailability more important than in other two soils. The analysis of water extracts by HPLC showed the appearance of two degradation products of glyphosate AMPA. The appearance of AMPA during incubation varied significantly depending on the speed of mineralization of glyphosate in each soil (Table 2). In sandy loam soil, there was only 12.7 % of AMPA present on day 3 after treatment, whereas 87.3 % of the initial radioactive glyphosate was present on the same day.	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting	
Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP standard degradation study modified OECD Guideline 307
Test compound	Glyphosate (CAS 1071-83-6) [Phosphonomethyl-^{14}C]-glyphosate was obtained from ARC-ISOBIO (Belgium) diluted in water. Its specific radioactivity was 385 GBq/mmol and its radiochemical purity 99 %. Non-radioactive glyphosate (purity 98.5 %) was obtained from GIL Cluzeau (France). AMPA (CAS 74341-63-2), 10 ng/µL in water, was obtained from Dr. Ehrenstorfer GmbH (Germany).
Test system and conditions	Three cultivated soils from the Lorraine region in eastern France were selected on the basis of their texture and pH. None of these soils had ever been exposed to glyphosate. The jars were incubated in the dark at 20 °C for 80 days. Analyses were performed in triplicates and one control of unspiked soil per type of soil was considered.
Statistical design	Analyses were performed in triplicates and one control of unspiked soil per type of soil was considered.
Relevance	
Environmental relevance	The degradation studies are in principle performed considering the current guidance documents. However, the studies are not documented well enough to be considered further.
Weight of evidence	
"Positive"/"Negative" evidence	The results are principally supported by other reliable studies.

Andréa et al. (2003)

Title: Influence of repeated applications of glyphosate on its persistence and soil bioactivity	
Author: Mara Mercedes de Andréa, Terezinha Bonanho Peres, Luiz Carlos Luchini, Sheila Bazarin, Solange Papini, Marcus Barifouse Matallo and Vera Lucia Tedeschi Savoy	
Reference: Pesq. agropec. bras., Brasília, v. 38, n. 11, p. 1329-1335, nov. 2003	
Year: 2003	
Results and conclusion: Degradation of isopropylamine salt of glyphosate after repeated applications was analysed in the laboratory. The ^{14}C-glyphosate applied to soil was immediately mineralized since the first application, mainly in the first week after each treatment. The amounts mineralized in the first week after each application were 31.70±4.29 %, 20.65±3.89 %, 22.03±1.02 % and 14.50±0.99 %, respectively. The immediate mineralization seem to decrease with increasing number of treatments. The detected amounts of $^{14}CO_2$ were formed from the degradation of glyphosate to AMPA, as well as from degradation of AMPA and express really complete degradation of the pesticide molecule.	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation in soil after repeated applications (no explicit DT_{50} calculation)
Protocol	Non-GLP study
Test compound	CAS 38641-94-0 [^{14}C]-glyphosate solution containing 372 mg and 26.8 kBq mL^{-1} by mixture of Nortox® formulated glyphosate certified by the Environmental Chemistry Laboratory of Instituto Biológico as 480 g/L of the isopropylamine salt of glyphosate, and [N-(^{14}C-phosphonomethyl)-glycine] from Amersham International with 2.70 GBq mmol^{-1}.
Test system and conditions	An Ultisol soil sample from the Centro Experimental do Instituto Biológico, Campinas, SP, Brazil, was collected from 0 to 15 cm of the soil profile from an area without occurrence of pesticide applications Each treatment consisted in 3.0 mg glyphosate and 0.22 kBq of ^{14}C-glyphosate per g soil, which agrees with the interval of recommended doses. Soil moisture was 60 % WMHC. The flasks were maintained in the dark at 25 ºC during all the experimental time.
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is small since the DT_{50} were not calculated and the provided information is not sufficient to analyse the results further.
Weight of evidence	
"Positive"/"Negative" evidence	The amount of degradation is principally in line with other reliable studies performed. The effect after repeated applications seems questionable.

Assalin et al. (2010)

Title: Studies on degradation of glyphosate by several oxidative chemical processes: Ozonation, photolysis and heterogeneous photocatalysis	
Author: MARCIA R. ASSALIN, SANDRA G. DE MORAES, SONIAC.N.QUEIROZ, VERA L. FERRACINI and NELSON DURAN	
Reference: Journal of Environmental Science and Health Part B (2010) 45, 89–94	
Year: 2010	
Results and conclusion: Degradation of aqueous solutions containing glyphosate was observed by oxidative advanced processes. Processes based on the formation of hydroxyl radical, such as Ti/UV and O_3/pH 10, were effective for the degradation of glyphosate and its degradation intermediates, AMPA, after a short treatment time. Under the experimental conditions used in this study the degradation of glyphosate followed a pseudo first-order kinetic law for both processes studied. The half–lives obtained for glyphosate degradation were 1.8 and 6.2 minutes for O_3/pH 10 and TiO_2/UV, respectively.	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation by oxidative chemical processes
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6, purity 99.8 %) and AMPA(CAS 1066-51-9 purity 99.1 %) obtained from Monsanto and used without futher purification.
Test system and conditions	A stock solution containing 1000 mg/L of glyphosate was prepared in deionised water and diluted to the required concentration (42.275 mg/L) for the degradation experiments. The original pH of this solution was about 6.5. The pH was adjusted to 10 by the addition of a NaOH solution for the ozonation experiment. An ozone concentration of 14 mg/L was applied for 30 minutes in a batch reactor. Samples (42.275 mg/L glyphosate solution, 400 mL) were submitted to ozonation at pH 6.5 and at pH 10 (pH adjusted with a sodium hydroxide solution) at room temperature. Titanium dioxide (80 % anatase and 20 % rutile, average particle size of 30 nm and BET-Method–Brunauer, Emmett and Teller [BET] surface of 50 ± 15 m^2/g) was used without any pretreatment. Illuminationwas provided by a high-pressure mercury lamp (Philips HPL-N, 125 W; λ > 290 nm) with the glass bulb removed. The lamp was fixed in the center of the reactor and cooled by a water jacket, at room temperature. The suspension was bubbled with oxygen (through a sintered glass disk placed in the bottom of the reactor) at a flow rate of about 6 ± 0.2 L/h for 30 minutes. Mineralization was followed by measuring the total organic carbon (TOC).
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is small since no environmental relevant DT_{50} were obtained due to the artificial experimental conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The amount of degradation observed cannot be compared with the results of standard tests.

Barrett and Mc Bride (2005)

Title: Oxidative Degradation of Glyphosate and Aminomethylphosphonate by Manganese Oxide	
Author: K. A. BARRETT AND M. B. MC BRIDE	
Reference: ENVIRONMENTAL SCIENCE & TECHNOLOGY, VOL. 39, NO. 23, 9223-9228	
Year: 2005	
Results and conclusion: The authors were unable to measure significant glyphosate and AMPA degradation in the presence of Mg^{2+}	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation by oxidative chemical processes
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6, 96 % purity) and AMPA (CAS 1066-51-9 purity 99 %)
Test system and conditions	Glyphosate reagent containing 10.5 mg/L glyphosate and 0.5 mM $MnCl_2$ was prepared in a background electrolyte solution of 0.01 M $NaNO_3$. On a molar basis, Mn was present at approximately 8 times the concentration of glyphosate. The effect of solution pH was assessed by adjusting the pH to 5.0, 6.0, or 7.0 with NaOH. Controls were prepared with no $MnCl_2$. Degradation of AMPA (99 % purity) in the presence of the manganese oxide was similarily studied.
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is small since no environmental relevant DT_{50} were obtained due to the artificial experimental conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The amount of degradation observed cannot be compared with the results of standard tests.

Bazot and Lebeau (2008)

Title: Oxidative Simultaneous mineralization of glyphosate and diuron by a consortium of three bacteria as freeand/or immobilized-cells formulations	
Author: S. Bazot & T. Lebeau	
Reference: Appl Microbiol Biotechnol (2008) 77:1351–1358	
Year: 2008	
Results and conclusion: Synthetic culture medium: glyphosate was mineralized between 72 and 480 h Sediment extract medium: no mineralisation of glyphosate	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation in soil
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6) obtained from Fluka, France, no information about purity
Test system and conditions	A bacterial consortium able to mineralize two herbicides, glyphosate (Pseudomonas 4ASW) and diuron (Arthrobacter sp. N4 and Delftia acidovorans), was cultivated in both a synthetic culture medium without phosphate and a sediment extract medium. In the aim at optimizing glyphosate and diuron mineralization, all the combinations, i.e., free and/or immobilized cells in Ca-alginate beads were tested.

Statistical design	Three replicates
Relevance	
Environmental relevance	The relevance is small since no environmental relevant DT_{50} were obtained due to the artificial experimental conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The amount of degradation observed cannot be compared with the results of standard tests.

Bonfleur et al. (2011)

Title: Mineralization and degradation of glyphosate and atrazine applied in combination in a Brazilian Oxisol	
Author: ELOANA J. BONFLEUR, ARQUIMEDES LAVORENTI and VALDEMAR L. TORNISIELO	
Reference: Journal of Environmental Science and Health Part B (2011) 46, 69–75	
Year: 2011	
Results and conclusion: Glyphosate mineralization rate was slightly higher in the presence of one dose of atrazine when compared with glyphosate alone. However, no significant differences were found when half or twice the atrazine dose was applied, meaning that differences in glyphosate mineralization rates cannot be attributed to the presence of atrazine	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation in soil (mineralisation) in the presence of atrazine
Protocol	Non-GLP study, modified OECD Guideline 307
Test compound	^{14}C-glyphosate purity 94 %, 0.2333 mCi mg^{-1} respectively. (CAS 1071-83-6)
Test system and conditions	Samples site National Center for Research on Beef Cattle (CNPGC-Embrapa) in Campo Grande, State of Mato Grosso, Brazil. soil type: dark red dystrophic Oxisol, clayey texture, maintained 16 years under cultivated pasture (Brachiaria brizantha). No information about previous applications of glyphosate. Soil samples were collected at 0-10 cm depth, Experiment conducted at 25 ± 2◦C, humidity weekly adjusted to 60 % of the retention capacity. Soil treatments consisted of the combination of a field dose of glyphosate (2.88 kg/ha) with 0, 1/2, 1 and 2 times a field dose of atrazine (3.00 kg/ha) and a field dose of atrazine with 0, 1/2, 1 and 2 times a field dose of glyphosate.
Statistical design	Four replicates
Relevance	
Environmental relevance	The relevance is small since atrazine is not used in the EU for many years.
Weight of evidence	
"Positive"/"Negative" evidence	The results are in the range of values obtained with standard GLP studies.

Castro et al. (2007)

Title: Biodegradation of the herbicide glyphosate by filamentous fungi in platform shaker and batch bioreactor	
Author: JOAO V. CASTRO, JR, MARIA C.R. PERALBA and MARCO A.Z. AYUB	
Reference: Journal of Environmental Science and Health Part B (2007) 42, 883–886	
Year: 2007	
Results and conclusion: The study demonstrated that cultures of the filamentous fungi Fusarium oxysporum can degrade the herbicide glyphosate, even at high concentration. The metabolism presented by growth of Fusarium strains in consortium was similar to the fungi in pure culture. The biodegradation conducted in the bioreactor was more efficient than in the platform shaker. All strains tested showed no improvement on biodegradation by changing the rate of oxygen. The metabolite AMPA was not observed in any of the assays studied, probably indicating the formation of other metabolites during the degradation of glyphosate	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation by Microorganisms (mineralisation) Czapeck medium
Protocol	Non-GLP study
Test compound	95 % pure glyphosate (N-phosphonomethylglycine) obtained from Milenia agricultural (CAS 1071-83-6)
Test system and conditions	Studies of the biodegradation of glyphosate as a sole source of phosphorous by fungal strains were carried out in 300mL Erlenmeyer flasks containing 100mL of Czapeck medium. The cultures were inoculated with a spore suspension (2.6×10^7 spores.mL^{-1}) and incubated at 30 °C on a shaking platform at 150 rev.min^{-1}. The fungal strains were inoculated as pure and consortium cultures. Samples were taken during the experiments to quantify the residue of herbicide. As control for non-biological degradation, assays were conducted without addition of microorganisms in the same way as for the fungal degradations above. All the assays were done in duplicate.
Statistical design	No information.
Relevance	
Environmental relevance	The relevance is small due to the artificial environment.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Doublet et al. (2009)

Title: Delayed degradation in soil of foliar herbicides glyphosate and sulcotrione previously absorbed by plants: Consequences on herbicide fate and risk assessment
Author: Jérémy Doublet, Laure Mamy, Enrique Barriuso
Reference: Chemosphere 77 (2009) 582-9.
Year: 2009
Results and conclusion: The fate in soil of herbicides residues in plants was different from that of control. Mineralization in soil of glyphosate in crops decreased compared to control, and amounts of ^{14}C-extractable residues, mainly composed by the metabolite aminomethylphosphonic acid (AMPA), and non-extractable residues (NER) increased. The experiments with contaminated plant parts incorporated show significantly reduced rates but not outside the known range.
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.

Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation of glyphosate in soil depdent on incorporated contaminated plant parts
Protocol	Non-GLP study, modified OECD 307 (adding contaminated plants)
Test compound	[Methyl-^{14}C]glyphosate purchased from Sigma Chemicals (St. Louis, USA; 81 MBq mmol^{-1}, 99.2 % purity, CAS 1071-83-6)
Test system and conditions	Soil samples were taken from the top layer (0–10 cm) of a French experimental site (Dijon, Burgundy). The soil is a clay-loam calcareous Cambisol with 37.7 % of clay (<2 µm), 29.6 % of silt (2–50 µm), 15.2 % of sand (50–200 µm), 16.7 % of $CaCO_3$, 1.63 % of organic carbon, pH in water of 8.2, and water field capacity of 26.1 % (determined at -1000 hPa). Ten 5 µL droplets of glyphosate or sulcotrione solutions were applied on the second youngest leaf of oilseed rape and/or maize plants. Seven DAT, treated leaves were washed (see above), then different aerial parts of plants (lamina, apex, petiole and stem) were incorporated into 11.4 or 57 g soil corresponding to 10 or 50 g dry soil (11.4 g for oilseed rape apex, 57 g for oilseed rape lamina and petiole and maize lamina and stem). Soil treated directly with herbicides was used as control.
Statistical design	No information
Relevance	
Environmental relevance	The results show an interesting degradation dependency which is not considered in the standard testing. However, the results can to some extend explain degradation variability.
Weight of evidence	
"Positive"/"Negative" evidence	The control experiments showed degradation in the range known from standard testing. The experiments which contaminated plant parts incorporated show significantly reduced rates but still in the range of results of reliable (standard) studies.

Ermakova et al. (2008)

Title: Microbial Degradation of Organophosphonates by Soil Bacteria	
Author: I. T. Ermakova , T. V. Shushkova, and A. A. Leont'evskii	
Reference: Microbiology, 2008, Vol. 77, No. 5, pp. 615–620	
Year: 2008	
Results and conclusion: Bacteria that can utilize glyphosate (GP) or methylphosphonic acid (MPA) as a sole phosphorus source have been isolated from soil samples polluted with organophosphonates (OP). No matter which of these compounds was predominant in the native habitat of the strains, all of them utilized methylphosphonate. Some of the strains isolated from GP-polluted soil could utilize both phosphorus sources. Strains growing on glyphosate only were not isolated. The isolates retained high destructive activity after long-term storage of cells in lyophilized state, freezing to -20 °C, and maintenance on various media under mineral oil. When phosphorusstarved cells (with 2 % phosphorus) were used as inoculum, the efficiency of OP biodegradation significantly increased (1.5-fold).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation of glyphosate in a mineral MS1 medium
Protocol	Non-GLP study
Test compound	Glyphosate (CAS 1071-83-6) no further information

Test system and conditions	The bacteria were cultivated in a mineral MS1 medium, sodium glutamate (Difco, United States) was used as the carbon source (10 g/l). Concentration of inorganic phosphorus was determined spectrophotometrically by formation of a complex of phosphomolibdate and malachite green in an acidic medium. Total phosphorus was determined by the same method after organophosphonate hydrolysis with ammonium persulfate. The content of MPA and GP in the culture liquid was calculated as the difference between total and inorganic phosphorus by introducing respective conversion rates for each compound.
Statistical design	No information
Relevance	
Environmental relevance	The relevance of the results is low due to the artificial environment.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard laboratory testing.

Getenga and Kengara (2004)

Title: Mineralization of Glyphosate in Compost-Amended Soil Under Controlled Conditions	
Author: Z. M. Getenga, F. O. Kengara	
Reference: Bull. Environ. Contam. Toxicol. (2004) 72:266-275	
Year: 2004	
Results and conclusion: All the mineralization curves for the soils with different treatments exhibited same patterns. The mineralization curves had only two phases, the initial rapid phase followed by a slow final phase, when the curves attained plateaus. The rapid phase lasted for about 20 days. The initial rapid phase phase of degradation was attributed to microbial action on the free glyphosate while the slower phase was due to the subsequent attack on the adsorbed glyphosate. The study showed that compost did not stimulate intense mineralization of glyphosate by microbes. The authors did not calculate the $DegT_{50}$, but according to the residue they can be estimated to be in the range of 50 to 100 d (which is rather low for glyphosate).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil where compost has been added
Protocol	Non-GLP study
Test compound	Glyphosate-phosphonomethyl-^{14}C-labeled (International Isotopes, Munich) with specific activity of 52 mCi/mmol, and radiochemical purity of 99 % (CAS 1071-83-6)
Test system and conditions	The soil was conditioned by being moistened to 75 % of the field water capacity before they were incubated at 30 °C in the darkness under aerobic conditions. Compost made from urban solid organic waste was added
Statistical design	Three replicates
Relevance	
Environmental relevance	Though the experiments do not follow standard testing the results are of relevance since in the agricultural environment soils are mixed with compost.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard laboratory testing.

Ghafoor et al. (2011)

Title: Measurements and modeling of pesticide persistence in soil at the catchment scale

Author: A. Ghafoor, N.J. Jarvis, T. Thierfelder, J. Stenström

Reference: Science of the Total Environment 409 (2011) 1900–1908

Year: 2011

Results and conclusion:
The purpose of the study was to study the influence of various soil physical, chemical and microbiological characteristics on pesticide persistence in the contrasting cultivated soils found in a small (13 km^2) agricultural catchment in Sweden and to develop and test a simple model approach that could support catchment scale modelling. Persistence of glyphosate was investigated in laboratory incubation experiments. Degradation rate constants were highly variable with coefficients of variation of 42 % for glyphosate. The degradation rates (0.006–0.05 day^{-1}) The mean rate constant was found to be 0.028 d^{-1} ($DegT_{50}$: 24.7 d). Results for sorption of glyphosate not adequately described. Multiple linear regression analysis and Mallows Cp statistic were employed to select the best set of independent parameters accounting for the variation in degradation. Detailed results are:

Soil characteristics

Soil No	Textural class	pH	Sand (%)	Silt (%)	Clay (%)	OC (%)	$CaCO_3$	Total N
1	Loam	7.6	49	32	18	1.6	9.2	0.16
2	Sand	6.2	87	8	4	1.2	0.1	0.08
3	Clay loam	7	43	27	30	2.3	0.1	0.22
4	Sandy loam	7.1	58	25	17	2.1	0.4	0.21
5	Sandy loam	6.9	68	17	15	2.1	0.2	0.21
6	Sandy loam	6.5	70	21	9	1.1	0.1	0.09
7	Loamy sand	6.5	85	9	6	1.6	0.1	0.15
8	Sandy loam	7.6	55	28	17	2.6	0.2	0.25
9	Silty clay	6.4	12	45	44	6.7	0.3	0.54
10	Silty clay	6.9	17	54	29	10.2	0.5	0.87
11	Clay	6.9	22	33	45	2.5	0.9	0.25
12	Sandy loam	7.3	56	24	20	5.4	0.4	0.53
13	Sandy loam	6	63	27	10	0.9	0.1	0.08
14	Loamy sand	6.1	83	11	6	1.3	0.1	0.13
15	Clay loam	7.5	35	39	25	3	0.1	0.28
16	Clay loam	7.1	31	40	29	1.9	0.2	0.19

Results (20 C and pF 2)

Soil No	k (day-1)	r^2
1	0.044±0.006	0.93
2	0.018±0.001	0.95
3	0.032±0.002	0.86
4	0.046±0.002	0.88
5	0.031±0.001	0.87
6	0.033±0.003	0.97
7	0.024±0.000	0.95
8	0.050±0.001	0.89
9	0.017±0.004	0.98
10	0.006±0.001	0.95
11	0.013±0.001	0.96
12	0.029±0.001	0.96
13	0.022±0.000	0.95
14	0.027±0.000	0.97
15	0.028±0.001	0.96
16	0.032±0.004	0.98

Proposed action:
No action since documentation of results insufficient (no raw data presented, no evaluation according to FOCUS degradation kinetics)

Type of information (critical, high/low weight, supporting, additional):
Supporting information, since the experiments were performed in principal similar to standard degradation studies but documentation of results (e.g. time dependent residues, kinetic analyses) is insufficient.

Reliability	
Endpoint	$DegT_{50}$ in soil
Protocol	Non-GLP standard degradation study OECD Guideline 307 adsorption study OECD 106 guideline
Test compound	Test compound: CAS 1071-83-6 Unlabelled: Glyphosate (N-(phosphonomethyl)glycine, 98 % purity) [P-methylene-^{14}C]glyphosate (5.155 MBq mg^{-1}, purity N99 %)
Test system and conditions	Degradation: Incubation experiments for each soil/pesticide combination were carried out on two replicate samples. Water contents were adjusted to and maintained at pF 2 throughout the experiment by the addition of de-ionized water as necessary. The samples were incubated in aerated glass tubes in the dark at 20 °C for 64 days. Duplicate samples (5 g) were taken after 0, 2, 4, 8, 16, 32 and 64 days of incubation for measurement of the residual concentrations of glyphosate. Sorption: Soil (four grams d.w. for glyphosate) was shaken to pre-equilibrate with 0.01 M $CaCl_2$ (39 mL) for 24 h at 20 °C.
Statistical design	Two replicate samples
Relevance	
Environmental relevance	The study was carried out using only soils from Östergötland, southern Sweden. However, the studies are performed close to standard testing guidelines on degradation and can be considered to calculate endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	The results on degradation and sorption are supported by other reliable studies.

Gimsing et al. (2004)

Title: Chemical and microbiological soil characteristics controlling glyphosate mineralisation in Danish surface soils

Author: Anne Louise Gimsing, Ole Kragholm Borggaard, Ole Stig Jacobsen, Jens Aamandb, Jan Sørensen

Reference: Applied Soil Ecology 27 (2004) 233–242

Year: 2004

Results and conclusion:
Glyphosate was rapidly adsorbed to iron and aluminium oxides, but were later released from these pools during mineralisation. In soils with high mineralisation rates the metabolite AMPA was formed and adsorbed. The rate of mineralisation was best correlated with the population size of Pseudomonas spp. bacteria in the soils. Phosphate addition had a stimulating effect on glyphosate degradation in soils with low mineralisation rates, but no effect or a negative effect on mineralisation in soils with high mineralisation rates. Finally, mineralisation rates were higher in soils from organically managed soils than in soils from conventional farming. The results indicate that the activity of glyphosate mineralising bacteria (e.g. Pseudomonas spp.) was a major factor controlling the fate of glyphosate in the soils.

Proposed action:
Consider as additional information. Recalculation of endpoints on degradation is not necessary

Type of information (critical, high/low weight, supporting, additional):
Supporting information

Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation)
Protocol	Non-GLP study modified OECD 307
Test compound	Glyphosate-monoisopropylamine solution (40 wt.%, density 1.218, Aldrich ChemicalCompany, 38641-94-0) ^{14}C glyphosate solution (7.40MBqml−1, 3.78 mM, Amersham Pharmacia Biotech, Hørsholm, Denmark, CAS 1071-83-6) water.
Test system and conditions	The soils used are from the A-horizon of five Danish agricultural soils representing the majority of soil types found in Denmark The glyphosate solution was mixed well into the soil, and a small test tube with 1 ml 1M NaOH was placed in a incubator at 15 °C.
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are close to standard testing studies. However, no DT_{50} were calculated for the compounds.
Weight of evidence	
"Positive"/"Negative" evidence	The results can be compared with standard laboratory testing. However, they were not evaluated according to the standard procedures.

Grundmann et al. (2008)

Title: Mineralization and Transfer Processes of ^{14}C-labeled Pesticides in Outdoor Lysimeters
Author: Sabine Grundmann & Ulrike Dörfler & Bernhard Ruth & Christine Loos & Tobias Wagner & Heidrun Karl & Jean Charles Munch & Reiner Schroll
Reference: Water Air Soil Pollut: Focus (2008) 8:177–185
Year: 2008
Results and conclusion: The glyphosate mineralization curves showed no lag phase – the microorganisms were able to mineralize glyphosate immediately. The cumulated amounts of mineralized ^{14}C-glyphosate amounted to 32–39 %. No accumulation of residues in the soil and no leaching of the residues to deeper soil layers could be observed after three applications. Glyphosate was rapidly degraded to AMPA in the soil. Glyphosate and AMPA were accumulated in soy bean nodules.
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary
Type of information (critical, high/low weight, supporting, additional): Supporting information
Reliability

Endpoint	Degradation of glyphosate in soil (mineralisation) Accumulation and leaching
Protocol	Non-GLP study
Test compound	CAS 1071-83-6 ^{14}C-glyphosate [N-(phosphonomethyl)glycine] had the ^{14}C-labeling on the phosphonomethyl group and was purchased from PerkinElmer, Rodgau, Germany (purity >98 %). Non-labeled glyphosate and metabolites were purchased from Dr. Ehrenstorfer (Augsburg, Germany)

Test system and conditions	The lysimeters consist of soil columns of 2 m height and a surface area of 1 m^2. To detect and quantify gaseous ^{14}C-losses from soil and plant surfaces, a two-chamber-system with special trapping facilities was designed. The chambers are placed on the surface of the lysimeters – a soil chamber and a plant chamber. Glyphosate was applied three times, in spring 2004 and in spring and autumn 2005 in an amount of 1 kg a.i. ha^{-1} (1.92 MBq/mg). During the experiment, mineralization and volatilization of the herbicides from soil and plants were measured during a time period of about 2–3 months after application
Statistical design	No information
Relevance	
Environmental relevance	The experiments are relevant though not performed close to standard lysimeter studies. Additionally, not sufficient information is provided to describe the situation (e.g. weather, irrigation).
Weight of evidence	
"Positive"/"Negative" evidence	The results support the information known for glyphosate from standard tests.

Haney et al. (2002)

Title: Bioremediation and Biodegradation: Effect of Roundup Ultra on Microbial Activity and Biomass from Selected Soils	
Author: R. L. Haney, S. A. Senseman, and F. M. Hons	
Reference: J. Environ. Qual. 31:730–735 (2002).	
Year: 2002	
Results and conclusion: Roundup Ultra appeared to be rapidly degraded by soil microbes regardless of soil type or organic matter content, even at high application rates, without adversely affecting microbial activity.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation)
Protocol	Non-GLP study
Test compound	CAS 38641-94-0 Isopropylamine salt of glyphosate as Roundup Ultra (480 g active ingredient/L)
Test system and conditions	Nine soils from Georgia and Texas were used in this study. The soils varied in soil pH (4.7 to 8.2), soil organic C (4.1 to 52.3 g C/kg soil), and clay content (6 to 45 %). The isopropylamine salt of glyphosate as Roundup Ultra (480 g active ingredient/L) was added to soil at a rate of 234 mg/kg. This amount was based on the recommended rate of RU being 0.84 kg/ha. Final moisture content was 20 % w/w at 30 °C
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are relevant though not performed with radio labelled compound. The authors describe the degradation only qualitatively (no DT_{50} calculated).
Weight of evidence	
"Positive"/"Negative" evidence	The results support the information known for glyphosate from standard tests.

Haney et al. (2002)

Title: Soil carbon and nitrogen mineralization as affected by atrazine and glyphosate	
Author: R. L. Haney,· S. A. Senseman, L. J. Krutz, F. M. Hons	
Reference: Biol Fertil Soils (2002) 35:35–40	
Year: 2002	
Results and conclusion: Soil C and N mineralization was sensitive to the addition of atrazine as well as atrazine mixed with glyphosate. The addition of low C:N ratio herbicides stimulates microbial activity and enhances the eventual mineralization of these compounds.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation) when atrazine is present
Protocol	Non-GLP study
Test compound	CAS 38641-94-0 Isopropylamine salt of glyphosate as Roundup Ultra (480 g active ingredient/L)
Test system and conditions	The soil used was a Weswood silt loam (fine, mixed, thermic Fluventic Ustochrept) with soil pH of 8.3 (1:2 soil/water), soil organic matter content of 10.6 g/kg soil, 115 g sand/kg, 452 g silt/kg, 310 g clay/kg. The isopropylamine salt of glyphosate as RoundUp Ultra (480 g active ingredient L^{-1}) was added to soil at rates of 2× (94 mg kg^{-1}), 4× (188 mg kg^{-1}), and 6× (282 mg kg^{-1}).
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are relevant though not performed with radio labelled compound. The authors describe the degradation only qualitatively (no DT_{50} calculated for glyphosate).
Weight of evidence	
"Positive"/"Negative" evidence	The results support the information known for glyphosate from standard tests.

Helander et al. (2012)

Title: Glyphosate in northern ecosystems
Author: Marjo Helander, Irma Saloniemi and Kari Saikkonen
Reference: Trends in Plant Science, October 2012, Vol. 17 (10): 569-575
Year: 2012
Results and conclusion: Glyphosate is the main nonselective, systemic herbicide used against a wide range of weeds. Its worldwide use has expanded because of extensive use of certain agricultural practices such as no-till cropping, and widespread application of glyphosate-resistant genetically modified crops. Glyphosate has a reputation of being nontoxic to animals and rapidly inactivated in soils. However, recent evidence has cast doubts on its safety. Glyphosate may be retained and transported in soils, and there may be cascading effects on nontarget organisms. These processes may be especially detrimental in northern ecosystems because they are characterized by long biologically inactive winters and short growing seasons. In this opinion article, we discuss the potential ecological, environmental and agricultural risks of intensive glyphosate use in boreal regions. The authors state that the half-life time of glyphosate may be much longer in northern ecosystems than generally presumed.

Proposed action: Consider as additional information as the article does not present experimental data but is a review article. No raw data and/or experimental design are given.	
Type of information (critical, high/low weight, supporting, additional): Low weight, as it is a review article no new experimental data are presented but cited and discussed.	
Reliability	
Endpoint	Sorption, degradation
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Jacobsen et al. (2008)

Title: Variation of MCPA, metribuzine, methyltriazine-amine and glyphosate degradation, sorption, mineralization and leaching in different soil horizons

Author: Carsten S. Jacobsen, Peter van der Keur, Bo V. Iversen d, Per Rosenberg, Heidi C. Barlebo c, Søren Torp d, Henrik Vosgerau e, Rene´ K. Juhler a, Vibeke Ernstsen, Jim Rasmussen, Ulla Catrine Brinch, Ole Hørbye Jacobsen

Reference: Environmental Pollution 156 (2008) 794–802

Year: 2008

Results and conclusion:
Glyphosate (K_d values determined in the range of 200 L/kg to 4000 L/kg) does not follow the simple rule that increased organic matter leads to increased sorption. The two most important component determining glyphosate sorption in the A-horizon is gravel and organic matter (the latter being negative). Glyphosate was often higher in the inorganic subsoil samples compared to the A-horizon samples. No calculated DT_{50} values were provided.
Detailed results on sorption are:

Soil characterisation:

Soil	Soil type	Sand (%) (fine + coarse)	Clay (%)	C.E.C. (in meq/100g)
Nedre Julianhede		69.7	3.8	
Nörlund		77.9	3.1	
Stubkaer		54.6	3.8	
Söbjerg		76.0	4.2	
Ruskaer		84.9	3.5	
Ilskov		83.3	4.4	
Skaaphusgaard		83.7	3.9	
Roejen Mosegard		80.1	3.4	
Röjen Kaer		82.0	3.3	
Röjen		79.8	3.3	
Sneptrup		86.3	3.0	
Simmelkjaer		88.9	3.4	
Neder Simmelkjaer		88.2	3.6	
Ommose		85.6	3.5	
Hallundbaek		86.8	3.8	

Adsorption

Soil type	OC %	pH ($CaCl_2$)	K_d	K_{oc}	K_f	K_{foc}	1/n
Nedre Julianhede	2.8	4.9	867				
Nörlund	2.1	5.3	237				
Stubkaer	4.8	5.8	1858				
Söbjerg	1.8	6.3	871				
Ruskaer	4.1	4.2	3758				
Ilskov	3.9	5.2	342				
Skaaphusgaard	2.6	5.1	n.a.				
Roejen Mosegard	6.4	5.6	108				
Röjen Kaer	2.3	4.9	690				
Röjen	2.7	4.7	656				
Sneptrup	2.2	4.5	400				
Simmelkjaer	1.8	4.1	586				
Neder Simmelkjaer	2.6	5.2	366				
Ommose	4.3	4.6	551				
Hallundbaek	1.6	5.5	257				

Proposed action:
Not to be considered further as the authors only provided K_d-values

Type of information (critical, high/low weight, supporting, additional):
Supporting information

Reliability	
Endpoint	Sorption of glyphosate in top soil and sub soil
Protocol	Non-GLP studies modified OECD 106 and OECD 307
Test compound	CAS 38641-94-0 ^{14}C-glyphosate (CAS 1071-83-6)
Test system and conditions	Sampling was performed at 15 locations placed on a 28 km long transect of the Karup outwash plain in northwest Jutland, Denmark OC content and particle size distribution were determined for the A, B and C horizons. A sample to solution ratio of 1:10 was used for glyphosate because this herbicide is highly adsorbed. The flasks were incubated on an orbital shaker at 10 °C for 96 h. Mineralization experiments were performed by adding ^{14}C-labelled pesticides in a total concentration of 1.0 mg pesticide/kg (dry weight) soil and incubating at 10 °C in the dark.
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are principally relevant. Unfortunately, the authors did not calculate DT_{50} for glyphosate.
Weight of evidence	
"Positive"/"Negative" evidence	The sorption studies support the information known for glyphosate from standard tests.

Karpouzas and Singh (2008)

Title: Microbial Degradation of Organophosphorus Xenobiotics: Metabolic Pathways and Molecular Basis
Author: Dimitrios G. Karpouzas and Brajesh K. Singh
Reference: ADVANCES IN MICROBIAL PHYSIOLOGY VOL. 51, 119-225
Year: 2008
Results and conclusion: In the present article, the microbial degradation and metabolic pathways for some OP compounds are reviewed. The chemical and molecular basis of OP degradation by microbes and the evolution and distribution of genes/enzymes are also reviewed. This article also examines applications and future use of OP-degrading microbes and enzymes for bioremediation, treatment of OP poisoning, and as biosensors.

Proposed action: Not to be considered further	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate and other organophosphorus xenobiotics
Protocol	No study
Test compound	Organophosphorus xenobiotics
Test system and conditions	-
Statistical design	-
Relevance	
Environmental relevance	The review is not suitable for considering in the dossier.
Weight of evidence	
"Positive"/"Negative" evidence	The conclusions cannot be compared with standard testing.

Kim et al. (2011)

Title: EFFECT OF SOIL METAL CONTAMINATION ON GLYPHOSATE MINERALIZATION: ROLE OF ZINC IN THE MINERALIZATION RATES OF TWO COPPER-SPIKED MINERAL SOILS	
Author: BOJEONG KIM, YOUNG SIK KIM, BO MIN KIM, ANTHONY G. HAY, and MURRAY B. MCBRIDE	
Reference: Environmental Toxicology and Chemistry, Vol. 30, No. 3, pp. 596–601, 2011	
Year: 2011	
Results and conclusion: For all but the highest Cu treatments (400 mg/kg) in the coarse-textured Arkport soil, mineralization began without a lag phase and declined over time. No inhibition of mineralization was observed for Zn up to 400 mg/kg in either soil, suggesting differential sensitivity of glyphosate mineralization to the types of metal and soil. Interestingly, Zn appeared to alleviate high-Cu inhibition of mineralization in the Arkport soil. The protective role of Zn against Cu toxicity was also observed in the pure culture study with Pseudomonas aeruginosa, suggesting that increased mineralization rates in high Cu soil with Zn additions might have been due to alleviation of cellular toxicity by Zn rather than a mineralization specific mechanism. Extensive use of glyphosate combined with its reduced degradation in Cu-contaminated, coarse-textured soils may increase glyphosate persistence in soil and consequently facilitate Cu and glyphosate mobilization in the soil environment,	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation) when heavy metals are
Protocol	Non-GLP study
Test compound	CAS 1071-83-6 labelled glyphosate (N-phosphono[^{14}C]methylglycine; specific activity 9.3 mCimM^{-1}; Sigma) and 0.1 mg unlabelled glyphosate to yield 10 mg/kg total glyphosate based on soil dry weight

Test system and conditions	An Arkport fine sandy loam and a Hudson silty clay loam, collected from the surface layer of uncontaminated agricultural research fields on the Cornell University campus (Ithaca, NY, USA) in May, 2003, were used for the present study. Calibrated amounts of $CuSO_4$ and $ZnSO_4$ solutions were sprayed on the soil samples to achieve target concentrations of 0, 100, 200, and 400 mg/kg of Cu and Zn both singly and in all combinations of these levels, based on soil dry weight. The data presented are the average values of individual measurements from triplicate samples. Glyphosate-treated soil samples were allowed to incubate for 80 d at 20 °C in the dark with the desired moisture level maintained (FC).
Statistical design	Three replicates
Relevance	
Environmental relevance	The conditions do not reflect the conditions for standard degradation experiments. The authors describe the degradation only qualitatively (no DT_{50} values calculated for glyphosate).
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard tests.

Klimek et al. (2001)

Title: Metabolism of the phosphonate herbicide glyphosate by a non-nitrate-utilizing strain of Penicillium chrysogenum	
Author: Magdalena Klimek, Barbara Lejczak, Pawel Kafarski and Giuseppe Forlani	
Reference: Pest. Manag. Sci 57:815-821	
Year: 2001	
Results and conclusion: Microbial activities were found to lead to a rapid and complete mineralisation of glyphosate in soil.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation of glyphosate by bacteria
Protocol	Non-GLP study
Test compound	Technical glyphosate (CAS 1071-83-6, acc. to HPLC >99 % pure)
Test system and conditions	The bacteria were cultivated in a mineral Czapek-Dox.
Statistical design	No information
Relevance	
Environmental relevance	The relevance of the results is low due to the artificial environment.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard laboratory testing.

Liu et al. (2010)

Title: The Environmental Risk Assessment of Herbicide Glyphosate on Various Chinese Cultivated Soils	
Author: Yihua Liu, Xiaoguang Wu, Mei Yang, Guonian Zhu	
Reference: Pest. Manag. Sci 57:815-821	
Year: 2001	
Results and conclusion: Laboratory experiments were performed to evaluate the degradation, adsorption, and leaching behavior of glyphosate in three agricultural soils with high sand content and different soil organic carbon content. Glyphosate degraded very fast in soils, the half-lives of glyphosate for the three soils were between 3.3 d-6.9 d, and the main metabolite of glyphosate was amino methylphosphonic acid (AMPA). The adsorption coefficient (KF) values for the three soils were were 93.99 (loam), 89.31 (clay) and 61.05 (sand), which may mean that the organic matter is not the key for glyphosate adsorption on soil. Leaching tests, performed in manually packed soil glass-plate, indicated that glyphosate moved very slowly on the three types of soil thin layer, it mainly stayed at the zone of 0-2 cm. Thus, the leaching behavior of glyphosate coincided well with the results of the batch sorption and degradation experiments. All the data showed that glyphosate had a low potential threat to groundwater.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation, adsorption, and leaching behavior of glyphosate in soil
Protocol	Non-GLP study
Test compound	Glyphosate isopropyl ammonium (95 %, technical grade), glyphosate standard (purity 99 %), Zhejiang Xinnan chemical company (Hangzhou, China)
Test system and conditions	*Degradation studies* All the working solutions were prepared by dissolving glyphosate isopropyl ammonium in distilled water. A quotient 20 g of each of the three types of soil was weighed and placed in a 250 mL flat-bottomed flask. The soil was commixed thoroughly with 1 mL working solution (200 mg/L). Distilled water was added to the point 60 % of the maximal holding capacity (MHC). 30 repetitions were set for each treatment. The flasks were covered with cotton plugs and placed at 25±1 ℃ in dark. Samples were taken according to a pre-determined schedule. *Adsorption studies* The triplicate samples of 5 g sieved soils were added to 250 mL conical flasks containing 50 mL glyphosate solution at concentrations of 2, 10, 50 and 100 mg/L (0.01 M $CaCl_2$ solution), respectively. After the addition of soil samples, the reaction mixtures were shaken on a horizontal shaker at 200 rpm at 25±1 ℃ until the equilibrium was established. After the desired time (24 h), 20 mL of sample was collected from each flask, centrifuged at 4000 rpm for 5 min and the supernatant was collected for pesticide residue analysis. *Mobility studies* 10 g soil was spreaded on the surface of a glass plate of 2×75×200 mm to prepare a thin layer of 0.50~0.75 mm. The plate was held at room temperature for 24 h to let dry. 500 μL of working solution (10 mg/L) was spotted in line at a site 1.5 cm away from one end of the plate. The plate was placed in a water-contained glass tank (holding 30º angle) until front boundary of the mobile phase (water) came to a location 13 cm away from the "starting line" (1 cm). After the water evaporated, the soil layer was cut into six sections and the glyphosate residue was then measured.
Statistical design	The pesticide degradation data were modeled using a simple first-order model: $Ct = C0e^{-kt}$, where Ct is the glyphosate concentration at time t, $C0$ is the initial concentration and k is the rate constant. The adsorption data were fitted to the Freundlich model.

Relevance	
Environmental relevance	The results are of relevance and were performed close to standard testing.
Weight of evidence	
"Positive"/"Negative" evidence	The results are in line with other reliable studies. No negative evidence.

Moneke et al. (2010)

Title: Biodegradation of glyphosate herbicide in vitro using bacterial isolates from four rice fields	
Author: A. N. Moneke, G. N. Okpala and C. U. Anyanwu	
Reference: African Journal of Biotechnology Vol. 9 (26), pp. 4067-4074, 28 June, 2010	
Year: 2010	
Results and conclusion: Time course of growth of the isolates on mineral salt medium containing glyphosate showed that both grew significantly ($P < 0.05$). The comparative effects of glyphosate as carbon and/or phosphorus source on the growth of the isolates showed that there was significant ($P < 0.05$) growth in the medium containing glucose and glyphosate. The effects of different concentrations of glyphosate on the growth of the isolates (P. fluorescens and Acetobacter sp) were evaluated. Significant ($P < 0.05$) growth was observed at lower concentrations (7.2-25 mg/ml) of glyphosate. No inhibition of growth was observed at high concentrations (100–250 mg/ml), indicating that the isolated bacteria can tolerate up to 250 mg/ml of glyphosate.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation in water using H_2O_2 and UV radiation
Protocol	Non-GLP study
Test compound	Isopropylamine salt of glyphosate, product Roundup® (containing 360 g active ingredient/L of glyphosate, Monsanto) CAS 38641-94-0
Test system and conditions	Soil samples were obtained from four rice fields located in Nigeria. These rice fields are known to have been previously exposed to glyphosate for long periods of time. Soil samples were collected from depths of 0-15 cm from three different sites in each of the four locations. These rice fields are known to have been previously exposed to glyphosate-based formulation (Roundup®) for long periods of time. Soil samples were collected from depths of 0-15 cm from three different sites in each of the four locations. Inoculate used for the study were prepared by inoculating isolates into nutrient broth and incubated at 30 °C for 24 h using sterile normal saline; the cells from the above cultures were re-suspended to a 0.5 McFarland nephelometer standard (Optical density of 0.17 at 660 nm).
Statistical design	Three replicates
Relevance	
Environmental relevance	The results are of minor relevance since the experiments do not describe any of the standard endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Pipke et al. (1988)

Title: Degradation of the phosphonate herbicide Glyphosate by Arthrobacter atrocyaneus ATCC 13752	
Author: Pipke, R., Amrhein, N.	
Reference: Applied and Environmental Microbiology (1988) Vol 54 (5): 1293-1296	
Year: 1988	
Results and conclusion: Arthrobacter atrocyaneus metabolized glyphosate to aminomethylphosphonic acid. The carbon of aminomethylphosphonic acid was entirely converted to CO_2. This is the first report on glyphosate degradation by a bacterial strain without previous selection for glyphosate utilization as a source of phosphorus.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation of glyphosate and AMPA by soil bacteria
Protocol	Non-GLP study
Test compound	^{14}C Glyphosate
Test system and conditions	The ability of Arthrobacter atrocyaneus ATCC 13752 to degrade glyphosate was evaluated in vitro. Samples were taken at the beginning of the experiments and after 80 h.
Statistical design	No information
Relevance	
Environmental relevance	The results cannot be used because the system is not comparable to standard
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Pizzul et al. (2009)

Title: Degradation of glyphosate and other pesticides by ligninolytic enzymes	
Author: Leticia Pizzul Æ Marı´a del Pilar Castillo Æ John Stenström	
Reference: Biodegradation (2009) 20:751–759	
Year: 2009	
Results and conclusion: In the presence of laccase and ABTS (Lac 2), 40.9 % of the glyphosate disappeared after 24 h, whereas 62.8 % of the glyphosate was degraded when Mn^{2+} and Tween 80 were added together with the enzyme (Lac 3). A synergistic effect of ABTS, Mn^{2+} and Tween 80 (Lac 4) was observed, where 90.1 % of glyphosate disappeared after 24 h. The metabolite AMPA was detected in all the cases where degradation of glyphosate occurred. No other metabolites were analysed in the present work, but the equal stoichiometry between AMPA formed and glyphosate degraded suggests that AMPA was not degraded and that there was no or negligible formation of other compounds. No degradation of glyphosate was observed with laccase alone.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation by ligninolytic enzymes
Protocol	Non-GLP study

Test compound	Non-labelled Glyphosate [N-(phosphomethyl)glycine] (CAS 1071-83-6), non labelled AMPA (CAS 1066-51-9) both supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) labelled glyphosate (2-^{13}C, 99 %; ^{15}N, 98 %) and AMPA (^{13}C, 99 %; ^{15}N, 98 %; methylene-D_2, 98 %) were supplied by LGC Standards (Borås, Sweden).
Test system and conditions	Horseradish peroxidase (EC 1.11.1.7), lignin peroxidase (EC 1.11.1.14) and laccase from Trametes versicolor (1.10.3.2) were purchased from Sigma–Aldrich (Steinheim, Germany). Manganese peroxidase (EC 1.11.1.13) from Nematoloma frowardii was obtained from JenaBios (Jena, Germany). The potential of MnP to degrade glyphosate was evaluated in vitro. All reactions were conducted in sterile, loosely capped, 8-ml glass vials. The vials were placed on a rotary shaker (150 rpm) at 35 °C and samples were taken at the beginning of the experiments and after 1, 4 and 7 days.
Statistical design	No information
Relevance	
Environmental relevance	The results cannot be used because the system the endpoint is not comparable to standard tests.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Reimer et al. (2005)

Title: Effect of Manure on Glyphosate and Trifluralin Mineralization in Soil
Author: M. Reimer, A. Farenhorst, and J. Gaultier
Reference: Journal of Environmental Science and Health Part B, 40:605–617, 2005
Year: 2005
Results and conclusion: Experiments were conducted with glyphosate in soil microcosms in the laboratory for a total of 332 days. The rate and amount of mineralization of glyphosate were significantly influenced by the additions of fresh manure to soil in the laboratory and by the history of manure applications in the field. However, the maximum difference in herbicide mineralization between soils that were free of manure application and those amended with manure in the field or in the laboratory was only 7.3 % of that initially applied. Therefore, it is conclude that liquid hog manure application to soil will have no significant effect on the mineralization of glyphosate and trifluralin under field conditions. Half lives were found for the degradation of glyphosate in the range of 18 to 34 d.
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.
Type of information (critical, high/low weight, supporting, additional): Additional information

Reliability	
Endpoint	Glyphosate degradation by ligninolytic enzymes
Protocol	Non-GLP study
Test compound	Labelled compound: [*phosphonomethyl*-^{14}C] glyphosate (specific activity 89 MBq mmol^{-1}; Sigma-Aldrich Co., St. Louis, MO) Non-labelled compound: analytical-grade glyphosate (99 % purity; Chem Service,West Chester, PA) (CAS 1071-83-6)

Test system and conditions	Surface soils (0–10 cm) of well-drained Orthic Black Chernozems (Canadian soil classification system) were collected from three study sites in western Manitoba, identified here by their vicinity to the nearest town: Birtle (101°05_W, 50°42_N), Decker (100°78_W, 50°27_N), and Neepawa (99°47_W, 50°23_N). For each site, representative soil samples were collected from lower slope positions in two adjacent fields: one field with a long-term history of hog manure applications and one field that had never received manure. Soil organic carbon and total nitrogen contents were determined. Fresh liquid hog manure was obtained from a farm near Fannystelle, MB. Microcosms were incubated at 20 °C and soil was brought to 70 % field capacity. Herbicide
Statistical design	Three replicates
Relevance	
Environmental relevance	The results are of relevance, but due to the manure applications they cannot be directly compared with standard testing.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests The results are principally supported by other reliable studies.

Roffignac et al. (2008)

Title: Efficiency of a bagasse substrate in a biological bed system for the degradation of glyphosate, malathion and lambda-cyhalothrin under tropical climate conditions	
Author: Laure de Roffignac, Philippe Cattan, Julie Mailloux, David Herzog and Fabrice Le Bellec	
Reference: Pest Manag Sci 64:1303–1313 (2008)	
Year: 2008	
Results and conclusion: Results showed that more than 99 % of glyphosate were degraded in 6 months. In the biological bed, the DT_{50} value for glyphosate 33 days. The degradation rate of aminomethylphosphonic acid (AMPA) residues from the degradation of glyphosate was slower than that of the other pesticides (DT_{50} 69 days).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation of glyphosate in a biobed under tropical conditions
Protocol	Non-GLP study
Test compound	Glyphosate (product Glyfos, 360 gL^{-1}) (CAS 1071-83-6)
Test system and conditions	The experiment took place at the CIRAD (Centre de Cooperation Internationale en Recherche Agronomique pour le D´eveloppement) research station at Vieux-Habitants, 97 119 Guadeloupe, France. The biological bed was a hole dug in the ground with concrete walls coated with impermeable paint and topped with a metal roof. The internal dimensions were 2m long, 1m wide and 0.80m deep. The biological bed was filled with biomix to a height of 65 cm, which is equivalent to a volume of 1300 L. The biomix used was a mixture of soil and bagasse in a proportion of 1 volume of soil to 3 volumes of bagasse. Glyphosate was sprayed in the biological bed at 295 × 103 µg/kg.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is small due to the tropical conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Rueppel et al. (1977)

Title: Metabolism and Degradation of Glyphosate in Soil and Water	
Author: Rueppel, M.L., Brightwell, B.B., Schaefer, J., Marvel, J.T.	
Reference: J. Agric. Food Chem., Vol. 25 (3): 517-528	
Year: 1977	
Results and conclusion: Using soil/water shake flasks, up to 50 % of each carbon of/L^{4}C was evolved as $^{14}CO_2$ in 28 days. In two of the three soils examined, glyphosate was 90 % dissipated in less than 12 weeks. Aminomethylphosphonic acid, the only significant soil metabolite of glyphosate, also undergoes rapid degradation in soil. Short-term shake flask metabolism experiments with both ^{13}C and ^{14}C-labeled glyphosate were carried out in order to permit facile, unequivocal spectral identification of glyphosate and its transient metabolite aminomethylphosphonic acid. Comparison of the metabolic samples to both reference standards and the spiked controls by means of ^1H, ^{31}P, and ^{13}C NMR, mass spectral analysis, ion-exchange chromatography, and thin-layer chromatography has unequivocally characterized both bound and unbound glyphosate and aminomethylphosphonic acid in soil. The parent herbicide glyphosate has also been shown to be stable to sunlight, nonleachable in soil, to have a low propensity for runoff, and to have a minimal effect on microflora.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation of glyphosate and AMPA in a soil/water shake flasks system
Protocol	Non-GLP study
Test compound	^{13}C and ^{14}C Glyphosate
Test system and conditions	Each flask contained 4.5 g (dry weight) of the appropriate soil, 1 mg of the desired radioactive compound, and distilled water (100 mL). Incubation time was 28 d. After the desired period (normally 7 days) of metabolism on an incubator shaker at 30 °C, flasks were flushed with air for 1.25 h to collect the evolved CO_2. The flask contents were then transferred to 250-mL centrifuge bottles, centrifuged at 8000 rpm for 15 min, and the supernatant transferred to a 100-mL volumetric flask for analysis by liquid scintillation counting and TLC/beta camera. The soil was washed once with 25 mL of H_2O, centrifuged, and the supernatant removed for analysis for radioactivity. The ^{14}C content of
Statistical design	No information
Relevance	
Environmental relevance	The results cannot be used because the system is not comparable to standard
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Santos et al. (2009)

Title: Biodegradation of glyphosate in rhizospheric soil cultivated with Glycine max, Canavalia ensiformis E Stizolobium aterrimum	
Author: SANTOS J.B., FERREIRA, E.A., FIALHO, C.M.T., SANTOS, E.A., GALON, L.; CONCENÇO, G.; ASIAZÚ, I. and SILVA, A.A.	
Reference: Planta Daninha, Viçosa-MG, v. 27, n. 4, p. 781-787, 2009	
Year: 2009	
Results and conclusion: Biodegradation of glyphosate was evaluated in rhizospheric soil cultivated with Glycine max (soybean, var. BRS245-RR), Canavalia ensiformis and Stizolobium aterrimum. After these species were cultivated for 60 days, soil samples were collected, placed in flasks and treated with ^{14}C-glyphosate. After 30 days of incubation, the total release rate of C-CO_2 was determined along with microbial biomass (MBC), metabolic quotient (qCO_2), and degradation percentage of the radio-labeled glyphosate released as ^{14}C-CO_2. A higher mass of rhizosphere-associated microorganisms was verified in the soil samples from pots cultivated with soybean, regardless of glyphosate addition. However, in the presence of the herbicide, this characteristic was the most negatively affected. Microorganisms from the C. ensiformis rhizosphere released a lower amount of ^{14}C-CO_2, while for those originated from S. aterrimum, the amount released reached 1.3 % more than the total carbon derived from the respiratory activity. The rhizospheric soil from S. aterrimum also presented higher glyphosate degradation efficiency per microbial biomass unit. However, considering qCO_2, the microbiota of the rhizospheric soil cultivated with soybean was more efficient in herbicide degradation.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Dependence of glyphosate degradation in soil from the presence of Glycine max, Canavalia ensiformis and Stizolobium aterrimum cultivations
Protocol	Non-GLP study
Test compound	Non-labelled compound: glyphosate (CAS 1071-83-6),
Test system and conditions	In the laboratory, the soil samples were sieved, shade-dried for 24 h, weighed (150 g) and then placed in 300 mL glass flasks. Sample moisture was adjusted to 70 % of field capacity and a glyphosate solution at the concentration of 1.76 mg/kg equivalent to 3.36 kg/ha of the technical product was added. Eight treatments (soil samples from soils cultivated with Glycine max, Canavalia ensiformis, Stizolobium aterrimum and non-cultivated soil) were evaluated, with these samples being treated and non-treated with glyphosate. The experiment was arranged in a completely randomized design in a factorial scheme (4 x 2) with four repetitions. The flasks were incubated for 32 days for evaluation of the C-CO_2 release rate, quantified every eight days. To determine soil basal respiration rate after incubation of the samples, the CO_2 evolved was captured in flasks containing 100 mL of NaOH (0.25 mol/L) under a continuous air flow system (free of CO_2 and moisture). After each incubation period, indirect titration of NaOH with HCl (0.25 mol/L) was carried out and the excess NaOH that did not react to evolved CO_2 was quantified (Anderson, 1982). At the end of the incubation period, the microbial biomass carbon was determined.
Statistical design	Four replicates
Relevance	
Environmental relevance	The results are of relevance, but due cannot be directly compared with standard testing.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Schnurer et al. (2006)

Title: Effects of Surface Sorption on Microbial Degradation of Glyphosate	
Author: YLVA SCHNURER, PER PERSSON, MATS NILSSON, ANDERS NORDGREN, AND REINER GIESLER	
Reference: Environ. Sci. Technol. 2006, 40, 4145-4150	
Year: 2006	
Results and conclusion: Additions of glyphosate, in combination with glucose and N, did not change the respiration rate in comparison with the same treatment but without glyphosate. In contrast, glyphosate additions combined with glucose and P decreased microbial growth, whereas the combination with goethite counteracted the negative effect. The results suggest that glyphosate was de-carboxylated in the sorbed state. Stimulating microbial growth by the addition of glucose and nitrogen resulted in further oxidation of glyphosate and only phosphate was detectable on the goethite surface after 13 days incubation. The results show that sorbed glyphosate is microbial degradable, and it retards microbial activity.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Effects of sorption on microbial degradation of Glyphosate
Protocol	Non-GLP study
Test compound	Non-labelled compound: glyphosate (CAS 1071-83-6),
Test system and conditions	The soil was collected in the Nyänget catchment (64°15N, 19°45E), Svartberget Experimental Forest, Vindeln, northern Sweden. The mean annual precipitation (1980-1995) was 590 mm, of which 40 % was snow. The mean annual temperature was 1.4 °C, with a January mean of -10.6 °C and a July mean of 14.3°C. The soil moisture content of the samples was adjusted to about 270 % of the organic matter (w/w) to optimize conditions for microbial growth. Respiration kinetics were recorded hourly at 20 °C, using a 96-unit respirometer (18, 21) with 250-mL plastic jars. Different treatments were examined using attenuated total reflectance Fourier transform (ATR-FTIR) spectroscopy.
Statistical design	Five replicates
Relevance	
Environmental relevance	The results are of relevance, but due cannot be directly compared with standard testing.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Schroll et al. (2006)

Title: Quantifying the Effect of Soil Moisture on the Aerobic Microbial Mineralization of Selected Pesticides in Different Soils	
Author: REINER SCHROLL, HANSHEINRICH BECHER, ULRIKE DÖRFLER, SEBASTIA GAYLER, SABINE GRUNDMANN, HANSPETER HARTMANN, AND JÜRGEN RUOSS	
Reference: Environ. Sci. Technol. 2006, 40, 3305-3312	
Year: 2006	
Results and conclusion: At soil water potential <20 MPa minimal pesticide mineralization occurred; a linear correlation ($P \leq 0.0001$) exists between increasing soil moisture (within a soil water potential range of -20 and -0.015 MPa), and increased relative pesticide mineralization; optimum pesticide mineralization was obtained at a soil water potential of -0.015 MPa, and when soil moisture approximated water holding capacity, pesticide mineralization was considerably reduced	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Effects of soil moisture on microbial degradation of Glyphosate
Protocol	Non-GLP study
Test compound	Labelled compound: phosphonomethylene-^{14}C >98 % purity (CAS 1071-83-6)
Test system and conditions	The agricultural soils used were characterized by large variations in soil texture (sand content 4-88 %) and organic matter content (0.97-2.70 % org. C. Soil-water retention curves were determined at the Institute for Soil Science of Technical University Munich. Biodegradation of ^{14}C-labeled chemicals was studied in a discontinuously aerated laboratory system. Soils were incubated in 100 mL double-wall flasks in the dark at 20 °C (1 °C.). Twelve different soil moistures ranging from 0.01 and 0.25 g g^{-1} (equivalent to 5 up to 100 % WHC) were selected to study the effect of increased soil water content on glyphosate mineralization.
Statistical design	Four replicates
Relevance	
Environmental relevance	The results are of relevance, but due cannot be directly used as the rate constants were not provided.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Shushkova et al. (2012)

Title: Biodegradation of Glyphosate by Soil Bacteria: Optimization of Cultivation and the Method for Active Biomass Storage	
Author: T. V. Shushkova, I. T. Ermakova1, A. V. Sviridov, and A. A. Leontievsky	
Reference: Microbiology, 2012, Vol. 81, No. 1, pp. 44–50	
Year: 2012	
Results and conclusion: Conditions for obtaining the active biomass of *Ochrobactrum anthropi* GPK 3 and *Achromobacter* sp. kg 16, bacteria which are able to degrade the herbicide glyphosate (N-phosphonomethylglycine), were investigated. In the batch culture, degradation was most effective in the medium with pH 6.0–7.0 and aeration at 10–60 % of air saturation supplemented with glutamate and ammonium chloride as sources of carbon and nitrogen, respectively. Due to the adaptation of the cells and induction of the relevant enzymatic systems, the inoculum grown in the presence of glyphosate exhibited 1.5–2-fold higher efficiency of xenobiotic degradation than that grown with other sources of phosphorus (orthophosphate and methylphosphonic acid). The efficiency of the toxicant decomposition increased with an increase in a specific load of glyphosate, which the cells were subjected to during the initial stage of growth. The specific load was regulated both by the initial cell concentration and the concentration of the phosphorus source, and the effect was probably determined by its availability to microorganisms. Storage of the liquid biopreparation as a paste with stabilizers (ascorbate, thiourea, and glutamate) at room temperature for 50 days resulted in high level of bacteria viability and a degrading activity approximately equal to that obtained when the bacteria were maintained on the agar medium containing glyphosate at 4 °C with monthly transfers to the fresh culture medium.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Influence of different cultivation conditions on the degradation of glyphosate by soil bacteria
Protocol	Non-GLP
Test compound	Glyphosate (500 mg/L) as the herbicide GroundBio (36 % aqueous solution of the isopropylamine salt, Technoexport, Russia)
Test system and conditions	The goal of the present work was to select optimal conditions for the cultivation of *Ochrobactrum anthropi* GPK 3 and *Achromobacter* sp. kg 16, providing maximal effectiveness of the herbicide degradttion, as well as to work out the storage conditions for the biomass intended for introduction into the soil.
Statistical design	No information
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard studies.

Sillanpää et al. (2011)

Title: Degradation of chelating agents in aqueous solution using advanced oxidation process (AOP)	
Author: Mika E.T. Sillanpää, Tonni Agustiono Kurniawan, Wai-hung Lo	
Reference: Sillanpää, M.E.T., *et al.* Degradation of chelating agents in aqueous solution using advanced oxidation process (AOP). Chemosphere 83(11): 1443-1460. doi:10.1016/j.chemosphere.2011.01.007 (2011)	
Year: 2011	
Results and conclusion: The performance of individual AOP is compared. The selection of the most suitable AOP seems to depend on the characteristics of effluents, technical applicability, discharge standard, regulatory requirements and environmental impacts.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Degradation of chelating agents in aqueous solution using advanced oxidation process
Protocol	Review
Test compound	No chelating agents
Test system and conditions	Review
Statistical design	No information
Relevance	
Environmental relevance	The results have no relevance for pesticide registration.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard studies.

Simonsen et al. (2008)

Title: Fate and availability of glyphosate and AMPA in agricultural soil	
Author: LOUISE SIMONSEN, INGE S. FOMSGAARD, B. VENSMARK and NIELS HENRIK SPLIID	
Reference: Journal of Environmental Science and Health Part B (2008) 43, 365–375	
Year: 2008	
Results and conclusion: The disappearance of glyphosate and the formation and disappearance of AMPA were monitored. The resulting curves were fitted based on FOCUS degradation kinetics. The best fit of the glyphosate degradation data was obtained using a first-order multi compartment (FOMC) model. DT_{50} values of 9 days (glyphosate) and 32 days (AMPA) indicated relatively rapid degradation. Detailed results are: Soil characteristics	
Parameter	**Value**
% Clay (<2 μm)*a*	13.3
% Silt (2–20μm)*a*	16.2
% Coarse silt (20–63 μm)*a*	24.7
% Fine sand (63–200 μm)*a*	26.9
% Coarse sand (200–2000 μm)*a*	16.2
% Humus *a*	2.7
Density (g/cm3)	2.626
% $CaCO_3$	n.d.
pH-H_2O	6.5
P (Al)*b*	7

K (Al)*b*	20
Mg (Al)*b*	10
Ca (Al)*b*	140
P (HCl)*b*	39.9

Quantities of glyphosate and AMPA in blank soil samples found by extraction with borate solution (50 % MWHC, 14.3 °C

Time (days)	***Glyphosate (ng/g dry soil)***	***AMPA (ng/g dry soil)***
0	0.81	10.46
7	0.61	7.33
14	0.50	5.86
21	0.41	5.59
35	0.46	5.03
49	0.41	4.04
77	0.32	3.87
105	0.31	4.45
179	0.19	1.16

Model parameters ± confidence interval and DT_x values for glyphosate fitted with first order multi component (FOMC) and the decline of AMPA fitted with single first order (SFO).

Compound	***Parameter***	***Value***
Glyphosate	M0	7.8 ± 0.58 nmol/g dry soil
	A	0.85 ± 0.28
	β	7.1 ± 4.7
	DT_{50}	9 days
	DT_{90}	101 days
	M_0	4.2 ± 0.28 nmol/g dry soil
AMPA	k	0.022 ± 0.0038 $days^{-1}$
	DT_{50}	32 days
	DT_{90}	106 days

Proposed action:
Consider as supporting information because it was not totally performed in line with FOCUS degradation kinetics (no DFOP or HS kinetics performed though residues > 10 % at the end of the study).

Type of information (critical, high/low weight, supporting, additional):
Supporting information

Reliability	
Endpoint	Glyphosate degradation by ligninolytic enzymes
Protocol	Non-GLP study
Test compound	Labelled compound ^{14}C-glyphosate solution (110 µL) and ^{13}C-^{15}N-glyphosate solution (180 µL) (CAS 1071-83-6)
Test system and conditions	The soil was sampled from the 0–2.5 cm layer of a field, where reduced tillage had been practiced, in Sandved (eastern Denmark). ^{14}C-glyphosate solution (110 µL) and ^{13}C-^{15}N-glyphosate solution (180 µL) were applied to each of 50 samples of 5 g dry soil.
Statistical design	Six replicates
Relevance	
Environmental relevance	The results are of relevance and were performed close to standard testing.
Weight of evidence	
"Positive"/"Negative" evidence	The results are principally supported by other reliable studies.

Stenrød et al. (2006)

Title: Spatial variability of glyphosate mineralization and soil microbial characteristics in two Norwegian sandy loam soils as affected by surface topographical features	
Author: Marianne Stenrød, Marie-Paule Charnay, Pierre Benoit, Ole Martin Eklo	
Reference: Soil Biology & Biochemistry 38 (2006) 962–971	
Year: 2006	
Results and conclusion: Considerable spatial heterogeneity in the degradation rate of glyphosate and general carbon utilization exists even across small areas within a single agricultural field. This horizontal variability was observed over several spatial scales, and could not be clearly explained. It evidently arose from differences in environmental factors affecting microbial activity and growth, and topographical features controlling redistribution of water and matter flow patterns were correlated to the investigated soil microbial variables.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Spatial variability of glyphosate mineralization
Protocol	Non-GLP study
Test compound	Non-labelled aqueous solutions of glyphosate were prepared from Roundup Ecow (30.05 % glyphosate active ingredient, density rZ1198 g lK1) (Monsanto Crop Sciences, Norway). Labelled: [P-methylen ^{14}C]glyphosate (radiochemical activity 5.155 MBq mgK1, radiochemical purity >99 %) (Institute of Isotopes, Budapest, Hungary) to give 84.85 kBq mL^{-1} (CAS 1071-83-6)
Test system and conditions	Soil was sampled from agricultural fields in Grue (South-East Norway) at N608280 E128020, and Malselv (North Norway) at N698150 E188330. Both locations had alluvial sandy loam soils, with potatoes as a main crop in rotation with barley. Two sites were used with two different sampling strategies to catch the spatial variability at two different scales. The site at Grue was used for assessing the variability at a metre scale and to observe horizontal variations. The site at Malselv was used for a decimetre scale study to evaluate horizontal and vertical variations in a soil profile. The statistical processing of the results included analysis of variance (ANOVA), principal component analysis (PCA), linear regression analysis and Tukey's test for comparison of means. A significance level of 5 % was used for hypothesis testing.
Statistical design	Complex statistical analysis
Relevance	
Environmental relevance	There is relevance is as they point out the variability of degradation even on a small scale.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Stenrød et al. (2005)

Title: Effect of freezing and thawing on microbial activity and glyphosate degradation in two Norwegian soils	
Author: Marianne Stenrød, Ole Martin Eklo, Marie-Paule Charnay and Pierre Benoit	
Reference: Pest Manag Sci 61:887–898 (2005)	
Year: 2005	
Results and conclusion: The glyphosate mineralization pattern was comparable with the overall microbial activity in the soils. Observed different levels of diversity might explain some of the difference in total glyphosate mineralization between soils. Organic C mineralization was found to be a good predictor of glyphosate mineralization for each soil individually, supporting other investigations. The two freeze–thaw treatments gave a similar total amount of glyphosate mineralized during the 84-day period but less than in the +5 °C treatment.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate mineralization under cold conditions
Protocol	Non-GLP study
Test compound	Non-labelled aqueous solutions of glyphosate were prepared from Roundup Ecow (30.05 % glyphosate active ingredient, density rZ1198 g lK1) (Monsanto Crop Sciences, Norway). [P-methylene-^{14}C]glyphosate (radiochemical activity 5.155MBqmg^{-1}, radiochemical purity >99 %; Institute of Isotopes, Budapest, Hungary) to give 8.00 kBqmg^{-1} (CAS 1071-83-6)
Test system and conditions	Soil was sampled from agricultural fields in Grue (South-East Norway) at N608280 E128020 and Malselv (North Norway) at N698150 E188330. Both locations had alluvial sandy loam soils, with potatoes as a main crop in rotation with barley. Bulk soil samples were taken from the top 10cm of the Ap-horizon in mid-October 2002. Bulk soil samples of both soils were adjusted to 70 % of WHC. The soil samples were subjected to one of four winter temperature simulation regimes; constant thaw (control at +5 °C), constant freezing (control at -5 °C), short-term temperature fluctuations (24 h at -5 °C followed by 24 h at +5 °C), and long-term temperature fluctuations (3 weeks at -5 °C followed by 3weeks at +5 °C) for a total of 12 weeks. Glyphosate mineralization during the incubation was monitored by measuring the $^{14}CO_2$ in the NaOH traps by LSC (Packard Tri-Carb 2900TR), using Hionic-Fluor
Statistical design	Three replicates
Relevance	
Environmental relevance	There is relevance is as they point out the dependency of degradation in cold winter periods.
Weight of evidence	
"Positive"/"Negative" evidence	The results are in line with other reliable studies.

Sviridov et al. (2011)

Title: New Approaches to Identification and Activity Estimation of Glyphosate Degradation Enzymes	
Author: A. V. Sviridov, N. F. Zelenkova, N. G. Vinokurova, I. T. Ermakova, and A. A. Leontievsky	
Reference: Biochemistry (Moscow), 2011, Vol. 76, No. 6, pp. 720_725.	
Year: 2011	
Results and conclusion: Glyphosate degradation can follow different pathways depending on physiological characteristics of metabolizing strains: in Ochrobactrum anthropi GPK3 the initial cleavage reaction is catalyzed by glyphosateoxidoreductase with the formation of aminomethylphosphonic acid and glyoxylate, whereas Achromobacter sp. MPS12 utilize C-P lyase, forming sarcosine. The proposed methodology has several advantages as compared to others described in the literature.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6),
Test system and conditions	Two strains of bacteria that degrade phosphonates: Ochrobactrum anthropi GPK3, isolated from soils contaminated with GP, and Achromobacter sp. MPS12A, isolated from sites of contamination with methylphosphonic acid (MPA) and adapted to growth in GP-containing medium. The organisms were under periodic cultivation in liquid mineral medium MS1 without phosphates. Sodium glutamate at concentration of 55 mM was used as a source of carbon. 3 mM GP as a Roundup component was used as a sole phosphorus source.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is less relevant since the authors did not calculate any DT_{50} values.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with reliable studies.

Yang et al. (2013b)

Title: Mild salinization stimulated glyphosate degradation and microbial activities in a riparian soil from Chongming Island, China
Author: C. Yang, S. Shen, M. Wang and J. Li
Reference: Journal of Environmental Biology, Vol. 34, 367-373, 2013
Year: 2013

Results and conclusion:
An incubation experiment was conducted to investigate the effects of simulated saltwater treatment with different percentages of artificial sea water on degradation dynamics of herbicide glyphosate and microbial activities in a riparian soil in Chongming Island, China. The results showed that 10 % sea water treatment showed significantly enhancing effects on degradation efficiency of glyphosate with the lowest residual concentration among all the treatments. However, glyphosate degradation was markedly decreased in the riparian soil with 20 % and 50 % sea water treatments.
The half-lives for 20 % and 50 % seawater treatments were prolonged by 12.1 and 39.0 %, respectively, as compared to control. Microbial investigation indicated that 10 % seawater treatment significantly stimulated microbial activities in the glyphosate-spiked riparian soil throughout the incubation period. At 42 day of incubation experiment, flourescein diacetate (FDA) hydrolysis rate, microbial adenosine triphosphate (ATP), and basal soil respiration (BSR) in the glyphosate-spiked riparian soil with 10 % seawater were 59.2, 42.5 and 31.8 % higher than those with no saltwater treatment, respectively. In contrast, saltwater treatment with 50 % sea water significantly inhibited microbial activities. Especially, FDA hydrolysis rate, microbial ATP and BSR were decreased by 66.4, 58.6 and 66.8 %, respectively, as compared to control. The results indicate that levels of simulated saltwater can exert variable effects on herbicide degradation dynamics and microbial parameters in the riparian soil. In the following the half-life's of glyphosate biodegradation in riparian soils affected by saltwater treatments are given:

Salt water treatments [%]	Half life [d]	R^2
0	14.1	0.9877
10	10.5	0.9386
20	15.8	0.9976
50	19.6	0.9898

Proposed action:
Consider as additional information. Recalculation of endpoints on degradation is not necessary.

Type of information (critical, high/low weight, supporting, additional):
Additional information

Reliability	
Endpoint	Glyphosate biodegradation in riparian soils affected by saltwater treatments and effect of saltwater microbial parameters in a riparian soil
Protocol	Not given
Test compound	Glyphosate

Test system and conditions	Site description : The study was conducted in Chongming Island of Yangtze River Estuary, located in the East of China, where frequent seawater incursion results in an increase in salinity of inland freshwater, especially in the Northeast next to the East China Sea. Collection and pre-treatment of the riparian soil for incubation experiment: Non-contaminated soil was obtained from the 0-30 cm depth' of a riparian wetland (121.26°E, 31.55° N) in the Southwest of Chongming Island, where soil salinity is relatively slight due to receiving less seawater incursion. Present research aims at the overall effects of saltwater treatment on biodegradation dynamics of glyphosate and the related microbial parameters. Accordingly, the riparian soil for incubation experiment was spiked with glyphosate only at the concentration of about 5 mg/kg, which is slightly higher than the maximum of field concentration in the riparian soils. The collected riparian soil was spiked with glyphosate following the modified procedure Brinch *et al.*, 2002. The prepared contaminated soil with glyphosate was stored at 4 °C in an airtight container for laboratory incubation experiment. Soil incubation and saltwater treatment: 200g dry weight equivalent glyphosate-contaminated riparian soil was weighed into a 500-ml flask and statically incubated at 30 °C in an incubator without illumination. Artificial seawater was prepared. Saltwater additions with 0, 10, 20 and 50 % seawater were made once per day for 10 days, and simultaneously the flasks were kept submerged with 2 cm depth above the soil surface. After that, the soil moisture was adjusted to 85-90 % of the maximum water holding capacity (WHC, which was 654 g/kg for the investigated soil) with addition of deionized water by weight method until the end of the incubation experiment. The incubation experiments were set up with three replicates per treatment. The incubation experiment ran for 50 d. Soil sample collection and analysis: Soil sampling was carried out at intervals of I, 7, 14, 21, 28, 35, 42, 50, 60 d of incubation period. The collected field-moist soil samples were homogenized and passed through a 2 mm sieve. A portion of the soil samples were stored in 4 °C for analysis for the microbial parameters, and the other portion of soil samples were freeze-dried for determination of the concentrations of the residual glyphosate as well as other chemical parameters. Residual glyphosate in riparian soil was determined following the method of Hu *et al.* (2008) with some modifications. Briefly, 10 g of frozen-dried soil sample were weighed into a stoppered centrifuge tube and extracted with 2 M NH_4OH under microwave assistance, derivatized by trifluoroacetic anhydride (TF AA) and trifluoroethanol (TFE), and then determined by gas chromatography with a nitrogen-phosphorus detector (GC-NPD). The glyphosate degradation kineties in the riparian soil with simulated saltwater treatments was described by a first-order kinetic model. Soil microbial biomass (MBC) was determined by fumigation with ethanol free $CHCl_3$ and extraction with 0.5m K_4SO_4. Basal soil respiration (BSR) was determined by measuring CO_2 evolution in the aerobic condition.
Statistical design	All analyses were performed in triplicate. Data were analyzed statistically by analysis of variance (ANOVA). Duncan's New Multiple Range Test (DMRT) was employed to assess differences between the treatment means. The effects of simulated saltwater incursion with different seawater addition levels on glyphosate degradation and related microbial parameters were declared as significant at 5 % probability levels. Standard errors were calculated for mean values of all determinations. All statistical analyses were performed with SPSSI 2.0software.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

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Detailed description of open literature –rate of degradation in soil field studies

Adams et al. (2007)

Title: The Absence of Glyphosate Residues In Wet Soil and the Adiacent Watercourse after a Forestry Application in New Brunswick	
Author: Gregory W. Adams, Troy Smith, and J. David Miller	
Reference: NORTH. J. APL. FOR. 24(3)	
Year: 2007	
Results and conclusion: In August 2005, 3 years after herbicide application, the site was fully occupied by a mix of vegetation similar to the vegetation before spraying. Glyphosate concentrations in all water samples were below the detection limit. Average moisture content of the soil samples was 84.2 ± 0.5 %. Recoveries from the site soil samples averaged 42.5 ± 2.9 %. One replicate from one of the three soil collection sites, collected 1 year after application, was positive at the detection limit (0.40 ± 0.20 µL/g; uncorrected value). The remaining samples were negative. In this study, we found that the time taken to 50 % degradation of the glyphosate (DT_{50}) in the water-saturated sediments was faster than expected (approximately 1 day considering the detection limit and recoveries).	
Proposed action: No action since documentation of results insufficient. Furthermore, the publication focuses on a site outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Supporting information, since the experiments were performed in principal similar to standard degradation studies but documentation of results (e.g. time dependent residues, kinetic analyses) is insufficient.	
Reliability	
Endpoint	DT_{50}, PECSW and PECSoil
Protocol	Non-GLP, no standard laboratory studies
Test compound	Test compound: CAS 40465-66-5 Unlabelled: Glyphosate, as a formulation, Vision concentrate (PCP 19899; Monsanto Canada, Winnepeg, MB, Canada)
Test system and conditions	Field study to analyse stream concentration after application of glyphosate. The application rate was 1.67 kg/ha glyphosate; the application took 2.5 hours. Water collections were taken at three locations along a stream 65 m from the perimeter of the treatment area spaced out along the perimeter of the treatment area. Three soil sample collection sites were identified in the treated area. These were all approximately 4 m^2, were comprised mainly of clay soil and organic matter, and were located where surface water would accumulate depending on the time of year. The use of GC-MS with single ion monitoring ensured a reliable detec tion of glyphosate residues. For water samples, the limit of quantification was 25 µg/L (25 ppb). Glyphosate recoveries from three water samples averaged 130 +/- 6.5 % at the limit of quantification (mean ± SE). Recoveries from the sand/organic soil laboratory method studies were 90.6 ± 9.2 % and the detection limit was 0.40 µg/g.
Statistical design	No information
Relevance	
Environmental relevance	The study is principally relevant since the experimental design is close to the scenarios considered in the registration procedure (Drift as entry route). However, no sufficient information about raw data is given. It is further not clear whether the DT_{50} are simply caused by advection in the fresh water system.
Weight of evidence	
	The results may be influenced by advection in the surface water.

Grey et al. (2009)

Title: Herbicide Dissipation from Low Density Polyethylene Mulch	
Author: Timothy L. Grey, William K. Vencill, Theodore M. Webster, and A. Stanley Culpepper	
Reference: Weed Science 2009 57:351–356	
Year: 2009	
Results and conclusion: Data indicated that glyphosate dissipation was rapid following irrigation. Glyphosate DT_{50} was 1 h in the irrigated study, but 84 and 32 h for the dry scenario, respectively. This indicated that glyphosate could be removed from LDPE mulch with rainfall or irrigation, primarily due to their high water solubility.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in mulch
Protocol	Non-GLP study
Test compound	Roundup WeathermaxTM, glyphosate, EPA Reg. No. 524-537, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167. 23 P. (CAS 1071-83-6) unlabelled
Test system and conditions	Field studies conducted in Ty, GA to evaluate the dissipation of herbicides from LDPE mulch. The dry experiments did not receive irrigation or rainfall. For the irrigated experiments, samples were collected at 1 HAT, irrigated at 3 HAT with 1 cm of water using an overhead irrigation system, then sampled at 5 HAT. This washing and sampling procedure was then repeated at 24, 48, 72, and 96 HAT. Irrigation water pH samples were periodically collected and ranged from 7.0 to 8.1.
Statistical design	Four Three replicates
Relevance	
Environmental relevance	The environmental relevance is limited since mulch was tested instead of soil.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard laboratory testing.

Laitinen et al. (2006)

Title: Fate of the herbicides glyphosate, glufosinate-ammonium, phenmedipham, ethofumesate and metamitron in two Finnish arable soils
Author: Pirkko Laitinen, Katri Siimes, Liisa Eronen, Sari Rämo, Leena Welling, Seija Oinonen, Leona Mattsoff and Marja Ruohonen-Lehto
Reference: Pest Manag Sci 62: 473–491
Year: 2006
Results and conclusion: Glyphosate had been used in the sandy loam site the previous autumn and about one-third of the applied dose was still detected in the following May. Our results from the field study also show clear overwinter persistence for glyphosate. About 10–20 % of applied glyphosate was detected in the subsequent June in both field sites, demonstrating that the time for 90 % (DT_{90}) dissipation of glyphosate in our study was about 11 months. Glyphosate had been used in the clay soil site 1.5 years prior to our study. This gave no background signal, corresponding to less than 10 % of the applied amount of glyphosate. However, AMPA was detected in both background samples and at the end of our field study, indicating that it is more persistent than glyphosate
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.

Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil under Finish conditions
Protocol	Non-GLP study
Test compound	CAS 40465-66-5 Glufosinate-ammonium CAS 1071-83-6 Glyphosate
Test system and conditions	Field trials were carried out for 26 months starting in May 1999 at two different geographical sites in southern Finland. The soil in Perni was a clay soil according to FAO texture classes, while the soil in Janakkala was a sandy loam soil. Both fields were intensively drained (at 1m depth, tiles about 10m apart). Air temperature and cumulative precipitation were recorded at both geographical sites during the growing seasons. Relative soil moisture was measured at three depths (8, 25 and 40 cm). At both geographical sites, two of the four 200m^2 plots were sown with glufosinate-ammonium-resistant sugar beet. Soil samples were taken from the tillage layer (0–28 cm) and subsoil (28–50 and 50–70 cm).
Statistical design	Eight to ten samples were collected into a single jar for each analysis.
Relevance	
Environmental relevance	The conditions in Finland do not reflect the conditions for standard degradation experiments and normalisation to standard conditions was not performed. However, the results are nevertheless of relevance as they describe glyphosate degradation over a cold winter period.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard tests.

Lane et al. (2011)

Title: Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate
Author: Matthew Lane, Nicola Lorenz, Jyotisna Saxena, Cliff Ramsier, Richard P. Dick
Reference: Pedobiologia-International Journal of Soil Biology (2010), doi:10.1016/j.pedobi.2011.12.005
Year: 2011
Results and conclusion: Glyphosate caused a significant decrease in the total microbial biomass in 46 soybean rhizosphere soil that had no previous exposure to glyphosate, at 7 days after glyphosate application. However, no significant changes were observed in the overall microbial community structure. In conclusion, the glyphosate application lowered the total microbial biomass in the 49 GR soybean rhizosphere soil that had no previous exposure to glyphosate, at 7 days after glyphosate application; caused no changes in the microbial community structure; and did not reduce the plant available K (soil exchangeable or plant tissue).
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary. Furthermore, the experimental site was outside the EU.
Type of information (critical, high/low weight, supporting, additional): Additional information
Reliability
Endpoint: Microbial activity and K dynamics after glyphosate treatment
Protocol: Non-GLP study
Test compound: Unlabelled glyphosate, CAS 1071-83-6

Test system and conditions	Field study and Greenhouse-experiment: Two soil with similar physical and chemical characteristics, yet different levels of previous exposure to glyphosate were used in this study. Both sites were within an eleven kilometre radius in eastern Delaware County, Ohio. The climate is characterized in winter with an average temperature of – 2.8 °C and an average daily minimum temperature is -7.8 °C. In summer, the average 136 temperature is 21.1 °C and the average daily maximum temperature is 27.8 °C. The total annual precipitation is 941 mm. Of this, 536 mm, or about 58 percent, usually falls in April through September. Glyphosate was applied up to three times a year while growing soybeans, and once a year while cultivating corn, for an average of two yearly glyphosate applications.
Statistical design	No information
Relevance	
Environmental relevance	The results are of minor relevance since the experiments do not describe any of the standard endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Newton et al. (2008)

Title: Dissipation of four forest-use herbicides at high latitudes	
Author: Mike Newton & Elizabeth C. Cole & Ian J. Tinsley	
Reference: Environ Sci Pollut Res DOI 10.1007/s11356-008-0039-7	
Year: 2008	
Results and conclusion: Dissipation rates did not follow first-order rates because freezing conditions slowed most microbial activity. All products dissipated to close to or below detection limits within the time of the study.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary. Furthermore, the experimental site is an extreme site outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation in soil under extremely cold conditions
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate CAS 1071-83-6
Test system and conditions	Test plots were in upland and river bottom sites at 65°N and 58°N latitudes. The northern site has extremely cold winters, with soils that freeze to a depth of 1-2 m, and precipitation of 275 mm/year. The southern site has heavy rain and snowfall, amounting to 2,250 mm/year evenly distributed. Soil seldom freezes deeply. On each test plot, glyphosate was applied at twice the normal operational use rate to facilitate detection.
Statistical design	No information
Relevance	
Environmental relevance	The results cannot be used because the climatic conditions extreme and not comparable to the standard degradation studies. Also no normalisation to standard conditions was done.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Siimes et al. (2006)

Title: Comparison of the behaviour of three herbicides in a field experiment under bare soil conditions	
Author: K. Siimes, S. Rämö b, L. Welling, U. Nikunen, P. Laitinen	
Reference: agricultural water management 84 (2006) 53 – 64	
Year: 2006	
Results and conclusion: Field study: Herbicides were detected mainly in the topsoil (0-3 cm). The field dissipation half-life time of glufosinate-ammoniumin the topsoil was about 1 week, whereas that of ethofumesate was over 10 weeks. Glyphosate analyses from soil media failed because organic fertilizer caused similar peaks in chromatography.	
Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional):	
Supporting information	
Reliability	
Endpoint	Degradation of Glyphosate and glufosinate-ammonium under bare soil conditions
Protocol	Non-GLP study
Test compound	Non-labelled compound: glyphosate (CAS 1071-83-6), glufosinate-ammonium (CAS 40465-66-5)
Test system and conditions	The experiment was carried out on a leaching field plot (width 51 m, mean length 70 m) located in Toholampi in Finland. After 3 years of barley and 1 year of potato cultivation, no crop was cultivated on the plot during the study. Ethofumesate, glyphosate or glufosinate-ammonium had never been used before on the study plot. The three herbicides, as commercial herbicide products, were sprayed on bare soil on 8th July 1999 using the maximum recommended rates for single application
Statistical design	No replicates
Relevance	
Environmental relevance	The results are of relevance, but due cannot be directly used as they were not analysed considering FOCUS deg kinetics.
Weight of evidence	
"Positive"/"Negative" evidence	The results for glufosinate ammonium are in line with reliable studies.

Torstensson et al. (2005)

Title: Efficacy and fate of glyphosate on Swedish railway embankments
Author: Lennart Torstensson, Elisabet Börjesson and John Stenström
Reference: Pest Manag Sci 61:881–886
Year: 2005
Results and conclusion: The persistence of glyphosate in the embankments investigated was studied for two application rates of RoundUp Bio (3 and 5 litre/ha). 50 % disappearance times (DT_{50}) for the different rates of glyphosate during the years of investigation were 3 ±1 month. The appearance of glyphosate and its metabolite AMPA [(aminomethyl)phosphonic acid] in the embankment, e.g. mobility and persistence, was also studied. Mobility was low in most cases, the main proportion of both glyphosate and AMPA being found in the upper 30-cm layer although minor amounts penetrated to lower depths. The 50 % disappearance time of glyphosate was generally <5months in railway embankments but cases with longer persistence were found. Transport to the groundwater was observed for glyphosate and AMPA in groundwater pipes along tracks. Downward transport appears to be dependent on the application rate, which should not exceed 3 litre/ha of RoundUp Bio to avoid groundwater contamination. A lower rate of glyphosate mixed with a low rate of another herbicide may achieve acceptable weed control and be environmentally safer.

Proposed action: Consider as supporting information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6), Non-labelled AMPA (CAS 1066-51-9)
Test system and conditions	The railway embankments used in this investigation are located across Sweden. Most of the embankments are constructed of gravel and coarse and finer sand. For studies of the presence of glyphosate and AMPA in the embankment, samples were taken from randomly chosen areas (25 × 40 cm^2) within the experimental plot, in one of the four replicates. The uppermost layer (0–10 cm) was sampled by cutting a sample from an area 9 cm × 9 cm and 10 cm depth. After that, the whole 10-cm layer within the sample area (25 × 40 cm^2) was removed. The procedure was repeated for each of the remaining layers to be sampled.
Statistical design	Four replicates
Relevance	
Environmental relevance	There is relevance for this specific type of application in railway tracks. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

Vinther et al. (2008)

Title: Field-Scale Variation in Microbial Activity and Soil Properties in Relation to Mineralization and Sorption of Pesticides in a Sandy Soil	
Author: F. P. Vinther, U. C. Brinch, L. Elsgaard L. Fredslund B.V. Iversen and S. Torp C.S. Jacobsen	
Reference: J. Environ. Qual. 37:1710–1718	
Year: 2008	
Results and conclusion: As for the soil properties and the microbial parameters, the pesticide parameters exhibited a considerable higher variation in the Bs horizon than in the Ap horizon. The mineralization was quite low for all three pesticides (0.8 to 12.8 %). The use of contour maps along with descriptive statistics, including CVs, may give a good impression of the spatial variation and distribution within a field. The results of this study indicate that spatial variation of soil properties, and in particular the content of soil organic C, has a major influence on the spatial variability of microbial parameters and parameters elated to glyphosate degradation and sorption in the soil. The local-scale variations within 100 m^2 areas were two to three times lower than the field-scale variation within the entire field of about 4 ha.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Labelled: (N-(phosphonomethyl)-glycine ^{14}C labelled at the P-methylene end to a specific activity of 172.1 μCi mg^{-1} (CAS 1071-83-6)

Test system and conditions	The study site was located in the northern part of Jutland (52°27′ E, 43°21′ N) on the Yoldia plains, composed mainly by deposits from the Yoldia. The soil texture (i.e., clay [<2 µm], silt [2–63 µm], and sand [63–500 µm]) was measured in the 102 individual soil samples. The moisture content was adjusted to 90 % of the soil water-holding capacity. The microcosms were incubated at 10 °C
Statistical design	Four replicates
Relevance	
Environmental relevance	There is relevance for this analysis. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

Weaver et al. (2007)

Title: Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil	
Author: Mark A Weaver, L Jason Krutz, Robert M Zablotowicz and Krishna N Reddy	
Reference: Pest Manag Sci 63:388–393 (2007)	
Year: 2007	
Results and conclusion: Field study: Following the second in-season glyphosate application, the microbial community was not clearly separated by glyphosate treatment. Lab study: mineralization of glyphosate followed first-order kinetics, and estimates for parameters *a* and *k* were significantly different between treatments. After incubation for 42 days, 32–37 % of the applied glyphosate was mineralized when applied at threefold field rates, with about 9 % forming bound residues. These results indicate that glyphosate has only small and transient effects on the soil microbial community, even when applied at greater than field rates.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled: Field study: Glyphosate-isopropylammonium (Roundup Ultra) (CAS 38641-94-0) Lab study: glyphosate (98 % purity; Chem Service) and ^{14}C-labeled glyphosate (54 mCi mmol^{-1} specific activity, 99 % radiolabelled purity; Amersham Life Sciences) (CAS 1071-83-6)
Test system and conditions	Field study: Glyphosate-resistant soybean (AG 4702RR) was planted in Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) on the Southern Weed Science Research farm in Stoneville, MS (USA). Glyphosate-isopropylammonium (Roundup Ultra) was applied at 4 weeks after Non-glyphosate treated plots were included as a control. Bulk surface soil (0–2.5 cm) was collected at time of planting, before initial glyphosate application and 14 days after each glyphosate application. Mineralization of ^{14}C-glyphosate was evaluated.
Statistical design	Four replicates
Relevance	
Environmental relevance	There is relevance for this analysis. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

Wang et al. (2010)

Title: Residue and field decline study of glyphosate-ammonium in ramie field	
Author: Wang Yan-hui (wangyh1984@163.com); Li Xin; Zhou Xiao-mao (zhouxm1972@126.com); Bai Lian-yang; Cai Hai-lin	
Reference: Nongyaoxue Xuebao Volume: 12 Issue: 2 Pages: 201-206 Published: JUN 2010	
Year: 2010	
Results and conclusion: A simple, sensitive and selective method using gas chromatography equipped with flame photometric detector (GC-FPD) was developed to determine residues of glyphosate-ammonium in soil and ramie root. Soil samples were extracted with 0.01 mol/L sodium hydroxide and other samples were extracted with water and acetone. Glyphosate was previously derived with trimethylorthoacetate (TMOA) in the presence of acetic acid. Combination of AG1-X8 anion exchange chromatography with Florisil cartridge cleanup process was favourable for the GCPFPD analysis. The recovery ranged from 73.6 % to 102.6 % and 85.9 % to 105.1 % with the relative standard deviations of 2.3 % to 8.1 % and 5.4 % to 13.0 %, respectively. The limit of detection (LOD) of the method was 0.5 x 10(-10) g. The limit of quantification (LOQ) was 0.05 mg/kg. The half-life of glyphosate-ammonium was 1.6-2.6 d, 1.0-1.8 d and 1.1-1.5 d in soil of Hunan, Guangxi and Fujian Province at two years, respectively. No glyphosate-ammonium residues were detected in ramie and soil samples at treatments of 2 250 3 375 g (a.i.) /ha at harvest season (60 days after the treatment).	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary. Furthermore, the experimental site is outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Analysis of glyphosate ammonium residues in soil
Protocol	Non-GLP study
Test compound	Non-labelled: glyphosate-ammonium
Test system and conditions	Only the abstract given
Statistical design	No information given
Relevance	
Environmental relevance	The authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be directly compared with standard studies.

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Vinther F.P. *et al.* 2008. Field-Scale Variation in Microbial Activity and Soil Properties in Relation to Mineralization and Sorption of Pesticides in a Sandy Soil. J. Environ. Qual. 37:1710–1718

Weaver M. *et al.* 2007. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. Pest Manag Sci 63:388–393

Wang *et al.* 2010. Residue and field decline study of glyphosate-ammonium in ramie field. Nongyaoxue Xuebao Volume: 12 Issue: 2 Pages: 201-206

Detailed description of open literature – Adsorption and desorption of the active substance and all relevant metabolites

Accinelli et al. (2006)

Title: Influence of Cry1Ac Toxin on Mineralization and Bioavailability of Glyphosate in Soil	
Author: CESARE ACCINELLI, WILLIAM C. KOSKINEN, AND MICHAEL J. SADOWSKY	
Reference: J. Agric. Food Chem. 2006, 54, 164-169	
Year: 2006	
Results and conclusion: Results from laboratory investigations indicate that soil incorporation of purified Cry1Ac toxin in the range of 0.25-1.0 µg g^{-1} does not influence glyphosate mineralization or its sorption in soil. These results are in contrast to results obtained in previous investigations done using a mixture of Cry toxins at a concentration of 10 µg g^{-1} (21). The concentration of Cry toxins in soil occurring during the growing season has been estimated not to exceed 1 µg g^{-1}, based on the average concentrations of Cry toxin in crop residues incorporated into the top soil or left at the soil surface (22). On the basis of these estimates and the results obtained here, the data indicate that concentrations of Cry1Ac comparable to those encountered under field conditions do not have the potential to increase persistence and sorption of glyphosate in soil. Following K_{foc}-values were determined for glyphosate: sandy loam (Italy): 6230 L/kg and 6408 L/kg (US soil).	
Proposed action: Consider as additional information. No standard test is performed but effects of Cry1Ac Toxin on the sorption behaviour of Glyphosate are reported. Though interpretation given in the publication is plausible, it needs further in depth investigations to assess the relevance for the endpoint mobility.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	High
Endpoint	$DegT_{50}$ in soil and sorption on soil
Protocol	Non-GLP, standard degradation study, modified OECD Guideline 307 and 106
Test compound	Unlabelled glyphosate (chemical purity > 98 %), ^{14}Clabeled glyphosate (N-phosphonomethyl-2-^{14}C-glycine; radiopurity > 99 %, specific activity: 1.18 106 MBq g^{-1}), CAS-no.: 1071-83-6

Test system and conditions	Two soils with different physicochemical properties, taken from areas of the Po Valley (Italy) and of south central Minnesota, were selected for this study. The Italian soil (IT, 0.7 % OC) and the American soil (MN, 0.94 % OC) were both classified as sandy loam At both locations; the soil was collected from fields that had not received glyphosate applications within the previous 5 years. A portion of the IT and MN soils was mixed with Cry1Ac toxin powder to obtain a final concentration of 100 µg g^{-1} soil. Aliquots of these two amended soils were mixed with a sufficient mass of IT and MN soils to obtain final soil concentrations of 0.25, 0.5, and 1.0 µg Cry1Ac toxin g^{-1} soil (air-dried basis). The soil moisture in treated soil samples was adjusted to the gravimetric content at -33 kPa using distilled water and incubated in the dark at 25 °C. Isotherms for sorption of glyphosate to IT and MN soils containing different Cry1Ac toxin concentrations were determined using the batch equilibrium method 20 °C for 14 h.
Statistical design	Three replicates were prepared for each soil type and toxin concentration, and controls consisted of soils with no toxin addition.
Relevance	
Environmental relevance	The sorption studies are in principle performed considering the current guidance documents and can be considered for the calculation of sorption parameters. The degradation studies are not documented well enough to be considered further.
Weight of evidence	
"Positive"/"Negative" evidence	Partly positive evidence.

Accinelli et al. (2005)

Title: Effects of Incorporated Corn Residues on Glyphosate Mineralization and Sorption in Soil
Author: CESARE ACCINELLI, WILLIAM C. KOSKINEN, JEFFREY D. SEEBINGER, ALBERTO VICARI, AND MICHAEL J. SADOWSKY
Reference: J. Agric. Food Chem. 2005, 53, 4110-4117
Year: 2005
Results and conclusion: Addition of corn residues did not change the relative differences in the sorptive capacities between two soils, regardless of the incorporated amount. There were no differences between corn residues from the two isolines. Incorporation of low amount of corn residues did not affect sorption of [^{14}C]glyphosate to the soils. In contrast, incorporation of the highest corn residue amount reduced the sorption capacities of both soils for glyphosate. The observed decrease of herbicide sorption on soil mixed with the highest level of corn residue is possibly due to coverage of the soil sorptive sites by the corn residues, and not due to a reduction of the sorptive capacity of the soil per se. It also was observed that the binding of glyphosate and its degradation products to corn residue increased over the incubation time. This might be attributed to decomposition of the wheat residues associated with a decline in cellulose concentration and an enrichment of lignin More detailed information on the effect of weathering on the sorptive properties of corn residues to glyphosate is needed.
Proposed action: Consider as additional information. No standard test is performed but effects of incorporated corn on the sorption behaviour of Glyphosate are reported. Though interpretation given in the publication is plausible, it needs further in depth investigations to assess the relevance for the endpoint mobility.
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing. Though interpretation given in the publication is plausible, it needs further in depth investigations to assess the relevance for the endpoint mobility.

Reliability	
Endpoint	K_f-values, 1/n, r^2
Protocol	Modified standard (OECD 106), corn added to soil followed by sorption study according to OECD 106. Non-GLP
Test compound	Test compound: Glyphosate (unlabeled, chemical purity > 98 %) and [^{14}C]-labelled glyphosate (*N*-phosphonomethyl-2-^{14}C-glycine; radio purity > 99 %, specific activity 1.18·106 MBq g^{-1}), CAS-no.: 1071-83-6
Test system and conditions	Corn residues of two different hybrids were incorporated into a sandy and sandy loam soil. Concentrations of corn were from 0.5 to 8 %.
Statistical design	Triplicates, two soils, two corn hybrids, isotherm-calculation Freundlich-equation used.
Relevance	
Environmental relevance	Environmental relevance is given: Conservation tillage systems are characterized by a significant presence of crop residues at the soil surface so that glyphosate is applied to a soil matrix rich in poorly decomposed crop residues as corn might be left.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with such a design not known. No negative evidence.

Albers et al. (2009)

Title: The influence of organic matter on sorption and fate of glyphosate in soil – Comparing different soils and humic substances
Author: Christian N. Albers, Gary T. Banta, Poul Erik Hansen, Ole S. Jacobsen
Reference: Environmental Pollution 157, 2865–2870
Year: 2009
Results and conclusion: Three approaches were followed to investigate the dynamics between glyphosate and soil organic matter. 1) Sorption studies with seven purified soil humic fractions showed that these could sorb glyphosate and that the aromatic content, possibly phenolic groups, seems to aid the sorption. 2) Sorption studies with six whole soils and with SOM removed showed that several soil parameters including SOM are responsible for the strong sorption of glyphosate in soils. 3) After an 80 day fate experiment, appr. 40 % of the added glyphosate was associated with the humic and fulvic acid fractions in the sandy soils, while this was the case for only appr. 10 % of the added glyphosate in the clayey soils. Glyphosate sorbed to humic substances in the natural soils seemed to be easier desorbed than glyphosate sorbed to amorphous Fe/Al-oxides. Detailed results are:

Soil characterisation:

Soil	Soil type	Sand (%) Fine	Medium	coarse	Clay+silt (< 0.063mm)	(%)
SIA	Clay soil	26	19	7	47	
SIB	Clay soil	43	21	5	32	
WZ	Dark clayey zone soil	28	16	3	54	
FIA	Sandy soil	16	35	12	6	
FIB	Sandy soil	11	74	12	4	
FIC	Sandy soil	22	74	4	0.2	

Adsorption

Soil type	SOM %	pH (H_2O)	Kd	Koc	Kf	Kfoc	1/n
SIA	2.8	7.0			22[1)]		
SIB	1.7	7.5			29[1)]		
WZ	5.2	6.3			87[1)]		
FIA	4.5	6.6			28[1)]		
FIB	1.6	6.5			28[1)]		
FIC	0.2	6.6			24[1)]		

[1)] read from figure in the publication; no data in table or other raw data available

Desorption

Soil type	OC (%)	pH ($CaCl_2$)	Desorption K_d	K_{oc}	K_f	K_{foc}	1/n
					39[1)]		
					82[1)]		
					119[1)]		
					26[1)]		
					36[1)]		
					42[1)]		

[1)] read from figure in the publication; no data in table or other raw data available

Proposed action:
Not to be considered for the endpoint sorption and mobility as raw data are not reported. Results are presented as figures only but are not reported as tabular numbers

Type of information (critical, high/low weight, supporting, additional):
High weight, supporting information to already existing.

Reliability	High
Endpoint	K_f-values, 1/n, R^2
Protocol	Modified standard (OECD 106), sorption to soils and soil organic matter Non-GLP.
Test compound	Glyphosate (purity >99 %), ^{14}C-glyphosate (purity >99 %), CAS-no.: 1071-83-6
Test system and conditions	Sorption studies with soils and soil organic matter, adsorption isotherms, substance 3 concentrations each, 70 h. single measurements; mineralisation determined for 80 days; 5 sampling points.
Statistical design	Single measurements, Freundlich isotherms
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative"	Partly positive evidence.

Alexa et al. (2008)

Title: Researches regarding extractable glyphosate residues from different soils	
Author: Ersilia ALEXA, Aurel LAZUREANU, Simion ALDA, Monica NEGREA and Olimpia IORDANESCU	
Reference: Comm. Appl. BioI. Sci, Ghent University. 73/4, 2008	
Year: 2008	
Results and conclusion: The extractable glyphosate residues from soil solution have been determined analytically (HPLC-FlD). Substrates used were Black Chemozem, Typical Gteysoil, Slight Vertisol, with moderate carbonatation. The glyphosate adsorption on the 3 soils is high (>80 %), depends on their physico-chemical characteristics and it increases: Gleysol, Black Chernozem, Vertisol. The analyzed soils are characterized through a high content of clay (36,5-41,8 %) and humus (3,35-4,09 %) which enhances the adsorption capacity of the glyphosate on soil particles, the glyphosate forming stable complexes with clays, which immobilize the active substance deactivating it. The quantity of extractable residues from the soil is low (<20 %), depends on the characteristics of the soil and decreases as follows: Gleysoil, Black Chernozem, Slight Vertisol. The glyphosate leaching capacity in the soil is reduced because of the intense adsorption of the herbicide molecules in the surface horizon (0-10 cm).	
Proposed action: Consider as additional information. Data quality and quantity are not sufficient, and raw data are not comprehensively reported. Thus, their use for endpoint and PEC-assessment is not possible.	
Type of information (critical, high/low weight, supporting, additional): Critical, no additional information to already existing.	
Reliability	Medium
Endpoint	Extractable amount of Glyphosate after several times of application.
Protocol	No standard test design, non-GLP
Test compound	Glyphosate, purity not given, CAS-no.: 1071-83-6
Test system and conditions	Active ingredient added to soils in concentration according to GAP. Incubation for 1, 3, 7, 14 and 21 days in air tight vessels. Extraction, determination of a.i. concentration in the extract
Statistical design	Not given in the paper, no Freundlich equation, no further information
Relevance	
Environmental relevance	Test substance representative and relevant; parameters influencing the endpoint have been considered adequately.
Weight of evidence	
"Positive"/"Negative" evidence	Minor positive evidence, no negative evidence.

Al-Rajab et al. (2008)

Title: Sorption and leaching of ^{14}C-glyphosate in agricultural soils

Author: Abdul Jabbar Al-Rajab, Samira Amellal, Michel Schiavon

Reference: Agron. Sustain. Dev. 28, 419–428

Year: 2008

Results and conclusion:
Aim: to assess the dynamic interactions between glyphosate sorption and leaching; and to identify the main factors that influence the two processes in three undisturbed agricultural soils using microlysimeters under outdoor conditions.
OECD 106: Glyphosate was strongly adsorbed, yielding empirical constants of Freundlich sorption isotherms (K_f) of 16.6 for the clay loam soil, 33.6 for the silt clay loam soil and 34.5 for the sandy loam soil, with nf close to 1 in all three cases. Glyphosate was also weakly desorbed, i.e. 5 to 24 % (w) of initially sorbed glyphosate. Sorption and desorption were only pH-dependent.
Outdoor microlysimeter: nearly 70 % of the initial glyphosate was present in the soil in a non-extractable form at the beginning of the experiment. Conversely, only less than 20 % of the initial glyphosate is present in the soil in a non-extractable form after 11 months. These findings suggest that the non-extractable residues become available and take part in biodegradation and leaching. The amounts of ^{14}C-glyphosate derivatives leached were less than 0.28 % of the initially applied glyphosate. AMPA metabolite generally represented up to 100 % of the residues present in the leachates. The results of leaching were highly influenced by the hydrodynamic properties and the biodegradation capacities of the soils.
Detailed results are:

Soil characterisation:

Soil	Soil type	Sand (%)	Clay (%)	C.E.C (in meq/100g)
A	Sandy loam		10.5	
B	Silt clay loam		30.6	
C	Clay loam		34.9	

Adsorption

Soil type	OC %	pH (H_2O)	K_d	K_{OC}	K_f	K_{foc}	1/n
A	0.82	5.1			16.6		0.9995
B	1.45	6.3			33.6		1.004
C	1.91	7.9			34.5		0.97

Desorption

Soil type	OC %	pH (H_2O)	Desorption				
			% desorbed	K_{OC}	K_f	K_{foc}	1/n
A	0.82	5.1	6.58; 7.37[1)]				
B	1.45	6.3	5.13; 6.91[2)]				
C	1.91	7.9	21.52; 24.33[3)]				

[1)] first number: % desorbed at initially sorbed Glyphosate concentration of 3.18 µg/g; second number: % desorbed at initially sorbed Glyphosate concentration of 131.7 µg/g

[2)] first number: % desorbed at initially sorbed Glyphosate concentration of 3.18 µg/g; second number: % desorbed at initially sorbed Glyphosate concentration of 131.7 µg/g

[3)] first number: % desorbed at initially sorbed Glyphosate concentration of 2.86 µg/g; second number: % desorbed at initially sorbed Glyphosate concentration of 115.0 µg/g

Proposed action: Not to be considered for the endpoint sorption and mobility. Raw data on mass balances and test item concentrations in the aqueous and solid phases are not reported. Though the study is plausible, the validity cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information	
Reliability	High
Endpoint	OECD 106: K_f-values, 1/n, R^2, % desorbed; Outdoor microlysimeter: amount in leachate and distribution in soil over time (11 months).
Protocol	Standard (OECD 106), non-GLP; outdoor lysimeter studies, non-GLP
Test compound	OECD 106: [Phosphonomethyl-^{14}C]-glyphosate (purity: 99 %); non-radioactive glyphosate (purity 98.5 %) Outdoor microlysimeter: [Phosphonomethyl-^{14}C]-glyphosate diluted in Roundup Express (isopropylamine salt) and water CAS-no.: 1071-83-6
Test system and conditions	OECD 106: 7 concentrations for isotherms, 3 soils; undisturbed outdoor microlysimeter (diameter: 10 cm, length: 25 cm): duration: 11 months, 3 soils, 7 sampling points, 21 lysimeter in total
Statistical design	OECD 106: triplicates, lysimeter: single lysimeter per sampling and soil. Stat Box computer software; Comparison of means by Newman-Keuls test at levels of 0.05, 0.01 and 0.001.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Autio et al. (2004)

Title: Adsorption of sugar beet herbicides to Finnish soils
Author: Sari Autio, Katri Siimes, Pirkko Laitinen, Sari Rämö, Seija Oinonen, Liisa Eronen
Reference: Chemosphere 55, 215–226
Year: 2004
Results and conclusion: Adsorption of glyphosate studied using the batch equilibrium method in 21 soil samples collected from different depths. None of the measured soil parameters could alone explain the adsorption mechanism of these five herbicides. The results can be used in model assessments of risk for leaching to groundwater resulting from weed control of sugar beet in Finland. Detailed results are:

Soil characterisation:

Soil	Soil type	Silt (%) (0.002-0.02 mm)	Clay (%) (<0.002 mm)	Soil type
Kokemäki (0-20 cm)	Silty loam	62	17	
Kokemäki (20-40 cm)	Silty loam	66	17	
Kotkanoja (0-20 cm)	Clay	15	46	
Kotkanoja (20-40 cm)	Clay	13	58	
Rehtijärvi (0-20 cm)	Sandy loam	8	13	
Rehtijärvi (20-40 cm)	Sandy loam	3	4	
Perniö (0-30 cm)	Clay	24	41	
Perniö (30-60 cm)	Clay	28	47	
Turenki (0-30 cm)	Sandy loam	15	4	
Turenki (30-60 cm)	Sandy loam	13	4	
Perniö (0-25 cm)	Clay	n.a.	41	
Perniö (25-50 cm)	Clay	n.a.	(>30 %)	
Turenki (0-20 cm)	Sandy loam	n.a.	21	
Turenki (20-45 cm)	Sandy loam	n.a.	8	
Perniö (0-30 cm)	Clay	24	41	
Turenki (0-30 cm)	Sandy loam	15	4	
Toholampi (0-25 cm)	Silt loam	16	5	
Toholampi (25-35 cm)	Silt loam	20	4	
Toholampi (35-60 cm)	Silt loam	30	8	
Jokioinen (0-30 cm)	Muddy clay	n.a.	57	
Jokioinen (0-30 cm)	Organic soil	13	79	

Adsorption

Soil type	OC %	pH ($CaCl_2$)	K_d	K_{OC}	K_f	K_{foc}	1/n
Kokemäki (0-20 cm)	2.42	5.4			-	-	-
Kokemäki (20-40 cm)	0.47	6.1			166	34926	0.97
Kotkanoja (0-20 cm)	2.88	5.8			55	1914	0.92
Kotkanoja (20-40 cm)	0.54	5.6			249	46436	0.91
Rehtijärvi (0-20 cm)	2.57	5.8			44	6039	0.90
Rehtijärvi (20-40 cm)	0.72	5.7			55	2139	1.00
Perniö (0-30 cm)	7.06	6.0			97	1374	1.03
Perniö (30-60 cm)	2.96	6.0			41	1370	1.02
Turenki (0-30 cm)	5.93	6.4			97	1643	0.85
Turenki (30-60 cm)	1.77	5.9			51	2900	0.86
Perniö (0-25 cm)	2.67	8.1			58	2193	0.93
Perniö (25-50 cm)	2.5	7.9			113	4500	0.87
Turenki (0-20 cm)	2.35	7.1			93	3946	0.90
Turenki (20-45 cm)	0.75	6.8			90	11986	0.86
Perniö (0-30 cm)	7.05	6.0			179	2544	1.26
Turenki (0-30 cm)	5.93	6.3			121	2045	0.98
Toholampi (0-25 cm)	7.90	5.4[1)]			159	2014	0.93
Toholampi (25-35 cm)	4.50	5.6[1)]			102	2273	1.05
Toholampi (35-60 cm)	1.30	5.4[1)]			37	2823	0.76

Jokioinen (0-30 cm)	12.60	6.9	84	664	0.91
Jokioinen (0-30 cm)	26.00	5.2	303	1165	1.14

[1)] pH-value (H_2O)

Proposed action:
Consider as additional information for the endpoint sorption and mobility. Raw data on mass balances and test item concentrations in the aqueous and solid phases are not reported. Furthermore, Freundlich isotherms are not presented for each soil but exemplary. Though the study is plausible, the validity cannot be proven.

Type of information (critical, high/low weight, supporting, additional):
High weight, supportive information to already existing.

Reliability	High
Endpoint	K_f, K_{foc}, 1/n,
Protocol	Standard (OECD 106, version from 1981; exception: water used instead of $CaCl_2$), Non-GLP
Test compound	Glyphosate (CAS no1071-83-6), unlabelled compound (purity not given)
Test system and conditions	21 soils tested, 2 test concentrations, batch equilibrium method, recoveries according the guideline requirements
Statistical design	Duplicates, Freundlich equation
Relevance	
Environmental relevance	Given. Environmental parameter measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Other reliable studies support the results. No negative evidence.

Barja and dos Santos Afonso (2005)

Title: Aminomethylphosphonic Acid and Glyphosate Adsorption onto Goethite: A Comparative Study

Author: B. C. BARJA AND M. DOS SANTOS AFONSO

Reference: Environ. Sci. Technol., 39, 585-592

Year: 2005

Results and conclusion:
The adsorption isotherms and surface coverage of AMPA and glyphosate in aqueous suspensions of goethite as a function of pH were measured. Adsorption isotherms were calculated using a nonlinear regression fitting program (Solver, Excel 5.0) to approximate a Langmuir shape. The Langmuir constant and maximum coverage of every system were reported. Values for maximum coverage were normalized with the area of the goethite for a better comparison.

Proposed action:
Consider as additional information as no standard protocol has been followed but basic research is published.

Type of information (critical, high/low weight, supporting, additional):
Low weight, may be additional information to already existing

Reliability	Low
Endpoint	Langmuir constant
Protocol	No standard, Non-GLP
Test compound	Glyphosate (99 %), CAS-no.: 1071-83-6, AMPA (99 %), CAS-no.: 1066-51-9
Test system and conditions	Suspensions of goethite brought to a fixed ionic strength and desired pH. Samples left to reach equilibrium for 24 h. A given number of microliters of 0.010M PMG or AMPA added to suspensions, pH readjusted until constant values were reached. Isotherms measured after 24 h.
Statistical design	No information on replicates, on tested concentrations to obtain isotherms.

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with comparable design not known. No negative evidence.

Benetoli et al. (2010)

Title: ADSORPTION OF GLYPHOSATE IN A FOREST SOIL: A STUDY USING MÖSSBAUER AND FT-IR SPECTROSCOPY	
Author: Luís Otávio de B. Benetoli, Henrique de Santana, Cristine E. A. Carneiro e Dimas A. M. Zaia, Ailton S. Ferreira e Andrea Paesano Jr., Cássia Thaïs B. V. Zaia	
Reference: Quim. Nova, Vol. 33, No. 4, 855-859	
Year: 2010	
Results and conclusion: The adsorption of glyphosate onto mineral particles of the forest reserve soil could be occurred through the interaction of the GPS carboxylic group with the metals in soil. Furthermore, glyphosate interacts with Fe^{3+} in soil solution.	
Proposed action:	
Consider as additional information as no standard protocol has been followed but basic research is published.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing	
Reliability	Medium
Endpoint	FT-IR data for adsorption of Glyphosate onto mineral particles of the soil
Protocol	Experimental setup partly similar to OECD 106, Non-GLP
Test compound	Glyphosate (analytical grade), CAS-no.: 1071-83-6
Test system and conditions	Study of adsorption of glyphosate onto soil mineral particles, using FT-IR and Mössbauer spectroscopy. Soil/KCl-solution, glyphosate added, shaken for 24 h, centrifuged; supernatant: voltammograms, solid: FT-IR and Mössbauer spectra.
Statistical design	2 soils tested, 4 replicates, no further information on statistics used.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with such a design not known. No negative evidence.

Borggaard (2011)

Title: Does Phosphate Affect Soil Sorption and Degradation of Glyphosate? – A Review	
Author: Ole K. BORGGAARD	
Reference: Trends Soil Sci Plant Nutr J, 2(1):16-27	
Year: 2011	

Results and conclusion: Although several factors may control transport of glyphosate (and AMPA) from the terrestrial to the aquatic environment, the similarity between glyphosate and phosphate in relation to sorption processes strongly indicates competition between the two species for sorption sites on soil solids. This may lead to glyphosate leaching in phosphate-rich soils, where sorption sites are occupied by phosphate provided the sorption mechanisms of the two sorbates are identical. On the other hand, while sorption may protect the herbicide against microbial degradation, soil solution glyphosate is bioavailable and can be biodegraded, i.e. blocking of sorption sites by phosphate may increase soil solution glyphosate, and hence degradation. In addition to this indirect effect, phosphate may directly interfere with the microbial glyphosate degradation. The review discusses the various processes.
Proposed action: Consider as additional information as the article does not present experimental data but is a review article. No raw data and/or experimental design are given.
Type of information (critical, high/low weight, supporting, additional): Low weight, as it is a review article no new experimental data are presented but cited and discussed.

Reliability	
Endpoint	Sorption, degradation
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Borggaard and Gimsing (2008)

Title: Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review
Author: Ole K Borggaard and Anne Louise Gimsing
Reference: Pest Manag Sci 64, 441–456
Year: 2008
Results and conclusion: The very wide use of glyphosate to control weeds in agricultural, silvicultural and urban areas throughout the world requires that special attention be paid to its possible transport from terrestrial to aquatic environments. The aim of this review is to present and discuss the state of knowledge on sorption, degradation and leachability of glyphosate in soils. Difficulties of drawing clear and unambiguous conclusions because of strong soil dependency and limited conclusive investigations are pointed out.
Proposed action: Consider as additional information as the article does not present experimental data but is a review article. No raw data and/or experimental design are given.
Type of information (critical, high/low weight, supporting, additional): Low weight. As it is a review article no data are published but cited from other publications and discussed. Additional information.

Reliability	
Endpoint	
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable

Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cáceres-Jensen et al. (2009)

Title: Adsorption of Glyphosate on Variable-Charge, Volcanic Ash–Derived Soils	
Author: L. Cáceres-Jensen, J. Gan, M. Báez, R. Fuentes and M. Escudey	
Reference: J. Environ. Qual. 38:1449–1457	
Year: 2009	
Results and conclusion: Three volcanic ash–derived soils were selected for Glyphosate adsorption studies. Glyphosate was rapidly and strongly adsorbed on the selected soils, and adsorption isotherms were well described by the Freundlich relationship with strong nonlinearity ($n_{fads} < 0.5$). The n_{fads} values were consistently higher than n_{fdes} values, suggesting strong hysteresis. Adsorption (K_{ads}) increased strongly when pH decreased. The presence of glyphosate changed the adsorption behaviour of phosphate at its maximum adsorption capacity. During the successive desorption steps, glyphosate at the highest level increased K_{ads} values for phosphate in the Andisol soils but had little effect in the Ultisol soil. This different behaviour was probably due to the irreversible occupation of some adsorption sites by glyphosate in the Ultisol soil attributed to the dominant Kaolinite mineral.	
Proposed action: To be considered as additional information as non-standard soils were used, but the influence of variable charge of the volcanic ash-derived soils on sorption were investigated.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information as non-European soils were investigated.	
Reliability	Medium
Endpoint	K_f, 1/n
Protocol	Similar to OECD 106, non-GLP
Test compound	Glyphosate, CAS-no.: 1071-83-6; no further information on purity
Test system and conditions	Batch equilibrium experiments, 24 h shaking, room temperature
Statistical design	7 concentrations, duplicate measurements, 2 soils, adsorption isotherms of phosphate were fitted to the Langmuir model
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Other reliable studies support the results, no negative evidence.

da Cruz et al. (2007)

Title: Adsorption of Glyphosate on Clays and Soils from Paraná State: Effect of pH and Competitive Adsorption of Phosphate
Author: Lútecia Hiera da Cruz, Henrique de Santana, Cássia Thaïs Bussamra Vieira Zaia and Dimas Augusto Morozin Zaia
Reference: BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY, Vol. 50 (3), pp. 385-394
Year: 2007

Results and conclusion: This work showed that the adsorption of glyphosate (GPS) depends on surface area for clays and amount of clays and CEC for soils. Organic matter (OM) had a secondary role in the adsorption of GPS on soils. The adsorption of GPS on soils from Londrina and Floraí counties and clays (montmorillonite, kaolinite) decreased when pH increased, however, for bentonite clay and soil from Tibagi county was kept constant. For the soils, the competitive adsorption between GPS and phosphate showed that displace of GPS by phosphate was related to the amount of clays, CEC and pH. GPS was not easily displaced by phosphate on the clays. The FT-IR spectra of the soils and clays showed that soil from Londrina resembled kaolinite. Thus, this could explain the results of adsorption of GPS and the competitive adsorption between GPS and phosphate.
Proposed action: Consider as additional information as the articles presents basic research. The OECD standard protocol is not followed in detail. No raw data are published, and thus the validity of the study cannot be proven.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.

Reliability	Low
Endpoint	Amount of Glyphosate (µmol) adsorbed to soil
Protocol	Similar to OECD 106, non-GLP but basic research on adsorption processes
Test compound	Glyphosate (95 %), CAS-no.: 1071-83-6
Test system and conditions	Small Eppendorf vials, small volumes tested, shaking for 24 h, ninhydrin-test for amount of absorbed Glyphosate, different pH-values; number of replicates not clearly reported.
Statistical design	The ANOVA test and Student-Newman-Keuls test (S-N-K test) were used for the comparisons between means at a significance level of $p<0.05$
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive relevance not clear, no negative relevance.

Damonte et al. (2007)

Title: Some aspects of the glyphosate adsorption on montmorillonite and its calcined form
Author: Marina Damonte, Rosa M. Torres Sánchez, María dos Santos Afonso
Reference: Applied Clay Science 36, 86–94
Year: 2007
Results and conclusion: Influences of pH and ionic strength on the aggregation of montmorillonite particles in the presence or absence of glyphosate (PMG) were studied. Adsorption isotherms and X-ray diffraction indicated that ligand exchange is the main mechanism of PMG adsorption. The surface coverage increased with the ionic strength and was more noticeable at high PMG concentration indicating inner-sphere surface complexation. At low PMG concentration the inner-sphere surface complexes are located of the external clay mineral surface while at high PMG concentration the surface complexes are also formed in the interlayer space.
Proposed action: Not to be considered as no data are published but isotherms are reported graphically only. Thus, the validity and quality of data cannot be proven.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, basic research on influences of environmental parameter on sorption processes

Reliability	Low
Endpoint	Sorption isotherms, no numbers given but graphical reporting
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (purity not given), CAS-no.: 1071-83-6

Test system and conditions	Sorption isotherms measured using batch method and under modification of ionic strength and pH-values.
Statistical design	Number of replicates, and of concentrations used for isotherm determination not reported
Relevance	
Environmental relevance	Environmental parameter modified during the project, thus environmental relevance given.
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

de Jonge et al. (2001)

Title: GLYPHOSATE SORPTION IN SOILS OF DIFFERENT PH AND PHOSPHORUS CONTENT

Author: H. de Jonge, L.W. de Jonge, O.H. Jacobsen, T. Yamaguchi, and P. Moldrup

Reference: Soil Science; 166, 230–238

Year: 2001

Results and conclusion:
This study quantifies the variation in glyphosate sorption and desorption to a coarse sandy soil and to a sandy loam soil with varying phosphorus content and pH. Using batch experiments, glyphosate adsorption and desorption isotherms were determined on soil samples taken from long-term field experiments that received different additions of phosphorus and lime. The isotherms were best fitted with an extended Freundlich model. The phosphate content in the soils had a significant influence on the sorption of glyphosate. With 0.5 M bicarbonate extractable P (pH 8.5) increasing from 6.2 to 58.7 in the loamy sand and 9.1 to 87.4 in the coarse sand, the extended Freundlich adsorption coefficient (K_f, MF, ads) decreased from
214.7 to 106 and from 154.0 to 83.5, respectively.
Detailed results are:
Soil characterisation:

Soil [1)]		Sand (%)	Clay (%)	C.E.C. (in meq/100g)
St. Jyndevad A	Coarse sand	89.6	4.2	
St. Jyndevad B	Coarse sand	89.6	4.2	
St. Jyndevad C	Coarse sand	89.6	4.2	
St. Jyndevad D	Coarse sand	89.6	4.2	
St. Jyndevad E	Coarse sand	89.6	4.2	
St. Jyndevad F	Coarse sand	89.6	4.2	
St. Jyndevad G	Coarse sand	89.6	4.2	
St. Jyndevad H	Coarse sand	89.6	4.2	
St. Jyndevad I	Coarse sand	89.6	4.2	
St. Jyndevad J	Coarse sand	89.6	4.2	
St. Jyndevad K	Coarse sand	89.6	4.2	
St. Jyndevad L	Coarse sand	89.6	4.2	
St. Jyndevad M	Coarse sand	89.6	4.2	
St. Jyndevad N	Coarse sand	89.6	4.2	
St. Jyndevad O	Coarse sand	89.6	4.2	
St. Jyndevad P	Coarse sand	89.6	4.2	
Askov A	Sandy loam	71.0	10.8	
Askov B	Sandy loam	71.0	10.8	
Askov C	Sandy loam	71.0	10.8	
Askov D	Sandy loam	71.0	10.8	
Askov E	Sandy loam	71.0	10.8	

Askov F	Sandy loam	71.0	10.8
Askov G	Sandy loam	71.0	10.8

[1)] Soils were from Danish long-term field experiments with varying phosphate additions. There differentiated herein as A, B, C, etc. Jynvard soil: 0 – 15.6 kg P /ha y; Askov soil: 0 – 57 kg P /ha y

Adsorption

Soil type	OC %	pH ($CaCl_2$)	K_d	K_{OC}	$K_{f\ MF\ ads}$	N_{ads}	D_{ads}
St. Jyndevad A	1.32	3.7			107.4	0.61	0.07
St. Jyndevad B	1.06	3.6			80.6	0.62	0.07
St. Jyndevad C	1.28	3.6			83.5	0.66	0.07
St. Jyndevad D	1.06	3.8			79.4	0.67	0.08
St. Jyndevad E	1.2	4.2			121.2	0.60	0.06
St. Jyndevad F	1.33	4.3			141.2	0.63	0.07
St. Jyndevad G	1.3	4.2			118.1	0.66	0.08
St. Jyndevad H	1.4	4.3			111.5	0.61	0.06
St. Jyndevad I	1.26	4.5			126.8	0.56	0.06
St. Jyndevad J	1.26	4.7			120.0	0.57	0.05
St. Jyndevad K	1.21	4.6			92.0	0.59	0.04
St. Jyndevad L	1.36	4.9			116.2	0.60	0.05
St. Jyndevad M	1.33	5.2			154.0	0.61	0.07
St. Jyndevad N	1.14	5.5			138.8	0.65	0.07
St. Jyndevad O	1.29	5.4			136.6	0.62	0.06
St. Jyndevad P	1.2	5.5			119.8	0.62	0.05
Askov A	1.28	6.2			214.7	0.55	0.06
Askov B	1.26	6.2			165.1	0.60	0.05
Askov C	1.25	6.3			137.6	0.67	0.07
Askov D	1.23	6.4			106.4	0.70	0.06
Askov E	1.21	6.3			171.7	0.57	0.05
Askov F	1.40	6.3			144.0	0.59	0.03
Askov G	1.44	6.3			151.3	0.65	0.08

Desorption

Soil type	OC %	pH	Desorption				
		($CaCl_2$)	K_d	K_{OC}	$K_{f.\ MF.\ des}$	N_{des}	D_{des}
St. Jyndevad A	1.32	3.7			364.9	0.17	0.06
St. Jyndevad B	1.06	3.6			246.7	0.28	0.12
St. Jyndevad C	1.28	3.6			312.0	0.18	0.06
St. Jyndevad D	1.06	3.8			286.3	0.21	0.09
St. Jyndevad E	1.2	4.2			415.1	0.17	0.08
St. Jyndevad F	1.33	4.3			458.0	0.18	0.09
St. Jyndevad G	1.3	4.2			404.5	0.20	0.09
St. Jyndevad H	1.4	4.3			367.6	0.22	0.10
St. Jyndevad I	1.26	4.5			367.8	0.21	0.10
St. Jyndevad J	1.26	4.7			361.1	0.23	0.10
St. Jyndevad K	1.21	4.6			290.3	0.25	0.10
St. Jyndevad L	1.36	4.9			355.0	0.23	0.10
St. Jyndevad M	1.33	5.2			436.7	0.23	0.09
St. Jyndevad N	1.14	5.5			418.3	0.24	0.09

St. Jyndevad O	1.29	5.4	409.2	0.27	0.10
St. Jyndevad P	1.2	5.5	317.1	0.27	0.10
Askov A	1.28	6.2	453.7	0.33	0.15
Askov B	1.26	6.2	394.0	0.25	0.03
Askov C	1.25	6.3	239.7	0.65	0.18
Askov D	1.23	6.4	240.6	0.52	0.13
Askov E	1.21	6.3	383.9	0.35	0.13
Askov F	1.40	6.3	362.0	0.34	0.11
Askov G	1.44	6.3	465.2	0.11	0.08

MF = modified Freundlich; N, D = shape-governing parameters

Proposed action:
To be considered as supportive information. Adsorption is not described according to OECD 106 but by an empirical modified Freundlich-model. Thus, results are not completely comparable to those obtained by following OECD 106.

Type of information (critical, high/low weight, supporting, additional):
Low weight, supporting information, basic research on sorption competitive processes

Reliability	High
Endpoint	K_f, 1/n, R^2
Protocol	Comparable to OECD 106, non-GLP
Test compound	Glyphosate (labelled and un-labelled, purity not reported), CAS-no.: 1071-83-6
Test system and conditions	Similar to OECD 106
Statistical design	Two soils, 5 concentrations, triplicate measurements, Langmuir-isotherms, Freundlich-isotherms, modified Freundlich-isotherms
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence as results are supported by similar publications; no negative evidence.

de Miranda Colombo and Masini (2011)

Title: Developing a fluorimetric sequential injection methodology to study adsorption/ desorption of glyphosate on soil and sediment samples

Author: Sandro de Miranda Colombo, Jorge C. Masini

Reference: Microchemical Journal 98, 260-266

Year: 2011

Results and conclusion:
This paper describes the development of a sequential injection method to automate the fluorimetric determination of glyphosate. The method was applied to study adsorption/desorption properties in a soil and in a sediment sample. Adsorption and desorption isotherms were properly fitted by Freundlich and Langmuir equations, leading to adsorption capacities of 1384 ± 26 and 295 ± 30 mg/kg for the soil and sediment samples, respectively. These values are consistent with the literature, with the larger adsorption capacity of the soil being explained by its larger content of clay minerals, while the sediment was predominantly sandy.

Proposed action:
To be considered as additional information as the study focused on the development of an analytical methodology but not on the standard testing of Glyphosate.

Type of information (critical, high/low weight, supporting, additional):
Low weight, supportive information because results fit into known data.

Reliability	Low

Endpoint	K_f, 1/n, R^2
Protocol	Similar to OECD 106, however, focus of the study is to develop an analytical methodology
Test compound	Glyphosate (no further information on purity), CAS-no.: 1071-83-6
Test system and conditions	1 soil, 1 sediment tested, 7 Glyphosate concentrations, duplicates
Statistical design	Freundlich equation applied
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, information supported by other reliable literature data.

de Santana et al. (2006)

Title: Effect in glyphosate adsorption on clays and soils heated and characterization by FT–IR spectroscopy	
Author: Henrique de Santana, Luís R.M. Toni, Luís O. de B. Benetoli, Cássia T.B.V. Zaia, Maurilio Rosa Jr., Dimas A.M. Zaia	
Reference: Geoderma 136(3-4): 738-750. doi:10.1016/j.geoderma.2006.05.012	
Year: 2006	
Results and conclusion: The main achievement of this paper is the determination of mineral structural changes in soils from three different sites of Paraná State, Brazil caused by heating and their effect on glyphosate (GPS) adsorption. Changes in soil structure due to heating probably play an important role in GPS adsorption. The non-adsorption of GPS on soil after burning could be a problem, as the unadsorbed GPS could either leach to groundwater or decrease the productivity of some crops.	
Proposed action: Consider as additional information as basic research is published. Data quality and quantity are not sufficient, and raw data are not comprehensively reported. Thus, their use for endpoint and PEC-assessment is not possible.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because basic research is reported, influence of environmental parameter on sorption processes given, no K_f-values reported.	
Reliability	Low
Endpoint	FT-IR-spectra
Protocol	Similar to OECD 106, non-GLP
Test compound	Glyphosate (analytical grade), CAS-no.: 1071-83-6
Test system and conditions	Similar to OECD 106, batch experiments at different temperatures (room temperature, 280 °C, 650 °C)
Statistical design	5 concentrations, single measurements only. ANOVA test and Student–Newman–Keuls test at a significance level of p =0.05 for comparison of results at different temperatures
Relevance	
Environmental relevance	Given as the influence of environmental parameter is measured.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Dideriksen and Stipp (2003)

Title: The adsorption of glyphosate and phosphate to goethite: A molecular-scale atomic force microscopy study	
Author: K. DIDERIKSEN and S. L. S. STIPP	
Reference: Geochimica et Cosmochimica Acta, Vol. 67, No. 18, pp. 3313–3327	
Year: 2003	
Results and conclusion: The adsorption of glyphosate and phosphate to the goethite {010} surface (Pbnm notation) was studied using an atomic force microscope (AFM). The microscope was capable of producing molecular scale images of surfaces exposed to glyphosate, phosphate and nitric acid. The relative maximum adsorption density of phosphate and glyphosate on the {010} surface expected from the AFM data was in agreement with that determined with X-ray photoelectron spectroscopy (XPS).	
Proposed action:	
Consider as additional information since no OECD standard protocol was followed but the article focuses on basic research.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on sorption processes, basic research	
Reliability	Medium
Endpoint	Sorption analysed using AFM
Protocol	No standard protocol, FT-IR-analysis of surface, no batch experiments, non-GLP
Test compound	Glyphosate (analytical grade), CAS-no.: 1071-83-6
Test system and conditions	No batch experiments but surface analysis using atomic force microscope.
Statistical design	Fourier transformation analysis
Relevance	
Environmental relevance	Given. The influence of environmental parameter was investigated.
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Dion et al. (2001)

Title: Competitive sorption between glyphosate and inorganic phosphate on clay minerals and low organic matter soils	
Author: H. M. Dion, J. B. Harsh, H. H. Hill Jr.	
Reference: Journal of Radioanalytical and Nuclear Chemistry, Vol. 249, No , 385–390	
Year: 2001	
Results and conclusion: Inorganic phosphate may influence the adsorption of glyphosate to soil surface sites. It has been postulated that glyphosate sorption is dominated by the phosphoric acid moiety; therefore, inorganic phosphate could compete with glyphosate for surface sorption sites. We examine sorption of glyphosate in low organic carbon systems where clay minerals dominate the available adsorption sites using ^{32}P-labeled phosphate and ^{14}C-labeled glyphosate to track sorption. We found glyphosate sorption strongly dependent on phosphate additions. Isotherms were generally of the L type, which is consistent with a limited number of surface sites. Most sorption on whole soils could be accounted for by sorption observed on model clays of the same mineral type as found in the soils.	
Proposed action: Consider as additional information since no OECD standard protocol was followed but the article focuses on basic research.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by inorganic phosphate	

Reliability	Medium
Endpoint	K-values obtained by Langmuir equation, 1/n
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Unlabeled and ^{14}C-labeled glyphosate (98.7 % purity), CAS-no.: 1071-83-6
Test system and conditions	Batch experiments, 3 soils and clay minerals
Statistical design	Langmuir-equation
Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Farenhorst et al. (2009)

Title: Variations in soil properties and herbicide sorption coefficients with depth in relation to PRZM (pesticide root zone model) calculations
Author: A. Farenhorst, D.A.R. McQueen, I. Saiyed, C. Hilderbrand, S. Li, D.A. Lobb, P. Messing, T.E. Schumacher, S.K. Papiernik, M.J. Lindstrom
Reference: http://dx.doi.org/10.1016/j.geoderma.2009.02.002
Year: 2009
Results and conclusion: Soil profiles were obtained from three landform elements in a strongly eroded agricultural field and segmented. Soil samples were analyzed for glyphosate sorption by soil (K_d, K_{OC}). Considering all soil profiles, glyphosate K_d values ranged from 19 to 547 L/kg and were predominantly controlled by variations in soil pH and clay content. PRZM predicted that glyphosate would be immobile in soils even under an extreme rainfall scenario of 384 mm at one day after herbicide application. PRZM output was particularly sensitive to input values of K_d, relative to input values of soil properties. We conclude that, when pesticide fate models such as PRZM are being used in policy analyses at larger-scales, data on K_d values in different landform elements and at the soil horizon level could be important for strengthening pesticide leaching predictions.
Proposed action: Consider as additional information since the publication focuses on a comparison of experimental and modelling results. Raw data on the experimental part are not sufficiently comprehensive for their use for endpoint and PEC-assessment.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information; PRZM-modelling results are additional as PRZM version 3.12.2 is not used in the EU for pesticide registration.

Reliability	Low for sorption; medium for modelling
Endpoint	K_d-values, PRZM-modelling output
Protocol	Non-GLP, batch experiments comparable to tier II OECD 106
Test compound	Glyphosate, purity not given, CAS-no.: 1071-83-6
Test system and conditions	Batch experiments similar to OECD 106, 24 h, room temperature
Statistical design	Duplicate measurements, soils from 3 landscapes, depth dependent soil sampling resulting in 90 individual samples. PRZM (pesticide root zone model, version 3.12.2)
Relevance	
Environmental relevance	Given; in depth analyses of subsoil layers.
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, is supported by other publications; no negative evidence.

Farenhorst et al. (2008)

Title: Herbicide Sorption Coefficients in Relation to Soil Properties and Terrain Attributes on a Cultivated Prairie	
Author: A. Farenhorst, S. K. Papiernik,I. Saiyed, P. Messing, and K. D. Stephens, J. A. Schumacher, D. A. Lobb and S. Li, M. J. Lindstrom, T. E. Schumacher	
Reference: J. Environ. Qual. 37:1201–1208	
Year: 2008	
Results and conclusion: The sorption of glyphosate in soil was quantified for 287 surface soils (0–15 cm) collected in a 10 × 10 m grid across a heavily eroded, undulating, calcareous prairie landscape. Other variables that were determined included soil carbonate content, soil pH, soil organic carbon content (SOC), soil texture, soil loss or gain by tillage and water erosion, and selected terrain attributes and landform segments. Regression equations were generated to estimate herbicide sorption in soils. The variation of glyphosate sorption across the field (upper slope: K_{OC}=11182; mid-slope: K_{OC}=14863; lower slope: K_{OC}=10891) was not much dependent on our measured soil properties and calculated terrain attributes. We conclude that the integration of terrain attributes or landform segments in pesticide fate modelling is of not much advantage for herbicides such as glyphosate that are strongly bound to soil regardless of soil properties.	
Proposed action: Not to be considered as soils outside the EU are used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on pesticide fate modelling; K_{OC}-values are in the range of those given in the dossier.	
Reliability	Medium
Endpoint	Pesticide leaching (by modelling); K_d-values
Protocol	Batch experiments comparable to tier II OECD 106
Test compound	Unlabeled Glyphosate (99 %) and ^{14}C-labeled Glyphosate (95 %); CAS-no.: 1071-83-6
Test system and conditions	Batch equilibrium analysis, 24 h, and room temperature.
Statistical design	287 individual samples; duplicate measurements per soil sample. Assessment of effects of landscape segments by SPSS version 13.0 (2004, SPSS Inc.), Sigma Stat version 2.03 (1992–1997, SPSS Inc.), or SAS version 8.01 (2000, SAS Inst.). Nonparametric Kruskal Wallis ANOVA.
Relevance	
Environmental relevance	Given; influencing endpoints analysed and considered adequately
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence; no negative evidence.

Ghafoor et al. (2012)

Title: Modelling pesticide sorption in the surface and subsurface soils of an agricultural catchment
Author: A. Ghafoor, N. J. Jarvis and J. Stenström
Reference: Published online in Wiley Online Library: 21 December 2012, (wileyonlinelibrary.com) DOI 10.1002/ps.3453
Year: 2012

Results and conclusion:
BACKGROUND: Sorption models that improve upon the koc concept are urgently needed for reliable spatial modelling of pesticide leaching. Sorption of glyphosate, bentazone and isoproturon was measured in surface and subsurface soils to test an 'extended' partitioning model that also accounts for inorganic sorbents and pH. Best-subset regression and Akaike information criteria were used to justify the inclusion of predictors and identify suitable models.
RESULTS: The extended partitioning model improved upon the k_{oc} concept for all three compounds: inorganic sorbents dominated sorption in subsurface soils, and their effects were only masked by organic matter in surface soils with organic carbon contents larger than ca 2 %. Interactions between organic and inorganic sorbents affected glyphosate sorption, but apparently not that of bentazone or isoproturon.
CONCLUSION: Information on clay, iron and aluminium oxides and soil pH, in addition to organic carbon, is needed for accurate prediction of pesticide leaching. The variables f_{oc}, f_{clay} and pH are generally available, whereas measurements of oxides of Al and Fe are rarely reported. The authors therefore emphasise the need to measure and report contents of oxides of Al and Fe in soil survey databases, because small variations in their concentrations may contribute significantly to large variations in sorption, especially of ionisable pesticides.

Proposed action:
To be considered as supportive information.

Type of information (critical, high/low weight, supporting, additional):
Supporting information, basic research on sorption

Reliability	
Endpoint	Freundlich sorption parameters, the variation in k_{oc}, relationships between soil physicochemical properties and pesticide sorption and modelling results
Protocol	Non-GLP
Test compound	Unlabelled isoproturon {N,N-dimethyl-N'-[4-(1-methylethyl)-phenyl]urea; 99 % purity}, bentazone [3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide; 97 % purity] and glyphosate [N-(phosphomethyl)glycine, 98 % purity] were used.
Test system and conditions	The study was carried out in the E21 monitoring catchment in Östergötland, southern Sweden. The total catchment area of 13 km^2 consists of 95 % agricultural land, with main crops of winter and spring sown cereals, rape, potatoes and peas. The soils, which are derived from glacial and post-glacial fluvial sediments and glacial till (moraine), have a wide range of texture, from loamy sand to clay. Soil samples were collected from 60 locations in the catchment (one location every 20 ha) on a grid pattern. Five soil samples from each location and depth were taken in the surface 0–20 cm, 20–45 cm and 45-70 cm, bulked, homogenised by passing through a 2 mm sieve, put into plastic bags and stored at 4° C until use. Sorption of the three test compounds was measured in topsoil samples at 16 of these locations, selected to cover the range of measured textures, organic matter contents and pH values. Sorption was also measured in samples taken from the two subsoil layers 20–45 and 45–70 cm at five of these 16 locations. Soil pH was measured on fresh samples after shaking the samples in deionised water (1:2.5) at room temperature. Particle size distributions were evaluated using the standard pipette method. Total organic C and N were measured using a Leco CN 2000 instrument (LECO Corp., St Joseph, MI). Ammonium lactate extractable phosphorus (PAL) was measured. Oxides of aluminium and iron (Alox and Feox) were determined in oxalate extracts of soils by ICS-AAS. Adsorption experiments were carried out according to the OECD 106 guideline. The sorption measurements were fitted to the Freundlich equation using non-linear regression (the nls procedure in the R software package).
Statistical design	Two replicates according to OECD 106 guideline, Freundlich equation using non-linear regression
Relevance	
Environmental relevance	Given

Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Gimsing and Borggaard (2002)

Title: Competitive adsorption and desorption of glyphosate and phosphate on clay silicates and oxides	
Author: A. L. GIMSING AND O. K. BORGGAARD	
Reference: Clay Minerals, 37, 509–515	
Year: 2002	
Results and conclusion: Competitive adsorption of glyphosate and phosphate on goethite and gibbsite and on illite, montmorillonite and two kaolinites differing in surface area was evaluated. The results show that glyphosate and phosphate are competing for the adsorption sites, but the degree of competition depends on the adsorbent. On goethite the competition is very much in favour of phosphate, on gibbsite the competition is closer, but still phosphate is favoured, while on illite, montmorillonite and kaolinite the competition is almost equal. The amounts of glyphosate and phosphate, which can be adsorbed also depends on the adsorbent: the oxides adsorb more than the clay silicates. The amount adsorbed on kaolinite was dependent on the specific surface area. Changes in the surface area did not affect the competition between glyphosate and phosphate for adsorption sites. The results indicate that differences among soils of different mineralogical composition regarding the adsorption of glyphosate and phosphate can be expected.	
Proposed action: Consider as additional information as data quality is low, no standard OECD protocol is followed and the validity of the study cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on sorption processes	
Reliability	Low
Endpoint	Amount of absorbed as function of time
Protocol	No OECD guideline followed; non-GLP
Test compound	Glyphosate purified from a glyphosate concentrate (purity?) and ^{14}C-Glyphosate (purity?); CAS-no.: 1071-83-6
Test system and conditions	Various minerals used to which Glyphosate was added, stirred over a certain time period, filtered, Glyphosate concentration in filtrate determined (sorption kinetics)
Statistical design	Experiments in triplicate
Relevance	
Environmental relevance	Given as the influence of environmental parameters on sorption were investigated.
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Gimsing and Borggaard (2002)

Title: EFFECT OF PHOSPHATE ON THE ADSORPTION OF GLYPHOSATE ON SOILS, CLAY MINERALS AND OXIDES
Author: ANNE LOUISE GIMSING and OLE K. BORGGAARD
Reference: Intern. J. Environ. Anal. Chem., Vol. 82, No. 8–9, pp. 545–552
Year: 2002

Results and conclusion: The effect of phosphate (ortho-phosphate) on the adsorption of the widely used glyphosate herbicide was evaluated with three typical Danish agricultural soils as well as pure oxides (goethite, FeOOH and gibbsite, $Al(OH)_3$ and silicates (illite and montmorillonite), which are considered the most important glyphosate and phosphate adsorbents in soils. All experiments showed competition between phosphate and glyphosate for adsorption sites but the various adsorbents exhibited great variation in affinity for glyphosate and phosphate. The current studies showed that the competition in soils is almost equal, but still phosphate affects the sorption of glyphosate in soil. The amount of glyphosate and phosphate adsorbed by the various kinds of adsorbents was found to decrease in the order: oxides>silicates>soils. For the soils tested aluminium oxides, and to a lesser extent iron oxides seem the most important components in determining a soil's ability to absorb phosphate and glyphosate, whereas the clay content and clay type seem of minor or little importance for adsorption of these species.
Proposed action: Consider as additional information as the article focuses on basic research. No information on isotherms is given. Data quality and quantity is not sufficient for their use in endpoint and PEC-assessment.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because basic research is published giving more in depth insight into sorption mechanisms

Reliability	Medium
Endpoint	K_d-values
Protocol	Close to OECD 106, non-GLP
Test compound	Unlabeled Glyphosate (99–100 % pure), ^{14}C-labeled Glyphosate (purity?); CAS-no.: 1071-83-6
Test system and conditions	Batch experiments, kinetics, room temperature
Statistical design	Triplicate measurements, 3 soils, comparison of % sorption between phosphate and Glyphosate
Relevance	
Environmental relevance	Given as influence of environmental parameter such as pH, soil parameter etc. on sorption was measured.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence

Gimsing et al. (2004)

Title: Influence of soil composition on adsorption of glyphosate and phosphate by contrasting Danish surface soils
Author: A. L. GIMSING, O. K. BORGGAARD & M. BANG
Reference: European Journal of Soil Science, 55, 183–191
Year: 2004
Results and conclusion: Adsorption of phosphate and glyphosate to five contrasting Danish surface soils was investigated by batch adsorption experiments. The different soils adsorbed different amounts of glyphosate and phosphate, and there was some competition between glyphosate and phosphate for adsorption sites, but the adsorption of glyphosate and phosphate seemed to be both competitive and additive. The competition was, however, less pronounced than found for goethite and gibbsite in an earlier study. The soil's pH seemed to be the only important factor in determining the amount of glyphosate and phosphate that could be absorbed by the soils; consequently, glyphosate and phosphate adsorption by the soils was well predicted by pH, though predictions were somewhat improved by incorporation of oxalate-extractable iron. Other soil factors such as organic carbon, the clay content and the mineralogy of the clay fraction had no effect on glyphosate and phosphate adsorption. The effect of pH on the adsorption of glyphosate and phosphate in one of the soils was further investigated by batch experiments with pH adjusted to 6, 7 and 8. These experiments showed that pH strongly influenced the adsorption of glyphosate. A decrease in pH resulted in increasing glyphosate adsorption, while pH had only a small effect on phosphate adsorption.

Proposed action: Consider as additional information as the article focuses on influence of soil parameter on sorption. Quality of data is not sufficiently high for their use for endpoint and PEC-assessment. The validity of the study cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only since basic research on competitive mechanisms is published.	
Reliability	Medium
Endpoint	K_d-values; correlation coefficients between glyphosate (Gly_{ads}) and phosphate adsorption (P_{ads}) and soil factors
Protocol	In analogy to OECD 106, non-GLP
Test compound	Un-labelled Glyphosate (99-100 % purity); ^{14}C-labeled Glyphosate (purity ?); CAS-no.: 1071-83-6
Test system and conditions	5 soils tested, batch experiment, sorption kinetics; concentration of radioactivity in the supernatant measured
Statistical design	5 soils, triplicate measurements, correlations determined
Relevance	
Environmental relevance	Given as influence of environmental parameter such as pH, soil parameter etc. on sorption was measured.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Gimsing et al. (2004)

Title: Modelling the Kinetics of the Competitive Adsorption and Desorption of Glyphosate and Phosphate on Goethite and Gibbsite and in Soils	
Author: Anne Louise Gimsing, Ole Borggaard, Peter Sestoft	
Reference: Environ. Sci. Technol. 38, 1718-1722	
Year: 2004	
Results and conclusion: Kinetics of the Competitive Adsorption and Desorption of Glyphosate and Phosphate on Goethite and Gibbsite and in Soils: We present and evaluate six simple, kinetic models that only take time and concentrations into account. Three of the models were found suitable to describe the competition in soil. These three models all assumed both competitive and additive adsorption, but with different equations used to describe the adsorption. For the oxides, three additional models assuming only competitive adsorption were also found suitable. This is in accordance with the observation that the adsorption in soil is both competitive and additive, whereas the adsorption on oxides is competitive. All models can be incorporated in transport models such as the convection-dispersion equation.	
Proposed action: Consider as additional information as the article focuses on influence of soil parameter on sorption and their modelling. Quality of data is not sufficiently high for their use for endpoint and PEC-assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because basic research on competitive mechanisms are published.	
Reliability	Medium
Endpoint	Glyphosate concentrations in $CaCl_2$-solution at various time points after start of experiment as related to applied (% adsorbed)
Protocol	In analogy to OECD 106, non-GLP
Test compound	Un-labelled Glyphosate (99-100 % purity); ^{14}C-labeled Glyphosate (purity ?); CAS-no.: 1071-83-6
Test system and conditions	5 soils tested, batch experiment, sorption kinetics; concentration of radioactivity in the supernatant measured

Statistical design	5 soils, triplicate measurements, 2 scenarios modelled: a) phosphate added, equilibrium between sorbed and dissolved phosphate, thereafter Glyphosate added; b) Glyphosate added, equilibrium between sorbed and dissolved Glyphosate, thereafter Phosphate added
Relevance	
Environmental relevance	Low weight, additional information because basic research on competitive mechanisms are published.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Gimsing et al. (2007)

Title: Sorption of glyphosate and phosphate by variable-charge tropical soils from Tanzania	
Author: A.L. Gimsing, C. Szilas, O.K. Borggaard	
Reference: Geoderma 138, 127-132	
Year: 2007	
Results and conclusion: Sorption of glyphosate and phosphate by four contrasting soils from Tanzania, an Andisol (Sasanda), two Oxisols (Lubonde, Mlingano) and an Ultisol (Nkundi), with variable-charge clay minerals was studied by batch sorption experiments, during which glyphosate and phosphate were added separately as well as together (competitive sorption). Agreement was found between glyphosate and phosphate sorption and between sorbed glyphosate/phosphate and contents of aluminium and iron extractable by oxalate and dithionite-citrate-bicarbonate (oxides and allophane/ imogolite). The Langmuir sorption maxima of glyphosate ranged from 15.5 mmol/kg (Nkundi) to 126 mmol/kg (Sasanda), while that of phosphate varied from 5.8 mmol/kg (Nkundi) to 78.5 mmol/kg (Sasanda). Additive as well as competitive sorption can dominate the reaction of variable-charge soils with glyphosate and phosphate.	
Proposed action: Not to be considered as soils outside the EU are used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only since basic research on competitive mechanisms is published.	
Reliability	High
Endpoint	Glyphosate and phosphate sorption isotherms, sorption maxima and affinities by means of the Langmuir equation
Protocol	Close to OECD 106, non GLP
Test compound	Un-labeled Glyphosate (99-100 % purity); ^{14}C-labeled glyphosate (purity ?); CAS-no.: 1071-83-6
Test system and conditions	5 soils tested, batch experiment, glyphosate concentrations in supernatant measured, sorption isotherms calculated
Statistical design	4 soils, number of Glyphosate concentrations not given, triplicate measurements, sorption data fitted to Langmuir equation, sorption maxima and affinities calculated using a least squares non-linear method
Relevance	
Environmental relevance	Given as influence of environmental parameter on sorption was measured.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Jacobsen et al. (2008)

Title: Variation of MCPA, metribuzine, methyltriazine-amine and glyphosate degradation, sorption, mineralization and leaching in different soil horizons

Author: Carsten S. Jacobsen, Peter van der Keur, Bo V. Iversen d, Per Rosenberg, Heidi C. Barlebo c, Søren Torp d, Henrik Vosgerau e, Rene´ K. Juhler a, Vibeke Ernstsen, Jim Rasmussen, Ulla Catrine Brinch, Ole Hørbye Jacobsen

Reference: Environmental Pollution 156 (2008) 794–802

Year: 2008

Results and conclusion:
Glyphosate (K_d values determined in the range of 200 L/kg to 4000 L/kg) does not follow the simple rule that increased organic matter leads to increased sorption. The two most important component determining glyphosate sorption in the A-horizon is gravel and organic matter (the latter being negative). Glyphosate was often higher in the inorganic subsoil samples compared to the A-horizon samples. No calculated DT_{50} values were provided.
Detailed results on sorption are:

Soil characterisation:

Soil	Soil type	Sand (%) (fine + coarse)	Clay (%)	C.E.C. (in meq/100g)
Nedre Julianhede		69.7	3.8	
Nörlund		77.9	3.1	
Stubkaer		54.6	3.8	
Söbjerg		76.0	4.2	
Ruskaer		84.9	3.5	
Ilskov		83.3	4.4	
Skaaphusgaard		83.7	3.9	
Roejen Mosegard		80.1	3.4	
Röjen Kaer		82.0	3.3	
Röjen		79.8	3.3	
Sneptrup		86.3	3.0	
Simmelkjaer		88.9	3.4	
Neder Simmelkjaer		88.2	3.6	
Ommose		85.6	3.5	
Hallundbaek		86.8	3.8	

Adsorption

Soil type	OC %	pH ($CaCl_2$)	K_d	K_{oc}	K_f	K_{foc}	1/n
Nedre Julianhede	2.8	4.9	867				
Nörlund	2.1	5.3	237				
Stubkaer	4.8	5.8	1858				
Söbjerg	1.8	6.3	871				
Ruskaer	4.1	4.2	3758				
Ilskov	3.9	5.2	342				
Skaaphusgaard	2.6	5.1	n.a.				
Roejen Mosegard	6.4	5.6	108				
Röjen Kaer	2.3	4.9	690				
Röjen	2.7	4.7	656				
Sneptrup	2.2	4.5	400				
Simmelkjaer	1.8	4.1	586				
Neder Simmelkjaer	2.6	5.2	366				
Ommose	4.3	4.6	551				
Hallundbaek	1.6	5.5	257				

Proposed action:
Not to be considered further as the authors only provided K_d-values

Type of information (critical, high/low weight, supporting, additional):
Supporting information

Reliability	
Endpoint	Sorption of glyphosate in top soil and sub soil
Protocol	Non-GLP studies modified OECD 106 and OECD 307
Test compound	CAS 38641-94-0 ^{14}C-glyphosate (CAS 1071-83-6)
Test system and conditions	Sampling was performed at 15 locations placed on a 28 km long transect of the Karup outwash plain in northwest Jutland, Denmark OC content and particle size distribution were determined for the A, B and C horizons. A sample to solution ratio of 1:10 was used for glyphosate because this herbicide is highly adsorbed. The flasks were incubated on an orbital shaker at 10 °C for 96 h. Mineralization experiments were performed by adding ^{14}C-labelled pesticides in a total concentration of 1.0 mg pesticide/kg (dry weight) soil and incubating at 10 °C in the dark.
Statistical design	Three replicates
Relevance	
Environmental relevance	The experiments are principally relevant. Unfortunately, the authors did not calculate DT_{50} for glyphosate.
Weight of evidence	
"Positive"/"Negative" evidence	The sorption studies support the information known for glyphosate from standard tests.

Jia et al. (2011)

Title: Adsorption of Glyphosate on Resin Supported by Hydrated Iron Oxide: Equilibrium and Kinetic Studies	
Author: Dongmei Jia, Chao Zhou, Changhai Li	
Reference: Water Environ. Res., 83, 784	
Year: 2011	
Results and conclusion: Hydrated iron oxide supported on a resin was prepared as a new sorbent for the removal of glyphosate from wastewater. Batch adsorption studies were performed on glyphosate aqueous solutions with different initial glyphosate concentrations and temperatures. Experimental data were analyzed using the Langmuir and Freundlich isotherms, and the adsorption data were best fit to the Langmuir isotherm model. The thermodynamic parameters DG, DH, and DS also were calculated for the adsorption processes. Adsorption rate constants were determined using the pseudo-first-order and pseudosecond-order rate equations and Kannan–Sundaram intraparticle diffusion models. Adsorption of glyphosate clearly followed the pseudo-second-order model and was controlled by both film diffusion and intraparticle diffusion.	
Proposed action: Not to be considered as a sorbent other than soil was used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because a sorbent other than soil is used	
Reliability	Low
Endpoint	Langmuir and Freundlich isotherms for sorption on hydrated iron oxide supported on resin
Protocol	Similar to OECD 106, but sorbent other than soil, non-GLP
Test compound	Glyphosate (purity 98 %, CAS 1071-83-6)
Test system and conditions	Batch experiments, 7 concentrations, 2 different temperatures, 1 sorbent
Statistical design	Langmuir and Freundlich isotherms

Relevance	
Environmental relevance	Given, influence of temperature and iron investigated.
Weight of evidence	
"Positive"/"Negative" evidence	Comparable other studies not known; no negative evidence.

Kah and Brown (2006)

Title: Adsorption of Ionisable Pesticides in Soils	
Author: M. Kah and C.D. Brown	
Reference: Rev Environ Contam Toxicol 188:149–217	
Year: 2006	
Results and conclusion: Review article: This review presents the state of knowledge on the adsorption of ionisable pesticides in soils. It first introduces the issues concerning adsorption and the characteristics of this particular kind of chemical. Subsequently, the review focuses on the influence of soil properties on adsorption and on potential to predict the behaviour of ionisable pesticides in soils. The standardization of experimental settings and the application of approaches specific to a particular class of pesticide or different type of soil might be necessary to describe the complexity of interactions among ionisable molecules.	
Proposed action: Consider as additional information as the article is a review. No raw data are published.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because review article	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate; CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Keshteli et al. (2011)

Title: ADSORPTION BEHAVIOR OF GLYPHOSATE IN SOME CITRUS GARDEN SOILS OF IRAN
Author: Rafiei Keshteli, M.*, Farahbakhsh, M., Savaghebi, G.R.
Reference: EJEAFChe, 10 (2), 1943-1951
Year: 2011
Results and conclusion: This investigation was performed to study adsorption of glyphosate in six citrus garden soils of north of Iran. The soil samples were thoroughly characterized for their physical and chemical properties, particularly organic matter and iron and aluminum oxides. Both Langmuir and Freundlich isotherms represented the adsorption data well in all cases although Langmuir equation showed a better estimate of glyphosate adsorption. Amounts of Freundlich adsorption coefficient (K_f) are in the range of 42.52-77.46 L/kg and Langmuir absorption coefficient (K_L) in the range of 0.326-1.089 L/kg. Maximum absorption coefficient in the soils studied was the soil that had the highest organic carbon content. K_f and K_L had shown significant correlations with soil organic carbon.

Proposed action: Not to be considered as non-European soils were used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because a non-European soil is used.	
Reliability	High
Endpoint	K_f, 1/n, R^2
Protocol	Batch experiment, OECD 106, non-GLP
Test compound	Glyphosate (96 % purity); CAS-no.: 1071-83-6
Test system and conditions	Batch experiments under standard conditions, 2 replicates each, 6 soils, 5 concentrations
Statistical design	Freundlich and Langmuir isotherms
Relevance	
Environmental relevance	Given, influencing parameter adequately considered.
Weight of evidence	
"Positive"/"Negative" evidence	Other reliable studies support the results; no negative evidence.

Khenifi et al. (2010)

Title: Adsorption of Glyphosate and Glufosinate by Ni_2AlNO_3 layered double hydroxide
Author: A. Khenifi, Z. Derriche, C. Mousty, V. Prévot, C. Forano
Reference: Applied Clay Science 47 (2010) 362–371
Year: 2010
Results and conclusion: The removal of organophosphate and organophosphonate herbicides from aqueous solution by Ni_2Al LDH material was investigated. Batch adsorption studies were conducted to evaluate the effect of various parameters such as contact time and initial herbicides concentrations. The adsorption kinetics was tested for Elovich, intraparticle diffusion, pseudo-second-order, and pseudo-first-order reactions and rate constants of kinetic models were calculated. The equilibrium adsorption data were analysed by Freundlich, Langmuir, and Tempkin using linear regression technique. Langmuir isotherms best fitted the data for adsorption equilibrium for both herbicides. Structural and textural analysis (XRD, FTIR, MEB) of Ni_2AlNO_3 LDH at different rates of adsorption evidence a mechanism of adsorption via an anion exchange reaction, Glyphosate and Glufosinate being adsorbed, in a 1rst step, at the surface of the cristallites and then intercalated in the interlayer domains. In detail: Adsorption experiments examining the removal of the anionic pesticide Glyphosate and Glufosinate from aqueous solutions by NiAl-LDH materials indicated two distinguishable adsorption paths, external surface adsorption and interlayer anion exchange. This was confirmed by the structural and textural analysis (XRD, FTIR, MEB) of Ni_2AlNO_3 LDH at different rates of adsorption. Batch kinetic studies performed by LDH system data tended to fit well the second-order model. The intraparticle diffusion was not the only rate-limiting step; the surface adsorption and intraparticle diffusion were concurrently operating during the Glyphosate and Glufosinate interactions. The adsorption isotherms are of H and L types for Gly and Glu respectively and they were well described by Langmuir model. The result indicates an important role of NiAl-LDH materials as potential adsorbents for removal of organophosphate and organophosphonate pollutants from water. Freundlich isotherm constants for the adsorption of Glyphosate and Glufosinate by NiAl-NO_3 LDH: Glyphosate: K_F= 39.7 mg/g, n = 0.36, R^2= 0.91 Glyphosinate: K = 27.3 mg/g, n = 0.44, R^2= 0.94
Proposed action: Consider as additional information.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.

Reliability	
Endpoint	Adsorption of Glyphosate and Glufosinate by Ni_2AlNO_3 (Freundlich, Langmuir, and Tempkin)
Protocol	Not given
Test compound	Glyphosate and glufosinate (99 % purity)
Test system and conditions	$Ni_2Al–NO_3$ LDH was prepared by the coprecipitation method under nitrogen atmosphere (in order to minimise the contamination with atmospheric CO_2) and vigorous magnetic stirring. An aqueous solution of Ni and Al nitrate with Ni/Al molar ratio equal to 2 and total metal ion concentration of 1 M was added drop-wise to a flask containing 100 mL of deionised water. A solution of sodium hydroxide (2 M) was simultaneously added to fix the pH of co precipitation at 10.0±0.1. The addition of the salt solution was completed in 5 h. The precipitate was washed by three dispersion and centrifugation cycles in deionised water, and finally air-dried. Elemental analyses (Ni, Al, P) were performed by ICP (inductively coupled plasma) emission spectrometry with a Perkin-Elmer Optima 3000XL atomic emission spectrometer. Water contents were determined using a TG-DTA92 thermogravimetric analyzer. The material displays the following chemical composition: $Ni_{2\ 07}Al(OH)_{6\ 14}NO_3.1.98H_2O$. Ni_2Al LDH intercalated with Glyphosate (Ni_2AlGly) or Glufosinate (Ni_2AlGlu) were prepared by coprecipitation method as described above except that an excess of 2.5 times equivalence of Glyphosate or Glufosinate over Al3+ content was initially added into the reactor. Finally, the obtained gelatinous precipitates were washed by three dispersion and centrifugation cycles in deionised water and air-dried. For comparison, anion exchange reactions were also performed on Ni_2AlNO_3 with Glyphosate and Glufosinate, using 1 mg/mL Ni_2AlNO_3 aqueous suspension in 300 mg/L of herbicides at a pH=7.0. Powder X-ray diffraction (PXRD) patterns were obtained with a Siemens D501 X-ray diffractometer using Cu-Kα radiation (λ= 1.5415 A°) and fitted with a graphite back-end monochromator. The samples were scanned from 2° to 70° (2θ) using steps of 0.08° and a counting time of 4 s per step. The attenuated total reflectance infrared spectra (ATR-FTIR) were collected on a FTIR Nicolet 5700 (Thermo Electon Corporation) spectrometer equipped with a Smart Orbit accessory. Thermogravimetric analyses (TGA) were recorded on a Setaram TG-DTA92 thermogravimetric analyzer coupled with a mass spectrometry analyzer (Thermostar 300 Balzers Instruments) in the temperature range of 25–1100 °C, with a heating rate of 5 °C/min, under air flow in an alumina crucible. Scanning electron microscopy (SEM) images were obtained on a JEOL 5190 microscope operating at an acceleration voltage of 15 keV. Structural modelization of Glufosinate and Glyphosate molecules were performed using the semiempirical ChemBioDraw Ultra version 11.0 MM2 simulation program. The adsorption isotherms and the kinetic study were measured at 25 °C using the batch equilibrium method in open bottles. Each experiment was repeated at least three times. The suspensions were kept in a vessel with continuous shaking. To get a homogeneous dispersion, the samples were dispersed in 25 mL of deionised/ decarbonated water and stirred for 24 h before the Glyphosate or Glufosinate molecules were added. The pH value was adjusted (pH=7.0) by hydrochloric acid (0.1 M) or NaOH (0.1 M). Different experiments by varying the contact time and the initial herbicide concentration with a final volume of 50 mL were carried out. Fifty milligram of the LDH mass was used for different batch equilibrium experiments. After a contact time of 24 h, the suspensions were centrifuged. The amount of Glyphosate or Glufosinate adsorbed by the LDH (Qe) was determined from the difference between the initial (Ci) and the final equilibrium concentration (Ce) per gram of adsorbent. The amount of Gly and Glu present in the supernatant was measured as elementary phosphorus by ICP. The typical experimental error is lower than 5 % for all the experimental results.
Statistical design	Experiments were repeated three times. The equilibrium adsorption data were analysed by Freundlich, Langmuir, and Tempkin using linear regression technique.

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Kjær et al. (2011)

Title: Reply to Comments on ''Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils'' by Petersen, C.T. and Hansen, S. [Chemosphere 84 (4) (2011) 471–479]	
Author: Jeanne Kjær, Vibeke Ernstsen, Lis Wollesen de Jonge, Preben Olsen	
Reference: Chemosphere 85, 1539–1541	
Year: 2011	
Results and conclusion: We appreciate the opportunity to respond to the comments of Dr. Petersen and Dr. Hansen (Petersen and Hansen, 2011) and to further elaborate on the modes and transport pathways of strongly sorbing pesticides such as glyphosate and pendimethalin. Please find our response to the specific comments of Petersen and Hansen (2011) (marked in Italics) outlined below.	
Proposed action: Not to be considered, no experimental results, but letter to the editor	
Type of information (critical, high/low weight, supporting, additional): Low weight, letter to the editor	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate; CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kogan Marcelo et al. (2003)

Title: Adsorption of Glyphosate in Chilean soils and its relationship with unoccupied phosphate binding sites	
Author: Kogan Marcelo *et al.*	
Reference:	
Year: 2003	
Results and conclusion: Glyphosate adsorption by Chilean soils and its relationship with unoccupied binding sites for phosphate adsorption was investigated. Experimental maximum adsorption capacity was 15000, 14300, and 4700 µg/g for the three soils under consideration. Maximum adjusted adsorption capacity with the Langmuir model was 213884, 17874, and 5670 µg/g.	
Proposed action: Not to be considered as non-European soils are tested	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because Chilean soils are investigated	

Reliability	High
Endpoint	K_f, 1/n R^2
Protocol	Close to OECD 106, tier III, non-GLP
Test compound	Glyphosate; CAS-no.: 1071-83-6
Test system and conditions	3 soils, 4 concentrations, batch experiment
Statistical design	Linear, Freundlich, Langmuir
Relevance	
Environmental relevance	Given, parameter assessed adequately.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Lashermes et al. (2010)

Title: Sorption and mineralization of organic pollutants during different stages of composting
Author: G. Lashermes, S. Houot, E. Barriuso
Reference: Chemosphere 79, 455–462
Year: 2010
Results and conclusion: The potential for compost microflora to degrade organic pollutants (OP), and compost sorption properties, were characterized at different stages of composting. The highest level glyphosate mineralization was found during the thermophilic stage. Glyphosate mineralization was probably linked to total microbial activity. Sorption on compost was linked to hydrophobicity of Glyphosate. Moreover, sorption did not decrease as compost maturity increased. The sorption coefficient was positively correlated to mineralization kinetics parameters, suggesting a positive effect of sorption on increasing mineralization rates.
Proposed action: Not to be considered as the article focuses on basic research and a specific topic. Data are not comparable to other used for endpoint and PEC-assessment.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as such a specific issue is not addressed in the monograph

Reliability	High
Endpoint	K_d-values; concentration in the supernatant after equilibrium; mineralisation (% $^{14}CO_2$-formation)
Protocol	Sorption: close to OECD 106; mineralisation: similar to parts of OECD 307 but $^{14}CO_2$-formation only, non-GLP
Test compound	Unlabelled Glyphosate (98 % purity), ^{14}C-Glyphosate (93.8 % purity); CAS-no.: 1071-83-6
Test system and conditions	Composting first, compost taken at various stages of composting for sorption studies; 4 concentrations, batch experiments, 5 replicates per measurement.
Statistical design	Outliers were removed from the analytical replicates using the Dixon test. Variance homogeneity was checked with the Levene test on the mineralization potential of OP during 92-d incubations and K_d values prior to the performance of ANOVA and LSD ($P < 0.05$) test to evaluate the effect of compost maturity on the parameters measured. Pearson's correlations were also calculated between the biochemical properties, Kd, and kinetic parameters. All statistical analyses were performed using XLStat software.

Relevance	
Environmental relevance	Given, environmental parameter considered adequately.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with comparable design not known; no negative evidence.

Lexow et al. (2005)

Title: Glyphosate mobility in piedmont soils of the Australes range in the south of Buenos Aires Province	
Author: C. Lexow, I. Morell, A.G. Bonorino	
Reference: Chapter 16, p 199-206, in: Groundwater and Human Development. IAH Selected Papers on Hydrogeology 6. Bocanega, Hernandez and Usunoff (Eds)	
Year: 2005	
Results and conclusion: This study of glyphosate soil adsorption took place on an experimental plot of an agricultural sector of Buenos Aires Province. By carrying out batch tests the partition coefficient K_d, which relates the concentration of glyphosate in the water phase to the adsorbed one in the soil, was obtained. This coefficient was standardized according to the organic matter content (K_{OC}), and optimized using models based on the Freundlich isotherm. The greatest degree of adsorption of glyphosate occurs at surface level and decreases with depth, owing more to variations in the structure and chemical composition of the clay sediments than to the effect of the organic matter. There is a very high adsorption of the glyphosate in the soil (K_f: 17.0 - 49.2) so it falls into the category of being non-leachable. This characteristic gives it potentially little impact as a polluting agent, provided the conditions of preferential flow that could significantly increase its mobility are not generated.	
Proposed action: Not to be considered as the study was performed under outdoor conditions in Argentina; not representative for EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because study was performed under outdoor conditions in Argentina	
Reliability	Low
Endpoint	K_d, K_{OC}, K_f, 1/n, R^2
Protocol	In analogy to OECD 106, non-GLP
Test compound	Glyphosate (purity not given), CAS-no.: 1071-83-6
Test system and conditions	Batch experiments, 6 concentrations, soil from 3 depths
Statistical design	Freundlich Isotherms, number of replicates not given
Relevance	
Environmental relevance	Environmental parameter presented and discussed, thus environmental relevance is given.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies confirm the results, positive evidence; no negative evidence.

Litz et al. (2011)

Title: Comparative studies on the retardation and reduction of glyphosate during subsurface passage	
Author: N.T. Litz , A. Weigert, B. Krause, S. Heise, G. Grützmacher	
Reference: Water Research 45, 3047-3054	
Year: 2011	

Results and conclusion:
The herbicide Glyphosate was detected in River Havel (Berlin, Germany) in concentrations between 0.1 and 2 mg/L. Laboratory (sorption and degradation studies) and technical scale investigations (bank filtration and slow sand filter experiments) were carried out.
Batch adsorption experiments with Glyphosate yielded a low K_f of 1.89 (1/n = 0.48) for concentrations between 0.1 and 100 mg/L. Degradation experiments at 8 °C with oxygen limitation resulted in a decrease of Glyphosate concentrations in the liquid phase probably due to slow adsorption (half life: 30 days). During technical scale slow sand filter (SSF) experiments Glyphosate attenuation was 70-80 % for constant inlet concentrations of 0.7, 3.5 and 11.6 mg/L, respectively. Relevant retardation of Glyphosate breakthrough was observed despite the low adsorption potential of the sandy filter substrate and the relatively high flow velocity. The VisualCXTFit model was applied with data from typical Berlin bank filtration sites to extrapolate the results to a realistic field setting and yielded sufficient attenuation within a few days of travel time. Experiments on an SSF planted with Phragmites australis and an unplanted SSF with mainly vertical flow conditions to which Glyphosate was continuously dosed showed that in the planted SSF Glyphosate retardation exceeds 54 % compared to 14 % retardation in the unplanted SSF. The results show that saturated subsurface passage has the potential to efficiently attenuate glyphosate, favourably with aerobic conditions, long travel times and the presence of planted riparian boundary buffer strips.

Proposed action:
To be considered as additional information for risk mitigation. Not to be considered for endpoint and PEC-assessment.

Type of information (critical, high/low weight, supporting, additional):
High weight, supporting information on risk mitigation strategies

Reliability	High
Endpoint	K_f, K_d, 1/n, concentrations of gylphosate and AMPA
Protocol	Batch experiments: according to OECD 106; degradation experiments: partly similar to OECD 307, enclosures and SSF experi ments: no standard protocols available, non-GLP
Test compound	Glyphosate (98.7 % purity); CAS-no.: 1071-83-6
Test system and conditions	Laboratory batch, enclosure and slow sand filter tests, filter material used. Laboratory experiments: Degradation: partly reducing conditions, 5 sampling points. Batch experiments: 4 concentrations, number of replicates not given. Enclosures: area of 1m², height of 1.85m (filtration length 1.00 m), situated within an infiltration pond (area: 90m²), 3 Glyphosate levels. SSF experiments: two vertical-flow experimental SSFs: one without vegetation cover (average area 60m², filter depth 0.8 m, filter volume 48m³) and the other with a 3 year old vegetation cover of Phragmites australis (average area 68 m², filter depth 1.2 m, filter volume 81.6m³)
Statistical design	VisualCXTFit model, Freundlich isotherms,
Relevance	
Environmental relevance	Given, influencing environmental parameter recorded and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Publication with identical experimental setup not known, however results are logically explained; no negative evidence.

Mamy and Barruiso (2006)

Title: Desorption and time-dependent sorption of herbicides in soils	
Author: Mamy L. and E. Barruiso	
Reference: European Journal of Soil Science; doi:10.1111/j.1365-2389.2006.00822 x	
Year: 2006	
Results and conclusion: Objective of the study was to maximise the exploitation of sorption and/or desorption data to characterise desorption along with the effect of ageing on retention. The experiments involved three soils and five herbicides (inter alia Glyphosate). Sorption isotherms were not linear and desorption was markedly hysteretic. Desorption was inversely related to adsorption, being small when sorption was great as it is the case for Glyphosate. Single, different desorption isotherms are obtained that depend on initial sorbed herbicide concentration. A theoretical approach allowed calculation of adapted desorption parameters for different sorption concentrations from only one desorption isotherm. Generalised equations were derived to describe sorption and desorption, and these equations could be implemented in pesticide-fate models to take into account sorption and desorption parameters as well as their time dependence.	
Proposed action: Not to be considered as desorption-constants are not routinely used in PEC-assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as desorption isotherms are not needed for fate assessment	
Reliability	Medium
Endpoint	K_f, 1/n, desorption constants, desorption isotherms; desorption isotherms after several times of ageing.
Protocol	a) according to OECD 106; b) desorption after ageing not according to a protocol; non-GLP
Test compound	Glyphosate (purity not given); CAS-no.: 1071-83-6
Test system and conditions	a) batch adsorption and desorption studies; b) desorption studies after certain times of ageing.
Statistical design	3 soils; Freundlich; 7 time intervals for ageing experiment. Generalised equations to describe sorption and desorption.
Relevance	
Environmental relevance	Given, environmental parameter described.
Weight of evidence	
"Positive"/"Negative" evidence	Completely comparable publication not known, but results are plausible; no negative evidence.

Mamy and Barriuso (2005)

Title: Glyphosate adsorption in soils compared to herbicides replaced with the introduction of glyphosate resistant crops

Author: Laure Mamy, Enrique Barriuso

Reference: Chemosphere 61, 844–855

Year: 2005

Results and conclusion:
This work compares glyphosate adsorption in soil with that of other herbicides frequently used in rape (trifluralin and metazachlor), sugarbeet (metamitron) and corn (sulcotrione). Herbicide adsorption was characterised in surface soils and in the complete soils profiles through kinetics and isotherms using batch equilibration methods. Pedological and molecular structure factors controlling the adsorption of all five herbicides were investigated. Glyphosate was the most strongly adsorbed herbicide, thus having the weakest potential for mobility in soils. Glyphosate adsorption was dependent on its ionisable structure in relation to soil pH, and on soil copper, amorphous iron and phosphate content. Trifluralin adsorption was almost equivalent to glyphosate adsorption, whereas metazachlor, metamitron and sulcotrione adsorption were lower. Trifluralin, metazachlor and metamitron adsorption increased with soil organic carbon content. Sulcotrione was the least adsorbed herbicide in alkaline soils, but its adsorption increased when pH decreased. Ranking the adsorption properties among the five herbicides, glyphosate and trifluralin have the lowest availability and mobility in soils, but the former has the broadest spectrum for weed control. Detailed results are:

Soil characterisation:

Soil	Soil type	Sand	Clay (g/kg)	C.E.C. (in cmol/kg)
Châlons (0-10 cm. composite sample)			88	64
Châlons (0-10 cm)			93	7.1
Châlons (10-20 cm)			95	6.2
Châlons (20-30 cm)			91	4.6
Dijon (0-10 cm. composite sample)			376	17.8
Dijon (0-10 cm)			377	20.6
Dijon (10-20 cm)			327	27.0
Dijon (20-30 cm)			363	28.6
Dijon (30-60 cm)			396	31.7
Dijon (60-90 cm)			307	21.8
Toulouse (0-10 cm. composite sample)			274	16.4
Toulouse (0-10 cm)			235	15.9
Toulouse (10-20 cm)			222	15.3
Toulouse (20-30 cm)			221	15.3
Toulouse (30-40 cm)			236	14.9
Toulouse (40-50 cm)			245	15.0

Adsorption

Soil type	OC (g/kg)	pH (H_2O)	Kd (L/kg)	Koc (L/kg)	K_f	K_{foc}	n_f
Châlons (0-10 cm. composite sample)	18.6	8.4					

Châlons (0-10 cm)	20.0	8.2	31.1	1552	34.8	0.80
Châlons (10-20 cm)	17.8	8.3	+/- 2.1	+/- 105	+/- 0.6	+/- 0.02
Châlons (20-30 cm)	13.2	8.5				
Dijon (0-10 cm. composite sample)	13.5	8.3				
Dijon (0-10 cm)	16.9	8.2				
Dijon (10-20 cm)	14.8	8.2	38.7	2375	41.9	0.08
Dijon (20-30 cm)	9.5	8.3	+/- 2.5	+/- 153	+/- 0.5	+/- 0.02
Dijon (30-60 cm)	7.7	8.4				
Dijon (60-90 cm)	6.9	8.6				
Toulouse (0-10 cm. composite sample)	10.1	6.3				
Toulouse (0-10 cm)	9.6	7.6				
Toulouse (10-20 cm)	10.1	7.7	427	44360	276	0.77
Toulouse (20-30 cm)	9.4	7.7	+/- 31	+/- 3341	+/- 13	+/- 0.02
Toulouse (30-40 cm)	8.6	7.9				
Toulouse (40-50 cm)	6.8	8.2				

Proposed action:
To be considered as additional information but not for endpoint and PEC-assessment. Raw data on mass balances and test item concentrations in aqueous and solid phases are not reported and thus, the validity of the study cannot be proven.

Type of information (critical, high/low weight, supporting, additional):
High weight, supportive information, K_f-values compared to those already known

Reliability	High
Endpoint	K_f, 1/n, K_{OC}, K_d
Protocol	According to OECD 106;
Test compound	[Methyl-^{14}C]glyphosate (97.7 % purity); CAS-no.: 1071-83-6
Test system and conditions	Batch method according to OECD 106
Statistical design	3 soils, up to 4 soil depths, 8 sampling times for kinetics, 2 replicates; 6 concentrations for isotherms, 2 replicates, Freundlich isotherms
Relevance	
Environmental relevance	Given, guideline requirements fulfilled.
Weight of evidence	
"Positive"/"Negative" evidence	K_f-values in the range of already known values; no negative evidence.

Morillo et al. (2002)

Title: The effect of dissolved glyphosate upon the sorption of copper by three selected soils	
Author: E. Morillo, T. Undabeytia, C. Maqueda, A. Ramos	
Reference: Chemosphere 47, 747–752	
Year: 2002	
Results and conclusion: The effect of the pesticide glyphosate (GPS) on adsorption processes of copper onto three soils of different characteristics has been studied. Cu adsorption decreases in general with increasing GPS concentration in solution, due principally to the lower equilibrium-pHs, although this is not the only variable affecting copper adsorption. For the same pH values, Cu adsorption is higher in two of the three soils in the presence of GPS, but for the third soil, Cu adsorption is higher in the absence of GPS. This behaviour is explained by the possibility of GPS adsorption on these soils and by the formation of Cu-GPS complexes in solution. The soils showing a higher Cu adsorption in the presence of GPS than in its absence for the same pH are able to adsorb this pesticide. In these soils, copper can be adsorbed directly on the soil surfaces, and also through the formation of bonds with GPS previously adsorbed. The third soil was not able to adsorb GPS. Consequently, all the pesticide remained in solution, forming strong Cu complexes with low tendency to be adsorbed on this soil. For this reason, the concentration of free Cu in solution is drastically reduced, and the adsorption of copper on this soil is lower.	
Proposed action: Not to be considered for endpoint and PEC-assessment as sorption of copper in combination with Glyphosate has been investigated.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only	
Reliability	Low
Endpoint	Adsorption isotherms of copper
Protocol	Comparable to OECD 106, tier II
Test compound	Copper, Glyphosate was added (no purity given); CAS-no.: 1071-83-6
Test system and conditions	Batch equilibrium method
Statistical design	3 soils, 5 Glyphosate, 4 copper concentrations
Relevance	
Environmental relevance	Given, parameter are reported.
Weight of evidence	
"Positive"/"Negative" evidence	Not directly comparable to publications dealing with Glyphosate sorption; no negative evidence.

Nourouzi et al. (2010)

Title: Adsorption of glyphosate onto activated carbon derived from waste newspaper	
Author: M. Mohsen Nourouzi, T.G. Chuah, Thomas S.Y. Choong	
Reference: Desalination and Water Treatment 24, 321-326	
Year: 2010	
Results and conclusion: This paper investigates the ability of activated carbon derived from waste newspaper (WNAC) to remove pesticide glyphosate from aqueous solution. The influence of initial pH was first studied. It was found that the WNAC presented the highest uptake capacity at pH 2.5. Adsorption isotherm models such as Langmuir, Freundlich and Redlich-Peterson were used to describe the adsorption of glyphosate by WNAC. The results show that the Langmuir adsorption isotherm model best fits the experimental data. The maximum adsorption capacity of WNAC is found to be 48.4 mg/g.	
Proposed action: Not to be considered for endpoint and PEC-assessment as activated carbon derived from waste newspaper is used. Thus, no standard scenario for pesticide environmental risk assessment in the EU is presented.	

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	Langmuir, Freundlich and Redlich-Peterson isotherms
Protocol	Comparable to OECD 106
Test compound	Glyphosate (analytical grade); CAS-no.: 1071-83-6
Test system and conditions	Batch equilibrium study using activated carbon derived from waste newspaper as a sorbent.
Statistical design	8 pesticide concentrations
Relevance	
Environmental relevance	Given; environmental parameter considered.
Weight of evidence	
"Positive"/"Negative" evidence	No comparable citations known, however, results are plausible; no negative evidence.

Ololade et al. (2014)

Title: Sorption of Glyphosate on Soil Components: The Roles of Metal Oxides and Organic Materials
Author: I. A. OLOLADE, N. A. OLADOJA, F. F. OLOYE, F. ALOMAJA, D. D. AKERELE, J. IWAYE AND P. AIKPOKPODION
Reference: Soil and Sediment Contamination, 23:571–585, 2014
Year: 2014
Results and conclusion: The sorption characteristics of glyphosate (GPS) on soil and their main components were investigated, indicating that the mineral phase is more important than the organic carbon in adsorption of GPS. Sorption isotherms were determined from each component using the batch equilibrium method at various concentrations (5, 10, 15, 20, 25, and 30 mg/L) and sorption affinity of GPS was approximated by the Freundlich equation. The sorption strength K_f [mg/kg(L/mg)$^{-n}$] across the various components ranged from 2.1–134.9 while the organic carbon-normalized Freundlich sorption capacity values, K_{foc}, ranged from 1.28–3.53 mg/kg-OC/(mg/L)^n. Infrared Fourier transform spectroscopy (FTIR) of the components showed significant structural differences. The results suggest that the presence of the oxides and hydroxides iron, in particular in soil solutions, enhanced GPS adsorption. They also suggest that reduction in OC % due to various treatments may enhance the remobilization of GPS into the aqueous phase (i.e., groundwater), though at different rates. Comparatively, contribution of surface area to the adsorption of GPS on the various components proved more significant than contents of organic carbon.
Proposed action: Not to be considered as non-European soils were used.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because a non-European soil is used.
Reliability

Endpoint	K_f, k_{foc}
Protocol	Not given
Test compound	Glyphosate (N-(phosphono-methyl-glycine) with >98 % certified purity

Test system and conditions	Surface soil (top 1–5 cm) was collected from University Campus, Ondo State, Nigeria, with a clean, methanol-rinsed, stainless-steel trowel. The soil samples were air-dried, passed through a 60-mesh screen, and stored in glass bottles for further use. The soil samples were characterized following the conventional methods (Tao *et al.*, 2006). Soil samples were prepared with four different reagents to extract target components. 0.1 mol/L $NH_2OH{\cdot}HCl$ and 0.01 mol/L HNO_3 for 30 min were used to remove manganese oxides (Li *et al.*, 2006). About 3.0 mL H_2O_2 (30 %) heated to 40 °C was employed to remove organic matters (OMs) based on a previous report (Mikutta *et al.*, 2005). 0.2 mol/L $(NH_4)_2C_2O_4$ was buffered at pH 3.0 with $H_2C_2O_4$ and shaken in the dark for 4 h, then employed to extract both Fe and Mn hydrous oxides (Pei *et al.*, 2006). All of the samples were centrifuged at 3800 rpm for 30 min, and supernatant was filtered (0.45 µm) into 50 mL polypropylene (PP) tube for the determination of Fe and Mn. The extracts were washed 3–4 times with distilled water and air-dried. Sorption capacity of GPS on the treated and untreated samples was determined using the batch equilibrium method. GPS is a non-residual herbicide with solubility in water of 12 g/L at 25 °C. Briefly, triplicate adsorption experiments were done using PP centrifuge tube (50 mL capacity) by mixing 0.5 g of air-dried sample with 20 mL of 0.5 mmol/L $CaCl_2$ solutions containing various concentrations of GPS (5, 10, 15, 20, 25, and 30 mg/L). Thesamples were shaken for 24 h at 25 ± 1 °C on a 2D-shaker at 250 rpm at pH maintained at 7± 0.1. The preliminary sorption kinetic test showed that the apparent sorption equilibrium was reached at 120 min for the original sample. The equilibrium pHs were maintained by adding aliquots of 0.1 M HCl or NaOH. The tubes were centrifuged at 9000 rpm for 30 min. The supernatant were taken out, filtered through a 0.22 µm nylon syringe filter, and analyzed by a UV-visible spectrophotometer (UV–VIS 1902PC, Searchtech Instrument). The glyphosate concentrations were determined by comparing them with the calibration graph obtained by preparing standards of glyphosate. The amount of GPS adsorbed was calculated from the differences between its concentration in solution before and after equilibration. Blanks and controls were prepared at the same time under the same conditions. Blanks were set up using the same solid-to-water ratios as the samples, but without adding GPS. One control sample with only the test substance in 0.5 M NaCl solution (without soil sample) was subjected to precisely the same steps as the test systems, in order to check the stability of the test substance in NaCl solution and its possible adsorption on the surfaces of the test vessels. All of the experiments, including controls and blanks, were carried out in duplicate. The GPS desorption experiment was performed on soil residues immediately after adsorption experiments. All of the supernatant solution was removed and replaced with 20 mL fresh 0.5 M NaCl prepared with distilled water. Soil residues were equilibrated using an end-over-end mechanical shaker for 120 min at 25 ± 1 °C at pH= 7. Thereafter, desorbed GPS was measured in filtrates as described in adsorption experiments after centrifuging the sample at 9000 rpm for 30 min and filtering through a 0.22µm nylon syringe filters.
Statistical design	Freundlich equation
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence

Pessagno et al. (2008)

Title: Glyphosate behavior at soil and mineral-water interfaces	
Author: Romina C. Pessagno, Rosa M. Torres Sánchez, María dos Santos Afonso	
Reference: Environmental Pollution 153, 53-59	
Year: 2008	
Results and conclusion: Adsorption isotherms and surface coverage of glyphosate (PMG), in aqueous suspensions of three Argentine soils with different mineralogical composition were measured as a function of PMG concentration and pH. Zeta potential curves for PMG/soils system were also determined. PMG formed surface complexes on goethite, kaolinite, illite, montmorillonite and soils with similar maximum surface coverage, and the extent of the complexation was dependent on the ligand concentration in solution and pH. The extent of PMG adsorption onto iron oxides was higher than onto soils or clays. The adsorption behavior of PMG on minerals and soils in aqueous suspensions were analyzed as a function of pH and surface coverage. The results suggest that the phosphonate moiety of PMG coordinates to the external surface site of solids with similar structures as iron oxides. The formation of inner-sphere surface complexes is suggested. These results are potentially important to provide a fundamental understanding of the degradability and bioavailability of PMG in soils and natural waters. PMG complexation with metal ions and its adsorption onto mineral surfaces might affect its degradation, distribution, and bioavailability in soils and groundwater. The study of the properties of these soils and mineral surface complexes is of high importance in order to assess the implications for control of PMG contamination. PMG belongs to a unique class of strongly chelating agents and the adsorption process makes herbicide more persistent in soil.	
Proposed action: Not to be considered as non-European soils were used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, non-European soils	
Reliability	Medium
Endpoint	Langmuir isotherms, maximum adsorption densities, zeta potential,
Protocol	Similar to OECD 106, tier III
Test compound	Glyphosate (CAS-no.: 1071-83-6; 99 % purity)
Test system and conditions	3 soils plus one after OM removal and iron removal, batch experiments,
Statistical design	Non-linear regression fitting program (Solver,Excel 10) to approximate a Langmuir shape
Relevance	
Environmental relevance	pH-dependencies and influence of soil material was tested, thus, influencing parameters were considered adequately; environmental relevance given.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Petersen and Hansen (2011)

Title: Letter to the Editor	
Author: Carsten T. Petersen, Søren Hansen	
Reference: Chemosphere 85, 1538	
Year: 2011	
Results and conclusion: Comments on ''Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils'' by J. Kjær, V. Ernsten, O.H. Jacobsen, N. Hansen, L.W. de Jonge, P. Olsen [Chemosphere 84(4) (2011) 471–479].	
Proposed action: Not to be considered; letter to the Editor	

Type of information (critical, high/low weight, supporting, additional): Low weight, letter to the Editor	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (CAS-no.: 1071-83-6)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Prata et al. (2003)

Title: GLYPHOSATE SORPTION AND DESORPTION IN SOILS WITH DISTINCT PHOSPHORUS LEVELS

Author: Fábio Prata; Vanessa Camponez do Brasil Cardinali; Arquimedes Lavorenti; Valdemar Luiz Tornisielo; Jussara Borges Regitano

Reference: Scientia Agricola 60 (1), 175-180

Year: 2003

Results and conclusion:
Glyphosate sorption in the three studied soils was influenced by the soil P level, and the amount of sorbed glyphosate became substantially reduced at P levels starting from 1000 mg dm^{-3}. This visualization of a reduction in glyphosate sorption, however lacks of practical importance, since these phosphorus levels would never be attained under field conditions in agricultural soils, which suggests that under this condition the competition between glyphosate and P for covalent binding sites in the soil must not occur. Thus, these results confirm that the extent of the binding forces in glyphosate is proportional to the soil capacity of adsorbing inorganic phosphate. However, even as a secondary role, organic matter plays also a very important role for glyphosate retention, especially in oxide-poor soils. Glyphosate competes with phosphorus for specific sorption sites of the soil, but this competition becomes only important when the soil P levels reach very high values, which are not attained under agricultural field conditions. The herbicide extraction is low and increases with P levels in the soil.
Glyphosate remains in the soil as a bound residue.
Detailed results are:
Soil characterisation:

Soil	Soil type	Sand (g/kg)	Clay (g/kg)	C.E.C. (in meq/100g)
Nvef	Rhodic Kandiudalf	250	550	
Law	Anionic Acrudox	590	350	
G	Typic Humaquept	200	540	

Adsorption[1)]

Soil type	OC (g/kg)	pH (H_2O)	K_d	K_{OC}	K_f	K_{foc}	1/n
Nvef	27.5	6.1			184,3		
Law	18.9	5.5			172,3		
G	78.5	4.2			222,1		

1) Applied amount of phosphate: 0 – 50000 kg P_2O_5/ha

Proposed action:
To be considered as non-European soils (Brasil) were used.

Type of information (critical, high/low weight, supporting, additional): High weight, supportive information on Glyphosate sorption dependencies on soil parameter	
Reliability	High
Endpoint	K_f-values, 1/n
Protocol	According to OECD 106, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity not given)
Test system and conditions	3 (soils) × 5 (soil P levels) factorial experiment, three replicates, 5 Glyphosate concentrations, batch experiments, 25 +/- 2 °C
Statistical design	Freundlich Isotherms; Regression analyses for: increasing levels of P and Freundlich constants, as well as for the total sorbed percentage. Analyses of variance and mean comparison tests (Tukey, $P < 0.05$) for the percentages of glyphosate extracted and desorbed
Relevance	
Environmental relevance	Influence of phosphate on sorption investigated; environmental relevance given.
Weight of evidence	
"Positive"/"Negative"	Other studies support the results; no negative evidence.

Prata et al. (2005)

Title: GLYPHOSATE BEHAVIOR IN A RHODIC OXISOL UNDER NO-TILL AND CONVENTIONAL AGRICULTURAL SYSTEMS	
Author: Fábio Prata, Arquimedes Lavorenti, Jussara Borges Regitano, Harry Vereecken, Valdemar Luiz Tornisielo & Adelino Pelissari	
Reference: R. Bras. Ci. Solo, 29:61-69	
Year: 2005	
Results and conclusion:	
The behaviour of glyphosate in a Rhodic Oxisol, collected from fields under no-till (NT) and conventional (CON) management systems in Ponta Grossa, Parana state (Brazil) was investigated. Glyphosate mineralization, soil-bound forms, sorption and desorption kinetics, sorption/desorption batch experiments, and soil glyphosate phythoavailability (to Panicum maximum) were determined. Sorption: The glyphosate sorption kinetics was practically instantaneous (over 90 % sorbed within 10 min). For both the NT and CON systems, glyphosate presented a high sorption rate, which difficult its mineralization. The molecules remained in the soil as bound-residue. Mineralisation: The NT system contributed to the acceleration of glyphosate mineralization. The main metabolite resulting from glyphosate degradation was aminomethylphosphonic acid (AMPA).	
Proposed action: Not to be considered as non-European soils (Brasil) were used.	
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information on Glyphosate behaviour in soils under different management systems.	
Reliability	High
Endpoint	$^{14}CO_2$-formation; sorption kinetics, Freundlich sorption and desorption constants
Protocol	According to OECD 106, non-GLP, close to OECD 307 but comprehensive for measurements mineralisation only, AMPA analysed but not used for kinetics
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity not given), ^{14}C-labelled Glyphosate, 2 label positions (> 97 % purity)

Test system and conditions	Mineralisation: completely randomized design with a 2 x 2 factorial scheme (two management systems and two ^{14}C radiolabelled positions in the glyphosate), with five replicates. $^{14}CO_2$ evolution measured in 7-day intervals during 63 days. Sorption: kinetics investigated in a batch experiment, 7 equilibration times up to 60 hours. Sorption/desorption using equilibrium batch experiments. Five different concentrations for sorption and one concentration for desorption. Same soils as for mineralisation.
Statistical design	Freundlich Isotherms; Regression analyses for results for the different endpoints and soil management systems. Analyses of variance and mean comparison tests (Tukey, $P < 0.05$).
Relevance	
Environmental relevance	Influence of soil management practices on mineralisation and sorption investigated; environmental relevance given.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Pessagno (2005)

Title: N-(PHOSPHONOMETHYL)GLYCINE INTERACTIONS WITH SOILS	
Author: Pessagno, R.C., dos Santos Afonso, M., Torres Sanchez, R.M.	
Reference: The Journal of the Argentine Chemical Society, 93 (4/6), 97-108	
Year: 2005	
Results and conclusion: The adsorption isotherms and surface coverage of glyphosate (NPhosphonomethylglycine, PMG) in aqueous suspensions of Argentine soils as a function of PMG concentration and pH were measured. Zeta potential curves for the PMG/soils system were also determined. The formation of inner sphere surface complexes of PMG on the soil surface, were analyzed as a function of pH and surface coverage.	
Proposed action: Not to be considered as a non-EU soila (Argentina) were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, non-European soils	
Reliability	Low
Endpoint	Maximum adsorption densities, Langmuir constants, zeta potential curves
Protocol	Similar to OECD 106, tier III
Test compound	Glyphosate (99 % purity); CAS-no.: 1071-83-6
Test system and conditions	3 soils and Goethite tested, batch experiments, pH-dependencies
Statistical design	Number of concentrations not given, number of replicates not reported
Relevance	
Environmental relevance	Given as pH-dependencies are determined.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Rampazzo et al. (2012)

Title: Adsorption of glyphosate and aminomethylphosphonic acid in soils
Author: N. Rampazzo, G. Rampazzo Todorovic, A. Mentler, and W.E.H. Blum
Reference: Int. Agrophys., 2013, 27, 203-209, doi: 10.2478/v10247-012-0086-7
Year: 2012
Results and conclusion: The results showed that glyphosate is initially adsorbed mostly in the upper 2 cm. It is than transported and adsorbed after few days in deeper soil horizons with concomitant increasing content of its metabolite aminomethylphosphonic acid. Moreover, Fe-oxides seem to be a key parameter for glyphosate and aminomethylphosphonic adsorption in soils. This study confirmed previous studies: the analysis showed lower contents of dithionite-soluble and Fe-oxides for the Chernozem, with consequently lower adsorption of glyphosate and aminomethylphosphonic as compared with the Cambisol and the Stagnosol. In detail: No-tillage plots show a higher bulk density and a lower total porosity than conventionally tillaged plots as due to a natural settlement of particles free from tillage practices. Shortly after Roundup Max application only a part of the applied glyphosate amount enter the upper 0-2 cm and is then transported and adsorbed in deeper horizons with time with concomitant increase of the aminomethylphosphonic acid content. The results showed distinguished contents of glyphosate and aminomethylphosphonic acid in different soils at the same soil depth, according to their chemical-mineralogical adsorption properties, especially Fe-oxides (Fed and Feo). Thus, iron-oxides in general seem to be a key parameter for glyphosate and aminomethylphosphonic acid adsorption in soils.
Proposed action: Consider as additional information.
Type of information (critical, high/low weight, supporting, additional): Supporting information.

Reliability	
Endpoint	Adsorption of glyphosate and aminomethylphosphonic
Protocol	Not given
Test compound	Glyphosate (Roundup Max)

Test system and conditions	The experiments were carried out at agricultural experimental fields, where different tillage systems: no-tillage (NT), direct drill, no plough, with a winter green vegetation cover and maize crop in spring, and conventional tillage (CT), plough with or without a winter green vegetation cover in 3 field replications are tested since 2007 (Kirchberg, Styria), 1999 (Pyhra and Pixendorf, Lower Austria). Three soils under different climatic conditions and featuring different physico-mineral composition were investigated: a sandy stagnic Cambisol (WRB, 2006; Nestroy *et al.*, 2000) at Kirchberg (Styria) fromtertiary carbonate free sediments, a loamy Stagnosol (WRB, 2006; Nestroy *et al.*, 2000) from carbonate free sediments (flysch, sandstone) at Pyhra (Lower Austria) and aChernozem(WRB, 2006; Nestroy *et al.*, 2000) from loess at Pixendorf (Lower Austria). Moreover, these three soil types were selected because of their contrasting physico-chemico-mineralogical parameters eg texture, carbonate content, pH-value, and Fe-oxides for a better understanding of their influence on the glyphosate behaviour and extraction from soils. The Roundup Max application was performed at all three sites according to the common agricultural practice ie 4 l Roundup Max (450 g glyphosate /l Roundup Max) were dissolved in 200 l of water and applied per ha (2 %herbicide solution). This corresponds to an application of 1 800 g glyphosate ha^{-1} or 180 mg glyphosatem-2. The application was carried out at sunny and not windy weather at the NT-plots. Soil bulk samples from all plots (NT and CT) were taken for physico-chemico-mineralogical analysis at each site at two soil depths (0-5 and 5-20 cm), collected from 10 different points/field replication. The samples were air-dried and sieved at 2 mm size (fine earth). Moreover, for further physical analysis undisturbed samples (cylinders with 200 cm^3) were taken separated from each NT and CT field replication at 5-15 cm soil depth each in 5 repetitions. In order to investigate the fate of glyphosate and AMPA in depth and time after Roundup Max application, soil bulk samples were taken at different time intervals after application at 10 points within each NT-field replication (pooled than to one sample per site). After each soil sampling soil samples were immediately transported to the laboratory in cooling boxes. In the laboratory all samples were stored at -18 °C until measurements. All physical, chemical and mineralogical analyses were carried out according to the standard methods.
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications; no negative evidence.

Selim et al. (2010)

Title: The sorption of Glyphosate and its metabolite amino-methyl-phosphonic acid (AMPA) on biopolymer chitin	
Author: Shady Selim, A. Klik, B. Grillitsch, M. Fürhacker, and A. Mentler	
Reference: Report ALVA – Jahrestagung 2010, „Vom Lebensmittel zum Genussmittel – was essen wir morgen“? May 31-June, 1 2010, Austria	
Year: 2010	
Results and conclusion: The main objective of this study was to demonstrate that chitin has a considerable adsorption capacity for glyphosate and its main metabolite AMPA. In the present study, the adsorption of glyphosate and its metabolite aminomethyl phosphonic acid (AMPA) onto chitin was analyzed. Results showed that chitin had an adsorption capacity and the adsorptive coefficient of glyphosate is higher than that of AMPA. The Freundlich equation fits the adsorption behaviour of glyphosate and AMPA better than the Langmuir model. Values were: Glyphosat: 974.4 mg/g (S_{max}, r^2=0.972, Langmuir); 436.7 mg/g (K_f, r^2=0.979, Freundlich) AMPA: 673.8 4 mg/g (S_{max}, r^2=0.977, Langmuir); 13.26 mg/g (K_f, r^2=0.980, Freundlich).	
Proposed action: Not to be considered as no soils are used for sorption.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on Glyphosate sorption to material other than soil	
Reliability	Low
Endpoint	Freundlich and Langmuir constants
Protocol	Close to OECD 106, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity not given)
Test system and conditions	Batch method with concentration of Chitin 10 g/L < 1 mm and concentrations of glyphosate and AMPA varied from 1µg/L to 500 µg/L at 23 C° isotherm, no further data presented
Statistical design	Not specified
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	Completely comparable publications not known, however, results are plausible; no negative evidence.

Shareef and Hamadamin (2009)

Title: Adsorption of Metalaxyl and Glyphosate on Six Erbilian Agricultural Soils	
Author: KAPIA M. SHAREEF and SHIREEN I. HAMADAMIN	
Reference: Asian Journal of Chemistry 21 (4), 2673-2683	
Year: 2009	
Results and conclusion: A study was conducted to determine the differences in the adsorption behaviour of two non-ionic pesticides, metalaxyl [N-(2,6-dimethyl phenyl)N-(methoxyacetyl)] and glyphosate [N-phosphonomethyl-glycine] on six agricultural soil samples from Erbil governorate. Data from batch equilibrium method revealed that the adsorption of metalaxyl and glyphosate on the selected soil samples followed the first order rate law. Glyphosate exhibited the faster rate of accumulation with 76.53 % adsorption on the soil solid matrix after 0.5 h as compared to that for metalaxy 66.06 %. Linear, Freundlich and Langmuir models were used to describe the adsorption of both pesticides. Values of distribution coefficient (K_d) indicated moderate to strong adsorption of metalaxyl (mean calculated K_d: 5.963 mL g^{-1}) and very strong adsorption of glyphosate (mean calculated K_d: 703.716 mL g^{-1}) and consequently there is no considerable risk of groundwater contamination. Wide variation in adsorption affinities of the soils to both pesticides was observed, K_d values for metalaxyl varied between 2.93 and 9.97 mL g^{-1} and for glyphosate between 5.16 and 456.34 mL g^{-1}. A linear correlation was found between the values of adsorption coefficients of both pesticides and soil organic carbon (R^2: 0.61 and 0.69 for metalaxyl and glyphosate, respectively).	

Proposed action: Not to be considered as non-European soils are used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Low
Endpoint	K_d, R^2, Langmuir and Freundlich isotherm
Protocol	Close to OECD 106, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity > 99.2 %)
Test system and conditions	Batch method, 6 soils, 7 time points for kinetics, 3 concentrations, number of replicates not given
Statistical design	Langmuir and Freundlich models; too less concentrations measured for reliable isotherms
Relevance	
Environmental relevance	Given; influencing parameter are reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Sheals et al. (2002)

Title: Adsorption of Glyphosate on Goethite: Molecular Characterization of Surface Complexes	
Author: Sheals J., Sjöberg S. and Persson P.	
Reference: Environ. Sci. Technol. 36, 3090-3095	
Year: 2002	
Results and conclusion: The adsorption of Glyphosate (PMG) on goethite (R-FeOOH) has been studied as a function of pH and PMG concentration. Adsorption was investigated with batch experiments, attenuated total reflectance Fourier transform infrared spectroscopy (ATRFTIR), and X-ray photoelectron spectroscopy (XPS). A minor quantity of bidentate complexes is thought to form both at near-neutral pH and when the surface concentration of PMG is low. The findings show that goethite has a relatively large capacity for PMG adsorption and thus aids the removal of bioavailable PMG from soil solution. The phosphonate group binds to the goethite component of soil to form predominantly monodentate inner-sphere complexes while the carboxylate group remains relatively "free" from complexation with goethite, leaving it subject to degradation and/or complexation with metal ions.	
Proposed action:	
Not to be considered as Goethite and no soil was used.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional in-depth information on soil sorption processes	
Reliability	Low
Endpoint	surface concentration of PMG as a function of total PMG concentration at three constant pH-values
Protocol	Partly comparable to OECD 106, non-GLP
Test compound	^{14}C-Glyphosate (CAS-no.: 1071-83-6; purity 95 %)
Test system and conditions	Batch method, different pH-values tested
Statistical design	No details given
Relevance	
Environmental relevance	Given; influencing parameter such as pH-value are tested.

Weight of evidence	
“Positive”/”Negative” evidence	Results supported by other publications; no negative evidence.

Shushkova et al. (2009)

Title: Sorption and Microbial Degradation of Glyphosate in Soil Suspensions	
Author: T. V. Shushkova, G. K. Vasilieva, I. T. Ermakova, and A. A. Leontievsky	
Reference: Applied Biochemistry and Microbiology, Vol. 45, No. 6, pp. 599–603	
Year: 2009	
Results and conclusion: Sorption and microbial destruction of glyphosate, the active agent of the herbicide Ground Bio, in suspensions of sod-podzol and gray forest soils has been studied. According to the adsorptive values (3560 and 8200 mg/kg, respectively) and the Freundlich constants (K_f: 15.6 and 18.7, respectively), these soils had a relatively high sorption capacity for the herbicide. Inoculation of a native suspension of sod-podzol soil with cells of a selected straindegrader *Ochrobactum anthropi* GPK 3 resulted in a 25.4 % decrease in the total glyphosate content (dissolved and extractable), whereas in a non-inoculated suspension, the loss did not exceed 5.5 %. The potential for the use of a selected bacterial strain in the glyphosate destruction processes in soil systems is demonstrated for the first time.	
Proposed action: Not to be considered as soils outside EU were tested.	
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information on the lower range of K_f-values and on selected microbial strains degrading Glyphosate	
Reliability	Medium
Endpoint	Sorption: K_f, 1/n, R^2; dissipation: amount of Glyphosate in soil suspension
Protocol	Mineralisation: no standard protocol, adsorption isotherms: similar to OECD 106; non-GLP
Test compound	Glyphosate-isopropylammonium salt (CAS-no.: 8641-94-0; product: Ground Bio)
Test system and conditions	Sorption: Batch method, 2 soils, 8 concentrations Dissipation: soil suspensions (sterile, non-sterile; bacterial straindegrader *O. anthropi* GPK 3 and indigenous microbial community.
Statistical design	Sorption: Freundlich Isotherms; Dissipation: triplicate measurements, percentage error less than 12 %, P = 0.95, no DT_{50} calculated
Relevance	
Environmental relevance	Given; influencing parameter such as soil properties reported.
Weight of evidence	
“Positive”/”Negative” evidence	No publication with same bacterial strain used is known, results are plausible; no negative evidence.

Si et al. (2013)

Title: Complex Interaction and Adsorption of Glyphosate and Lead in Soil

Author: Y.-B. SI, Y. XIANG, C. TIAN, X.-Y. SI, J. ZHOU AND D.-M. ZHOU

Reference: Soil and Sediment Contamination, 22:72–84, 2013

Year: 2013

Results and conclusion:
In the study, the adsorption and co-adsorption of Pb and glyphosate were determined on two soils [a red (RS) soil, Udic Ferrisol, and a yellow-brown (YB) soil, Udic Luvisol] of distinctly different chemical characteristics at varying pH conditions. Results indicate that the adsorption of lead and glyphosate strongly depends on soil types: the RS soil, characterized by a relatively high iron/aluminum content but a low pH and organic matter content, shows a much lower adsorption capacity for Pb but a higher sorption for glyphosate than the YB soil. The co-existence of Pb and glyphosate in soils resulted in complex interactions among Pb, glyphosate, Pb-glyphosate complexes, and soil minerals. The presence of glyphosate decreased Pb adsorption on the two soils, which was attributed primarily to the formation of soluble Pb-glyphosate complexes having relatively low affinities to soil surfaces. On the other hand, addition of Pb increased the adsorption of glyphosate on both soils, which was attributed to: (1) a decreased solution pH due to the ion exchange between Pb^{2+} and H^{+} on soil surfaces; and (2) increased sorption sites where Pb was adsorbed and acted as a bridge between glyphosate and the soil. The present study illustrates that the complex interactions among glyphosate, Pb, and soil may have important implications for the mobility and bioavailability of Pb in soil and should thus be considered in future environmental risk assessments.
The Freundlich fitting parameters corresponding to glyphosate adsorption isotherms on the RS and YB soils in the absence or presence of Pb are given in the following:

Soil	Pb^{2+} [mg/L]	k_f	1/n	R
RS	0	1304.60±75.03	0.308±0.016	0.987
	50	1341.60±54.82	0.313±0.011	0.993
	200	1420.30±39.13	0.315±0.008	0.998
YB	0	368.18±9.67	0.377±0.007	0.999
	50	376.73±10.40	0.387±0.008	0.999
	200	385.05±11.33	0.432±0.009	0.999

Proposed action:
Not to be considered as non-European soils were used.

Type of information (critical, high/low weight, supporting, additional):
Low weight, additional information because a non-European soil is used.

Reliability	
Endpoint	K_f, 1/n
Protocol	Not given
Test compound	Glyphosate

Test system and conditions	A surface red soil (RS soil, Udic Ferrisol) and a surface yellow-brown soil (YB soil, Udic Luvisol) were used for the experiments and obtained at a depth of 0–20 cm from Yingtan County, Jiangxi Province, and Feidong County, Anhui Province, in China. Effect of Glyphosate and pH on Lead Adsorption on Soils: Lead adsorption isotherms were determined in batch experiments by mixing the soil (5.0 g) with the Pb stock solution in a background electrolyte concentration of 0.01 mol/L $NaNO_3$. The experiment was performed at three levels of glyphosate (0, 50, and 200 mg/L) at the same background solution. The added Pb concentrations varied from 0, 50, 100, 150, 200, 300, 400, to 500 mg/L. The final volume was made up to 25 mL, which gave a solid to solution ratio of 1:5 (w/v). All experiments were performed in duplicate. The sample tubes were subsequently shaken for 2 h at 25 °C, centrifuged and then filtered through a filter paper. Solution pH was measured following equilibrium. The Pb concentration in the centrifuged solution was determined by atomic absorption spectrophotometry (AAS) using a ThermoElemental SOLAAR M5 spectrometer (Thermo Electron Corp., Verona, WI, USA). The amount of Pb adsorbed was calculated by the difference between that added in the initial solution and that found after equilibrium. Similar experiments were performed to study the effect of pH on Pb adsorption in the presence or absence of glyphosate. In this case, a fixed, final Pb concentration of 200 mg/L was used, and the final solution pH was adjusted from 3 to 9 by adding different volumes of either 0.01 mol/L NaOH or 0.01 mol/L HNO_3 solution. Effect of Pb and pH on Glyphosate Adsorption on Soils: Glyphosate adsorption isotherms in the presence or absence of Pb were determined by mixing the glyphosate and the soil in a background electrolyte solution of 0.01 mol/L $NaNO_3$. The final volume was 25 mL, and the final glyphosate concentrations were 0, 200, 400, 600, 800, 1000, and 1200 mg/L, respectively. Three Pb concentration levels (0, 50, and 200 mg/L) were used to study the effect of Pb on glyphosate adsorption. All experiments were performed in duplicate. Following the equilibration at at 25 °C for 2 h, samples were centrifuged and filtrated through a filter paper. The clear supernatant solution was thus obtained, and pH determined following equilibrium. The glyphosate concentration in the supernatant solution was determined by an Agilent 1100 high-performance liquid chromatography (HPLC) (Agilent Technologies Co. Ltd., Santa Clara, CA, USA) using a C18 Hypersil ODS column (250 mm × 4.6 mm i.d., with a 5 μm particle size). In brief, 1.0 mL supernatant of glyphosate was first derived by reacting with 130 mmol/L p-toluene-sulphonyl chloride in acetonitrile (1:1 by volume) for 5 min at 50◦C. Derivatized samples (20 μL) were then injected into the chromatographic column. The mobile phase consisted of 50 mmol/L sodium phosphate (pH 2.3) in 15 % (v/v) acetonitrile. The analysis proceeded at a flow rate of 1.0 mL/min, and glyphosate detected at 240 nm (Kawai *et al.*, 1991; Abdullah *et al.*, 1995; Forlani *et al.*, 1999). The limit of detection was estimated to be 2×10−10 g, and the minimum determination concentration of glyphosate in soil samples was 0.02 mg/kg. The ranges of average recoveries and coefficient variation of the method were 94.2 %~98.3 % and 0.66 %~3.63 %, respectively. The amount of glyphosate adsorbed was calculated by the difference between that added in the initial solution and that found after equilibrium. Similar experiments were performed to study the effect of pH on glyphosate adsorption in the presence or absence of Pb. In this case, a fixed, final glyphosate concentration of 200 mg/L was used, and the final solution pH was adjusted from 3 to 9 by adding different volumes of either 0.01 mol/L NaOH or 0.01 mol/L HNO_3 solution.
Statistical design	All experiments were performed in duplicate. The Freundlich equation was used to describe the adsorption data. Data from the recovery rate were analyzed by calculation of the means and standard deviations. All experimental data were processed using the statistical software SPSS 11.5. The speciations of Pb and glyphosate at varying pH conditions were calculated using the computer program WinSGW.
Relevance	
Environmental relevance	Given

Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Sørensen et al. (2006)

Title: Sorption, desorption and mineralisation of the herbicides glyphosate and MCPA in samples from two Danish soil and subsurface profiles	
Author: Sebastian R. Sørensen, Anne Schultz, Ole S. Jacobsen, Jens Aamand	
Reference: Environmental Pollution 141, 184-194	
Year: 2006	
Results and conclusion: The vertical distribution of the sorption, desorption and mineralisation of glyphosate and MCPA was examined in samples from two contrasting soil and subsurface profiles, obtained from a sandy agricultural site and a non-agricultural clay rich site. The highest mineralisation of [^{14}C–methylen] glyphosate, with 9.3-14.7 % degraded to $^{14}CO_2$ within 3 months, was found in the deepest sample from the clay site. In the deeper parts of the sandy profile high sorption and low desorption of glyphosate coincided with no or minor mineralisation indicating a limited glyphosate bioavailability. The herbicide was not mineralised under anoxic conditions. Based on the present study the potential for natural attenuation of glyphosate is apparent in both profiles. In sandy locations, such as Fladerne Bæk, glyphosate will most likely not be mobile since preferential flow patterns probably are insignificant and sorption to matrix components will retain the herbicide in the top soil. If the herbicide should bypass the soil zone, however, and hence enter the deeper part of the tested profile, it appeared that the mineralisation potential may be low, but that the majority of the glyphosate will be associated with the matrix.	
Proposed action: Not to be considered as K_d-values for one concentration only are reported. No raw data on mass balances and concentrations in aqueous and solid phases are given, and thus the validity of the study cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): High weight; supportive information on depth-dependent sorption and mineralisation of Glyphosate	
Reliability	Medium
Endpoint	Mineralisation: $^{14}CO_2$-formation. Sorption: K_d-values
Protocol	Mineralisation: similar to OECD 307 (but mineralisation tested only); sorption: according to OECD 106
Test compound	Glyphosate (CAS-no.: 1071-83-6; 97.5 % purity); [^{14}C-methylen] Glyphosate (~95 % radiochemical purity).
Test system and conditions	Mineralisation: airtight glass flasks containing a vial with NaOH to capture $^{14}CO_2$. The aquifer sediments saturated to water holding capacity with natural groundwater. Anoxic mineralisation experiments performed in butyl rubber sealed serum flasks equipped with a base trap similar to the aerobic experiments. Sorption: batch experiment, 96 h, one concentration
Statistical design	Number of replicates not reported; 2 soils sampled at 8 different depths.
Relevance	
Environmental relevance	Given; influencing parameter reported.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Spanoghe et al. (2005)

Title: Rainfastness and adsorption of herbicides on hard surfaces	
Author: Pieter Spanoghe, Johan Claeys, Luc Pinoy and Walter Steurbaut	
Reference: Pest Manag Sci 61:793–798	
Year: 2005	
Results and conclusion: Herbicides are still used to control weeds on hard surfaces, including municipal, private and industrial sites. In this study, three kinds of hard surface were evaluated: asphalt, concrete surface and gravel (fine and coarse). Three herbicides were applied: glyphosate, diuron and diflufenican. At different times after treatment with the herbicides, rainfall was simulated and substance concentration determined in run-off. After this run-off event, the materials were immersed in water to measure desorption which, together with the compound in the run-off, gave a measure of the dislodgable residues. The polar herbicide glyphosate lost 75 % in run-off from asphalt but was adsorbed strongly to soil and concrete pavement.	
Proposed action: Not to be considered as building material and no soils were tested.	
Type of information (critical, high/low weight, supporting, additional): High weight; supportive information on behaviour of Glyphosate having been applied onto building material and subjected to run-off events.	
Reliability	High
Endpoint	Concentration in run-off; K_d-values for building material
Protocol	No standard protocol; non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; product Canyon® was applied).
Test system and conditions	a) runoff-experiments: The hard surfaces were treated with a suspension concentrate containing 112 g/L glyphosate, 71 g/L diuron and 15 g/L diflufenican (Canyon®). Rainfall was simulated and thereafter, run-off was collected from the drain. b) adsorption experiment: kinetics measured; Freundlich isotherm was obtained by adding building material to solutions of differing concentrations of the herbicide. Concentration in the supernatant was analysed. c) desorption experiment: building material obtained after a) was immersed in water for 72 h.
Statistical design	Number of replicates not reported; for Freundlich-isotherms 4 concentrations were measured.
Relevance	
Environmental relevance	Given; influencing parameter reported.
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other publications; no negative evidence.

Strange-Hansen et al. (2004)

Title: Sorption, mineralization and mobility of N-(phosphonomethyl)glycine (glyphosate) in five different types of gravel
Author: Rikke Strange-Hansen, Peter E Holm, Ole S Jacobsen and Carsten S Jacobsen
Reference: Pest Manag Sci 60:570–578
Year: 2004

Results and conclusion:
Cumulative mineralization of [methyl-^{14}C]glyphosate in batch studies was highest in coarse gravel, amounting to 14 % after 4 days at 30 °C and 32 % after 31 days. Mineralization was slowest in the sandy reference soil, amounting to only 2 % after 31 days. The adsorption coefficient (K_d) of glyphosate in gravel ranged from 62 to 164 litre/kg, while that in the sandy reference soil was 410 litre/kg. The results indicate that the relatively low K_d in gravel allows a relatively high rate of glyphosate mineralization by the biomass. When K_d is high, in contrast, mineralization is slow. Lowering the temperature to 10 °C decreased mineralization by 50 % in one of two gravels. The leaching of glyphosate was screened in simple columns of gravel or soil in which precipitation events (20mm over a 2-h period) were simulated on three occasions, starting either immediately after or 2 days after application of glyphosate. [^{14}C]Glyphosate was applied as a tracer mixed with the commercial product Roundup Garden at the recommended rate of 2.4 kg glyphosate/ha, equivalent to 1µgg^{-1} soil. The highest concentration of [^{14}C] compounds (expressed in terms of glyphosate concentration) in leachate from the columns exceeded 1300 µg litre^{-1}, and was detected in rounded gravel after the first rain event. No glyphosate was detected in leachate from the sandy reference soil.

Proposed action:
Not to be considered as raw data on mass balances and on concentrations in the aqueous and solid phases are not reported. K_f-values cannot be calculated from the published data. Furthermore, the validity of the study cannot be proven.

Type of information (critical, high/low weight, supporting, additional):
Low, additional information

Reliability	Medium
Endpoint	K_d = distribution coefficient; [^{14}C]carbon dioxide
Protocol	According to the OECD guidelines (106), but with a modified soil: solution ratio of 2 instead of 5; non-GLP,
Test compound	[methyl-^{14}C]Glyphosate (specific activity 1.08MBq mmol^{-1}; radio-chemical purity >99 %); unlabelled glyphosate (purity 98 %); for column experiments, Roundup Garden (commercial SL formulation containing 120 g litre^{-1} glyphosate (isopropylamine salt)); five types of gravel and a sandy agricultural reference soil; CAS-no.: 1071-83-6
Test system and conditions	Batch studies: 1) Sorption: glyphosate/[^{14}C]glyphosate concentration of 0.6 mg/kg (192 Bq), rotated for 96 h; 2) Mineralization in flasks: glyphosate/[^{14}C]glyphosate concentration of 16.9 mg/kg (0.1mM) and 833 Bq, final moisture level equivalent to 80 % of WHC, incubated at 30 ◦C in the dark for 31 days, repeated on two types of gravel incubated at 10, 20 and 30 ◦C; 3) Leaching studies: Two columns for each substrate, and each column was exposed to two different simulated precipitation events (Table 3). [^{14}C]Glyphosate (1733 Bq in short columns, and 5666 Bq in tall columns) mixed with Roundup Garden (recommended rate of 2.4 kg glyphosate/ha), Over the next 6 days the columns were subjected to three simulated precipitation events at 20 ◦C, trapping the [^{14}C]carbon dioxide, total effluent collected 1 day after each precipitation event, determination of residual [^{14}C] compounds.
Statistical design	Analyses in triplicate; K_d value represents one measurement of glyphosate in the solution, assuming linearity between glyphosate adsorption and glyphosate concentration in the solution
Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications.

Todorovic (2009)

Title: BEHAVIOR OF ORGANIC POLLUTANTS IN THE SOIL ENVIRONMENT. SPECIAL FOCUS ON GLYPHOSATE AND AMPA
Author: Gorana Rampazzo Todorovic
Reference: "Qualità of the Environment" series assembles the scientific communications presented during the "Air, Water, and Soil Quality" International Congress held at Imola (Imola) on 24th and 25th of June 2009. ISBN 10: 88-901261-7-5 ISBN 13: 978-88-901261-7-8
Year: 2009
Results and conclusion: The aim of the meeting was to take account of the present quality of the air water-soil system, comparing Italian realities with those in other countries of the European Union and to make known the most efficient measures and instruments for fighting ecosystem degradation and the waste of resources. In this document, the state of the art regarding the main mechanisms, processes and factors governing the fate and behaviour of organic contaminants in the soil-groundwater system is reviewed. The behaviour of organic contaminants in soils is generally governed by a variety of complex dynamic physical, chemical and biological processes, including sorption–desorption, volatilization, chemical and biological degradation, uptake by plants, run-off, and leaching. These processes directly control the transport of contaminants within the soil and their transfer from the soil to water, air or food. The relative importance of these processes varies with the chemical nature of the contaminant and the properties of the soil. Both the direction and rate of these processes depend on the chemical nature of the organic contaminant and the chemical, biological, and hydraulic properties of the soil. Better understanding of the behaviour of glyphosate is needed (e.g. adsorption conditions, environmental influence, specific soil parameters, soil microbes behaviour) for a better risk assessment of environmental pollution.
Proposed action: Not to be considered as the article is a review and no raw data are published.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only.

Reliability	Medium
Endpoint	Soil properties and the K_d-values for Glyphosate for different soils and silica Sand based on literature data
Protocol	-
Test compound	-
Test system and conditions	Information about: Dissipation ways of the organic pollutants; Soil parameters governing the glyphosate fate in environment; Glyphosate sorption in soil; Glyphosate sorption on iron oxides; Biodegradation of glyphosate; Glyphosate biodegradation pathways; Environmental fate of metabolites; Influence of glyphosate on shallow aquifers/aquatic/marine ecosystems
Statistical design	-
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Summary of results of other publications.

Vereecken (2005)

Title: Review Mobility and leaching of glyphosate: a review
Author: Harry Vereecken
Reference: Pest Manag Sci 61:1139–1151
Year: 2005

Results and conclusion: The purpose of this review is to present and discuss the state of knowledge with respect to the mobility and leaching of glyphosate from agricultural soils. Specific attention is given to the adsorption behaviour of glyphosate and the analysis of available studies on glyphosate transport. In addition, there are a number of experimental and numerical studies indicating that other strongly sorbing substances may be transported rapidly to the subsurface. The experimental studies analysed in the paper encompass column-, lysimeter and field-scale experiments on glyphosate transport. The experimental findings, combined with transport studies on other strongly sorbing pesticides in the literature, support the hypothesis that transport of glyphosate may be caused by an interaction of high rainfall events shortly after application on wet soils showing the presence of preferential flow paths. Concentrations of glyphosate in European groundwater have been reported occasionally but monitoring is still limited.	
Proposed action: Consider as additional information. The article presents an overview on the state of the art with respect to mobility and leaching of glyphosate in agricultural soils.	
Type of information (critical, high/low weight, supporting, additional): Medium; overview and summary of endpoints	
Reliability	Medium
Endpoint	Freundlich exponent, K_f; Freundlich distribution coefficient; summary data for field sites with respect to leaching of glyphosate
Protocol	-
Test compound	-
Test system and conditions	Information about: adsorption of Glyphosate (on clay minerals; on soil organic matter, on soil oxides and hydroxides); mobility and leaching of Glyphosate (on the laboratory scale, on the lysimeter scale, on the field scale); occurrence in groundwater
Statistical design	-
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications.

Waiman et al. (2012)

Title: A simple and rapid spectrophotometric method to quantify the herbicide glyphosate in aqueous media. Application to adsorption isotherms on soils and goethite
Author: Carolina V. Waiman, Marcelo J. Avena, Mariano Garrido, Beatriz Fernández Band, Graciela P. Zanini
Reference: Geoderma 170, 154–158
Year: 2012
Results and conclusion: This article presents a simple, fast and low cost UV–vis spectrophotometric method to quantify glyphosate. This method can be used to perform adsorption isotherms on soils and metal oxides. It comprises a derivatization step and further measurement of the absorbance at 265 nm. The trueness of the results is validated using Ultra Performance Liquid Chromatography with tandem mass spectrometry detection (UPLC-MS/MS) as a reference method. The proposed spectrophotometric method is able to quantify glyphosate in the concentration range from 0.084 to 21.8 mg/L. This range is suitable to construct reliable adsorption isotherms. Examples of adsorption isotherms on goethite at pH 4.5 and a soil sample at pH 4.5, 6.0 and 8.0 are given. Interferences caused by dissolved organic matter can be corrected at least up to an organic matter concentration of 12 mg/L.
Proposed action: Not to be considered as the article focuses on method development. Raw data on mass balances and concentrations in the aqueous and solid phases are not sufficient; the validity of the results cannot be proven.
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information

Reliability	Medium
Endpoint	K_f and 1/n
Protocol	Standard, OECD
Test compound	Analytical-standard glyphosate (PESTANAL, 99.729 %), CAS-no.: 1071-83-6. Analytical reagent-grade disodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$), 9-fluorenyl methoxycarbonyl chloride (FMOC-Cl) for synthesis; one soil and goethite
Test system and conditions	Adsorption isotherms: batch equilibration technique at room temperature; concentration range from 7 mg L^{-1} to 190 mg L^{-1}; Isotherms were performed at pH 4.5, 6.0 and 8.0. Quantification of glyphosate was performed by UV–vis spectrophotometry after a derivatization step with FMOC-Cl in alkaline media
Statistical design	Freundlich isotherms
Relevance	
Environmental relevance	Given. The parameters influencing the endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Waimann et al. (2013)

Title: A real time in situ ATR-FTIR spectroscopic study of glyphosate desorption from goethite as induced by phosphate adsorption: Effect of surface coverage
Author: C. V. Waiman, M. J. Avena, A. E. Regazzoni, G. P. Zanini
Reference: Journal of Colloid and Interface Science 394 (2013) 485–489
Year: 2013
Results and conclusion: The desorption of glyphosate from goethite as induced by the adsorption of phosphate was investigated by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy in combination with adsorption isotherms. Desorption of glyphosate was very low in the absence of phosphate. Addition of phosphate promoted glyphosate desorption. At low initial surface coverages, added phosphate adsorbed on free surface sites, mainly, displacing a small amount of glyphosate. At high initial surface coverages, on the contrary, phosphate adsorption resulted in a significant glyphosate desorption. In the latter conditions, the ratio desorbed glyphosate to adsorbed phosphate was 0.60. The desorption process can be explained by assuming that phosphate adsorbs first forming a monodentate mononuclear complex, which rapidly evolves into a bidentate binuclear complex that displaces glyphosate.
Proposed action: Consider as additional information.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.

Reliability	
Endpoint	Glyphosate desorption from goethite as induced by phosphate adsorption
Protocol	Not given
Test compound	Glyphosate, phosphate

Test system and conditions	Goethite synthesis and characterization: Goethite particles were synthesized as described by Puccia et al, following the methodology proposed by Atkinson *et al.* Adsorption isotherms: Glyphosate and phosphate adsorption isotherms were obtained by batch equilibration experiments. They were performed by adding 0.2 mL of the stock goethite suspension (22.10 g/L) to 15 mL polypropylene centrifuge tubes, to which 9.8 mL of an aqueous solution of known concentration of either glyphosate or phosphate was added; the concentration of the background electrolyte (KCl) was 0.1 M. The pH of these dispersions was adjusted to 4.5 and kept constant by adding a few microliters of either KOH or HCl solutions. The tubes were shaken overnight with an end-overend rotator, and then, the supernatants separated by centrifugation. The concentration of glyphosate in the supernatants was measured by the UV-Vis spectrophotometric method proposed by Waiman *et al.* The concentration of phosphate was quantified by the molybdenum blue method proposed by Murphy and Riley. UV–Vis spectra were recorded with an Agilent 8453 UV–Vis diode array spectrophotometer equipped with a 1 cm Hellma quartz cell. The amount of glyphosate and phosphate adsorbed by goethite was calculated solving the mass balance of the systems. ATR-FTIR spectroscopy: ATR-FTIR spectra were obtained using a Nicolet Nexus 470 FTIR spectrometer equipped with a DTGS detector, a SMART-ARK ATR accessory and a ZnSe crystal (area: 10 × 72 mm, incident angle: 45º, total reflections: 12).
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Wang (2005)

Title: Effects of phosphate on the adsorption of glyphosate on three different types of Chinese soils	
Author: WANG Yu-Jun, ZHOU Dong-Mei, SUN Rui-Juan	
Reference: Journal of Environmental Sciences Vol. 17, No.5, 711-715	
Year: 2005	
Results and conclusion: This paper studied the effects of phosphate on the adsorption of glyphosate on three different types of Chinese soils including two variable charge soils and one permanent charge soil. The results indicated that Freundlich equations used to simulate glyphosate adsorption isotherms gave high correlation coefficients (0.990-0.998) with K values of 2751, 2451 and 166 for the zhuanhong soil (ZH soil, Laterite), red soil (RS, Udic Ferrisol) and Wushan paddy soil (WS soil, Anthrosol), respectively. The more the soil iron and aluminium oxides and clay contained, the more glyphosate adsorbed. The presence of phosphate significantly decreased the adsorption of glyphosate to the soils by competing with glyphosate for adsorption sites of soils. Meanwhile, the effects of phosphate on adsorption of glyphosate on the two variable charge soils were more significant than that on the permanent charge soil. When phosphate and glyphosate were added in the soils in different orders, the adsorption quantities of glyphosate on the soils were different, meaning a complex interaction occurred among glyphosate, phosphate and the soils.	
Proposed action: Not to be considered as non-EU soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by inorganic phosphate	
Reliability	Medium
Endpoint	K values obtained by Freundlich equations; correlation coefficients
Protocol	Partly similar to OECD 106, non-GLP

Test compound	Glyphosate (purity not given), CAS-no.: 1071-83-6; phosphate (analytical grade):
Test system and conditions	Batch experiments, 3 soils, Glyphosate adsorption isotherms on the soils with and without phosphate were performed
Statistical design	Measurements in duplicate; Freundlich isotherms
Relevance	
Environmental relevance	Applying glyphosate in soil containing higher content of phosphate will possibly increase the environmental risk of glyphosate transfening from soil to groundwater and surface water.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence

Wang et al. (2006)

Title: Cosorption of zinc and glyphosate on two soils with different characteristics	
Author: Yu-Jun Wang, Dong-Mei Zhou, Rui-Juan Sun, Long Cang, Xiu-Zhen Hao	
Reference: Journal of Hazardous Materials A137, 76–82	
Year: 2006	
Results and conclusion: Cosorption of Zn and glyphosate on a Red soil (RS, Udic Ferrosols) and a Wushan soil (WS, Anthrosol) was studied. In comparison with the WS, the RS has less adsorption capacity for Zn and higher for glyphosate. The presence of glyphosate decreased Zn adsorption on the two soils, which are resulted from the decreased equilibrium solution pH caused by the added glyphosate, and also the formation of water-soluble complexes of glyphosate with solution Zn^{2+} that had lower affinity to soil surface in comparison with Zn^{2+} itself. Such effect is more significant on the RS than on the WS, mainly because of the less adsorption quantity of Zn on the former one. On the contrary, the presence of Zn increased the adsorption quantities of glyphosate on the RS and WS, which is resulted from the decreasing pH value of the equilibrium solution caused by Zn^{2+} exchange with H^+ ions of soil surface. Such results suggest that glyphosate in field may increase the mobility and bioavailability of Zn and correspondingly increase its environmental risk.	
Proposed action: Not to be considered as non-EU soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by zinc	
Reliability	Medium
Endpoint	No K values, only adsorption isotherms
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Glyphosate (purity not given); CAS-no.: 1071-83-6
Test system and conditions	Glyphosate adsorption isotherm on two soils in the absence and presence of Zn; 7 concentrations of glyphosate and 3 concentrations of Zn
Statistical design	Measurements performed in replicate, no further statistic
Relevance	
Environmental relevance	Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Wang et al. (2009)

Title: Adsorption Kinetics of Glyphosate and Copper(II)-Alone and Together on Two Types of Soils
Author: Yu-Jun Wang, Yu-Xia Cui, Dong-Mei Zhou, Shen-Qiang Wang, An-Yun Xiao, Ru-Hai Wang
Reference: Soil Sci. Soc. Am. J. 73(6): 1995-2001. doi:10.2136/sssaj2008.0360
Year: 2009
Results and conclusion: Adsorption kinetics of glyphosate and Cu(II) alone and together were studied using a continuous flow experimental setup on two soils with different characteristics at pH5.5. Four kinetic models, i.e., the Lagergren first-order, pseudo-second-order, Elovich, and power function equations, were successfully used to describe their adsorption kinetics. Among the four models, the Lagergren first-order kinetic model fit the experimental data of glyphosate and Cu(II) adsorption the best. Glyphosate significantly increased the adsorption quantity of Cu(II) on the Red soil (a Hapludult or Udic Ferrosol), due to the fact that Cu(II) was adsorbed on the sites where glyphosate had been strongly adsorbed. Glyphosate decreased the adsorption of Cu(II) on the Wushan soil (a Haplaquept or Anthrosol), however, because adsorption of glyphosate on this soil was weak and the complex of glyphosate and Cu(II) tended to be highly soluble in water, thus preventing Cu(II) from exchanging with Ca^{2+} and Mg^{2+} ions on the soil surface. On the other hand, the presence of Cu(II) decreased the adsorption of glyphosate on both soils, which may be attributed to the lower affinity of the Cu(II)–glyphosate complex to the soils than glyphosate alone.
Proposed action: Not to be considered as non-EU soils were tested.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by inorganic Cu(II)

Reliability	Medium
Endpoint	Correlation coefficients (R^2); Kinetic parameters and the normalized standard deviation
Protocol	Non-GLP
Test compound	Glyphosate (purity not given), CAS-no.: 1071-83-6
Test system and conditions	Column experiments; one glyphosate concentration; with or without Cu(II); soils: surface (0–20 cm) Red soil (low pH (4.95) and high Fe oxide content) and a surface (0–20 cm) Wushan soil (high organic matter content, CEC, and pH (7.20)
Statistical design	Lagergren first-order, pseudo-second-order, Elovich, and power function equations
Relevance	
Environmental relevance	Not given; parameter influencing endpoints are not measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Xu et al. (2009)

Title: Land Use and Riparian Effects on Prairie Wetland Sediment Properties and Herbicide Sorption Coefficients
Author: Dani Xu, Sheila Meyer, Jeanette Gaultier, and Annemieke Farenhorst, Dan Pennock
Reference: J. Environ. Qual. 38:1757–1765
Year: 2009

Results and conclusion: Bottom sediments were sampled in 0- to 5- and 5- to 10-cm sections from 17 wetlands under five different land use classes. Sediments were analyzed for total organic carbon (TOC), total inorganic carbon (TIC), pH, electrical conductivity, exchangeable cations (EXCAT), total cation exchangeable capacity (CEC), and percent clay (%clay). Sediment herbicide sorption partition coefficient (K_d) was measured for trifluralin, atrazine, 2,4-D, and glyphosate. The sorption of the herbicides in the sediment increased in the order of 2,4- D < atrazine < glyphosate < trifluralin. The sorption of 2,4-D, atrazine, and trifluralin was positively correlated to TOC, EXCAT, and CEC but negatively correlated to %clay. Glyphosate sorption was negatively correlated to pH, TIC, EXCAT, and %clay. Overall, wetland sediments that were recently cultivated (ECNR and E4G) had lower TOC, TIC, EC, EXCAT, CEC, and K_d values (2,4-D, trifluralin, and atrazine) than sediments that had not been recently cultivated (ECR, E20G, and SP).
Proposed action: Not to be considered as raw data on mass balances and concentrations in the aqueous and solid phases are not given. Thus, the validity of the study cannot be proven.
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information about the influence of TOC etc of the sorption

Reliability	High
Endpoint	Sorption partition coefficient K_d (L/kg); K_{OC}; Data as mean ± SE
Protocol	similar to OECD 106 , non-GLP
Test compound	Glyphosate (99 % purity; Chem Service, West Chester, PA), and (phosphonomethyl-^{14}C) glyphosate (95 % purity, specific activity 344.1 MBq mmol^{-1}; Sigma-Aldrich Co.); CAS-no.: 1071-83-6
Test system and conditions	Batch equilibrium experiments, set to 20 °C until equilibrated (24 h)
Statistical design	In duplicate, Kolmogorov-Smirnov normality test, nonparametric Kruskal-Wallis test
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Yu and Zhou (2005)

Title: Adsorption characteristics of pesticides methamidophos and glyphosate by two soils
Author: Ying Yu, Qi-Xing Zhou
Reference: Chemosphere 58, 811–816
Year: 2005
Results and conclusion: Contributions of organic matter and minerals in soil were evaluated by comparing changes in adsorption of methamidophos (MDP) and glyphosate (GPS) before and after removal of organic matter from argaltoll (mollisol) and typustalf (alfisol) soils. Adsorption isotherms of MDP and GPS by the two soils comforted to Freundlich equation, and the adsorption capacity of GPS by argaltoll soil was higher than that of MDP. Due to the removal of organic matter from soils, K_f values of MDP and GPS adsorbed by argaltoll soil, which were calculated from Freundlich equations and the measure of adsorption capacity, decreased by 46.1 % and 75.0 %, and these by typustalf soil decreased by 34.9 % and 52.5 %, respectively. Results from this study suggested that soil organic matter made greater contributions to adsorption of GPS, but soil minerals could provide more available adsorption sites for MDP.
Proposed action: Not to be considered as non-EU soils were tested.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information about the influence of organic matter

Reliability	High

Endpoint	Freundlich coefficients K_f, n_f (measure of the nonlinearity of the isotherm; indicates concentration dependence of adsorption), correlation coefficient rf and standard error Se
Protocol	Similar to OECD 106, non-GLP
Test compound	Commercial products: the emulsified oil containing 40 % of MDP (pH 6.4) and water agent containing 10 % of GPS salt (pH 6.1) (from the Chemical Plant of the Zhejiang Technical University and the Jiangnan Chemical Plant in Zhenjiang, Jiangsu Province); CAS-no.: 1071-83-6
Test system and conditions	Batch-equilibration technique; two unpolluted surface soils; with or without organic matter; different concentration of MDP or GPS; shaken at 25 ± 1 °C for 24 h
Statistical design	Three replicates; Freundlich isotherms;
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Yu et al. (2005)

Title: Effects of methamidophos and glyphosate on copper sorption-desorption behavior in soils	
Author: YU Ying, ZHOU Qixing, HE Zhenli	
Reference: Science in China Ser. C Life Sciences Vol.48 Supp. 1 67–75	
Year: 2005	
Results and conclusion: A batch-equilibration technique was employed to study the impact of two organophosphorus pesticides methamidophos (MDP) and glyphosate (GPS) on copper (Cu^{2+}) sorption-desorption for phaeozem and burozem collected from Northeastern China. The addition of the two pesticides decreased Cu^{2+} sorption, increased Cu^{2+} desorption and prolonged the equilibrium time of Cu^{2+} sorption-desorption. But GPS appeared to exert a stronger influence on Cu^{2+} sorption-desorption due to its stronger complexion with Cu^{2+}. When MDP was added, Cu^{2+} sorption-desorption was linearly correlated with MDP treatment concentrations. But in the presence of GPS, Cu^{2+} sorption first underwent a rapid decrease period, and then slowly tended towards a steady period. The reverse pattern could be found for Cu^{2+} desorption in the presence of GPS. Without pesticides and with the existence of MDP, Cu^{2+} sorption-desorption kinetics was well conformed to two-constant equation and Elovich equation. But that was not the case for Cu^{2+} desorption kinetics in the presence of GPS although its sorption could be also described by these two equations.	
Proposed action: Not to be considered as non-EU soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on complex reaction	
Reliability	Medium
Endpoint	Partition coefficient Kp (L/kg), Standard deviation
Protocol	Similar to OECD 106, non-GLP
Test compound	Emulsified oil containing 40 % MDP (pH 6.37) (Zhejiang Industry University Chemical Plant); 10 % GPS salt solution (pH 6.12) Jiangnan Chemical Plant); CAS-no.: 1071-83-6
Test system and conditions	Batch-equilibration technique; two unpolluted soils different concentrations of MDP or GPS; sorption of Cu^{2+}; shaken for 24 h; desorption experiment; kinetic study equilibrated for 1, 2, 5, 10, 15, 20, 40, 80, 120, 240 min
Statistical design	Three replicates

Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Zhao et al. (2009)

Title: Glyphosate mobility in soils by phosphate application: Laboratory column experiments	
Author: Bingzi Zhao, Jiabao Zhang, Jiandong Gong, Hui Zhang, Congzhi Zhang	
Reference: Geoderma 149: 290-297	
Year: 2009	
Results and conclusion: (1) phosphate application might induce system pH decrease, and (2) the overall glyphosate mobility by phosphate application might depend on the relative contribution of two competing processes: increase in glyphosate adsorption from pH decrease versus reduced glyphosate adsorption from competitive adsorption between phosphate and glyphosate for sorption sites available. To test these two hypotheses, laboratory batch and column experiments using a miscible displacement approach were conducted on two Primosols and one Anthrosols, to investigate, respectively, (1) pH-dependent glyphosate adsorption onto the three studied soils, and (2) glyphosate leaching and mobility in soil columns as influenced by phosphate application. Our results showed that glyphosate adsorption consistently decreased with increase in system pH. The effect of phosphate application on glyphosate mobility varied with soil type. We conclude that phosphate application can cause system pH change with various extents in the soil, which subsequently contribute to glyphosate mobility in different degree.	
Proposed action: To be considered as additional information for the endpoint sorption and mobility. The article presents basic research and understanding of pH and phosphate influence. Furthermore, raw data on mass balances and concentrations in the aqueous and solid phases are not reported and thus, the validity cannot be proven.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information of mechanisms of glyphosate mobility in the presence of phosphate	
Reliability	Medium
Endpoint	Breakthrough curves (BTCs)
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Glyphosate (99.9 % purity); CAS-no.: 1071-83-6
Test system and conditions	1) batch experiments, shaken for 20 h at 25 °C; three soils 2) column leaching experiments, 6 column experiments including with or without phosphate (two concentrations), and 3 levels of glyphosate introduced;
Statistical design	Duplicate samples
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Zhou et al. (2004)

Title: Adsorption and cosorption of cadmium and glyphosate on two soils with different characteristics	
Author: Dong-Mei Zhou, Yu-Jun Wang, Long Cang, Xiu-Zhen Hao, Xiao-San Luo	
Reference: Chemosphere 57, 1237–1244	
Year: 2004	
Results and conclusion: Adsorption and cosorption of cadmium and glyphosate on a Wushan soil (WS soil, Anthrosol) and a Zhuanhong soil (ZH soil, Udic Ferrisol) as affect by solution pH were studied by means of batch adsorption experiments. It indicated that the adsorption quantity of Cd or glyphosate was highly relevant to soil characteristics. The WS soil had higher adsorption capacity of Cd than the ZH soil, due to its high organic matter content and cation exchange capacity (CEC). In contrast, the adsorption quantity of glyphosate on the WS soil was less than that on the ZH soil, because the WS soil has lower iron and aluminum oxides content but higher pH than the ZH soil. The herbicide glyphosate affected Cd adsorption on the two soils when they coexisted in a same soil solution, which was attributed to a glyphosate-induced pH-decrease and the corresponding decline in negative surface charges of the soil. Besides that, glyphosate reacted with solution Cd to form the water-soluble complexes that had lower affinity to soil surface in comparison with Cd itself. On the other hand, the presence of Cd in the soil solution also affected the adsorption of glyphosate on the soils. The presence of Cd increased adsorption quantity of glyphosate on the WS and ZH soils, which was resulted from the decrease of equilibrium solution pH caused by Cd^{2+} exchange with H^{+} ions of soil surface. In addition to that, glyphosate adsorption possibly takes place on sites where Cd was previously adsorbed and acted as a bridge between the soil and glyphosate.	
Proposed action: Not to be considered as non-European soils were tested.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on adsorption influenced by inorganic cadmium and by pH	
Reliability	High
Endpoint	Freundlich equations and their correlation coefficients
Protocol	Similar to OECD 106; non-GLP
Test compound	Glyphosate (purity not given); CAS-no.: 1071-83-6
Test system and conditions	1) Cadmium adsorption isotherms in the presence and absence of glyphosate; 2) Effect of pH on Cd adsorption in the presence and absence of glyphosate; 3) Glyphosate (different concentrations) adsorption isotherm on the soils in the presence and absence of cadmium, shaken for 2h at 25 °C; 4) Effect of pH on glyphosate adsorption in the presence and absence of cadmium
Statistical design	Two replicates; Freundlich equations; two soils
Relevance	
Environmental relevance	Given; parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

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Detailed description of open literature – Column leaching

Barrett and McBride (2007)

Title: PHOSPHATE AND GLYPHOSATE MOBILITY IN SOIL COLUMNS AMENDED WITH ROUNDUP	
Author: Katherine A. Barrett and Murray B. McBride	
Reference: Soil Science;172:17–26	
Year: 2007	
Results and conclusion: Sorption of glyphosate and competitive desorption of phosphate in soils has been measured in column leaching experiments. Soils representing a wide range of physical and chemical properties, including total and soluble P, were included in the study. The results suggest that glyphosate sorption does not necessarily result in PO^{3-} dissolution and that there is only limited competition for sorption sites between glyphosate and PO_4^{3-}. Strong glyphosate sorption on high-organic matter soils indicates bonding of this anion by a metal bridge to organic functional groups.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required. Data on sorption/desorption are sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to endpoint leaching, soil column leaching	
Reliability	Medium
Endpoint	Concentration of glyphosate in the leachate, total P and PO_4^{3-} in the leachate.
Protocol	No standard protocol followed, non-GLP
Test compound	Commercial Roundup (containing 27 % active ingredient), CAS-no.:1071-83-6
Test system and conditions	Soil column studies with homogenized soils. Air-dried soil packed into polycarbonate tubes (width of 5.5 cm and height of 10 cm, with soil depth of approximately 6 cm). Application of roundup, ageing for 24 h, 100 mL leachate.
Statistical design	Duplicates, StatView software, $P < 0.05$ are considered to be significantly different.
Relevance	
Environmental relevance	Given. Influencing parameter are reported and considered.
Weight of evidence	
"Positive"/"Negative" evidence	Results of other studies with identical design not known. No negative evidence.

Barrett and McBride (2006)

Title: Trace Element Mobilization in Soils by Glyphosate	
Author: K. A. Barrett and M. B. McBride	
Reference: Soil Sci. Soc. Am. J. 70: 1882–1888	
Year: 2006	
Results and conclusion: No significant increases in elemental leaching were detected in mineral and organic soils with normal background concentrations of heavy metals and phosphor.	
Proposed action: Not to be considered for the endpoint sorption and leaching as desorption of trace elements is measured.	
Type of information (critical, high/low weight, supporting, additional): Low weight may be additional information to already existing.	
Reliability	Medium
Endpoint	Trace element concentrations in soil column leachates.
Protocol	Non-GLP, no standard protocol
Test compound	Reagent-grade glyphosate [N-(phosphonomethyl)glycine, 96 %), CAS-no.:1071-83-6
Test system and conditions	Tendency for glyphosate to mobilize Cu and other elements was tested in soil leaching experiments by applying glyphosate alone or complexed with Cu to mineral and organic soil columns (10 cm height, 1.8 cm i.d.) and measuring the concentrations of these elements in the leachates.
Statistical design	ANOVA analysis of variance, 6 soils tested, single test (?)
Relevance	
Environmental relevance	Given, environmental parameter reported.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with identical design and objective not known. No negative evidence.

Candela et al. (2007)

Title: Laboratory studies on glyphosate transport in soils of the Maresme area near Barcelona, Spain: Transport model parameter estimation	
Author: L. Candela, J. Álvarez-Benedí, M.T. Condesso de Melo, P.S.C. Rao	
Reference: Geoderma 140, 8–16	
Year: 2007	
Results and conclusion: Batch and column experiments were performed using two soils collected from the Maresme area near Barcelona, Spain. Measured batch sorption isotherms for glyphosate conformed to the Freundlich model. The sorption coefficients ranged from 93 to 154, suggesting that it is strongly bound to soil. Glyphosate breakthrough curves measured during steady, saturated water flow in soil columns showed asymmetrical behavior, which was attributed to non-equilibrium sorption and the presence of a sink due to irreversible sorption loss. Loss of glyphosate increased with residence times, with longer columns or with slower pore-water velocities and breakthrough occurred earlier than predicted by retardation factors calculated from batch data. A two-site equilibrium/kinetic sorption model coupled with first-order sinks was fitted to the observed glyphosate breakthrough curves to estimate the rate parameters for non-equilibrium sorption and irreversible sorption. The transport parameters obtained by numerical simulation suggest that glyphosate sorption is a kinetic process depending on the pore-water velocities and the residence time of soil solution.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required.	
Type of information (critical, high/low weight, supporting, additional): Sorption experiments: low weight, no additional information to already existing. Column experiments: low weight, supportive information give insight into the sorption kinetic processes	

Reliability	Medium
Endpoint	K_f-values, 1/n, R^2; column break through curves
Protocol	Adsorption batch experiment: comparable to OECD 106, however, OECD not cited; column experiment: similar to soil leaching (OECD 312), non-GLP
Test compound	Glyphosate, HPLC grade, CAS-no.:1071-83-6
Test system and conditions	Batch experiments comparable to OECD 106, shaking for 24 hours, 25 °C. Column transport experiments: 5 columns, 2 different lengths, 2 soils, various pore water velocities.
Statistical design	Batch experiments: five concentrations, duplicate measurements, 2 soils. Sorption isotherms
Relevance	
Environmental relevance	Given, influencing parameter well considered.
Weight of evidence	
"Positive"/"Negative" evidence	Other reliable studies support the information on sorption behaviour. No negative evidence.

Dousset et al. (2004)

Title: Transfer of hexazinone and glyphosate through undisturbed soil columns in soils under Christmas tree cultivation	
Author: S. Dousset, C. Chauvin, P. Durlet, M. Thevenot	
Reference: doi:10.1016/j.chemosphere.2004.06.007	
Year: 2004	
Results and conclusion: The leaching of glyphosate (N-(phosphono-methyl-glycine)) through structured soil columns was studied using one loamy sand and two sandy loams from sites currently under Christmas tree cultivation in the Morvan. The three soils were cultivated sandy brunisol. After 160 mm of simulated rainfall applied over 12 days, less than 0.01 % of applied Glyphosate appeared in the leachate. The mobility was greater in the soils with higher gravel contents, coarser textures, and lower organic carbon contents. Moreover, glyphosate migration seems negatively correlated not only to soil organic carbon, but also to aluminium and iron contents of soils. The surface water contamination with glyphosate via the horizontal subsurface flow in upper centimetres of soil appears unlikely.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on soil column leaching	
Reliability	Medium
Endpoint	Residues in leachate
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (> 98 % purity), CAS-no.:1071-83-6, AMPA, CAS-N.:1066-51-9
Test system and conditions	Undisturbed soil column leaching experiments, 15 cm inner diameter, 20 cm length, leaching experiment 48 h after Glyphosate application
Statistical design	3 sampling sites, 2 columns per site
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Information supported by other publications; no negative evidence.

Dousset et al. (2007)

Title: Facilitated Transport of Diuron and Glyphosate in High Copper Vineyard Soils	
Author: Sylvie Dousset, Astrid Jacobson, Jean Baptiste des Sogne, Nathalie Guichard, Philippe Baveye and Francis Andreux	
Reference: Environ. Sci. Technol., 41, 8056–8061	
Year: 2007	
Results and conclusion: The effect of copper on the leaching of glyphosate through a granitic and a calcareous soil was studied using sieved-soil columns. Each soil was enriched with copper sulphate to obtain soil copper concentrations of 125, 250, 500, and 1000 mg/kg. Glyphosate leaching was influenced by soil pH and copper concentration. In the calcareous soil glyphosate leaching decreased as copper levels increased. In the granitic soil glyphosate leaching increased as copper levels increased. The shapes of the copper elution curves in presence of glyphosate were similar to shapes of the glyphosate curves, suggesting the formation of Cu-glyphosate complexes that leach through the soil. Increasing copper concentrations reduce glyphosate leaching through calcareous soils, and conversely, increases glyphosate leaching through granitic soils. Our findings suggest that the risk of groundwater contamination by glyphosate increases in granitic soils with elevated copper concentrations.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required. Data on sorption/desorption are sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information because leaching through soil columns is not needed in the monograph due to comprehensive information on sorption. K_d-values are in the same range as already obtained results; furthermore, no K_f-values have been measured.	
Reliability	Medium for soil leaching; low for sorption
Endpoint	K_d-values, concentrations in leachate, break-through curves
Protocol	Batch experiments: comparable to tier II of OECD 106; soil column leaching: comparable to OECD 312. All non-GLP.
Test compound	Glyphosate (N-(phosphono-methyl-glycine; >98 %purity), ^{14}C-glyphosate (99.7 % radiochemical purity), CAS-no.:1071-83-6
Test system and conditions	Batch experiments to determine K_d-values for several Cu-concentrations; soil column leaching experiments using columns of 6 cm length and inner diameter of 6.8 cm.
Statistical design	2 soils, triplicate measurement per Cu-concentration for the batch-experiments; single measurements per Cu-concentration for soil column leaching experiments.
Relevance	
Environmental relevance	Given, influences of environmental variables on sorption and leaching are investigated.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Gjettermann et al. (2011)

Title: Kinetics of Glyphosate Desorption from Mobilized Soil Particles	
Author: B. Gjettermann, C. T. Petersen, S. Hansen, C. Bender Koch, M. Styczen	
Reference: Soil Sci. Soc. Am. J. 75 (2), 434–443	
Year: 2011	
Results and conclusion: Desorption of glyphosate [N-(phosphonomethyl)glycine] on mobilized particles from column leaching was investigated. Particles leached by free drainage from the bottom and particles mobilized by splash erosion and collected next to the top of the column. Glyphosate concentrations in the leachate were determined and values of the Damköhler number were estimated. It was concluded that desorption kinetics are important for evaluating the significance of dissolved and particle-facilitated transport of glyphosate. To quantify particle-facilitated glyphosate transport, the water and solid phases in the leachate should consequently be separated within a few minutes after leaching.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required. Data on sorption/desorption are sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, details on dissolved and particle bound transport are not needed for endpoint calculation	
Reliability	Medium
Endpoint	Desorption from leached particles, soil column leaching, desorption constant
Protocol	Soil column leaching partly comparable to OECD 312, particle transport, non-GLP
Test compound	Glyphosate solution: 4.4 % ^{14}C-labeled glyphosate, 93.5 % unlabeled glyphosate, (CAS-no.:1071-83-6) and 2.1 % AMPA (CAS-no.: 1066-51-9); purities not given
Test system and conditions	Two soil columns (50-cm height, 30-cm diameter) each from tilled and non-tilled plots, fresh leachate samples investigated within 30 min of sampling, and desorption from splash-eroded particles in suspension followed for 48 h.
Statistical design	4 columns in total, desorption constants calculated, regression equations, Damköhler number (Da, measure of the relative importance of kinetics to equilibrium processes in transport)
Relevance	
Environmental relevance	Given, influencing parameter were tested and considered.
Weight of evidence	
"Positive"/"Negative" evidence	Other literature of same topic as published by the same authors in 2009 supports the results; no negative evidence.

Gjettermann et al. (2011)

Title: Evaluation of Sampling Strategies for Pesticides in a Macroporous Sandy Loam Soil	
Author: B. GJETTERMANN, M. STYCZEN, C. B. KOCH, S. HANSEN, AND C. T. PETERSEN	
Reference: Soil and Sediment Contamination, 20:986-994	
Year: 2011	
Results and conclusion: It is not straightforward to sample and demonstrate the presence and transport of pesticides in heterogeneous soil. Following leaching experiments with four differently structured 50-cm-long soil columns (tilled and untilled soil), the objective of this study as to investigate the extent that visual tracing of the dye Brilliant Blue could support in soil sampling for two strongly sorbing pesticides (^{14}C-labeled glyphosate and pendimethalin). About 830 samples were collected. No pesticide was found below 10-25 cm depth by random sampling, even though 0.21-0.31 % of the applied amounts were leached, and 0.18 % of the soil volume was sampled. With similar sampling efforts, the pesticides could generally be traced throughout the columns by sampling from stained soil volumes, only. None of the two particular sampling strategies for pesticides produced accurate mass balances or balances that were obviously better than the other. No pesticide was detected outside stained soil volumes, except for glyphosate in one sample. Below 30 cm, stained soil comprised on average 5 % of the total soil volume, leaving 95 % as expectedly pesticide-free. The results suggest that much more efficient sampling for sorbing pesticides can be obtained by using the dye and focusing on stained soil volumes.	
Proposed action: Not to be considered as publication deals with a specific analytical procedure to trace pesticides in loamy soils.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Low
Endpoint	Glyphosate concentration in soil from soil columns after leaching and Brillant Blue tracing
Protocol	No protocol
Test compound	Glyphosate (CAS-no: 1071-83-6, purities not given) and AMPA, Roundup Bio, ^{14}C-glyphosate, and blank formulation. Distribution: ^{14}C-glyphosate (4.4 %), unlabeled glyphosate (93.5 %), and AMPA (2.1 %)
Test system and conditions	Soil column leaching experiments, analysis of Glyphosate in soil layers of the soil columns, visual tracing by Brillant Blue, collection of 830 soil samples, and application of both analytical methods, establishment of mass balances
Statistical design	Not further detailed
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other experiments. No negative evidence.

Gjettermann et al. (2009)

Title: Particle-facilitated Pesticide Leaching from Differently Structured Soil Monoliths	
Author: B. Gjettermann, C. T. Petersen, C. B. Koch, N. H. Spliid, C. Grøn, D. L. Baun, M. Styczen	
Reference: J. Environ. Qual. 38:2382–2393	
Year: 2009	
Results and conclusion: The leaching of soil particles and surface applied ^{14}C-labeled glyphosate from intact soil columns were investigated, and the relative significance of particle-facilitated pesticide transport was quantified. Pesticide leaching was driven by preferential water flow in macropores. For the plowed structure 10 % of the leached glyphosate was bound to particles whereas significantly less glyphosate was bound to particles in leachate from minimally disturbed columns. Thus, the results suggest that soil structure affected the mode of transport of glyphosate. It is likely that glyphosate sorbed strongly when applied on recently plowed soil (K_d = 503 L/kg for the soil), and that it could be mobilized and transported independently of soil particles more easily when applied on the minimally disturbed soil covered in part with crop residues (K_d < 1 L/kg for straw). Significantly less amounts of soil particles were leached from minimally disturbed (119–247 mg) than from recently plowed (441–731 mg) columns.	
Proposed action: To be considered as it is supportive information for modelling purposes.	
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information	
Reliability	High
Endpoint	K_d-values; soil column leaching, macropore leaching, preferential flow, relation to tillage practices;
Protocol	Sorption of Glyphosate and AMPA: based on OECD 106; Soil column leaching: no standard, partly comparable to OECD 112, non-GLP
Test compound	Glyphosate solution: 4.4 % ^{14}C-labeled glyphosate, 93.5 % unlabeled glyphosate, CAS-no.: 1071-83-6, and 2.1 % AMPA (CAS-no.: 1066-51-9); purities not given
Test system and conditions	Sorption: same soils as for column leaching,; Soil columns (50-cm height, 30-cm diameter) from: recently plowed (four columns) and an untilled (five columns) sandy loam soil; Glyphosate concentration in filtered and non-filtered leachate analysed.
Statistical design	Sorption: 4 concentrations for isotherms; linear adsorption isotherms of sorbed chemical as function of chemical in solution; distribution coefficient (by slope of the isotherm) calculated by linear regression analysis. Colum leaching: 4 and 5 columns, respectively; relative significance of particle-facilitated transport (fraction of leached glyphosate being bound to soil particles)
Relevance	
Environmental relevance	Given, influencing parameter were tested and considered.
Weight of evidence	
"Positive"/"Negative" evidence	Other literature on preferential flow supports information; no negative evidence.

Magga et al. (2008)

Title: Soil column experiments used as a means to assess transport, sorption, and biodegradation of pesticides in groundwater	
Author: ZOI MAGGA, DIMITRA N. TZOVOLOU, MARIA A. THEODOROPOULOU, THEODORA DALKARANI, KONSTANTINOS PIKIOS and CHRISTOS D. TSAKIROGLOU	
Reference: Journal of Environmental Science and Health Part B 43, 732–741	
Year: 2008	
Results and conclusion: Soil column experiments are used to investigate the fate of three pesticides of high, intermediate, and low solubility in groundwater: N-phosphonomethyl glycine (glyphosate); O,O-diethyl-S-[(ethylthio) methyl] phosphorodithioate (phorate); (2,4-dichlorophenoxy)acetic acid (2,4-D). Feed solutions are prepared by adding each pesticide (100 mg/L glyphosate, 50 µg/L phorate, 50 mg/L 2,4-D) along with conservative tracer, KBr, in synthetic groundwater. The concentration of the pesticides in effluents is detected by ion chromatography (glyphosate, 2,4-D) and GC-FID (phorate). The Br-breakthrough curves are employed to estimate the dispersion coefficient and mean pore velocity in each column. Solute transport and reactive models accounting for equilibrium/non-equilibrium sorption and biodegradation are coupled with inverse modelling numerical codes to estimate the kinetic parameters for all pesticides.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required. Data on sorption/desorption are sufficient for the assessment	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information in the context of discussing leaching modelling (e.g. FOCUS Pelmo) results, K_d-values estimated from the soil column leaching experiment, not directly measured as it is the case in a study according to OECD 106	
Reliability	Medium
Endpoint	Concentrations in leachate, dispersion coefficients, breakthrough curves, kinetic parameter e.g., rate constant of non-equilibrium sorption, half-saturation growth constant, estimated K_d-values
Protocol	Partly comparable to OECD 312, non-GLP
Test compound	Glyphosate (purity not given), CAS-no.:1071-83-6
Test system and conditions	Transport and leaching through packed soil columns (i.d. 5 cm, length 80 cm) under controlled laboratory conditions
Statistical design	One column
Relevance	
Environmental relevance	Given, environmental parameter discussed.
Weight of evidence	
"Positive"/"Negative" evidence	No contradiction to other results; no negative evidence.

Strange-Hansen et al. (2004)

Title: Sorption, mineralization and mobility of N-(phosphonomethyl)glycine (glyphosate) in five different types of gravel	
Author: Rikke Strange-Hansen, Peter E Holm, Ole S Jacobsen and Carsten S Jacobsen	
Reference: Pest Manag Sci 60:570–578	
Year: 2004	
Results and conclusion: Cumulative mineralization of [methyl-^{14}C]glyphosate in batch studies was highest in coarse gravel, amounting to 14 % after 4 days at 30 °C and 32 % after 31 days. Mineralization was slowest in the sandy reference soil, amounting to only 2 % after 31 days. The adsorption coefficient (K_d) of glyphosate in gravel ranged from 62 to 164 litre/kg, while that in the sandy reference soil was 410 litre/kg. The results indicate that the relatively low K_d in gravel allows a relatively high rate of glyphosate mineralization by the biomass. When K_d is high, in contrast, mineralization is slow. Lowering the temperature to 10 °C decreased mineralization by 50 % in one of two gravels. The leaching of glyphosate was screened in simple columns of gravel or soil in which precipitation events (20 mm over a 2-h period) were simulated on three occasions, starting either immediately after or 2 days after application of glyphosate. [^{14}C]Glyphosate was applied as a tracer mixed with the commercial product Roundup Garden at the recommended rate of 2.4 kg glyphosate/ha, equivalent to 1µgg^{-1} soil. The highest concentration of [^{14}C] compounds (expressed in terms of glyphosate concentration) in leachate from the columns exceeded 1300 µg litre^{-1}, and was detected in rounded gravel after the first rain event. No glyphosate was detected in leachate from the sandy reference soil.	
Proposed action: To be considered as additional information only since the soil column leaching studies are not required Data on sorption/desorption are sufficient for the assessment	
Type of information (critical, high/low weight, supporting, additional): Low, additional information	
Reliability	Medium
Endpoint	K_d = distribution coefficient; [^{14}C]carbon dioxide
Protocol	According to the OECD guidelines (106), but with a modified soil: solution ratio of 2 instead of 5; non-GLP
Test compound	[methyl-^{14}C]Glyphosate (specific activity 1.08MBq mmol^{-1}; radiochemical purity >99 %); unlabelled glyphosate (purity 98 %); CAS-no.:1071-83-6 for column experiments: Roundup Garden (commercial SL formulation containing 120 g litre^{-1} glyphosate (isopropylamine salt), CAS-no.: 38641-94-0
Test system and conditions	Batch studies: 1) Sorption: glyphosate/[^{14}C]glyphosate concentration of 0.6 mg/kg (192 Bq), rotated for 96 h; 2) Mineralization in flasks: glyphosate/[^{14}C]glyphosate concentration of 16.9mg/kg (0.1mM) and 833 Bq, final moisture level equivalent to 80 % of WHC, incubated at 30 ◦C in the dark for 31 days, repeated on two types of gravel incubated at 10, 20 and 30 °C; 3) Leaching studies: Two columns for each substrate, and each column were exposed to two different simulated precipitation events. [^{14}C]Glyphosate (1733 Bq in short columns, and 5666 Bq in tall columns) mixed with Roundup Garden (recommended rate of 2.4 kg glyphosate/ha), Over the next 6 days the columns were subjected to three simulated precipitation events at 20 °C, trapping the [^{14}C]carbon dioxide, total effluent collected 1 day after each precipitation event, determination of residual [^{14}C] compounds. Five types of gravel and a sandy agricultural reference soil
Statistical design	Analyses in triplicate; K_d value represents one measurement of glyphosate in the solution, assuming linearity between glyphosate adsorption and glyphosate concentration in the solution.

Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications.

Vischetti et al. (2006)

Title: Biochemical parameter changes in urban-waste compost used as biofilter for pesticide decontamination	
Author: COSTANTINO VISCHETTI, PIERO PERUCCI, CRISTIANO CASUCCI, ELGA MONACI and STEFANO DUMONTET	
Reference: Intern. J. Environ. Anal. Chem. Vol. 86, Nos. 3–4, 15 March–10 April 2006, 195–205	
Year: 2006	
Results and conclusion: Here, water that was contaminated by three different pesticides, the insecticide chlorpyrifos (Chl), the fungicide metalaxyl (Meta) and the herbicide glyphosate (Gly), was percolated through 2 kg of UWC (Urban-waste compost) material. The pesticide residues in the leached water and the modifications induced in some of the UWC biochemical and microbiological parameters (including microbial biomass carbon (MBC) and nitrogen (MBN), and fluorescein diacetate (FDA) hydrolysis, alkaline monophosphatase (AMP) and dehydrogenase (DH) activities) were investigated over 2 months of incubation at 20 °C. The UWC showed a good retention capacity towards the three pesticides tested, with the highest efficiency for Gly. Chl caused an initial detrimental effect on the MBC content and a decrease in the FDA hydrolysis capacity, while Meta and Gly increased the MBC content throughout the incubation. The results demonstrate that UWC can be successfully used as a biofilter to reduce pesticide spills and to clean up water contaminated with pesticides. The evaluation of the modifications induced on the UWC MBC and MBN, and FDA hydrolysis, AMP and DH activities suggest different biodegradation potentials of the UWC microorganisms vs. the three pesticides studied.	
Proposed action: Not to be considered; basic research, no endpoint correction needed	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN); Alkaline monophosphatase (AMP) activity; Dehydrogenase [DH] activity; recoveries of the pesticides
Protocol	Standard, non-GLP
Test compound	Analytical standard of glyphosate (Gly), (CAS-no.: 1071-83-6)
Test system and conditions	81 PVC columns, filled with 2 kg of UWC, treated twice a day with 4 L of deionised water containing Chl, Meta and Gly at different field doses; leached water was collected from each column after each leaching event and analysed to determine the pesticide residues. The modifications of the UWC microbiological and biochemical parameters induced by the pesticides were evaluated
Statistical design	Three replicates for each pesticide, for each sampling time; SYSTAT programme was used for the analysis of variance and Duncan's range test on the means
Relevance	
Environmental relevance	Given. Parameters influencing endpoints are measured.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence; results supported by other publications.

Zhao et al. (2009)

Title: Glyphosate mobility in soils by phosphate application: Laboratory column experiments	
Author: Bingzi Zhao, Jiabao Zhang, Jiandong Gong, Hui Zhang, Congzhi Zhang	
Reference: Geoderma; article in press	
Year: 2009	
Results and conclusion: (1) phosphate application might induce system pH decrease, and (2) the overall glyphosate mobility by phosphate application might depend on the relative contribution of two competing processes: increase in glyphosate adsorption from pH decrease versus reduced glyphosate adsorption from competitive adsorption between phosphate and glyphosate for sorption sites available. To test these two hypotheses, laboratory batch and column experiments using a miscible displacement approach were conducted on two Primosols and one Anthrosols, to investigate, respectively, (1) pH-dependent glyphosate adsorption onto the three studied soils, and (2) glyphosate leaching and mobility in soil columns as influenced by phosphate application. Our results showed that glyphosate adsorption consistently decreased with increase in system pH. The effect of phosphate application on glyphosate mobility varied with soil type. We conclude that phosphate application can cause system pH change with various extents in the soil, which subsequently contribute to glyphosate mobility in different degree.	
Proposed action: Not to be considered for the endpoint sorption and mobility as the study aims at basic research and understanding of pH and phosphate influence.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information of mechanisms of glyphosate mobility in the presence of phosphate	
Reliability	Medium
Endpoint	Breakthrough curves (BTCs)
Protocol	Partly similar to OECD 106, non-GLP
Test compound	Glyphosate (99.9 % purity); CAS-no.: 1071-83-6
Test system and conditions	1) batch experiments, shaken for 20 h at 25 °C; 2) column leaching experiments, 6 column experiments including with or without phosphate (two concentrations), and 3 levels of glyphosate introduced rate, three soils
Statistical design	Duplicate samples
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Zhou et al. (2010)

Title: Differential Transport of Atrazine and Glyphosate in Undisturbed Sandy Soil Column	
Author: Y. ZHOU, Y. WANG, D. HUNKELER, F. ZWAHLEN AND J. BOILLAT	
Reference: Soil and Sediment Contamination, 19:365–377	
Year: 2010	
Results and conclusion: Laboratory studies were conducted to determine the behaviour of atrazine and glyphosate within the root zone of an undisturbed sandy soil in Jianghan Plain, central China. Chloride as a tracer for water movement was applied to the soil as KCl for 26 hours before pesticide application for another 160 hours. Glyphosate, atrazine, and Cl concentrations (conc.) were determined as a function of time in breakthrough curves (BTCs). Atrazine BTC was fitted better in convection-dispersion equation equilibrium model. For glyphosate, however, a two-site non-equilibrium model was chosen. Leaching rate of atrazine from sandy soil was much higher than that of glyphosate and it took longer for glyphosate to leach through the column due to stronger sorption and degradation to its major metabolite, AMPA (aminomethylphosphonic acid, CH_6NO_3P), which was detected (up to 8890 ng/l) in the final leachate.	

Proposed action: To be considered as additional information only since the soil column leaching studies are not required and data on sorption/desorption are sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, supporting information of leaching of glyphosate and AMPA	
Reliability	High
Endpoint	Breakthrough curves BTC; kinetic parameters: retardation factor R and partitioning coefficient β
Protocol	Standard, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6) from Landi (Switzerland) with 360 g/l certified purity.
Test system and conditions	Leaching column experiment, Glyphosate, AMPA and atrazine concentrations in the leachate samples were analyzed
Statistical design	Breakthrough curves to a two-site (or bicontinuum) sorption model with degradation. The two-site model is a one-dimensional advective-dispersive transport model with a first-order bicontinuumdescription of soil, which allows estimation of the rate parameters for non-equilibrium sorption and irreversible sorption.
Relevance	
Environmental relevance	Given. Parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

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Detailed description of open literature – Lysimeter studies or field leaching studies

Al-Rajab et al. (2008)

Title: Sorption and leaching of ^{14}C-glyphosate in agricultural soils
Author: Abdul Jabbar Al-Rajab, Samira Amellal, Michel Schiavon
Reference: Agron. Sustain. Dev. 28, 419–428
Year: 2008
Results and conclusion: Aim: to assess the dynamic interactions between glyphosate sorption and leaching; and to identify the main factors that influence the two processes in three undisturbed agricultural soils using microlysimeters under outdoor conditions. OECD 106: Glyphosate was strongly adsorbed, yielding empirical constants of Freundlich sorption isotherms (K_f) of 16.6 for the clay loam soil, 33.6 for the silt clay loam soil and 34.5 for the sandy loam soil, with n_f close to 1 in all three cases. Glyphosate was also weakly desorbed, i.e. 5 to 24 % (w) of initially sorbed glyphosate. Sorption and desorption were only pH-dependent. Outdoor microlysimeter: nearly 70 % of the initial glyphosate was present in the soil in a non-extractable form at the beginning of the experiment. Conversely, only less than 20 % of the initial glyphosate is present in the soil in a non-extractable form after 11 months. These findings suggest that the non-extractable residues become available and take part in biodegradation and leaching. The amounts of ^{14}C-glyphosate derivatives leached were less than 0.28 % of the initially applied glyphosate. AMPA metabolite generally represented up to 100 % of the residues present in the leachates. The results of leaching were highly influenced by the hydrodynamic properties and the biodegradation capacities of the soils.
Proposed action: To be considered for endpoint sorption as it is supportive information though raw data in a common sense of GLP-study reports are not available. Information is highly reliable and plausible.
Type of information (critical, high/low weight, supporting, additional): High weight, supportive information

Reliability	High
Endpoint	OECD 106: K_f-values, 1/n, R^2, % desorbed; Outdoor microlysimeter: amount in leachate and distribution in soil over time (11 months).
Protocol	Standard (OECD 106), non-GLP; outdoor lysimeter studies, non-GLP.
Test compound	OECD 106: [Phosphonomethyl-^{14}C]-glyphosate (purity: 99 %); non-radioactive glyphosate (purity 98.5 %) Outdoor microlysimeter: [Phosphonomethyl-^{14}C]-glyphosate diluted in Roundup Express (isopropylamine salt) and water CAS-no.: 1071-83-6
Test system and conditions	OECD 106: 7 concentrations for isotherms, 3 soils; undisturbed outdoor micro-lysimeter (diameter: 10 cm, length: 25 cm): duration: 11 months, 3 soils, 7 sampling points, 21 lysimeter in total.
Statistical design	OECD 106: triplicates, Lysimeter: single lysimeter per sampling and soil. Stat Box computer software; Comparison of means by Newman-Keuls test at levels of 0.05, 0.01 and 0.001.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Al-Salamah (2004)

Title: Simulating the fate and transport of pesticide in unsaturated soil: a case study with glyphosate-isopropylammonium
Author: I. S. Al-Salamah
Reference: Geo-Environmen J. F. Martin-Duque, C. A. Brebbia, A. E. Godfrey & J. R. Diaz de Teran (Editors) © 2004 WIT Press, www.witpress.com, ISBN 1-85312-723-X
Year: 2004
Results and conclusion: A simultaneous transport of water and glyphosate was studied experimentally and numerically. The glyphosate redistributed along the soil column experimentally and numerically. The predicted glyphosate concentrations were improved by increasing the dispersivity up to 75 mm. The observed glyphosate concentration proved that the mass flow mechanism is important for migration of the glyphosate in the sandy soil. The results of this study indicated that transport models need to include the effect of temperature and temperature gradient to describe the movement of water and glyphosate.
Proposed action: To be considered as additional information only since the climatic does not fit with European climatic conditions.
Type of information (critical, high/low weight, supporting, additional): Additional

Reliability	
Endpoint	Experimental simulation of glyphosate isopropyl-ammonium leaching
Protocol	Non-GLP standard study
Test compound	Glyphosate-isopropylammonium (CAS 38641-94-0)
Test system and conditions	Soil materials were sampled from a surface layer (0.0-0.3 m depth) from the Agriculture and Veterinary Collage farm, King Saud University, Al-Qassim. The soil was composed of sand (96.3 % sand, 1.9 % clay, and 1.8 % silt) materials. Four soil columns were packed at bulk densities of 1514 kg m^{-3}. The soil columns were buried vertically within a bare soil field with exposing the upper end to the natural atmosphere of Al-Qassim region. Water and glyphosate solution were poured at the open of soil column at different time. The soil temperatures and the soil moisture conditions at both ends of soil column were recorded.
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is small since the climatic does not fit with European climatic conditions.
Weight of evidence	
"Positive"/"Negative" evidence	The results are principally supported by other studies performed with leaching models

Aronsson et al. (2010)

Title: Leaching of N, P and glyphosate from two soils after herbicide treatment and incorporation of a ryegrass catch crop	
Author: H. Aronsson, M. Stenberg & B. Ulén	
Reference: Soil Use and Management; doi: 10.1111/j.1475-2743.2010.00311.x	
Year: 2010	
Results and conclusion: The overall aim was to determine the effects of different cropping systems with catch crops on losses of N, P and glyphosate. Soil type affected glyphosate leaching to a larger extent than the experimental treatments. Glyphosate was not leached from the sand at all, while it was found at average concentrations of 0.25 lg⁄L in drainage water from the clay soil on all sampling occasions.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): High weight, may be supporting information to already existing	
Reliability	Medium
Endpoint	Glyphosate concentration in leachate (drainage)
Protocol	Non-GLP
Test compound	Site 1: glyphosate applied as Glyphomax Bio, 3.5 or 4.0 L⁄ha Site 2: glyphosate applied as Round-up Bio, 3.5 L⁄ha. CAS-no.: 1071-83-6
Test system and conditions	During 2005–2007, studies were carried out in two field experiments in southwest Sweden with separately tile-drained plots on a sandy soil (three replicates) and on a clay soil (two replicates). Drainage water was sampled continuously in proportion to water flow and analysed for N, P and glyphosate. Catch crops were sampled in late autumn and spring and soil was analysed for mineral N content. The yields of following cereal crops were determined.

Statistical design	Analysis of variance: mixed procedure in SAS 9.1 for the statistical analysis of differences in yields, catch crop biomass and N and P contents, soil mineral N, leaching of N and P and concentrations of glyphosate between treatments. The t-test at P = 0.05 was used for pair wise comparisons by the PDIFF statement. Block was used as the random variable in analysis of a single year.
Relevance	
Environmental relevance	Given; relevant substance assessed; field studies in southwest of Sweden, environmental parameter
Weight of evidence	
"Positive"/"Negative" evidence	Further leaching and monitoring studies support the results. No negative evidence.

Augustin and Seibel (2002)

Title: Herbicide treatment of urban areas _ a possible source of surface water contamination	
Author: Bernd Augustin and Helmut Seibel	
Reference: GESUNDE PFLANZEN, 54. Jahrg., Heft 7	
Year: 2002	
Results and conclusion: A rough concrete surface with an inclination of 1-2 % for rainwater elimination was treated with Roundup Ultra® (Glyphosat), Basta® (Glufosinate) and Vorox G® (Glyphosat-Diuron). Run-off-water was collected after artificial rain (2 mm) given in different periods after herbicide application (1 and 24 h; 10 days). Chemical analysis showed that the run-off-water contained considerable quantities of Glyphosate and Glufosinat even 10 days after herbicide treatment and 17 mm of artificial and natural rainfall. The results are discussed considering recent detection of Glyphosate contamination of surface water in Germany.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing.	
Reliability	Low
Endpoint	Concentration in leachate
Protocol	For chemical analysis: DFG-method 405, non-GLP. General set-up without specific protocol but research project.
Test compound	Roundup Ultra® (Glyphosat), Vorox G® (Glyphosat-Diuron); CAS-no.: 1071-83-6
Test system and conditions	A rough concrete surface with an inclination of 1-2 % for rainwater elimination was treated with Roundup Ultra® (Glyphosat), Basta® (Glufosinate) and Vorox G® (Glyphosat-Diuron). Run-off-water was collected after artificial rain (2 mm) given in different periods after herbicide application (1 and 24 h; 10 days).
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	Results support monitoring data. However, monitoring studies and campaigns are of more reliability and relevance. No negative evidence.

Barrett and McBride (2007)

Title: PHOSPHATE AND GLYPHOSATE MOBILITY IN SOIL COLUMNS AMENDED WITH ROUNDUP	
Author: Katherine A. Barrett and Murray B. McBride	
Reference: Soil Science;172:17–26	
Year: 2007	
Results and conclusion: Sorption of glyphosate and competitive desorption of phosphate in soils has been measured in column leaching experiments. Soils representing a wide range of physical and chemical properties, including total and soluble P, were included in the study. The results suggest that glyphosate sorption does not necessarily result in PO_4^{3-} dissolution and that there is only limited competition for sorption sites between glyphosate and PO_4^{3-}. Strong glyphosate sorption on high-organic matter soils indicates bonding of this anion by a metal bridge to organic functional groups.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to endpoint leaching, soil column leaching	
Reliability	Medium
Endpoint	Concentration of glyphosate in the leachate, total P and PO_4^{3-} in the leachate.
Protocol	No standard protocol followed, non-GLP
Test compound	Commercial Roundup (containing 27 % active ingredient); CAS-no.: 1071-83-6
Test system and conditions	Soil column studies with homogenized soils. Air-dried soil packed into polycarbonate tubes (width of 5.5 cm and height of 10 cm, with soil depth of approximately 6 cm). Application of roundup, ageing for 24 h, 100 mL leachate.
Statistical design	Duplicates, StatView software, $P < 0.05$ are considered to be significantly different.
Relevance	
Environmental relevance	Given; influencing parameter are reported and considered.
Weight of evidence	
"Positive"/"Negative" evidence	Results of other studies with identical design not known. No negative evidence.

Barrett and McBride (2006)

Title: Trace Element Mobilization in Soils by Glyphosate
Author: K. A. Barrett and M. B. McBride
Reference: Soil Sci. Soc. Am. J. 70: 1882–1888
Year: 2006
Results and conclusion: No significant increases in elemental leaching were detected in mineral and organic soils with normal background concentrations of heavy metals and phosphor.
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing.

Reliability	Medium
Endpoint	Trace element concentrations in soil column leachates.
Protocol	Non-GLP, no standard protocol
Test compound	Reagent-grade glyphosate [N-(phosphonomethyl)glycine, 96 %); CAS-no.: 1071-83-6
Test system and conditions	Tendency for glyphosate to mobilize Cu and other elements was tested in soil leaching experiments by applying glyphosate alone or complexed with Cu to mineral and organic soil columns (10 cm height, 1.8 cm i.d.) and measuring the concentrations of these elements in the leachates.
Statistical design	ANOVA analysis of variance, 6 soils tested, single test (?)
Relevance	
Environmental relevance	Given, environmental parameter reported.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies with identical design and objective not known. No negative evidence.

Bergström et al. (2011)

Title: Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil
Author: Lars Bergström, Elisabet Börjesson, and John Stenström
Reference: J. Environ. Qual. 40:98–108
Year: 2011
Results and conclusion: Influence of adsorption on glyphosate degradation was confirmed: very slow degradation rate in clay soil (half-life 110–151 d). Kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation rate can be faster than that of glyphosate. The kinetics also suggests that the sarcosine pathway can be just as significant as via AMPA. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59 % of the initial amount of glyphosate added to the clay soil 748 d after application. Despite large amounts of precipitation in the autumn and winter after application, however, these residues were mainly located in the topsoil, and only 0.009 and 0.019 % of the initial amount of glyphosate added leached during the whole study period in the sand and clay, respectively. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil.
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.
Type of information (critical, high/low weight, supporting, additional): High weight, may be supporting information to already existing.

Reliability	High
Endpoint	Lysimeter studies: concentrations in leachate and soil; adsorption: K_f, 1/n, R^2; degradation: DT_{50}
Protocol	Lysimeter studies: no guideline; adsorption: OECD 106; degradation: similar to OECD 307, Non-GLP
Test compound	Glyphosate, ^{14}C-Glyphosate, purity not given; CAS-no.: 1071-83-6
Test system and conditions	Batch laboratory and lysimeter transport studies were performed to assess the potential for leaching of the compounds in two agricultural soils. Unlabeled and ^{14}C-labeled glyphosate were added at a rate corresponding to 1.54 kg a.i./ha on undisturbed sand and clay columns. Leachate was sampled weekly during a period of 748 d for analyses of glyphosate, AMPA, total ^{14}C, and particle-bound residues. Topsoil and subsoil samples were used for determination of glyphosate adsorption, glyphosate degradation, and formation of AMPA and its degradation.

Statistical design	Lysimeter studies: 7 lysimeters, 2 soils; adsorption: two soils (top and subsoil), duplicates; degradation: 2 soils, duplicates, first order kinetics both for Glyphosate and AMPA. Least squares fits of data on adsorption and on residual values of glyphosate and AMPA were fitted to their respective equations by nonlinear regression.
Relevance	
Environmental relevance	Given; environmental parameter adequately tested.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support information. No negative evidence.

Borggaard and Gimsing (2008)

Title: Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review	
Author: Ole K Borggaard and Anne Louise Gimsing	
Reference: Pest Manag Sci 64, 441–456	
Year: 2008	
Results and conclusion: The very wide use of glyphosate to control weeds in agricultural, silvicultural and urban areas throughout the world requires that special attention be paid to its possible transport from terrestrial to aquatic environments. The aim of this review is to present and discuss the state of knowledge on sorption, degradation and leachability of glyphosate in soils. Difficulties of drawing clear and unambiguous conclusions because of strong soil dependency and limited conclusive investigations are pointed out.	
Proposed action: Not to be used as it is a review article.	
Type of information (critical, high/low weight, supporting, additional): Low weight. As it is a review article no data are published but cited from other publications and discussed. Additional information.	
Reliability	
Endpoint	Review, no data published
Protocol	Not applicable
Test compound	Glyphosate (CAS-no.:1071-83-6), AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Brown and van Beinum (2009)

Title: Pesticide transport via sub-surface drains in Europe	
Author: Colin D. Brown, Wendy van Beinum	
Reference: Journal of Environmental Pollution; doi:10.1016/j.envpol.2009.06.029	
Year: 2009	
Results and conclusion: Results of 23 field drainage experiments undertaken at sites across Europe were collated and analysed by residual maximum likelihood. Both maximum concentration of pesticide in drain-flow (n = 167) and seasonal loss of pesticide to drains (n = 97) were significantly related to strength of pesticide sorption to soil, half-life of the pesticide in soil, the interval between application and first drain-flow and the clay content of the soil. The statistical models accounted for 71 % of the variability in both maximum concentration and seasonal load. Next, the dataset was used to evaluate the current methodology for assessment of aquatic exposure used in pesticide registration in Europe. Simulations for seven compounds with contrasting properties showed a good correspondence with field measurements. Finally, the review examines management approaches to reduce pesticide transport via sub-surface drains. Despite a large amount of work in this area, there are few dependable mitigation options other than to change application rate or timing or to restrict use of a compound in the most vulnerable situations.	
Proposed action: Not to be considered as it is a review article.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only as article is a review	
Reliability	Low (review article)
Endpoint	Maximum concentration of pesticide in drain-flow and seasonal loss of pesticide to drains;
Protocol	No standard protocol
Test compound	Glyphosate (CAS-no.: 1071-83-6)
Test system and conditions	Review article on results of 23 field drainage experiments.
Statistical design	Statistical technique: residual maximum likelihood (REML). Similar to multiple regression the method identifies a combination of factors that best explains the values for maximum concentration and seasonal loss.
Relevance	
Environmental relevance	Given; influencing parameter are considered and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Review article analyzing the results of other experiments; no negative evidence

Candela et al. (2010)

Title: Glyphosate transport through weathered granite soils under irrigated and non-irrigated conditions — Barcelona, Spain
Author: Lucila Candela, Juan Caballero, Daniel Ronen
Reference: http://dx.doi.org/10.1016/j.scitotenv.2010.03.006
Year: 2010

Results and conclusion: The transport of Glyphosate and AMPA has been studied in the Mediterranean Maresme area of Spain, north of Barcelona, where groundwater is located at a depth of 5.5 m. The unsaturated zone of weathered granite soils was characterized in adjacent irrigated and non-irrigated experimental plots where 11 and 10 boreholes were drilled, respectively. At the non irrigated plot, the first half of the period was affected by a persistent and intense rain-fall. After 69 days of application residues of Glyphosate up to 73.6 µg g^{-1} were detected till a depth of 0.5 m under irrigated conditions, AMPA, analyzed only in the irrigated plot was detected till a depth of 0.5 m. According to the retardation coefficient of Glyphosate as compared to that of Br^- for the topsoil and subsoil (80 and 83, respectively) and the maximum observed migration depth of Br^- (2.9 m) Glyphosate and AMPA should have been detected till a depth of 0.05 m only. (Furthermore for Glyphosate: surface soil (K_f=93), subsoil (K_f=154)) Such migration could be related to the low content of organic matter and clays in the soils; recharge generated by irrigation and heavy rain, and possible preferential solute transport and/or colloidal mediated transport.
Proposed action: Not to be considered for the endpoint sorption. Consideration for the monitoring chapter as it supports the information for soils of low content of organic matter and clays.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to the endpoint sorption and mobility. Might be supportive information to the chapter "monitoring data".

Reliability	Medium
Endpoint	Glyphosate concentrations in deeper soil layers and leachate, mobility and leaching
Protocol	Standard, OECD
Test compound	Glyphosate (Roundup®, 36 % p/v; CAS-no.: 1071-83-6), AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Outdoor plot experiments under irrigated and non-irrigated conditions, experiments run for approximately 90 days. Vadose zone soil and water sampling. Glyphosate and AMPA residue analyses in soil and water. Extraction efficiency >95 % for both analytes.
Statistical design	Not given in detail
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, supportive information; no negative evidence.

Fomsgaard et al. (2003)

Title: Leaching of Pesticides Through Normal-Tillage and Low-Tillage Soil—A Lysimeter Study. II. Glyphosate
Author: Inge S. Fomsgaard, Niels Henrik Spliid and Gitte Felding
Reference: JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, Part B— Pesticides, Food Contaminants, and Agricultural Wastes, Vol. B38, No. 1, pp. 19–35
Year: 2003
Results and conclusion: Leaching of glyphosate and/or its metabolite AMPA was studied in four lysimeters, two of them being replicates from a low tillage field (lysimeter 3 and 4), the other two being replicates from a normal tillage field (lysimeter 5 and 6). The mean yearly concentration of leached glyphosate and/or AMPA was significantly below 0.1 mg/l from both sets of lysimeters, and thus no significant difference between the two lysimeter sets was shown. However, in both sets of lysimeters several single findings at concentrations above 0.1 mg/l were seen (Glyphosate: up to 0.52 µg/L; AMPA: up to 0.22 µg/L), which might be due to the leaching of particle-bound compounds. A significant difference between the soil residual concentrations of AMPA was seen, the higher concentration was found in the set of lysimeter where low-tillage had been practiced. This might be due to differences in extraction efficiencies or due to residues resulting from earlier, more frequent sprayings with Round Up in the low tillage soil.

Proposed action: To be considered as supportive information, gives valuable information on leaching under outdoor conditions	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information	
Reliability	High
Endpoint	Concentration in lysimeter leachate
Protocol	Close to BBA test guideline (1990): guideline for the Testing of Plant Protection Products in Registration Procedure, Part IV, 4-3: Lysimeter tests for the translocation of plant protection products into the subsoil.
Test compound	Lysimeter 3 and 4 were sprayed with a mixture of ^{14}C-labelled glyphosate and unlabelled glyphosate (Roundup 2000 together with the additive Team Up), lysimeter 5 and 6 were sprayed with unlabelled glyphosate. Analysis for Glyphosate (CAS-no.:1071-83-6) and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	The soil was a sandy loam soil with 13–14 % clay. The lysimeters had a surface area of 0.5 m^2 and a depth of 110 cm. The spraying took place September 18, 1997. The total amount of glyphosate sprayed onto each lysimeter was 40 mg, corresponding to 0.8 kg active ingredient per ha. The lysimeters were installed in an outdoor system in Research Centre Flakkebjerg and were thus exposed to normal climatic conditions of the area. A mean of 260 l drainage water were collected from lysimeter 3 and 4 and a mean of 375 litres from lysimeter 5 and 6.
Statistical design	2 soils, 2 lysimeters per soil
Relevance	
Environmental relevance	Given; environmental and climatic parameters were recorded.
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, other studies are not in conflict to the results reported here; no negative evidence.

Grundmann et al. (2008)

Title: Mineralization and Transfer Processes of ^{14}C-labeled Pesticides in Outdoor Lysimeters	
Author: Sabine Grundmann & Ulrike Dörfler & Bernhard Ruth & Christine Loos & Tobias Wagner & Heidrun Karl & Jean Charles Munch & Reiner Schroll	
Reference: Water Air Soil Pollut: Focus (2008) 8:177–185	
Year: 2008	
Results and conclusion: The glyphosate mineralization curves showed no lag phase – the microorganisms were able to mineralize glyphosate immediately. The cumulated amounts of mineralized ^{14}C-glyphosate amounted to 32–39 %. No accumulation of residues in the soil and no leaching of the residues to deeper soil layers could be observed after three applications. Glyphosate was rapidly degraded to AMPA in the soil. Glyphosate and AMPA were accumulated in soy bean nodules.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the study was not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Degradation of glyphosate in soil (mineralisation) Accumulation and leaching
Protocol	Non-GLP study

Test compound	^{14}C-glyphosate [N-(phosphonomethyl)glycine] had the ^{14}C-labeling on the phosphonomethyl group and was purchased from PerkinElmer, Rodgau, Germany (purity >98 %). Non-labelled glyphosate and metabolites were purchased from Dr. Ehrenstorfer (Augsburg, Germany); CAS 1071-83-6
Test system and conditions	The lysimeters consist of soil columns of 2 m height and a surface area of 1 m^2. To detect and quantify gaseous ^{14}C-losses from soil and plant surfaces, a two-chamber-system with special trapping facilities was designed. The chambers are placed on the surface of the lysimeters – a soil chamber and a plant chamber. Glyphosate was applied three times, in spring 2004 and in spring and autumn 2005 in an amount of 1 kg a.i. ha^{-1} (1.92 MBq/mg). During the experiment, mineralization and volatilization of the herbicides from soil and plants were measured during a time period of about 2-3 months after application.
Statistical design	No information
Relevance	
Environmental relevance	The experiments are relevant though not performed close to standard lysimeter studies. Additionally, not sufficient information is provided to describe the situation (e.g. weather, irrigation).
Weight of evidence	
"Positive"/"Negative" evidence	The results support the information known for glyphosate from standard tests.

Hagner et al. (2013)

Title: The effects of biochar, wood vinegar and plants on glyphosate leaching and degradation	
Author: M. Hagner, O.-P. Penttinen, K. Tiilikkala, H. Setälä	
Reference: European Journal of Soil Biology 58 (2013) 1-7	
Year: 2013	
Results and conclusion: A pot experiment was established to explore the potential impacts of biochar, wood vinegar, and plants on the environmental fate of glyphosate. In the presence of plants (Lolium perenne), and irrespective of the presence of biochar or wood vinegar, leaching of glyphosate through the soil was multiple compared to the plant free systems. However, the addition of biochar to the soil decreased the leaching of glyphosate irrespective of plants. Soils treated with biochare-wood vinegar mixture showed the lowest glyphosate leaching, both with and without plants. Biochar, wood vinegar or plants, alone, had no effect on the degradation of glyphosate in soil. When the plants were present the degradation of glyphosate was highest in soils treated with biochare-wood vinegar mixture. The results imply that biochar in particular can be applied as a soil improving agent to reduce the potential environmental risks to aquatic environments caused by glyphosate.	
Proposed action: To be considered as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information from a field study	
Reliability	
Endpoint	Effects of plants, biochar and wood vinegar on the environmental fate of glyphosate (glyphosate leaching and degradation)
Protocol	GEP
Test compound	Glyphosate

Test system and conditions	The soil used in the experiment had no previous history of glyphosate application. It derived from an arable field used over 20 years in organic potato farming in Tammela, Finland. The biochar used here was derived from birch wood (incl. bark) and pyrolysed in a batch retort by Tisle Suomi Ltd. (Mikkeli, Finland) at 450 °C for a holding time of 23 h. The study was performed in a glasshouse at MTT Agrifood Research Finland in Jokioinen, Finland, in the summer of 2010. The experiment was conducted in 1500 ml flowerpots (Ø 11 cm, height 19 cm) with four holes (Ø 0.5 cm) at the bottom. The four treatments, each with 20 replicates consisted of soil mixed with 1) biochar, 2) wood vinegar, 3) biochar and wood vinegar, or 4) a control system with neither biochar nor wood vinegar. Coarse gravel (Ø 0.5e1.5 cm, 100 g dry) was put at the bottom of each pot to maintain capillary action and to prevent water holes from blocking up. The application rate of biochar to the pots corresponded to 51 t/ha, assuming 10 cm incorporation depth (3.3 % biochar content by dry mass). Wood vinegar concentrations applied to the pots corresponded to 2000 L/ha (0.26 %). To get data on the highest possible risks and benefits of the substances, relatively high concentrations of wood vinegar and biochar were used in the experiments. Before adding 800 g of treated soil to the pots, biochar (sieved through a 2 mm sieve) and wood vinegar were homogenously mixed with the soil in a bucket, and the water content of the mixture and that of the control soil was adjusted to 20 % of wet mass. The pots were randomly placed on a moist filter bed that ensured constant soil moisture during incubation. To provide optimal growth conditions for Lolium perenne, the pots were kept in a glasshouse with constant air temperature (23 ± 2 °C) throughout the experiment. The experiment ran for 82 days, during which time soil and water leachate samples were taken three times: at 4, 46 and 80 days. After the first sampling, seeds of L. perenne were sown (150 per pot) in half of the pots of each treatment to determine the effects of plants on the fate of glyphosate. All the pots were covered with plastic film for 7 days to maintain soil moisture conditions. After seed germination, the plastic film was removed and all pots were fertilised dose of 100 kg/ha). When the grass reached 20 cm in height (Day 36), half of the pots of each treatment (with and without plants) were treated with glyphosate (Roundup Bio; Monsanto, Copenhagen, Denmark) mixed with water (1:100) corresponding to 2000 g active ingredient/ha (ca. 2000 mg/pot). Glyphosate was sprayed according to the Good Experimental Practice GEP protocol used by the Agrifood Research Finland (MTT). The GEP standard was adopted by EEC in the Directive 93/71/EEC. Annex II in this directive specifies the requirements that are referred to as GEP. The GEP standard suites well to a variety of agricultural practices and experimentations. Grasses in glyphosate treated pots withered and died during 3e21 days after spraying the glyphosate. As part of initially added wood vinegar was obviously degraded, four days after the addition of glyphosate, a second addition of wood vinegar (500 L/ha) was made for pots that already contained wood vinegar. This was done to ensure that enough wood vinegar is present in the soil to stimulate glyphosate degradation by soil microbes. The second and third sampling events (46 and 80 d) were made 10 and 44 days after the addition of glyphosate.

Statistical design	Repeated measures MANOVA was used to examine treatment effects of biochar, wood vinegar, vegetation and glyphosate on microbial respiration, numbers of nematodes, the soil C/N ratio and leachate properties (pH, conductivity, TOC) at the second and third sampling events. Soil and leachate samples were analysed separately. In case of leachates, a significant interaction effect was observed between time and biochar and the effects of biochar on pH, conductivity, TOC of leachates in different sampling events were studied separately using Simple-effects model. Transformations (log, ln) were used to normalise the data. Samples taken 5 days after the study started differed from those taken 40 and 80 d after the start due to the addition of grasses (Day 6) and glyphosate (Day 36) to some of the pots. Therefore the effects of biochar and wood vinegar on nematodes, microbial respiration, TOC, conductivity and pH of the samples taken during day 5 were performed separately using MANOVA. Soil and leachate samples were analysed separately. All analyses were carried out using SPSS c.15 for Windows. As glyphosate concentrations in the leachates and soils were calculated from pooled samples (representing average of five replicates), statistical analyses were not performed.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Kjær et al. (2011)

Title: Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils	
Author: Jeanne Kjær, Vibeke Ernsten, Ole H. Jacobsen, Nis Hansen, Lis Wollesen de Jonge, Preben Olsen	
Reference: Chemosphere 84, 471–479	
Year: 2011	
Results and conclusion: Leaching of glyphosate was evaluated in an 8-month field study focussing on preferential flow and particle-facilitated transport, both of which may enhance the leaching of such a pesticides in structured soils. Glyphosate mainly sorbs to mineral sorption sites. The pesticide was applied to a structured, tile-drained soil, and the concentration was then measured in drainage water sampled flow-proportionally. Glyphosate leached from the root zone, with the average concentration in the drainage water being 3.5 µg/L. Particle-facilitated transport (particles >0.24 µm) accounted for only a small proportion of the observed leaching (13-16 %). Drain-connected macropores located above or in the vicinity of the drains facilitated very rapid transport of pesticide to the drains. That the concentration of glyphosate in the drainage water remained high (>0.1 µg/L) for up to 7 d after a precipitation event indicates that macropores between the drains connected to underlying fractures were able to transport strongly sorbing pesticides in the dissolved phase. Lateral transport of dissolved pesticide via such discontinuities implies that strongly sorbing pesticides such as glyphosate could potentially be present in high concentrations (> 0.1 µg/L) in both water originating from the drainage system and the shallow groundwater located at the depth of the drainage system.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information from a field study	
Reliability	Medium
Endpoint	Concentrations in drainage water, leaching pattern
Protocol	No standard protocol, field study, non-GLP

Test compound	Glyphosate (1.44 kg/ha active ingredient; 4.0 L/ha Roundup Bio), CAS-no.: 1071-83-6
Test system and conditions	For a period of 8 months following application of the glyphosate the concentration of the pesticide and bromide was measured on a weekly basis in drainage water, sampled flow-proportionally. In addition, more intense sampling of drainage water was performed in connection with three flow events triggered by precipitation in order to enable detailed description of the transport of water and pesticides. Sampling lasted for 2, 13 and 9 d, respectively.
Statistical design	Not applicable
Relevance	
Environmental relevance	Given; field study, environmental parameter recorded and reported.
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Kjær et al. (2005)

Title: Vadose Zone Processes and Chemical Transport: Leaching of Glyphosate and Amino-Methylphosphonic Acid from Danish Agricultural Field Sites	
Author: Jeanne Kjær, Preben Olsen, Marlene Ullum, and Ruth Grant	
Reference: J. Environ. Qual. 34 (2), 608–620	
Year: 2005	
Results and conclusion: The risk of leaching was evaluated for glyphosate and its degradation product AMPA under field conditions at one sandy and two loamy sites. Over a 2-yr period drainage water, groundwater, and soil water were sampled and analyzed for pesticides. At a sandy site, the strong soil sorption capacity and lack of macropores seemed to prevent leaching of both glyphosate and AMPA. At one loamy site, which received low precipitation with little intensity, the residence time within the root zone seemed sufficient to prevent leaching of glyphosate, probably due to degradation and sorption. Minor leaching of AMPA was observed at this site, although the concentration was generally low, being on the order of 0.05 µg/L or less. At another loamy site, however, glyphosate and AMPA leached from the root zone into the tile drains (1 m below ground surface [BGS]) in average concentrations exceeding 0.1 µg/L, which is the EU threshold value for drinking water. The leaching of glyphosate was mainly governed by pronounced macropore flow occurring within the first months after application. AMPA was frequently detected more than 1.5 yr after application, thus indicating a minor release and limited degradation capacity within the soil. Leaching has so far been confined to the depth of the tile drains, and the pesticides have rarely been detected in monitoring screens located at lower depths. This study suggests that as both glyphosate and AMPA can leach through structured soils, they thereby pose a potential risk to the aquatic environment.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing monitoring data	
Reliability	Low
Endpoint	Concentration in drainage water, groundwater, soil water
Protocol	Not applicable, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6), AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Field trials
Statistical design	Mean values and maximum values are reported
Relevance	
Environmental relevance	Environmental data, e.g., climatic data reported; thus environmental relevance is given.

Weight of evidence	
"Positive"/"Negative" evidence	Other studies report similar data, positive evidence; No negative evidence.

Klier et al. (2008)

Title: Modelling the Environmental Fate of the Herbicide Glyphosate in Soil Lysimeters	
Author: Christine Klier & Sabine Grundmann & Sebastian Gayler & Eckart Priesack	
Reference: Water Air Soil Pollut: Focus (2008) 8:187–207	
Year: 2008	
Results and conclusion: The results showed that the conducted laboratory experiments were useful to generate appropriate input values in dependence on environmental conditions for the subsequent fate modelling of glyphosate. Glyphosate transport measurements in the risk assessment study and the mathematical modelling results indicate that due to the high sorption of glyphosate to the soil matrix and the high microbial capacities for glyphosate degradation in the lysimeter soil, leaching risk can be considered to be low, but cannot be entirely excluded.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Supporting	
Reliability	
Endpoint	Experimental simulation of glyphosate leaching
Protocol	Non-GLP standard study
Test compound	^{14}C-radiolabelled glyphosate (5 mCi; 108 mg m^{-2} a.i.) (CAS 1071-83-6)
Test system and conditions	Field study combined with modelling: Meteorological driving parameters used in the study from the years 2003 to 2005 were measured at the automatic weather station at the lysimeter facility. Soil moisture was controlled during the study by tensiometers. The soil is classified as Haplic Arenosol and soil origin was Neumarkt in middle Bavaria. In the glyphosate transport study soil water flow was simulated according to the model HYDRUS 6.0 The bottom boundary condition used in this application considers free drainage. Glyphosate fate was simulated using LEACHP. For the fate in plants the model PLANTX by Trapp was used.
Statistical design	Not reported
Relevance	
Environmental relevance	The relevance is high since the results are of comparable quality as standard FOCUS simulations.
Weight of evidence	
"Positive"/"Negative" evidence	The results are principally supported by other studies performed with leaching models.

Laitinen et al. (2009)

Title: Glyphosate and phosphorus leaching and residues in boreal sandy soil	
Author: Pirkko Laitinen, Sari Rämö, Unto Nikunen, Lauri Jauhiainen, Katri Siimes, Eila Turtola	
Reference: Plant Soil 323, 267–283	
Year: 2009	
Results and conclusion: Persistence and losses of glyphosate were monitored in a field with low phosphorus status and possible correlation between glyphosate and phosphorus leaching losses was studied. Glyphosate and its metabolite AMPA residues in soil samples were analysed after a single application in autumn. Twenty months after the application the residues of glyphosate and AMPA in the topsoil (0–25 cm) corresponded to 19 % and 48 %, respectively, of the applied amount of glyphosate, and traces of glyphosate and AMPA residues were detected in deeper soil layers (below 35 cm). These results indicate rather long persistence for glyphosate in boreal soils. Surface runoff and subsurface drain-flow were collected continuously all year round for 20 months and analysed for glyphosate, AMPA, dissolved phosphate, total phosphorus and total suspended solids. The glyphosate concentrations in the surface runoff water were highest, with 99 % of the total leaching losses obtained, during the periods of snow melting and soil thawing in the first winter following the autumn application. The total leaching of glyphosate was 5.12 g/ha and that of AMPA 0.48 g/ha, corresponding to about 0.51 % and 0.07 %, respectively, of the applied amount of glyphosate. No residues of glyphosate and AMPA were detected in the subsurface drain-flow. The correlations between concentrations of glyphosate and dissolved orthophosphate as well as glyphosate and total phosphorus in surface runoff were significant ($p < 0.01$).	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information on Glyphosate behaviour in the environment	
Reliability	High
Endpoint	Concentrations in Soil, water (drain-flow) and root samples
Protocol	Not applicable; field trial, non-GLP
Test compound	Commercial product Roundup containing glyphosate 360 g/L Glyphosate
Test system and conditions	Experimental field study. 4 out of 16 plots were applied. Soil, water (drain-flow) and root samples analysed for Glyphosate (CAS-no.: 1071-83-6) and AMPA (CAS-no.: 1066-51-9)
Statistical design	Correlations between Glyphosate and phosphate
Relevance	
Environmental relevance	Depth dependent soil parameter reported; weather and other relevant environmental parameter recorded; thus environmental relevance given.
Weight of evidence	
“Positive”/”Negative” evidence	Supported by other studies; no negative relevance.

Laitinen et al. (2007)

Title: Glyphosate translocation from plants to soil – does this constitute a significant proportion of residues in soil?
Author: Pirkko Laitinen, Sari Rämö, Katri Siimes
Reference: Plant Soil 300 ,51–60
Year: 2007
Results and conclusion: Translocation of glyphosate (N-(phosphonomethyl) glycine) to plant roots and its impact on detected herbicide residues in sandy loam soil were studied in a glasshouse pot experiment in Finland. Quinoa (Chenopodium quinoa, Willd) plants in two different growing phases (6–8 and 12–14 leaf stages, groups A and B, respectively) were sprayed with non-labelled glyphosate. Bare soil pots were included as controls (group C). Glyphosate and AMPA concentrations were measured in soil and plant roots at various times after application. Glyphosate fate was simulated with the PEARL 3.0 model. Simulated concentrations in bare soil pots were very close to the observed ones. However, the model lacks a process description for herbicide transport within a plant and, therefore, the observed and simulated glyphosate residues in soil after canopy applications did not correlate. Simulations highlight the importance of the translocation process in glyphosate fate. We conclude that also in field studies part of the detected glyphosate soil residues must originate from plant roots, and translocation process should be included both in leaching assessments and pesticide fate models.
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on a possible realistic case but not a worst case for leaching and sorption

Reliability	Low
Endpoint	Concentrations in soil, upper plant and roots
Protocol	No standard protocol; basic research, non-GLP
Test compound	Glyphosate
Test system and conditions	Pot trials, glasshouse
Statistical design	Parallel residue analyses for soil samples; single extraction for root samples due to limited availability of material. PEARL 3.0 model (FOCUSPEARL 3.3.3) for modelling
Relevance	
Environmental relevance	Given; parameter reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Landry et al. (2005)

Title: Leaching of glyphosate and AMPA under two soil management practices in Burgundy vineyards (Vosne-Romanée, 21-France)
Author: David Landry, Sylvie Dousset, Jean-Claude Fournier, Francis Andreux
Reference: Environmental Pollution 138, 191-200
Year: 2005

Results and conclusion: Some drinking water reservoirs under the vineyards of Burgundy are contaminated with herbicides. Thus the effectiveness of alternative soil management practices, such as grass cover, for reducing the leaching of glyphosate and its metabolite, AMPA, through soils was studied. The leaching of both molecules was studied in structured soil columns under outdoor conditions for 1 year. The soil was managed under two vineyard soil practices: a chemically treated bare calcosol, and a vegetated calcosol. After 680 mm of rainfall, the vegetated calcosol leachates contained lower amounts of glyphosate and AMPA (0.02 % and 0.03 %, respectively) than the bare calcosol leachates (0.06 % and 0.15 %, respectively). No glyphosate and only low amounts of AMPA (< 0.01 %) were extracted from the soil. Glyphosate, and to a greater extent, AMPA, leach through the soils; thus, both molecules may be potential contaminants of groundwater. However, the alternative soil management practice of grass cover could reduce groundwater contamination by the pesticide.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information for risk mitigation discussion	
Reliability	High
Endpoint	Concentrations in leachates; additionally: mineralisation of ^{14}C-glyphosate
Protocol	Mineralisation comparable to OECD 307; leaching: no standard protocol; non-GLP
Test compound	Unlabelled Glyphosate (98 % certified purity), [^{14}C]Glyphosate (99.7 % radiochemical purity), CAS-no.: 1071-83-6
Test system and conditions	Structured soil columns (15 cm inner diameter, 25 cm length) under outdoor conditions. Soil from 0 -20 cm layer of a) a grassed calcosol with rye-grass between the vine rows, b) chemically weeded bare calcosol. Duration: May 2001 – May 2002. Weekly rainfall recorded. Additionally: mineralisation studies with soil taken from the upper 30 cm, duration 42 days.
Statistical design	Mineralisation: 4 replicates for each soil and blank; leaching: one undisturbed soil column per field-plot.
Relevance	
Environmental relevance	Given, influencing parameter recorded.
Weight of evidence	
"Positive"/"Negative" evidence	Completely comparable other studies not known; no negative relevance.

Litz et al. (2011)

Title: Comparative studies on the retardation and reduction of glyphosate during subsurface passage
Author: N.T. Litz , A. Weigert, B. Krause, S. Heise, G. Grützmacher
Reference: Water Research 45, 3047-3054
Year: 2011

Results and conclusion:
The herbicide Glyphosate was detected in River Havel (Berlin, Germany) in concentrations between 0.1 and 2 mg/L. Laboratory (sorption and degradation studies) and technical scale investigations (bank filtration and slow sand filter experiments) were carried out.
Batch adsorption experiments with Glyphosate yielded a low K_f of 1.89 (1/n = 0.48) for concentrations between 0.1 and 100 mg/L. Degradation experiments at 8 °C with oxygen limitation resulted in a decrease of Glyphosate concentrations in the liquid phase probably due to slow adsorption (half life: 30 days). During technical scale slow sand filter (SSF) experiments Glyphosate attenuation was 70-80 % for constant inlet concentrations of 0.7, 3.5 and 11.6 mg/L, respectively. Relevant retardation of Glyphosate breakthrough was observed despite the low adsorption potential of the sandy filter substrate and the relatively high flow velocity. The VisualCXTFit model was applied with data from typical Berlin bank filtration sites to extrapolate the results to a realistic field setting and yielded sufficient attenuation within a few days of travel time. Experiments on an SSF planted with Phragmites australis and an unplanted SSF with mainly vertical flow conditions to which Glyphosate was continuously dosed showed that in the planted SSF Glyphosate retardation exceeds 54 % compared to 14 % retardation in the unplanted SSF. The results show that saturated subsurface passage has the potential to efficiently attenuate glyphosate, favorably with aerobic conditions, long travel times and the presence of planted riparian boundary buffer strips.

Proposed action:
To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.

Proposed action:
To be considered as supplementary information for risk mitigation.

Type of information (critical, high/low weight, supporting, additional):
High weight, supporting information on risk mitigation strategies

Reliability	High
Endpoint	K_f, K_d,1/n, concentrations of gylphosate and AMPA
Protocol	Batch experiments: according to OECD 106; degradation experiments: partly similar to OECD 307, enclosures and SSF experiments: no standard protocols available, non-GLP
Test compound	Glyphosate (98.7 % purity), CAS-no.:1071-83-6)
Test system and conditions	Laboratory batch, enclosure and slow sand filter tests, filter material used. Laboratory experiments: Degradation: partly reducing conditions, 5 sampling points. Batch experiments: 4 concentrations, number of replicates not given. Enclosures: area of 1m^2, height of 1.85 m (filtration length 1.00 m), situated within an infiltration pond (area: 90m^2), 3 Glyphosate levels. SSF experiments: two vertical-flow experimental SSFs: one without vegetation cover (average area 60 m^2, filter depth 0.8 m, filter volume 48m^3) and the other with a 3 year old vegetation cover of Phragmites australis (average area 68 m^2, filter depth 1.2 m, filter volume 81.6 m^3)
Statistical design	VisualCXTFit model, Freundlich isotherms,
Relevance	
Environmental relevance	Given, influencing environmental parameter recorded and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Publication with identical experimental setup not known, however results are logically explained; no negative evidence.

Malone et al. (2004)

Title: Residual and Contact Herbicide Transport through Field Lysimeters via Preferential Flow	
Author: R. W. Malone, M. J. Shipitalo, R. D. Wauchope, and H. Sumner	
Reference: J. Environ. Qual. 33:2141–2148	
Year: 2004	
Results and conclusion: Four monolith lysimeters (8.1 m^2 by 2.4 m deep) were used to investigate leaching of contact and residual herbicides under a worst-case scenario. Glufosinate, atrazine, alachlor, and linuron were applied in 1999 before corn (Zea mays L.) planting and glyphosate, alachlor, and metribuzin were applied in 2000 before soybean [Glycine max (L.) Merr.] planting. A high-intensity rainfall was applied shortly after herbicide application both years. Most alachlor, metribuzin, atrazine, and linuron losses occurred within 1.1 d of rainfall initiation and the peak concentration of the herbicides coincided (within 0.1 d of rainfall initiation in 2000). More of the applied metribuzin leached compared with alachlor during the first 1.1 d after rainfall initiation (2.2 % vs. 0.035 %, $P < 0.05$). In 1999, 10 of 24 discrete samples contained atrazine above the maximum contaminant level (atrazine maximum contaminant level [MCL] = 3 µg/L) while only one discrete sample contained glufosinate (19 µg/L, estimated MCL = 150 µg/L). The results indicate that because of preferential flow, the break-through time of herbicides was independent of their sorptive properties but the transport amount was dependent on the herbicide properties. Even with preferential flow, glyphosate and glufosinate were not transported to 2.4 m at concentrations approaching environmental concern.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, performed in USA	
Reliability	Medium
Endpoint	Concentration in leachate; preferential flow, transport through lysimeter
Protocol	Not according to BBA-guideline but different design
Test compound	Glyphosate, CAS-no. 1071-83-6
Test system and conditions	Outdoor lysimeter study, 7 pesticides applied onto the same lysimeter, rainfall simulation experiments, concentration in leachate/percolate
Statistical design	4 lysimeters used
Relevance	
Environmental relevance	Given; weather conditions and artificial rainfall recorded.
Weight of evidence	
"Positive"/"Negative" evidence	No completely comparable publication known, but results are plausible; no negative concern.

Pappas and Smith (2007)

Title: Effects of dredging an agricultural drainage ditch on water column herbicide concentration, as predicted by fluvarium techniques
Author: E.A. Pappas and D.R. Smith
Reference: Journal of Soil and Water Conservation, Volume 62, Number 4
Year: 2007
Results and conclusion: In artificially drained agricultural areas, dredging of drainage ditches is often necessary to ensure adequate field drainage. Stream-simulator (fluvarium) experiments were performed to evaluate the potential of associated bed material changes to impact water column concentrations of atrazine, metolachlor, and glyphosate. In the first experiments, water having high herbicide concentrations flowed across bed sediment collected from a ditch immediately before or after dredging. Afterward, water having initially zero herbicide concentrations flowed across these sediments. Results indicate that the bed sediments remaining after dredging, which had coarser texture and lower organic matter, may contribute to overall higher water herbicide levels in the short term by removing significantly less glyphosate from contaminated water and contributing marginally higher sustained levels of herbicide to uncontaminated water, applicable where sediments exhibit similar dredging characteristic effects. In this case, dredging when herbicide levels are expected to be lowest can help minimize increased transport of some herbicides.
Proposed action: Not to be considered as a specific hydraulic technique and its consequences for Glyphosate levels was investigated.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information

Reliability	Medium
Endpoint	Glyphosate concentrations in water column and sediment
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (as roundup), CAS-no.: 1071-83-6
Test system and conditions	Stream simulator experiments (fluvarium run)
Statistical design	2 fluvarium runs each
Relevance	
Environmental relevance	Given, parameter are reported.
Weight of evidence	
"Positive"/"Negative" evidence	No other comparable publication known; no negative evidence.

Queiroz et al. (2011)

Title: GLYPHOSATE TRANSPORT IN RUNOFF AND LEACHING WATERS IN AGRICULTURAL SOIL
Author: Gabriela Marina Pompeo Queiroz, Marcos Rivail da Silva, Renata Joaquim Ferraz Bianco, Adilson Pinheiro, Vander Kaufmann
Reference: Quim. Nova, Vol. 34, No. 2, 190-195
Year: 2011
Results and conclusion: Glyphosate was determined in runoff and leaching waters in agricultural soil that received an application of active ingredient and was exposed to simulated intensive rain conditions. The concentrations decreased during the simulation period and the concentrations of the runoff were higher than those achieved in the samples of leaching waters. The concentrations were lower than the pattern in the Brazilian Regulation MS N. 518/2004 for drinking water. The transported load of the applied active ingredient by the leaching was of 15.4 % (w/w) and for the runoff was of 1.7 % (w/w).
Proposed action: Not to be considered; non-European site.

Type of information (critical, high/low weight, supporting, additional): Low weight, additonal information on Glyphosate concentrations in runoff and leachate waters in Brasil	
Reliability	Low
Endpoint	Concentrations in leaching and runoff waters after application of Glyphosate
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity 99.7 %)
Test system and conditions	Outdoor trials, 1 m^2 plots, lysimeters
Statistical design	? (english summary only, text Spanish)
Relevance	
Environmental relevance	? (english summary only, text Spanish)
Weight of evidence	
"Positive"/"Negative" evidence	? (english summary only, text Spanish)

Schmidt and Boas (2006)

Title: Accompanying experiments on weed control on public footways using the roller wiper 'Rotofix'	
Author: Heinz Schmidt, Peter Boas	
Reference: Nachrichtenbl. Deut. Pflanzenschutzd., 58 (2), 46–49	
Year: 2006	
Results and conclusion: Accompanying investigations on the roller wiper 'Rotofix' were carried out in model and road trials to identify seepage of glyphosate in relation to different footway surfaces and joint width and to identify rain wash of the herbicide and its loss to road drainage in public road environment. The model trial used boxes with various footway surfaces (small set paving, pavement flags, water-bound surface). After treatment the plots were irrigated (8.5 and 14 mm) and the seeping water sampled at various time intervals and analysed for glyphosate and AMPA. Glyphosate peaked with 10.6 mg/l. The concentration was found in the seepage water from the flag-paved footway immediately after irrigation. Three days later, however, it had already decreased by more than three times. Results were different with the loamy footway (water-bound surface) and the small set paving. Peaks were identified on the third day after application, but were clearly below the pavement flag level. In the road trial, four samples were taken from road drainage. First results showed low concentrations of appr. 0.0002 mg/L glyphosate and up to 0.0005 mg/L AMPA. The higher AMPA level is assumed to be not only due to Rotofix application. It can be concluded that non-licensed herbicide applications on public and private roads and squares might result in risk to surface waters. However, the roller-wiper "Rotofix" can be used after licensed by local Authorities.	
Proposed action: Not to be considered as it presents risk management conclusions.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additonal information on Glyphosate concentrations in seeping water after application by a special technique on pavement	
Reliability	Low
Endpoint	Concentrations in seeping waters after application of Glyphosate
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; 10 % Roundup-Ultra)
Test system and conditions	Plot trials (worst-case scenarios), outdoor trials of selected roads (Berlin; after spring application performed by the city of Berlin), monitoring of selected receiving waters (Berlin)
Statistical design	Not specified

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Completely comparable publications not known, however, results are plausible; no negative evidence.

Spliid et al. (2006)

Title: Leaching and degradation of 21 pesticides in a full-scale model biobed	
Author: Niels Henrik Spliid, Arne Helweg, Kirsten Heinrichson	
Reference: Chemosphere 65 (2006) 2223–2232	
Year: 2006	
Results and conclusion: During the total study period of 563 days, no traces of 10 out of 21 applied pesticides were detected in the percolate. Glyphosate was not detected.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation by in a biobed
Protocol	Non-GLP study
Test compound	Labelled 2-^{13}C, ^{15}N-glyphosate was from Cambridge Isotope Laboratories (Andover, Massachusetts, US). (CAS 1071-83-6),
Test system and conditions	The degradation and leaching of 21 pesticides (5 g of each) was followed in an established full-scale model biobed. Percolate was collected and analysed for pesticide residues, and the biobed material was sampled at three different depths and analysed by liquid chromatography double mass spectrometry (LC-MS/MS).
Statistical design	Six replicates
Relevance	
Environmental relevance	The relevance is low as the results are not relevant for standard PEC-simulations.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Stadlbauer et al. (2005)

Title: Lysimeteruntersuchungen zur Verlagerung von Glyphosate im Lichte der Anwendung von Pflanzenschutzmitteln zur Beseitigung von winterharten Gründecken [Lysimeter investigations on the removal of glyphosate in the light of applications of pesticides for removal of winter green cover]	
Author: Stadlbauer H, Fank J., Lorbeer G	
Reference: paper read at: 11. Lysimetertagung, Lysimetrie im Netzwerk der Dynamik von Ökosystemen, 5. und 6. April 2005. Raumberg-Gumpenstein, Austria, 131-136	
Year: 2005	
Results and conclusion: Not to be considered as	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on leaching behaviour	
Reliability	Low
Endpoint	Concentrations of Glyphosate and AMPA in soil and leachate
Protocol	No standard protocol given; non-GLP
Test compound	Glyphosate, isopropylammonium salt ("Round-Up", CAS-no: 38641-94-0), AMPA (CS-no.: 1066-51-9)
Test system and conditions	Lysimeter studies, size of lysimeter not given, application of Round-up (3.872 L/ha) applied in March 2002, duration until April 2004, leachate sampling, soil sampling in different depths shortly after application
Statistical design	1295 leachate samples analysed for Glyphosate and AMPA; soil samples from 3 depths at four sampling times analysed for Glyphosate and AMPA
Relevance	
Environmental relevance	Given, weather conditions and soil parameter recorded.
Weight of evidence	
"Positive"/"Negative" evidence	Results are supported by other studies; no negative evidence.

Stone and Wilson (2006)

Title: Preferential Flow Estimates to an Agricultural Tile Drain with Implications for Glyphosate Transport	
Author: Wesley W. Stone and John T. Wilson	
Reference: J. Environ. Qual. 35:1825–1835	
Year: 2006	
Results and conclusion: Agricultural subsurface drains, commonly referred to as tile drains, are potentially significant pathways for the movement of fertilizers and pesticides to streams and ditches in much of the Midwest. This paper uses chloride concentrations to estimate preferential flow contributions to a tile drain during two storms in May 2004. Chloride, a conservative anion, was selected as the tracer because of differences in chloride concentrations between the two sources of water to the tile drain, preferential and matrix flow. A strong correlation between specific conductance and chloride concentration provided a mechanism to estimate chloride concentrations in the tile drain throughout the storm hydrographs. A simple mixing analysis was used to identify the preferential flow component of the storm hydrograph. During two storms, preferential flow contributed 11 and 51 % of total storm tile drain flow; the peak contributions, 40 and 81 %, coincided with the peak tile drain flow. Positive relations between glyphosate [N-(phosphonomethyl)glycine] concentrations and preferential flow for the two storms suggest that preferential flow is an important transport pathway to the tile drain.	
Proposed action: Not to be considered as field trials were performed outside EU and focus on Chloride as tracer.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only as publication focuses on Chloride as tracer	
Reliability	Low
Endpoint	Concentration of Glyphosate in drain flow
Protocol	No standard protocol available
Test compound	Glyphosate (CAS-no.: 1071-83-6)
Test system and conditions	Tests focused on Chloride as tracer; no detailed information on Glyphosate given
Statistical design	Not given for glyphosate
Relevance	
Environmental relevance	Not applicable as publication focuses on Chloride.

Weight of evidence	
"Positive"/"Negative" evidence	Not known as publication focuses on Chloride.

Strange-Hansen et al. (2004)

Title: Sorption, mineralization and mobility of N-(phosphonomethyl)glycine (glyphosate) in five different types of gravel
Author: Rikke Strange-Hansen, Peter E Holm, Ole S Jacobsen and Carsten S Jacobsen
Reference: Pest Manag Sci 60:570–578
Year: 2004
Results and conclusion: Cumulative mineralization of [methyl-^{14}C]glyphosate in batch studies was highest in coarse gravel, amounting to 14 % after 4 days at 30 °C and 32 % after 31 days. Mineralization was slowest in the sandy reference soil, amounting to only 2 % after 31 days. The adsorption coefficient (K_d) of glyphosate in gravel ranged from 62 to 164 litre/kg, while that in the sandy reference soil was 410 litre/kg. The results indicate that the relatively low K_d in gravel allows a relatively high rate of glyphosate mineralization by the biomass. When K_d is high, in contrast, mineralization is slow. Lowering the temperature to 10 °C decreased mineralization by 50 % in one of two gravels. The leaching of glyphosate was screened in simple columns of gravel or soil in which precipitation events (20 mm over a 2-h period) were simulated on three occasions, starting either immediately after or 2 days after application of glyphosate. [^{14}C]Glyphosate was applied as a tracer mixed with the commercial product Roundup Garden at the recommended rate of 2.4 kg glyphosate/ha, equivalent to 1µgg^{-1} soil. The highest concentration of [^{14}C] compounds (expressed in terms of glyphosate concentration) in leachate from the columns exceeded 1300 µg litre^{-1}, and was detected in rounded gravel after the first rain event. No glyphosate was detected in leachate from the sandy reference soil.
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, data on sorption/desorption is sufficient for the assessment.
Type of information (critical, high/low weight, supporting, additional): Low, additional information

Reliability	Medium
Endpoint	K_d = distribution coefficient; [^{14}C]carbon dioxide
Protocol	According to the OECD guidelines (106), but with a modified soil: solution ratio of 2 instead of 5; non-GLP
Test compound	[methyl-^{14}C]Glyphosate (specific activity 1.08 MBq mmol^{-1}; radio-chemical purity >99 %); unlabelled glyphosate (purity 98 %); for column experiments, Roundup Garden (commercial SL formulation containing 120 g litre^{-1} glyphosate (isopropylamine salt)); five types of gravel and a sandy agricultural reference soil, CAS-no.: 1071-83-6
Test system and conditions	Batch studies: 1) Sorption: glyphosate/[^{14}C]glyphosate concentration of 0.6 mg/kg (192 Bq), rotated for 96 h; 2) Mineralization in flasks: glyphosate/[^{14}C]glyphosate concentration of 16.9mg/kg (0.1 mM) and 833 Bq, final moisture level equivalent to 80 % of WHC, incubated at 30 °C in the dark for 31 days, repeated on two types of gravel incubated at 10, 20 and 30 °C; 3) Leaching studies: Two columns for each substrate, and each column was exposed to two different simulated precipitation events (Table 3). [^{14}C]glyphosate (1733 Bq in short columns, and 5666 Bq in tall columns) mixed with Roundup Garden (recommended rate of 2.4 kg glyphosate/ha), Over the next 6 days the columns were subjected to three simulated precipitation events at 20 ◦C, trapping the [^{14}C]carbon dioxide, total effluent collected 1 day after each precipitation event, determination of residual [^{14}C] compounds
Statistical design	Analyses in triplicate; K_d value represents one measurement of glyphosate in the solution, assuming linearity between glyphosate adsorption and glyphosate concentration in the solution.

Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications.

Styczen et al. (2011)

Title: Macroscopic Evidence of Sources of Particles for Facilitated Transport during Intensive Rain	
Author: Styczen M., Petersen C.T., Bender Koch C., Gjettermann B.	
Reference: Vadose Zone J. 10:1151–1161	
Year: 2011	
Results and conclusion: Particulate transport of pesticides, heavy metals, and phosphate in soil is of interest when evaluating water contamination risk; however, researchers differ in their view of how and where the contaminated particles are mobilized. The main line of thought is that the particles originate in the soil, as a function of concentration differences between aggregate surfaces and surrounding immobile water, and move into mobile water by diffusion. Furthermore, material can be stored at the air–water interface. Low electrical conductivity enhances the formation of mobile particles. Other researchers consider the generation to take place close to the soil surface, as a side effect of splash erosion. By combining data on particle concentrations and the amounts of particle-bound glyphosate [N-(phosphonomethyl) glycine] from three leaching experiments with information about glyphosate on splashed material and the distribution of glyphosate in the soil, we concluded that the particles from the top 0.5 cm of the soil column contribute more than proportionally (up to 50 %) to the particles in the leachate. The development in particle concentration with time and in columns with different properties indicated that particle generation took place both inside the column and as a result of the splash process. The leached particles that are generated inside the column probably stem from the flow-active part of the plow layer, a volume that differs from column to column and between tillage treatments.	
Proposed action:	
Not to be considered as basic research on sorption and transport mechanisms; will not lead to a modification of an endpoint	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only as publication focuses on basic research on sorption and transport mechanisms	
Reliability	Low
Endpoint	Concentration of particle-bound Glyphosate on leached and splash-eroded particles; concentration in soil layers at the end of the study
Protocol	Similar to OECD 312
Test compound	Commercial product Roundup Bio (isopropylammonium salt?, CAS-no.: 38641-94-0), ^{14}C-labeled glyphosate (CAS-no.: 1071-83-6), and blank formulation plus AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Undisturbed soil columns, 0-60 cm soil depth, 30 cm inner diameter, sampled from two experimental plots with different tillage treatments, product applied. Leachate sampling, soil subdivided into pieces at the end of the study
Statistical design	T-tests (two treatments with repetitions) were used to evaluate the amount of water draining from the columns as well as to compare particle concentrations and amounts for the two treatments.
Relevance	
Environmental relevance	Given; influencing parameter are considered and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Other publication support the results, no contradictions; no negative evidence.

Tesfamariam et al. (2009)

Title: Glyphosate in the rhizosphere – Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants	
Author: Tsehaye Tesfamariam, S. Bott, I. Cakmak, V. Römheld, G. Neumann	
Reference: Europ. J. Agronomy 31, 126–132	
Year: 2009	
Results and conclusion: Contradictory results are reported concerning the bio-availability of glyphosate residues in soils and the potential risks for intoxication of non-target organisms. This study addresses the question whether plant residues of glyphosate-treated weeds or direct soil application of glyphosate bears an intoxication risk for subsequently cultivated sunflower seedlings. Also the potential role of different waiting times between glyphosate application and sunflower cultivation was considered. Generally, the detrimental effects were more pronounced after glyphosate weed application (90 % biomass reduction) compared with direct soil application (55–70 % biomass reduction) at waiting time 0 d. The inhibitory effects on seedling growth were associated with a corresponding increase in shikimate accumulation in the root tissue as physiological indicator for glyphosate toxicity. Glyphosate intoxication of sunflower seedlings was also associated with an impairment of the manganese-nutritional status, which was still detectable after a waiting time of up to 21 d, particularly on the Arenosol in the variants with glyphosate weed application. These findings indicate an important and yet un-investigated role of glyphosate in plant residues in determining the risk of non-target plant intoxication.	
Proposed action: Not to be considered as article aims at role of Glyphosate in plant residues and thus no need for recalculation of an environmental fate endpoint is needed.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only as article aims at role of Glyphosate in plant residues	
Reliability	Low
Endpoint	Plant growth
Protocol	Similar to OECD 208
Test compound	Roundup Ultramax® glyphosate formulation (CAS-no.: 1071-83-6)
Test system and conditions	Treated weeds: model plant perennial rye grass, Lolium perenne L.; soil application; subsequently cultivated after Glyphosate application: sunflower (Helianthus annuus L.), The experiments were conducted as greenhouse studies on two soils with contrasting properties (acidic, sandy Arenosol, calcareous loess subsoil)
Statistical design	All treatments comprised 4 replicates and pots were arranged in the greenhouse in a completely randomized block design. Analysis of variance was performed with SPSS statistics software package (SPSS Inc., IL, USA).
Relevance	
Environmental relevance	Given; influencing parameter are considered and discussed.
Weight of evidence	
"Positive"/"Negative" evidence	Other publication support the results, no contradictions; no negative evidence.

Ulén et al. (2012)

Title: Particulate-facilitated leaching of glyphosate and phosphorus from a marine clay soil via tile drains	
Author: B. ULÉN, G. ALEX, J. KREUGER, A. SVANBÄCK & A. ETANA	
Reference: Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2012; 62: Supplement 2, 241-251	
Year: 2012	
Results and conclusion: The objective of the present study was to quantify and evaluate leaching of glyphosate (Gly) in parallel with P. Leaching losses of autumn-applied Gly (1.06 kg/ha) via drainage water were examined by flow-proportional sampling of discharge from 20 drained plots in a field experiment in eastern Sweden. Samples were analysed for Gly in particulate-bound (PGly) and dissolved (DGly) form. The first 10 mm water discharge contained no detectable Gly, but the following 70 mm had total Gly (TotGly) concentrations of up to 6 µg/L, with 62 % occurring as PGly. On average, 0.7 g TotGly ha^{-1} was leached from conventionally ploughed plots, compared with 1.7 g TotGly ha^{-1} from shallow-tilled plots (cultivator to 12 cm working depth). Higher Gly losses occurred in snowmelt periods in spring, but then with the majority (60 %) as DGly. All autumn concentrations of PGly in drainage water were significantly correlated (p<0.001) to the concentrations of particulate-bound phosphorus (PP) lost from the different plots (Pearson correlation coefficient 0.84), while PP concentrations were in turn significantly correlated to water turbidity (Pearson correlation coefficient 0.81). Leaching losses of TotGly were significantly lower (by 1.3 g/ha; p<0.01) from plots that had been structure-limed three years previously and ploughed thereafter than from shallow-tilled plots. Turbidity and PP concentration also tended to be lowest in discharge from structure-limed plots and highest from shallow-tilled plots. This difference in TotGly leaching between soil management regimes could not be explained by differences in measured pH in drainage water or amount of discharge. However, previously structure-limed plots had significantly better aggregate stability, measured as readily dispersed clay (RDC), than unlimited plots. The effects of building up good soil structure, with strong soil aggregates and an appropriate pore system in the topsoil, on mitigating Gly and P losses in particulate and dissolved form should be further investigated.	
Proposed action: To be considered as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information from a field study	
Reliability	
Endpoint	Leaching of glyphosate and phosphorus
Protocol	Not given
Test compound	Glyphosate (Glypro Bio, 1.06 kg/ha active substance)

Test system and conditions	Experimental plots and soil characteristics: In 2006, an experimental field with a sub-surface drainage water collection system was constructed on a flat plain close to the Lake Bornsjön reservoir by Stockholm Water Company. It encompasses 28 drained plots, 20 of which were used in the present experiment. In order to match the experimental plots to farm machinery, their dimensions are 20 m × 24 m (0.048 ha) and the drains are placed centrally, with 8 m spacing, in order to effectively drain the soil. Drainage water flows to a sampling and measuring station and is recorded with tilting vessels and data logger. The data logger controls the flow-proportional sampling by means of small tube pumps in the basement of the station. After a certain volume of water has passed, the suction tube is first cleaned by reverse pumping and thereafter a small volume is sampled. The flow-proportional (composite) sampling took place in dark glass vessels (2.5 L) at relatively cold temperature and in darkness for a maximum of one week prior to freezing the water samples and transport to the laboratory before analysis. Glyphosate application and cultivation practices: In preceding years the crops were: winter wheat in 2007, spring barley in 2008 and 2009 and oats in 2010. No Gly had been applied to the actual experimental plots for the previous three years. Phosphorus fertilization (mean year 1988-2006) was 11 kg/ha year^{-1}, always applied in mineral form in spring. This is a moderate load, since the area has special restrictions. When starting the experiment the aim was to avoid P limitation of the crop and therefore 20 kg/ha year^{-1} were applied in 2007-2011 for all plots except four. Glyphosate was applied on 22 September 2010 as the commercial product Glypro Bio, at a rate equal to 1.06 kg/ha active substance. This amount, which represents a normal dose in Swedish production systems, was applied in evening at air temperature 11°C and no wind. Twelve days later, the conventional and structure-limed plots were stubble-harrowed and eight plots were shallow-tilled (12 cm) twice and reconsolidated with a rib-roller. After a further 10 days, the conventionally ploughed plots (8) and the structure-limed plots (4) were mouldboard-ploughed and the soil was inverted to a depth of 23 cm. Sampling and analysis: On 28 March, 186 days after glyphosate application in autumn, turbidity was observed once again in the flow-proportionally sampled water and additional water was collected for Gly analysis, which was performed on the 14 most turbid samples. The same analytical procedure was used for both PGly and DGly and involved ion-exchange and derivatization, using a modified version of Mogadati *et al.* (1996), followed by final identification and quantification by gas chromatography-mass spectrometry (GC-MS). The limit of detection (LOD) was 0.03, 0.1 and 0.2 mg/L for DGly, PGly and AMPA, respectively, with occasional higher LODs due to background interference. The limit of quantification (LOQ) was 2-3 times higher.
Statistical design	The mean and standard deviation were calculated for the experimental parameters determined in all flow-proportional samples from replicate plots for the different treatments. If no residue of Gly or AMPA was detected in a given sample, the value 0 was used for calculating the mean. Pearson correlation and regression linear relationships were determined between the parameters total glyphosate (TotGly=PGly+DGly), TotP, PGly, PP and turbidity for the autumn period (27 September-15 November) and between TotP and turbidity for the spring period (21 March-11 April). Any differences in glyphosate concentrations between the different soil treatments were analysed using Bonferroni post test assuming equal variance and a significance level of <B0.05. Leaching losses from the different plots in the autumn period were calculated by multiplying discharge by measured flow-proportional concentrations in the periods between sample collections. In the spring period, transport of TotGly was estimated from measured values from 14 plots on 28 March. Without any measured values the transport was estimated from TotP transport using the relationship between TotP and TotGly as determined for the 14 samples.

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Ulén et al. (2013)

Title: Spatial variation in herbicide leaching from a marine clay soil via subsurface drains
Author: B. M. Ulén, M. Larsbo, J. K. Kreuger and A. Svanbäck
Reference: Published online in Wiley Online Library: 13 June 2013, (wileyonlinelibrary.com) DOI 10.1002/ps.3574
Year: 2013
Results and conclusion: BACKGROUND: Subsurface transport via tile drains can significantly contribute to pesticide contamination of surface waters. The spatial variation in subsurface leaching of normally applied herbicides was examined together with phosphorus losses in 24 experimental plots with water sampled flow-proportionally. The study site was a flat, tile-drained area with 60 % marine clay in the topsoil in southeast Sweden. The objectives were to quantify the leaching of frequently used herbicides from a tile drained cracking clay soil and to evaluate the variation in leaching within the experimental area and relate this to topsoil management practices (tillage method and structure liming). RESULTS: In summer 2009, 0.14, 0.22 and 1.62 %, respectively, of simultaneously applied amounts of MCPA, fluroxypyr and clopyralid were leached by heavy rain five days after spraying. In summer 2011, on average 0.70 % of applied bentazone was leached by short bursts of intensive rain 12 days after application. Peak flow concentrations for 50 % of the treated area for MCPA and 33 % for bentazone exceeded the Swedish no-effect guideline values for aquatic ecosystems. Approximately 0.08 % of the glyphosate applied was leached in dissolved form in the winters of 2008/2009 and 2010/2011. Based on measurements of glyphosate in particulate form, total glyphosate losses were twice as high (0.16 %) in the second winter. The spatial inter-plot variation was large (72–115 %) for all five herbicides studied, despite small variations (25 %) in water discharge. CONCLUSIONS: The study shows the importance of local scale soil transport properties for herbicide leaching in cracking clay soils. In detail: Concentrations of the herbicides bentazone, clopyralid, fluroxypyr, MCPA and glyphosate were measured in subsurface drain discharge from a clay field during a four-year study. Despite hydrological conditions not representing a worst case scenario for leaching, the relative leaching losses of all herbicides studied were large compared to values reported in the literature. Measured concentrations of bentazone and MCPA exceeded Swedish guideline values based on predicted no effect on aquatic ecosystems for 50 and 33 % of the plots for MCPA and bentazone, respectively. All substances studied (except sulphonylure as which were not detected), irrespective of sorption strength, showed similar leaching patterns. These observations clearly demonstrate that preferential transport in macropores is the dominant transport process at this site. The variation in relative leaching losses between plots within the same treatment was greater than that between different substances. Crack stabilisation by gyttja, especially in the deeper subsoil, was suggested as an important explanatory factor for this large spatial variation in pesticide leaching, although it was not possible to investigate differences in gyttja content between plots. Continuous macropores connecting the soil surface to the subsoil may be a factor contributing to the generally large pesticide losses observed after shallow tillage. However, careful studies of soil macropore systems, including topsoil and subsoil properties, are needed to explain the unpredictability in leaching at this site.
Proposed action: To be considered as additional information.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information from a field study

Reliability	
Endpoint	Leaching of pesticides: Bentazone, MCPA, Fluroxypyr, Clopyralid, Glyphosate, Thifensulfuron-methyl and Tribenuron-methyl
Protocol	Not given

Test compound	Bentazone, MCPA, Fluroxypyr, Clopyralid, Glyphosate, Thifensulfuron-methyl and Tribenuron-methyl
Test system and conditions	The field site is located in a flat valley (mean slope less than 0.05‰) with a clay soil of marine origin in eastern Sweden. The experimental field (1.3 ha) with 28 plots (24 m × 20 m) was tile-drained in 2006 to 0.9 m depth (8 m spacing).9 Twenty-four of these plots were used in the experiment. The plots are situated in two rows of 14 plots at varying distance from an open ditch that acts as the recipient of drainage water from the surrounding valley. Three management practices were randomly assigned to the plots: Conventional autumn ploughing, shallow autumn tillage and structure-liming (i.e. liming carried out to reduce phosphorus leaching and to improve crop yield by improving soil structure). The leaching of seven different pesticides (Bentazone, MCPA, Fluroxypyr, Clopyralid, Glyphosate, Thifensulfuron-methyl and Tribenuron-methyl) with contrasting properties was studied. Water discharge from each plot was measured with tilting vessels in an underground basement where sampling of drainage water also took place. The water was sampled flow-proportionally, with every subsample representing 0.003 mm discharge in summer and 0.04 mm discharge in the rest of the year. The bulk samples were collected weekly (or for the first flow events following application more frequently). Accordingly, the actual peak concentrations were not captured. Immediately after collection all samples were frozen and sent to the Organic Risk Pollutants Laboratory, Department of Aquatic Sciences and Assessment, SLU, where they were analysed when detectable concentrations of herbicides were expected. The concentration of thifensulfuron-methyl and tribenu-ronmethyl (in 2008) was determined with solid-phase extraction followed by liquid chromatography and mass spectrometry (LCMS) and the concentration of clopyralid, fluroxypyr and MCPA (in 2009) by the same solid-phase extraction and by derivatisation and gas chromatography/mass spectrometry (GC/MS).14 Fluroxypyr and MCPA(in 2010)and bentazone(in 2011) were analysed by mass spectrometric determination (LC-MS/MS).15 Dissolved glyphosate(DissGly) and its main metabolite AMPA were analysed in winter 2008/2009 and 2010/2011, which involved ion exchange and derivatisation, followed by final identification and quantification by GC/MS. In winter 2010/2011, glyphosate analysis included particulate glyphosate (PartGly), which was trapped using a cellulose acetate filter with pore size 0.45 µm. The median value for limit of detection (LOD; in µg/L) was: 0.003 for bentazone, 0.005 for clopyralid, 0.01 for fluroxypyr, 0.003 for MCPA, 0.006 for thifensulfuron-methyl and tribenuron-methyl, 0.03 for dissolved glyphosate, 0.1 for particulate glyphosate and 0.2 for AMPA. Measured concentrations were below the LOD values for dissolved glyphosate, particulate glyphosate and AMPA in 20, 22 and 45 % of the samples, respectively. Dissolved reactive phosphorus (DRP) and particulate phosphorus (PP) were determined for all samples which were analysed for any pesticides.
Statistical design	Mean, SD, range, maximum
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Vogel and Linard (2011)

Title: AGRICULTURAL HERBICIDE TRANSPORT IN A FIRST-ORDER INTERMITTENT STREAM, NEBRASKA, USA	
Author: J. R. Vogel, J. I. Linard	
Reference: APPLIED ENGINEERING IN AGRICULTURE, Vol. 27(1): 63-74	
Year: 2011	
Results and conclusion: In this study, the transport of 6 herbicides and 12 herbicide degradates was examined during the 2004 growing season in an intermediate-scale agricultural watershed (146 ha) that is drained by a first- order intermittent stream, and the mass load for each herbicide in the stream was estimated. The herbicide load during the first week of storm events after application ranged from 17 % of annual load for trifluralin to 84 % of annual load for acetochlor. The maximum weekly herbicide load in the stream was generally within the first 3 weeks after application for those compounds that were applied within the watershed during 2004, and later for herbicides not applied within the watershed during 2004 but still detected in the stream. The apparent dominant mode of herbicide transport in the stream determined by analysis amongst herbicide and conservative ion concentrations at different points in the hydrograph and in base flow samples- was either overland runoff or shallow subsurface flow, depending on the elapsed time after application and type of herbicide. The load as a percentage of use (LAPU) for the parent compounds in this study was similar to literature values for those compounds applied by the farmer within the watershed, but smaller for those herbicides that had rainfall as their only source within the watershed.	
Proposed action: To be considered as additional information only since the soil since these studies are not required. Furthermore, available data is sufficient for the assessment and the studies were not performed close to standard tests.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as focus was on transport processes in agricultural areas	
Reliability	Medium
Endpoint	Herbicide concentrations in stream base flow, rain, and lysimeter samples; timing of maximum load in the stream, along with the type of transport at that time
Protocol	Non-GLP; field experiments, lysimeter
Test compound	Atrazine, acetochlor, trifluralin, glyphosate, pendimethalin, metolachlor, alachlor, and dimethenamid; herbicide degradates
Test system and conditions	This study was conducted in a small agricultural watershed. The application of test compounds was spread among 14 individual cropped areas within the watershed, with no particular upstream to downstream pattern in their distribution. Sampling intervals varied throughout the experiment, with the shortest intervals (2 min) during the time nearest the peak concentration in the stream at the sampling point. Time of travel was determined by calculating the time lapse from release of the dye to peak concentration at the sampling point.
Statistical design	Calculated by interpolation between times of streamflow and sampling events, (when necessary, modelled results for discharge from SWAT); pre-processing software, Better Assessment Science for Integrating point and Non-point Sources (BASINS) (USEPA, 2004), was used to create the default parameter files necessary for initial simulations
Relevance	
Environmental relevance	Given; influencing parameters are reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

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Detailed description of open literature – Photochemical degradation in water

Chen et al. (2012)

Title: Photocatalytic mineralization of glyphosate in a small-scale plug flow simulation reactor by UV/TiO_2	
Author: JIAN Q. CHEN, ZHI J. HU and NAN X. WANG	
Reference: Journal of Environmental Science and Health, Part B (2012) 47, 579-588	
Year: 2012	
Results and conclusion: The present work involves the photocatalytic mineralization of glyphosate on a plug flow reactor by UV/TiO_2. The effect of catalyst loading shows an optimal value (0.4 g/L) which is necessary to mineralize glyphosate. The kinetic rate of glyphosate mineralization decreases with the increasing initial concentration of glyphosate, and the data can be described using the first-order model. An alkaline environment is conducive to glyphosate mineralization. The mineralization efficiency increases with elevated flow rate to 114 mL min^{-1}, which is followed by a decrease with a further increase in flow rate due to the reduction of the residence time. The presence of external oxidants ($K_2S_2O_8$, H_2O_2 and $KBrO_3$) and photosencitizer (humic acid) can significantly enhance glyphosate mineralization. Photocatalysis oxidation ability of the three studied oxidants decrease in the order of: $S_2O_8^{2-} > BrO_3^- > H_2O_2$. Finally, the Langmuir–Hinshelwood (L-H) model was used to rationalize the mechanisms of reactions occurring on TiO_2 surfaces and L-H model constants were also determined.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Degradation in aqeous medium by photocatalytic mineralization
Protocol	Non-GLP study
Test compound	98 % pure glyphosate (N-phosphonomethylglycine) obtained from Yangnong Chemical Group Co., Ltd, Jiangsu, China (CAS 1071-83-6)
Test system and conditions	A certain amount of TiO_2 catalyst and glyphosate solution were loaded into the quartz jacket and then fitted into the constant temperature water bath (20±1◦C), which was fixed on the base of the heater and magnetic stirrer. Prior to an UV irradiation, the suspension was magnetically stirred for 15 min in order to achieve a maximal adsorption of the glyphosate on TiO_2 surface. The suspension was kept stirring during the entire mineralization process. Aliquots of 1.5 mL suspension were sampled from a sample outlet using a syringe at specific time intervals and the supernatant was obtained by high speed centrifugation for 10 min. The supernatants were then stored at 4°C until determination of phosphate (one of inorganic products of glyphosate mineralization). Solution pH (3.20– 11.11), initial concentration (2.0 × 10−4–8.0 × 10−4 mol/L), catalyst loading (0-0.8 g/L), and the presence of oxidants, photosensitizer, organic compounds, inorganic metal cations and anions were investigated for their effects on the photo mineralization efficiency.

Statistical design	No information
Relevance	
Environmental relevance	The relevance is small due to the artificial environment.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

Manassero et al. (2010)

Title: Glyphosate degradation in water employing the H_2O_2/UVC process	
Author: A. Manassero, C. Passalia, A.C. Negro, A.E. Cassano, C.S. Zalazar	
Reference: water research 44 (2010) 3875-3882	
Year: 2010	
Results and conclusion: Conversion of glyphosate increases significantly from pH = 3.7. From this value on, the increase becomes much less noticeable. The reaction rate depends on the initial herbicide concentration and has an optimum plateau of a hydrogen peroxide to glyphosate molar concentration ratio between 7 and 19. The expected non linear dependence on the irradiation rate was observed.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Glyphosate degradation in water using H_2O_2 and UV radiation
Protocol	Non-GLP study
Test compound	glyphosate (95 % provided by Red Surcos), CAS 1071-83-6 AMPA (>99 %, SigmaeAldrich) CAS 1066-51-9
Test system and conditions	The photodegradation of glyphosate was carried out in a cylindrical reactor made of Teflon TM, with two parallel, flat windows made of quartz (Reactor = 110 cm^3). Each window was irradiated with a tubular, germicidal lamp (lambda 253.7 nm) placed at the focal axis of a parabolic reflector made of mirror finished aluminium. Glyphosate was analyzed by ion chromatography with a suppressed conductivity detector.
Statistical design	No information
Relevance	
Environmental relevance	The results are of minor relevance since the experiments do not describe any of the standard endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared directly with standard tests.

Zhang et al. (2012)

Title: Enhanced photocatalytic performance of titania nanotubes modified with sulfuric acid	
Author: Guo-Wen Zhang, Guo-Hua He, Wei-Liang Xue, Xiong-Fa Xu, Dan-Ni Liu, Yue-Hua Xu	
Reference: Journal of Molecular Catalysis A: Chemical 363– 364 (2012) 423– 429	
Year: 2012	
Results and conclusion: Titania nanotubes modified with sulfuric acid (S-TNTs) were synthesized through hydrothermal treatment and impregnation method, and characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), photoluminescence spectra (PL), Fourier transform infrared spectroscopy (FT-IR), BET surface area and X-ray photoelectron spectroscopy (XPS). The photocatalytic activities of S-TNTs were investigated by the glyphosate degradation. The lower the PL intensity, the higher the photocatalytic activity. The sulfuric acid modification enhanced the photocatalytic activity of TNTs, and 7 %S-TNTs calcined at 400°C showed the highest photocatalytic activity. The photocatalytic performance of as-prepared S-TNTs was strongly related with the sulfuric acid concentration and the degree of crystallinity. The nanotube morphology, the specific surface area, as well as the crystallite size also had important impact on the photocatalytic activity of S-TNTs.	
Proposed action: Consider as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	
Endpoint	Degradation in aqeous medium by photocatalytic mineralization
Protocol	Non-GLP study
Test compound	Glyphosate (CAS 1071-83-6)
Test system and conditions	Aqueous slurries were prepared by adding 0.1 g photocatalyst to 500 ml of 1.0 × 10−4 mol/L glyphosate aqueous solution at neutral pH. Irradiations were performed with a 125 W high-pressure mercury lamp, and Fig. 1 shows the wavelength and intensity of this 125 W high-pressure mercury lamp. The high-pressure mercury lamp irradiates lights of many wavelengths including visible lights (400–600 nm). The aqueous slurries were stirred and bubbled with oxygen for 30 min prior to the irradiation. And then, at 10 min intervals, the suspension was extracted and centrifuged to separate the photocatalyst particles. The final oxidation product PO_4^{3-} concentration of the supernatant liquid was analyzed using the Mo– Sb–Ascorbic acid colorimetry.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is small due to the artificial environment.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with standard testing.

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Chen, J.Q., Hu, Z.J. and Wang, N.X. 2012. Photocatalytic mineralization of glyphosate in a small-scale plug flow simulation reactor by UV/TiO_2. Journal of Environmental Science and Health, Part B 47, 579–588.

Manassero, A., Passalia, C., Negro, A.C., Cassano, A.E., Zalazar, C.S. 2010. Glyphosate degradation in water employing the H_2O_2/UVC process. Water research 44: 3875-3882.

Zhang *et al.* 2012. Enhanced photocatalytic performance of titania nanotubes modified with sulfuric acid. Journal of Molecular Catalysis A: Chemical 363– 364 (2012) 423– 429.

Detailed description of open literature – Water/sediment studies

Degenhardt et al. (2012)

Title: Dissipation of glyphosate and aminomethylphosphonic acid in water and sediment of two Canadian prairie wetlands	
Author: DANI DEGENHARDT, DAVID HUMPHRIES, ALLAN J. CESSNA, PAULMESSING, PASCAL H. BADIOU, RENATA RAINA, ANNEMIEKE FARENHORST and DAN J. PENNOCK	
Reference: Journal of Environmental Science and Health, Part B (2012) 47, 631–639	
Year: 2012	
Results and conclusion: Glyphosate has been detected in a range of surface waters but this is the first study to monitor its fate in prairie wetlands situated in agricultural fields. An ephemeral wetland (E) and a semi-permanent wetland (SP) were each divided into halves using a polyvinyl curtain. One half of each wetland was fortified with glyphosate with the added mass simulating an accidental direct overspray. The results showed that the acute toxic effects of glyphosate contamination will be limited even for a worst-case point-source scenario resulting from direct overspray because glyphosate dissipated very rapidly in the water column of both an ephemeral and semipermanent wetland (field DT_{50} values of 1.3 and 4.8 d, respectively). Degradation of glyphosate to its major metabolite AMPA in the water-column and sorption of the herbicide to bottom sediment were more important pathways for the dissipation of glyphosate from the water column than movement of the herbicide with infiltrating water. Based upon the maximum concentration of glyphosate detected in the sediment, sorption to bottom sediment accounted for approximately 67 % and 10 % of the total glyphosate added to the wetland E and wetland SP, respectively.	
Proposed action: Consider as additional information. Recalculation of endpoints on dissipation from water is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Supporting information.	
Reliability	
Endpoint	Dissipation of glyphosate in water and sediment
Protocol	Non-GLP study
Test compound	Glyfos (Cheminova, Denmark), containing 360 g acid equivalent (a.e.)/L glyphosate as its isopropylamine salt (CAS 1071-83-6), unlabelled
Test system and conditions	Two wetlands were selected for the study, they were situated within a cultivated field and consisted of a smaller ephemeral wetland (E) and a larger semipermanent wetland (SP). Glyfos was applied and water and sediment samples were collected from day 1 to 77.
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared to results from reliable water/sediment studies.

Mercurio et al. (2014)

Title: Glyphosate persistence in seawater
Author: P. Mercurio, F. Flores, J.F. Mueller, S. Carter, A.P. Negri
Reference: Mar. Pollut. Bull. (2014), http://dx.doi.org/10.1016/j.marpolbul.2014.01.021
Year: 2014

Results and conclusion:
The biodegradation of glyphosate using standard simulation flask tests with native bacterial populations and coastal seawater from the Great Barrier Reef was quantified. The half-life for glyphosate at 25 °C in low-light was 47 days, extending to 267 days in the dark at 25 °C and 315 days in the dark at 31 °C, which is the longest persistence reported for this herbicide. AMPA, the microbial transformation product of glyphosate, was detected under all conditions, confirming that degradation was mediated by the native microbial community. This study demonstrates glyphosate is moderately persistent in the marine water under low light conditions and is highly persistent in the dark. Little degradation would be expected during flood plumes in the tropics, which could potentially deliver dissolved and sediment-bound glyphosate far from shore.

Proposed action:
Consider as additional information. Furthermore, the experimental site was outside the EU.

Type of information (critical, high/low weight, supporting, additional):
Additional information

Reliability

Endpoint	$DegT_{50}$ in water
Protocol	Non-GLP study according to the OECD methods for "simulation tests" (OECD, 2005)
Test compound	The glyphosate standard was obtained from Sigma–Aldrich.
Test system and conditions	The tests were conducted in natural seawater containing a native bacterial community and no addition of nutrients or artificial inoculum to best mimic ecological conditions. The tests were conducted under three scenarios: (1) 25 °C in the dark which corresponds to the mean annual seawater temperature on the GBR (AIMS, 2013); (2) 25 °C in low light conditions and (3) 31 °C in the dark which is a summer maximum temperature for nearshore areas of the mid-northern regions of the GBR. Three temperature-regulated incubator shakers (Thermoline TLM-530) were used in the experiments. A series of 6 × 900 mm LED strips (Superlight LED Lighting, Generation 3 High-Output LED Turbostrip) were fitted to one shaker, providing an even light environment of 40 µmol photons m^{-2} s^{-1} over a 12:12 light day cycle. This is equivalent to 1.7 mol photons m^{-2} day^{-1} which is within the range of light environments measured in shallow 3–6 m depths on turbid nearshore reefs of the GBR during the wet season. The position of flasks was randomised after every sampling period and flasks were consistently shaken at 100 rpm. All glassware was washed at 90 °C with laboratory detergent, rinsed and oven dried at 100 °C, acid washed (10 % HCl), rinsed × 5 with RO then Milli-Q water until pH neutral, oven dried a second time at 100 °C, baked in a muffle furnace at 350 °C for 30 minutes, and capped with aluminium foil until use. The glyphosate standard was purchased from Sigma–Aldrich, added to 2 mL of the carrier solvent ethanol (to assist in solubility), and made to 5 mg/L concentration with Milli-Q water. Coastal water was collected from19°16′ (S), 147° 03′ (E) and filtered to 20 µm to introduce the total bacterial diversity from this environment. The seawater was added to 500 mL Erlenmeyer flasks to a final volume of 300 mL and sample treatments were spiked with a final concentration of 10 µg/L glyphosate. The same volume of carrier was added to control sample flasks and was 0.0004 % (v/v). Each flask was stoppered with autoclaved silicone bungs to allow for aerobic conditions. The physical/chemical characteristics of the filtered seawater were measured for: pH, DIC, DOC, DIN, DON, TSS, bacterial counts and particle size distribution. Flow cytometry was used to quantify the microbial populations in the seawater used in the experiment. Samples were fixed with 5 % formaldehyde and stored at 4 °C. Sub-samples were stained using Sybr Green, diluted to 1:10,000, and allowed to develop in the dark for 30 min. Samples were run using a BD Accuri C6 cytometer (BD Biosciences, CA, USA) equipped with a red and blue laser (488 nm, 50mWmaximum solid state; 640 nm, 30mWdiode) and standard filter setup. Flow rate was 14 µL min^{-1}, 10-µm core. The natural microbial community populations and their abundances were measured for the initial seawater as well as treatments for the experiment using the Accuri CFlow plus software. For each sampling period, 5 mL control and glyphosate samples were collected and stored at 4 °C. The glyphosate and degradation product concentrations were

	determined by HPLC-MS/MS using an ABSciex 4000Q Trap mass spectrometer (ABSciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface and coupled to a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan).
Statistical design	Replicates were used. The half-lives ($T_{½}$) for glyphosate were calculated assuming first order kinetics.
Relevance	
Environmental relevance	Reliable water/sediment studies have shown that, in addition to microbial degradation, a major contributor to the aquatic dissipation of glyphosate is adsorption to the sediment. The study of Mercurio *et al.* (2014) is less relevant since the authors investigated degradation of glyphosate in natural seawater containing a native bacterial community but without any sediment, which does not reflect the conditions of the presence of glyphosate in the aquatic environment. The calculated $DegT_{50}$ values in water cannot be compared to $DissT_{50}$ values in water derived from relibale water/sediment studies.
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared to results from reliable water/sediment studies.

Tsui and Chu (2008)

Title: Environmental fate and non-target impact of glyphosate-based herbicide (Roundup) in a subtropical wetland	
Author: M.T.K. Tsui, L.M. Chu	
Reference: Chemosphere 71, 439–446	
Year: 2008	
Results and conclusion: For both ponds, glyphosate concentrations in the water decreased rapidly after 1-3 DPT, but then decreased gradually over time. Both physical adsorption to the bottom sediments and microbial degradation are thought to contribute to these decreases. Interestingly, the persistence of glyphosate in the freshwater pond was longer than in the estuarine system, which is likely due to the considerably higher concentrations of chelating metals (i.e. Cu and Fe) present in the sediment (4.5 and 11-fold higher, respectively) which potentially reduced the bioavailability of glyphosate to the microbial decomposers. Lastly, fishes used in the in situ bioassays (both in applied and unapplied areas) showed similar survival rates, indicating that the use of Roundup at the provided application rate posed no serious hazard.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary. Furthermore, the experimental site was outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6),
Test system and conditions	Glyphosate degradation in pond experiments. The two study sites were estuarine and freshwater ponds in Mai Po. The air temperature recorded for both experiments ranged from 23 to 26 °C.
Statistical design	Two replicates
Relevance	
Environmental relevance	The relevance is less relevant since the authors did not calculate any DT_{50} values.

Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with reliable studies.

References

Degenhardt *et al.* 2012. Dissipation of glyphosate and aminomethylphosphonic acid in water and sediment of two Canadian prairie wetlands. Journal of Environmental Science and Health, Part B (2012) 47, 631–639

Mercurio, P., Flores, F., Mueller, J.F., Carter, S., Negri, A.P. 2014. Glyphosate persistence in seawater. Mar. Pollut. Bull. (2014), http://dx.doi.org/10.1016/j.marpolbul.2014.01.021

Tsui, M.T.K, Chu, L.M. 2008. Environmental fate and non-target impact of glyphosate-based herbicide (Roundup) in a subtropical wetland. Chemosphere 71: 439–446.

Detailed description of open literature – Impact on water treatment procedures

Boucherie et al. (2010)

Title: "Ozone" and "GAC filtration" synergy for removal of emerging micropollutants in a drinking water treatment plant?	
Author: C. Boucherie, C. Lecarpentier, N. Fauchon, M. Djafer and V. Heim	
Reference: Water Science & Technology: Water Supply—WSTWS | 10.5 | 2010	
Year: 2010	
Results and conclusion: Ozonation plays an essential role in water disinfection to inactivate viruses, bacteria and some parasites (Giardia). Ozone treatment rates to attain disinfection goals also result in oxidation reactions of emerging pollutants. Pharmaceuticals – except Ciprofloxacin – are very reactive to ozone: they are removed as early as the transfer compartment outlet even at an ozone treatment rate of less than 1 g/m^3. Glyphosate, AMPA, Amitrole and Diuron– the four major pesticides in the Seine, Marne and Oise rivers–are reactive to ozone. Twenty-one pesticides are only partially reactive to ozone and an additional "GAC filtration" is needed to remove them. Further investigations have been planned to study the removal of Phthalates, Nonylphenols and Hormones by combining the "Ozone" and "GAC filtration" process units.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information	
Reliability	Medium
Endpoint	PH, temperature, alkalinity (AT), UV254, ozone gas and liquid residual; concentration of micropollutants
Protocol	Non-GLP
Test compound	Bezafibrate, Carbamazepin, Ciproflexacin, Diclofenac, Erythromycin, Fenofibrate, Ketoprofen, Metoprolol, Ofloxacin, Paracetamol, Phenazone, Propanolol, Roxithromycin, Spiramycin, Sulfachloropyridazine, Sulfamerazine Sulfamethoxazole, Tylosine, Acetochlore Alachlore Amitrole AMPA, Atrazine, Azoxystrobine, Bentazone, Bromuconazole, Carbendazime, Carbetamide, Carbofuran, Chloridazone, Chlortoluron, DCPMU, DEA, DEDIA, Deethylterbumeton, DIA, Dichloroprop, Difenoconazole, Dimetachlore, Diuron, Ethofumesate, Fluquinconazole, Flusilazole, Glyphosate, Hydroxyatrazine, Imazamethabenz-methyl, Isoproturon, MCPA, Mecoprop, Metazachlore, Metolachlore, Piclorame, Prochloraze, Propazine

Test system and conditions	The pilot unit consists of an ozonation-deozonation step linked to a GAC filtration column (see Figure 3). The system is continuously fed by SFW from the Neuilly-sur-Marne drinking water plant. Bromide or micropollutants are injected into the feeding line via a static mixer. Pharmaceutical tests:7 tests have been carried out with an ozone treatment level ranging from 0 to 2.1 g/m^3 and with the following experimental conditions: 7.3 < pH < 7.4, 17.2 < T(°C) < 17.6, 0.097 < UV254 (cm^{-1}) < 0.130 and AT = 4.0 meq/L. Pesticides tests: 6 tests have been carried out with an ozone treatment level ranging from 0 to 2.3 g/m^3 and with the following experimental conditions: pH = 7.3, 16.9 < T(°C) > 17.7, 0.143 < UV254 (cm^{-1}) < 0.184 and AT = 4.4 meq/L.
Statistical design	2 to 6 measurements
Relevance	
Environmental relevance	Environmental parameter are measured but not reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications.

Bozkaya-Schrotter et al. (2009)

Title: Treatment of nanofiltration membrane concentrates: Organic micropollutant and nom removal	
Author: B. Bozkaya-Schrotter, C. Daines, A. Brunel, J.-C. Schrotter, P. Breant	
Reference: Desalination and Water Treatment 9, 36–42	
Year: 2009	
Results and conclusion: This study aims to achieve complete elimination of pesticides and the elimination of 60 % of the natural organic matter (NOM) retained during nanofiltration step. The investigation included testing conventional water treatment techniques – adsorption, coagulation, ozonation – and the combination of ozonation and adsorption processes. Eight pesticides detected most commonly in French surface waters were selected as model micropollutants: atrazine, sulcotrione, bentazone, isoproturon, diuron, glyphosate, amitrole and acetochlore. Simultaneous combination of ozonation and powdered activated carbon (PAC) adsorption proved to be an efficient method for the elimination of the polar and ozone resistant pesticides at low carbon and ozone concentrations. This combination also achieved faster NOM removal than PAC adsorption only. It was observed that even with the use of high PAC concentrations, addition of low ozone dosages were necessary to degrade highly polar pesticides together with the NOM. No significant modification of the carbon activity and surface properties was observed at low ozone concentration levels, ca. 3 mg/L.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight; additional information	
Reliability	Medium
Endpoint	Removal %- ; elimination %- figures; no absolute data
Protocol	Standard, Quantitative analysis of pesticides was carried out by a laboratory specialised in environmental analyses
Test compound	Atrazine, sulcotrione, bentazone, diuron, glyphosate, acetochlore, isoproturon

Test system and conditions	1) Adsorption of pesticides from the concentrate: Selected amount of concentrate sample and the adsorbent are placed in a funnel and agitated for contact times between 2 and 30 min. Once the selected contact time is elapsed sample is filtered and analysed; 2) Oxidation by ozone and combination of PAC and O_3: Concentrate sample is placed into a 1 L glass funnel and ozone is introduced in the system. Ozone dosage was varied between 3 and 30 mg/L of concentrate. Funnel is then agitated, concentrate is collected and analyzed. Oxidation by ozone is combined with adsorption process by adding PAC in the system prior to injection of ozone. The amount of PAC varied between 30 and 3000 mg/L.
Statistical design	No data
Relevance	
Environmental relevance	Given; parameter influencing endpoints are measured and partly reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Brosillon et al. (2006)

Title: Chlorination kinetics of glyphosate and its by-products: Modeling approach	
Author: Stephan Brosillon, Dominique Wolbert, Marguerite Lemasle, Pascal Roche, Akbar Mehrsheikh	
Reference: WATER RESEARCH 40, 2113 – 2124	
Year: 2006	
Results and conclusion:	
Chlorination reactions of glyphosate, glycine, and sodium cyanate were conducted in well-agitated reactors to generate experimental kinetic measurements for the simulation of chlorination kinetics under the conditions of industrial water purification plants. The contribution of different by-products to the overall degradation of glyphosate during chlorination has been identified. The kinetic rate constants for the chlorination of glyphosate and its main degradation products were either obtained by calculation according to experimental data or taken from published literature. The fit of the kinetic constants with experimental data allowed us to predict consistently the concentration of the majority of the transitory and terminal chlorination products identified in the course of the glyphosate chlorination process. The simulation results conducted at varying aqueous chlorine/glyphosate molar ratios have shown that glyphosate is expected to degrade in fraction of a second under industrial aqueous chlorination conditions. Glyphosate chlorination products are not stable under the conditions of drinking water chlorination and are degraded to small molecules common to the degradation of amino acids and other naturally occurring substances in raw water. The kinetic studies of the chlorination reaction of glyphosate, together with calculations based on kinetic modeling in conditions close to those at real water treatment plants, confirm the reaction mechanism that we have previously suggested for glyphosate chlorination.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information	
Reliability	High
Endpoint	Dissipation of glyphosate and the formation of its chlorination products versus time; Chlorination kinetic model
Protocol	Non-GLP
Test compound	Non-labelled glyphosate (96 %), (N-phosphonomethyl)glycine and Glycine (99 %), 9-Fluorenylmethyl chloroformate (FMOC) (97 %)

Test system and conditions	Kinetics experiments: Model solutions of glyphosate (104 M), and glycine (104 M), in water buffered at pH 7 or 8 with a borate solution (0.2 M) were placed in a 100mL well-agitated reactor. The samples were chlorinated at HOCl/substrate molar ratios of approximately 1, 4, 20, 50, 100, and 200 using a 1M solution of NaOCl in water; incubated at room temperature in the dark for 24 h. For the kinetic measurements, 104M solutions of glyphosate, glycine, or sodium cyanate were chlorinated in a 1000mL well-agitated reactor using HOCl/substrate molar ratios of approximately 4 and 50; portions of reaction mixture were analyzed at scheduled times.
Statistical design	Chlorination kinetics were simulated, kinetic rate constants for the reaction sequence were either obtained by calculation according to the experimental data or taken from published literature.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence; results supported by other publications.

Garcia et al. (2013)

Title: The application of microfiltration-reverse osmosis/nanofiltration to trace organics removal for municipal wastewater reuse
Author: N. Garcia, J. Moreno, E. Cartmell, I. Rodriguez-Roda and S. Judd
Reference: Environmental Technology, 2013, Vol. 34, No. 24, 3183–3189, http://dx.doi.org/10.1080/09593330.2013.808244
Year: 2013
Results and conclusion: The fate of organic micropollutans (MPs) in a membrane system based on microfiltration (MF) and reverse osmosis/nanofiltration (RO/NF) has been investigated for the case of wastewater reuse. Both an operating full-scale water reuse plant and a pilot plant were employed, with 22 individual organic compounds at their ambient concentrations studied for the former and the latter employing two target compounds over a range of feed concentrations. Results revealed removal efficiencies higher than 75 % for most compounds in the full-scale plant, though mass flow studies on all streams revealed a significant imbalance of material for some compounds. Rejection efficiencies measured for candidate commercial NF and RO membranes tested at pilot scale challenged with a pharmaceutically active compound (ibuprofen, IBU) and an endocrine disrupting chemical (nonylphenol, NP) exceeded 99 %. Permeate concentrations were 0.005–0.14 μg/L for IBU and below the limit of detection for NP. A mass balance of the MPs for the full-scale plant across the MF and RO stages revealed a significant imbalance associated with the challenge of accurate determination of low concentrations. Differences in pilot plant and full-scale data were otherwise attributed to the impact of membrane ageing (and specifically hydrolysis) on RO rejection of the MPs examined.
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.
Type of information (critical, high/low weight, supporting, additional): Additional information

Reliability	
Endpoint	Fate of organic micropollutans (MPs) in a membrane system based on microfiltration (MF) and reverse osmosis/nanofiltration (RO/NF)
Protocol	Non-GLP

Test compound	EDTA, NP, estrone (E1), 17β-oestradiol (E2), 17α-ethynylestradiol (EE2), tributyltin, naphthalene (NAPHT), IBU, ofloxacin (OFLX), oxytetracyc (OXTCY), erythromycin, propranolol, fluoxetine , triclosan, diclofenac (DFC), 2244-tetrabromodiphenyl ether (BDPE47), 22445-pentabromodiphenyl ether (BDPE99), 22446-pentabromodiphenyl ether (BDPE100), 224455-hexabromodiphenyl ether (BDPE153), bis-(2-ethylhexyl) phthalate (DEHP), glyphosate (GLYPH) and mecoprop (MCPP)
Test system and conditions	A full-scale 1200m^3/d capacity UK-based MF-RO plant was used. It is fed with secondary-treated municipal wastewater from the neighbouring wastewater treatment works, and generates desalinated water for industrial reuse. The plant comprises a 150µmscreen for protecting the MF. The hollow fibre (HF) MF operates with regular backflushing and cleaning in place (CIP) with hypochlorite, acid and alkali for maintenance of permeability. The MF filtrate is held in an intermediate storage tank prior to treatment by the 2:1 array-configured RO process. Scaling in the RO is ameliorated by upstream dosing with antiscalant and acid. The plant operates at mean recoveries of 86 % at the MF stage and 73 % for the RO. Performance data for specific commercial RO membrane modules were obtained from an RO pilot plant installed at the Castell-Platja d'Aro WWTP (Catalonia, Spain). The 4.3m^3/d plant treats municipal wastewater, with a MBR fitted upstream to protect the RO. The MBR is assumed to provide biotreated and microfiltered municipal wastewater in a manner analogous to the full-scale reuse plant where classical activated sludge treatment precedes the MF stage. The RO process comprised a pressure vessel housing a single element, fed from an intermediate 200 L holding tank and protected by a cartridge filter. The rig permitted either discharge or recycling of the concentrate, the latter providing increased feedwater or retentate concentrations as encountered through RO staging. Three standard 40–40 in −4 in for 100 mm) in diameter and 40 in (or 1m) long – commercial membrane modules of differing rejection properties were employed for the study: two RO membranes (HR and LE) and one nanofiltration (NF) membrane (NF270), all provided by the Dow Chemical Company. The membranes were selected so as to provide a range of selectivity. Prior to each experiment, each membrane was conditioned using permeates from the MBR permeate tank for 16–20 h at a pressure of 4 bar for the NF membrane and 9 bar for the two RO membranes. The holding tank was then spiked with 10µg/L of the target compounds of IBU and NP as 50mL aliquots from a 40mg/L standard solution. Trials were undertaken by incrementally increasing the feedwater concentration by passing 50 % of the feedwater through the ROprocess and returning the concentrate to the holding tank. This process was repeated seven times for each of the three membranes tested, providing a range of concentration factor values between unity and 4.5. This enabled the overall feedwater to be increased in accordance with retentate concentration across a full-scale RO array. Permeate recoveries of 12–15 % were maintained throughout. MP removals at the full-scale installation were determined through sampling of the various streams, and specifically the feed and backwash/reject streams of both the MF and RO processes. Grab samples from this site were taken 2–3 times daily over a three-day period, and contaminants assayed by Anglian Water Laboratories (Huntingdon, UK) according to standards methods based on GC-ICP-MS. 22 MP 'priority' compounds were assayed (see "Test compound").
Statistical design	
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Gardner et al. (2013)

Title: Performance of UK wastewater treatment works with respect to trace contaminants	
Author: M. Gardner, V. Jones, S. Comber, M. D. Scrimshaw, T. Coello-Garcia, E. Cartmell, J. Lester, B. Ellor	
Reference: Science of the Total Environment 456-457 (2013) 359–369	
Year: 2013	
Results and conclusion: The study examined the performance of 16 wastewater treatment works to provide an overview of trace substance removal in relation to meeting the objectives of the Water Framework Directive (WFD). Collection and analysis of over 2400 samples including sewage influent, process samples at different stages in the treatment process and final effluent has provided data on the performance of current wastewater treatment processes and made it possible to evaluate the need for improved effluent quality. Results for 55 substances, including metals, industrial chemicals and pharmaceuticals are reported. Data for sanitary parameters are also provided. A wide range of removal efficiencies was observed. Removal was not clearly related to the generic process type, indicating that other operational factors tend to be important. Nonetheless, removals for many substances of current concern were high. Despite this, current proposals for stringent water quality standards mean that further improvements in effluent quality are likely to be required. In detail: The water-soluble regulated and emerging chemicals, such as EDTA, glyphosate and mecoprop, exhibited poor fractional removal (0.3–0.45). The more hydrophobic chemicals such as PAHs and flame retardants were more effectively removed than soluble chemicals such as EDTA, mecoprop, E1 and glyphosate.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Removal efficiencies for 55 substances, including metals, industrial chemicals and pharmaceuticals during waste water treatment
Protocol	Non-GLP
Test compound	55 substances, including metals, industrial chemicals and pharmaceuticals

Test system and conditions	The 16 WwTWs selected represent a cross-section of works types currently in operation in the UK and include AS, TF, membrane bioreactor (MBR) and oxidation ditches (OD) works. These works were a subset of the larger set of 160 works that had been selected for the previous effluent quality study (Gardner *et al.*, 2012) as representative of UK WwTWs. Influent to the works was combined sewage, hence including 'black' and 'grey' water. The population equivalents for these works ranged from 3424–205, 935 which was representative of the size profile of works present in the UK serving over 70 % of the national population (Gardner *et al.*, 2012). The total number of samples taken at any given WwTWs was approximately 150. Therefore, over the 16 WwTWs, approximately 2400 samples were taken, involving over 150,000 determinations. Spot samples were collected throughout the works to include influent, settled sewage, final effluent and sludge. Where tertiary treatment was present, an additional sample was taken post the secondary stage (secondary sewage). All works were sampled on a monthly basis for a one-year period, throughout 2010/2011. To assess within-day variability, a minimum of two samples were taken from each site over a 12-hour period (08.00 h–20.00 h). Hence, although the exact number of samples differed between works, samples were collected at each sampling point within each plant on approximately 12 occasions, with a minimum of two samples being collected on each sampling occasion. Additionally, at least one sludge process sample was taken on each visit; sludge sampling varied depending on the types of process employed at each works and accessibility issues. Data processing involved taking the average concentration for each sampling day. Mean, median and percentiles were calculated from the daily average values for each WwTWs and determinand. Fractional removal data were calculated from overall median values at each process stage and across the whole works. Prior to principle components analysis (PCA), the dataset was reviewed in order to ensure suitability for assessment using this technique. Initially, compounds with a high proportion of 'less than' (not detected) values were excluded, and subsequently, bivariate correlation between chemicals was also checked (Sharma, 1996). To assess the correlation between variables, both Pearson and Spearman correlation factors were used since some of the variables were not normally distributed. One of the variables in each correlated pair with a correlation factor >0.9 (Field, 2009) was excluded in order to create a dataset without redundant variables. The number of variables (chemicals) included in the PCA analysis was then reduced, as the number of sites in relations to variables resulted in a non-positive definite correlation matrix (Field, 2009). At this point, the criterion for inclusion was based on compounds identified as of interest at a national scale in the UK (Gardner *et al.*, 2012). The statistical analyses were performed with PASW Statistics 18 (free from SPSS) and Scout, 2008 (free from the US EPA).
Statistical design	See "Test system and conditions"
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Gasperi et al. (2010)

Title: Occurrence and removal of priority pollutants by lamella clarification and biofiltration	
Author: Johnny Gasperi, Vincent Rocher, Soléne Gilbert, Sam Azimi and Ghassan Chebbo	
Reference: Water Research 44 (2010) 3065 – 3076	
Year: 2010	
Results and conclusion: This study investigates the occurrence of all priority substances (n = 41) listed in the Water Framework Directive and additional substances (n = 47) in raw sewage, as well as the removal performance of lamella clarification and biofiltration techniques. Once the efficiency of both types of techniques has been assessed for typical wastewater parameters, the differences in each technique's ability to remove pollutants becomes obvious; nevertheless, pollutant removal in quantitative terms still depends on the physicochemical properties of the compounds used and operating conditions within the selected facility. For lamella clarification, the removal of organic chemicals was found to be primarily correlated with their sorption potential and, hence, strongly dependent upon log K_{ow} of the compound under study. Compounds with a strong hydrophobic character (log K_{ow} > 4.5) are removed to a significant extent (approx. 85 %), while hydrophilic compounds (log K_{ow} < 3.5) are poorly removed (<20 %). For biofiltration, the removal of chemicals appears to be compound-dependent, although this outcome involves several mechanisms, namely: i) physical filtration of total suspended solids, ii) volatilisation, iii) sorption, and iv) biotransformation of substances. Even if the complex processes within a biofilter system do not yield an accurate prediction of pollutant removal, two groups of chemicals can still be clearly identified: i) hydrophobic or volatile compounds, for which moderate to high removal rates are observed (from 50 % to over 80 %); and ii) hydrophilic, non-volatile and refractory compounds for which a low removal rate would be expected (<20 %).	
Proposed action: Not to be considered for listing in the summarizing table as raw data are insufficient.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific targets, namely wastewater treatment at a certain place and time.	
Reliability	High
Endpoint	Glyphosate concentration in final effluent of wastewater treatment
Protocol	No standard protocol; for further details see under test system and conditions
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	The occurrence of 88 substances at 3 sampling points, corresponding to raw sewage (RS), decanted effluents (DE) and final effluents (FE), were analyzed. In 2008, three sampling campaigns were carried out (March, September and December). At each site, 24-h composite samples were collected using automatic refrigerated samplers (at 4°C).
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Results are valid for that particular place and time; no negative evidence.

Ghanem et al. (2007)

Title: Concentrations and specific loads of glyphosate, diuron, atrazine, nonylphenol and metabolites thereof in French urban sewage sludge

Author: Aline Ghanem, Philippe Bados, Arantza Rua Estaun, Luis Felippe de Alencastro, Salima Taibi, Jacques Einhorn, Christian Mougin

Reference: Chemosphere Volume 69 Issue 9, 1368-1373

Year: 2007

Results and conclusion:
Indirect soil pollution by heavy metals and organics may occur when sewage sludge is used as fertilizer. It is essential to define the nature and amounts of pollutants contained in sewage sludge in order to assess environmental risk. Here, we present results from a one-year monitoring of herbicides (glyphosate, diuron and atrazine) and their major degradates in sewage sludge sampled from three wastewater treatment plants and one composting unit in the vicinity of Versailles, France. The concentrations of these compounds were determined, as well as these of the surfactant nonylphenol. We demonstrated the presence of glyphosate and aminomethylphosphonic acid at the mg/kg (dry matter) level in all samples. Diuron was detected at the µg/kg (d m.) level, whereas its degradate and triazine compounds were below the limits of quantification. Nonylphenol amounts were higher than the future European limit value of 50 mg/kg (d.m.).

Proposed action:
To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.

Type of information (critical, high/low weight, supporting, additional):
Medium weight, supporting information of monitoring of sewage sludge

Reliability	High
Endpoint	Specific load Lsp (load of a specific chemical in the sewage sludge per inhabitant connected per year (mg/cap/y)), Concentration of herbicides and nonylphenol in centrifuged sludge samples
Protocol	Non-GLP
Test compound	Glyphosate (99.5 %), diuron (99.0 %), atrazine (99.0 %), and their respective metabolites; (2-^{13}C, 99 %; ^{15}N, 98 %)-glyphosate, and (^{13}C, 99 %; ^{15}N, 98 %; Methylene-D_2, 98 %)-AMPA; Atrazine-D_5 and diuron-D_6 in acetone solutions at 100 µg ml^{-1}
Test system and conditions	The concentrations of glyphosate, diuron, atrazine, nonylphenol and their main metabolites have been monitored monthly from July 2004 to June 2005 in sludge samples obtained from the WWTPs (wastewater treatment plants).
Statistical design	The method for glyphosate and AMPA analysis showed mean recoveries of 70 % (RSD < 9 %) for glyphosate and 63 % (RSD < 5 %) for AMPA
Relevance	
Environmental relevance	Not reported
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Ghanem et al. (2006)

Title: Fate of herbicides and nonylphenol in soil–plant–water systems amended with contaminated sewage sludge	
Author: Aline Ghanem, Jacqueline Dubroca, Veronique Chaplain, Christian Mougin	
Reference: Environ Chem Lett (2006), DOI 10.1007/s10311-006-0034-5	
Year: 2006	
Results and conclusion: We studied the fate of sludge spiked with ^{14}C-labelled diuron, glyphosate and nonylphenol applied to the soil by the way of contaminated sewage sludge in the soil plant-water system. Here we show that the mineralization of the chemicals in mixture is reduced by 40-80 % by comparison with a direct soil contamination. The persistence of the chemicals in soils is increased in the presence of sludge. We showed also that the chemicals present in the sludge are mobile and partly transferred to soil leachates and plant seedlings. These results allow postulating that these compounds may induce an ecotoxicological impact on the soil ecosystem.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information of fate of herbicides contained in sludge, after spreading onto agricultural soil	
Reliability	Medium
Endpoint	Only figures of release of $^{14}CO_2$ (time-dependent mineralization); mass balance: non extractable ^{14}C, ^{14}C extracted by NaOH, ^{14}C extracted by organic solvents, and $^{14}CO_2$; transfer of radioactive chemicals to soil leachates and higher plants in %
Protocol	Non-GLP
Test compound	High purity glyphosate, diuron and nonylphenol, phosphonomethyl-^{14}C glyphosate (2084 MBq mmol^{-1}) and ring-^{14}C-U-nonylphenol (1998 MBq mmol^{-1}), ring-^{14}C-U-diuron (898 MBq mmol^{-1}); silt loam; sludge have been collected in urban WWTPs
Test system and conditions	3 experimental conditions have been retained for incubations in model ecosystems: soil alone and soil amended by sludge at two ratios. 1) model ecosystem = control, 0.7 kg of soil were spiked with labelled glyphosate, diuron and nonylphenol (370 kBq each) and unlabelled chemicals to ensure final amounts of 163, 480 and 203 µg chemical per model ecosystem. 2) model ecosystem = 'worst case' of contamination, sludge (28.5 dry sludge corresponding to 30 T dw ha^{-1}) mixed with soil; spiked with the same amounts of chemicals as the soil alone. 3) model ecosystem = agronomic reality, the soil with 5.7 g dry sludge (equivalent to 6 T dw ha^{-1}) with final amounts of chemicals being 33, 96 and 40 µg per model ecosystem; concentrations of added glyphosate, diuron and nonylphenol were 6, 17 and 7 ppm (dry sludge); spiked samples were 'aged' for 3 days at 4°C under nitrogen; stream (0.5 l min^{-1}) of wet air, $^{14}CO_2$ trapping in 1 N NaOH solutions; incubated for 91 days at 23°C under 16 h light and 8 h darkness. Leachates were collected after 45, 60 and 90 days of incubation by watering with 190 ml water (equivalent to 20 mm rainwater).Radish and wheat seedlings (sowing after 15 days of incubation), harvested after a further 15- and 45-days-period of growth, and dried.
Statistical design	No information
Relevance	
Environmental relevance	No information about parameters influencing endpoints.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Hanke et al. (2010)

Title: Relevance of urban glyphosate use for surface water quality	
Author: Irene Hanke, Irene Wittmer, Simone Bischofberger, Christian Stamm, Heinz Singer	
Reference: Chemosphere 81, 422–429	
Year: 2010	
Results and conclusion: Relative contributions of agricultural and urban uses to the glyphosate contamination of surface waters were studied in a small catchment (25 km^2) in Switzerland. Monitoring in four sub-catchments with differing land use allowed comparing load and input dynamics from different sources. Agricultural as well as urban use was surveyed in all subcatchments allowing for a detailed interpretation of the monitoring results. Water samples from the river system and from the urban drainage system (combined sewer overflow, storm sewer and outflow of wastewater treatment plant) were investigated. The concentrations at peak discharge during storm events were elevated throughout the year with maximum concentrations of 4.15 µg/L. Glyphosate concentrations mostly exceeded those of other commonly used herbicides such as atrazine or mecoprop. Fast runoff from hard surfaces led to a fast increase of the glyphosate concentration shortly after the beginning of rainfall not coinciding with the concentration peak normally observed from agricultural fields. The comparison of the agricultural application and the seasonal concentration and load pattern in the main creek from March to November revealed that the occurrence of glyphosate cannot be explained by agricultural use only. Extrapolations from agricultural loss rates and from concentrations found in the urban drainage system showed that more than half of the load during selected rain events originates from urban areas. The inputs from the effluent of the wastewater treatment plant, the overflow of the combined sewer system and of the separate sewer system summed up to 60 % of the total load.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight; only basic information	
Reliability	High
Endpoint	Lowest base flow concentration; total load; concentration
Protocol	Non-GLP
Test compound	Glyphosate, AMPA
Test system and conditions	Surface water and water from the urban drainage system were sampled by automatic devices at every sampling site except for the WWTP, where daily flow proportional composites were used. Samples were taken at high temporal resolution during 16 out of 35 rain events from March to November 2007.
Statistical design	Three aliquots every 5 min were collected during the first 6 h of an event, followed by a reduced sampling frequency of one composite sample per hour (four aliquots every 15 min); The relative standard deviations (RSDs) for the surface water sample were 12 % for glyphosate and 14 % for AMPA (N = 6). The RSDs of the WWTP samples were 5 % for glyphosate and 13 % for AMPA (N = 6). The recoveries were in the range of 80–121 % for glyphosate and 90 to 118 % for AMPA.
Relevance	
Environmental relevance	Given; parameter influencing endpoints are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Hedegaard and Albrechtsen (2014)

Title: Microbial pesticide removal in rapid sand filters for drinking water treatment – Potential and kinetics	
Author: M. J. Hedegaard, H.-J. Albrechtsen	
Reference: Water Research 48 (2014) 71-81	
Year: 2014	
Results and conclusion: Filter sand samples, taken from aerobic rapid sand filters used for treating groundwater at three Danish waterworks, were investigated for their pesticide removal potential and to assess the kinetics of the removal process. Microcosms were set up with filter sand, treated water, and the pesticides or metabolites mecoprop (MCPP), bentazone, glyphosate and p-nitrophenol were applied in initial concentrations of 0.03-2.4 mg/L. In all the investigated waterworks the concentration of pesticides in the water decreased – MCPP decreased to 42-85 %, bentazone to 15-35 %, glyphosate to 7-14 % and p-nitrophenol 1-3 % – from the initial concentration over a period of 6-13 days. Mineralisation of three out of four investigated pesticides was observed at Sjælsø waterworks Plant II – up to 43 % of the initial glyphosate was mineralised within six days. At Sjælsø waterworks Plant II the removal kinetics of bentazone revealed that less than 30 min was needed to remove 50 % of the bentazone at all the tested initial concentrations (0.1-2.4 mg/L). Increased oxygen availability led to greater and faster removal of bentazone in the microcosms. After 1 h, bentazone removal (an initial bentazone concentration of 0.1 mg/L) increased from 0.21 %/g filter sand to 0.75 %/g filter sand, when oxygen availability was increased from 0.28 mg O_2/g filter sand to 1.09 mg O_2/g filter sand. Bentazone was initially cleaved in the removal process. A metabolite, which contained the carbonyl group, was removed rapidly from the water phase and slowly mineralised after 24 h, while a metabolite which contained the benzene-ring was still present in the water phase. However, the microbial removal of this metabolite was initiated over seven days.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium, additional information	
Reliability	
Endpoint	Removal of mecoprop, bentazone, glyphosate and p-nitrophenol in rapid sand filters
Protocol	Non-GLP
Test compound	^{14}C-labelled pesticides (mecoprop, bentazone, glyphosate and p-nitrophenol)
Test system and conditions	Three different experimental laboratory set-ups were used: Degradation potential of filter sand: Filter sand from three Danish waterworks – Islevbro, Sjælsø Plant I and Sjælsø Plant II – was investigated for the removal potential of the pesticides mecoprop (MCPP), bentazone, glyphosate, and the degradation product p-nitrophenol. Removal kinetics: Bentazone removal at different initial concentrations was investigated with filter sand from Sjælsø waterworks Plant II. The removal was investigated intensively over 1 h, which is the residence time of the water in the rapid sand filter, and the experiment lasted for seven days to investigate for mineralisation. Effect of oxygen: Bentazone removal in the filter sand from Sjælsø waterworks Plant II was investigated under enhanced oxygen concentrations. The removal was investigated intensively in the initial phase of the experiment (the first few hours), and the experiment lasted for two days.
Statistical design	Not given in the paper
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Jönsson et al. (2013)

Title: Removal and degradation of glyphosate in water treatment: a review

Author: J. Jönsson, R. Camm and T. Hall

Reference: Journal of Water Supply: Research and Technology – AQUA | 62.7 | 2013

Year: 2013

Results and conclusion:
Glyphosate is a broad spectrum, non-selective herbicide, widely used for the post-emergence control of annual and perennial weeds in a variety of applications. Although of low toxicity, its presence in drinking water is undesirable and can cause drinking water compliance failure in the EU if found at concentrations > 0.1 µg/L. Treatment methods such as ozonation and activated carbon are currently used for pesticide degradation and removal. This article provides a review of the reported efficiency in removal and degradation of glyphosate and aminomethylphosphonic acid (AMPA) by some commonly employed treatment options. Additional experiments have been carried out where knowledge gaps have been identified. Oxidants used in water treatment, particularly Cl_2 and O_3, are highly effective in degrading glyphosate and AMPA. Removal by coagulation and activated carbon is ineffective as a barrier against contamination in drinking water. UV treatment is also ineffective for glyphosate and AMPA degradation but the combination of UV/H_2O_2 provided significant degradation of glyphosate, but not AMPA, under the conditions investigated. UV/TiO_2 treatment can degrade significant amounts of glyphosate but the irradiation time needed is long. Removal or degradation by bank filtration, slow sand filtration, ClO_2 and membranes is variable but can provide significant removal under the right conditions.

Summary of removal efficiencies of glyphosate and AMPA:

Treatment process	Glyphosate removal [%]	AMPA removal [%]
Bank and dune filtration	20-50	25-95
Aluminium coagulant and clarification	15-40	20-25
Iron coagulant and clarification	40-70	20-85
Chlorination	24->99	40->95
Chlorine dioxide	17-93	>99
Ozonation	60->99	25-95
Activated carbon adsorption	10-90	20-70
Membrane filtration	>90 (NF/RO), >50 (UF)	>95 (NF/RO)

Proposed action:
To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.

Type of information (critical, high/low weight, supporting, additional):
Medium, additional information

Reliability	
Endpoint	Removal and degradation of glyphosate and AMPA in water treatment
Protocol	Not given
Test compound	Glyphosate and AMPA
Test system and conditions	Batch tests were carried out to investigate the degradation of glyphosate and AMPA by oxidation using Cl_2, ClO_2, O_3, O_3/H_2O_2, and by adsorption using PAC (powdered activated carbon). Furthermore, a review of water treatment removal and degradation by bank filtration, chemical coagulation, clarification/filtration, slow sand filtration, chlorination, degradation of glyphosate by chlorine dioxide, ozone, UV, AOPs, activated carbon, pressure driven membrane process and air stripping was performed.
Statistical design	Not given

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Mehrsheikh et al. (2006)

Title: Investigation of the mechanism of chlorination of glyphosate and glycine in water
Author: Akbar Mehrsheikh, Marian Bleeke, Stephan Brosillon, Alain Laplanche, Pascal Roche
Reference: WATER RESEARCH 40, 3003 – 3014
Year: 2006
Results and conclusion: The chlorination reactions of glyphosate and glycine in water were thoroughly studied. Utilizing isotopically enriched (^{13}C and ^{15}N) samples of glycine and glyphosate and 1H, ^{13}C, ^{31}P, and ^{15}N NMR spectroscopy we were able to identify all significant terminal chlorination products of glycine and glyphosate, and show that glyphosate degradation closely parallels that of glycine. We have determined that the C1 carboxylic acid carbon of glycine/glyphosate is quantitatively converted to CO_2 upon chlorination. The C2 methylene carbon of glycine/glyphosate is converted to CO_2 and methanediol. The relative abundance of these two products is a function of the pH of the chlorination reactions. Under near neutral to basic reaction conditions (pH 6–9), CO_2 is the predominant product, whereas, under acidic reaction conditions (pH<6) the formation of methanediol is favoured. The C3 phosphonomethylene carbon of glyphosate is quantitatively converted to methanediol under all conditions tested. The nitrogen atom of glycine/glyphosate is transformed into nitrogen gas and nitrate, and the phosphorus moiety of glyphosate produces phosphoric acid upon chlorination. In addition to these terminal chlorination products, a number of labile intermediates were also identified including N-chloromethanimine, N-chloroaminomethanol, and cyanogen chloride. The chlorination products identified in this study are not unique to glyphosate and are similar to those expected from chlorination of amino acids, proteins, peptides, and many other natural organic matters present in drinking water.
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC calculation is affected by the results of the article.
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information about the mechanism of chlorination of glyphosate

Reliability	High
Endpoint	Proposed mechanism of glycine chlorination and of glyphosate chlorination
Protocol	Non-GLP
Test compound	Isotopically labelled glyphosate and glycine
Test system and conditions	Isotopically enriched (^{13}C and ^{15}N) glyphosate and glycine were utilized to investigate the various chlorination products formed from these compounds using ^{13}C, ^{15}N, ^{31}P, and 1H NMR spectroscopy. Chlorination was conducted in un-buffered D_2O at initial pHs of 8, 7, and 5. Additionally, the chlorination reactions were carried out in a 0.48M borate buffer in D_2O at pH 8 and 9. Chlorination products of glycine and glyphosate were monitored by HPLC using the corresponding ^{14}C-labeled test materials in unbuffered water at initial pHs of 9, 8, 7, 6, and 5 with aqueous chlorine at chlorine to substrate molar ratio of 100:1. Additionally, the chlorination reactions were carried out in a 0.05M borate buffer at pH 8 and 9 or a 0.05M phosphate buffer at pH 7, 6, and 5 in separate experiments.
Statistical design	No data

Relevance	
Environmental relevance	Supplementary data associated with this article can be found in the online version at doi:10.1016/j.watres.2006.06.027.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Nowack (2002)

Title: Aminopolyphosphonate removal during wastewater treatment	
Author: Bernd Nowack	
Reference: Water Research 36, 4636–4642	
Year: 2002	
Results and conclusion: Phosphonates are not biodegraded during wastewater treatment but are removed by adsorption processes. Field measurements from different wastewater treatment plants affirm that they are removed almost completely during wastewater treatment. Adsorption of nitrilotrismethylenephosphonic acid onto activated sludge, amorphous iron oxide and humic acids (HAs) was studied under controlled conditions. The adsorption onto HAs decreases sharply with increasing pH with negligible adsorption at pH above 6.5. Adsorption onto amorphous iron oxide follows a Langmuir behaviour. The presence of 1mM Ca doubles the maximum surface capacity at pH 7. Adsorption onto activated sludge is not very pH sensitive and is explained to a large extent by adsorption onto amorphous iron oxides, but the contribution of organic matter or other mineral phases cannot be ruled out.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight; experiments were not done with glyphosate	
Reliability	Medium
Endpoint	Not applicable
Protocol	Not applicable
Test compound	The phosphonates, nitrilotrismethylenephosphonic acid (NTMP), ethylenediaminetetramethylenephosphonic acid (EDTMP) and diethylenetriaminepentamethylenephosphonic acid (DTPMP)
Test system and conditions	1) Samples were taken from the WWTP of Weil, Germany. This WWTP receives wastewater from several textile factories that use phosphonates in their dyeing and bleaching processes and operates with chemical phosphate precipitation/flocculation. 24 hours flow proportional samples from the influent and effluent were taken; 2) A field experiment was carried out in the WWTP Ikast, Denmark. DTPMP was added at a rate of 7.6 kg/d to the wastewater stream from textile industry (1000-3000m^3/d), which is treated separately from the municipal wastewater. Daily water samples were taken by the personnel of the WWTP; 3) Adsorption experiments with hydrous ferric oxide: Increasing concentrations of NTMP were added to samples of the HFO-suspension, stirred for 2 h; filtered; analyzed for dissolved NTMP.; 4) Adsorption experiments with HA-SiO_2: addition of NTMP or metal-NTMP complexes; adsorption isotherms, pH was adjusted to 3.6, 4.8 and 6.5 and NTMP was added at concentrations between 0.1 and 15 mM
Statistical design	Not applicable
Relevance	
Environmental relevance	Given

Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence

Olofsson et al. (2013)

Title: Comprehensive mass flow analysis of Swedish sludge contaminants
Author: U. Olofsson, E. Brorström-Lundén, H. Kylin, P. Haglund
Reference: Chemosphere 90 (2013) 28–35
Year: 2013
Results and conclusion: A screening of metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants in sludge from seven Swedish sewage treatment plants (STPs) was performed in this study. This extensive screening provides information on mass flows of 282 compounds used in the Swedish society to sewage sludge. It reveals constant relative contaminant concentrations (ng mg/kg d.w.), except for some pesticides and perfluorinated compounds, indicating that these originate from broad usage and diffuse dispersion rather than (industrial) point sources. There was a five order of magnitude difference in the sum concentrations of the most and least abundant species (metals and polychlorinated dibenzo-p-dioxins and -furans, respectively). Lower total concentrations were found in sludge from STPs processing primarily food industry or household sewage. Proportions of the amounts used (in Sweden) found in sludge were lower for compounds that are present in consumer goods or are diffusely dispersed into the environment (0.01-1 % recovered in sludge) than for compounds used as detergents or PPCPs (17–63 %). In some cases, the recovery seemed to be affected by evaporation (e.g. octamethylcyclotetrasiloxane) or biotransformation (e.g. adipates) losses, while polychlorinated alkanes and brominated diphenyl ethers were recovered to disproportionately high degree (ca. 4 %); likely due to incomplete statistics for imported goods. Concentration of glyphosate in sewage sludge: 0.6 µg g^{-1} n.d., <2 µg g^{-1}, 0.1 µg g^{-1}, <2 µg g^{-1}, n.q., 0.7 µg g^{-1}.
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.
Type of information (critical, high/low weight, supporting, additional): Additional information

Reliability	
Endpoint	Concentration metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants in sludge
Protocol	The analyses were performed by several qualified laboratories, each following strict quality guidelines. Generally, internal standard quantification was used to compensate for losses during cleanup and analysis. For non-accredited analyses the extraction efficiencies were checked (e.g. using re-extraction) and found to be sufficient (better than 95 %).
Test compound	Metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants
Test system and conditions	A screening of metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants in sludge from seven Swedish sewage treatment plants (STPs) was performed.
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Saitúa et al. (2012)

Title: Drinking water obtaining by nanofiltration from waters contaminated with glyphosate formulations: Process evaluation by means of toxicity tests and studies on operating parameters	
Author: Hugo Saitúa, Fernando Giannini and Antonio Perez Padilla	
Reference: Journal of Hazardous Materials 227– 228 (2012) 204– 210	
Year: 2012	
Results and conclusion: Glyphosate formulations toxicity depends on all its components but commercial products only specify the active principle in their label. To treat contaminated waters and to verify if the unknown components which add toxicity have been removed represent a challenge. Nanofiltration and permeate analysis by toxicity tests with fish are an interesting alternative to evaluate the process. Permeates of solutions with concentrations five times above the lethal doses (48 mg/l) did not present toxicity, pointing that all toxic compounds were removed at the same time. Glyphosate rejection over an 80 % despite its molecular weight is lower than membrane MWCO, this could be associated to a predominant Donnan exclusion mechanism, combined with dielectric exclusion due to the solute high charge density. Glyphosate concentration did not show any effect over rejection. It increased when pressure was incremented from 2.5 to 4 bar and then remained constant in a 4–10 bar range. Because of dissociation of the glyphosate and the surface charged of the membrane depend on pH value, the rejection increase from 72.5 to 92.5 % when pH increase from 4 to 8.5. Studies with river water showed the same behavior with a slight decrease in rejection.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	No fate related endpoint
Protocol	Non GLP
Test compound	glyphosate acid (CAS 1071-83-6) and glyphosate isopropylamine (IPA, CAS 38641-94-0)
Test system and conditions	This work consisted in treatment of synthetic and natural waters contaminated with glyphosate commercial formulations, using a NF pilot plant. Process efficiency was evaluated analyzing the permeate by acute toxicity tests with fish. It was studied feed concentration, pressure, pH and ionic strength influence on glyphosate rejection in synthetic water and also in river water. The latter aspect was further studied by considering the changes in the relevant surface water characteristics, such as the pH, the concentration of dissolved organic compounds and the conductivity.
Statistical design	No information
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Schoonenberg et al. (2010)

Title: Reverse osmosis followed by activated carbon filtration for efficient removal of organic micropollutants from river bank filtrate	
Author: F. Schoonenberg, Kegel, B. M. Rietman and A. R. D. Verliefde	
Reference: Water Science & Technology – WST | 61.10 |	
Year: 2010	
Results and conclusion: The aim of this research was to assess the robustness of a drinking water treatment plant equipped with reverse osmosis and subsequent activated carbon filtration for the removal of these pollutants. The total removal efficiency of 47 organic micropollutants was investigated. Results indicated that removal of most organic micropollutants was high for all membranes tested. Some selected micropollutants were less efficiently removed (e.g. the small and polar NDMA and glyphosate, and the more hydrophobic ethylbenzene and napthalene). Very high removal efficiencies for almost all organic micropollutants by the subsequent activated carbon, fed with the permeate stream of the RO element were observed except for the very small and polar NDMA and 1,4-dioxane. RO and subsequent activated carbon filtration are complementary and their combined application results in the removal of a large part of these emerging organic micropollutants. Based on these experiments it can be concluded that the robustness of a proposed treatment scheme for the drinking water treatment plant Engelse Werk is sufficiently guaranteed.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Medium
Endpoint	The results for solute removal by RO were compared to calculations obtained using a predictive model, based on solute structure (QSAR (quantitative structure-activity relationship))
Protocol	Non GLP
Test compound	NDMA, 1,4-dioxane, NMOR, Diglyme, Glyphosate, Triglyme, Caffeine, TBA, MTBE, Phenazon, Metamitron, Terbutaline, Sulfamethoxazol, Sotalol, Pentoxifylline, ETBE, TAME, 2,4-dinitrophenol, Carbendazim, Monuron, Metribuzin, Metoxuron, Pirimicarb, Bisphenol-S, Metoprolol, TCEP, Benzene, Isoproturon, Chlorotoluron, Atrazine, Diethylphthalate, Diuron, Carbamazepine, Bentazon, Metobromuron, Dimethenamid, Ethylbenzene, Naphthalene, 2-MIB, Ibuprofen, Mecoprop (MCPP), Bisphenol-A, Linuron, Estrone, Dibutylphthalate, Diclofenac, Bezafibrate
Test system and conditions	1) the total removal efficiency of a wide selection of organic micropollutants by the combination RO-ACF was investigated. Rejection experiments were carried out on 4 different commercial 4-inch spiral wound reverse osmosis membranes. 2) the same selection of organic micropollutants was also spiked in the feed of an activated carbon column, which was fed with the permeate of the best performing membrane.
Statistical design	No information
Relevance	
Environmental relevance	Information on the analytical protocol can be found in Sacher *et al.* (2001).
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Song et al. (2013)

Title: Composite hollow fiber nanofiltration membranes for recovery of glyphosate from saline wastewater
Author: J. Song, X.-M. Li, A. Figoli, H. Huang, C. Pan, T. He, B. Jiang
Reference: Water Research 47 (2013) 2065-2074
Year: 2013
Results and conclusion: A high performance versatile composite hollow fiber nanofiltration (NF) membrane is reported for the separation of glyphosate from saline waste streams. Preparation of SPEEK based on an amorphous poly (ether ether ketone, PEEK) was investigated. The membrane was prepared by coating sulfonated polyether ether ketone (SPEEK) onto a polyethersulfone (PES) ultrafiltration (UF) hollow fiber membrane. The composite membrane was characterized by water permeability, scanning electron microscopy, and rejection toward sodium sulfate (Na_2SO_4), sodium chloride (NaCl), and calcium chloride ($CaCl_2$). About 90 % rejection toward sulfate anions and only 10 % rejection for calcium cations were obtained. A water permeability around 10-13 LMHBar and 90 % rejection for polyethylene glycol (PEG) with a molecular weight of 4000-6000 Da were observed. In the separation of glyphosate from saline wastewater, the membrane rejected less than 20 % of NaCl and higher than 90 % of glyphosate at an operating pressure of 5 bars and pH = 11.0. An economic analysis indicated that the cost for recovery of glyphosate was comparably low to the value gained by an increase in the productivity. The results may lead to a new promising low energy solution for the environmental problem faced by the herbicide industry.
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.
Type of information (critical, high/low weight, supporting, additional): Additional information

Reliability	
Endpoint	Recovery of glyphosate by a high performance versatile composite hollow fiber nanofiltration (NF) membrane
Protocol	Not given
Test compound	Glyphosate
Test system and conditions	A composite hollow fiber nanofiltration membrane was prepared by coating commercial ultrafiltration membrane with sulfonated amorphous PEEK for the separation of glyphosate from highly saline wastewater. Optimization of the membrane preparation parameters was carried out. Membranes with satisfactory properties were obtained by coating SPEEK of 1.5 wt. % solution onto a commercial UF membrane. Glyphosate concentration was measured according to the Chinese Standard Regulation (GB12686-2004) by UV-Vis spectrophotometry.
Statistical design	Repeated NF tests
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Suhadolc et al. (2010)

Title: Single application of sewage sludge – Impact on the quality of an alluvial agricultural soil	
Author: Metka Suhadolc, Reiner Schroll, Alexandra Hagn, Ulrike Dörfler, Michael Schloter, Franc Lobnik	
Reference: Chemosphere in press; doi:10.1016/j.chemosphere.2010.08.024	
Year: 2010	
Results and conclusion: The effects of sewage sludge on soil quality with regard to its nutrient and heavy metal content, microbial community structure and ability to maintain specific soil function (degradation of herbicide glyphosate) were investigated in a three months study using an alluvial soil (Eutric Fluvisol). Dehydrated sewage sludge significantly increased soil organic matter (up to 20.6 % of initial content), total and available forms of N (up to 33 % and 220 % of initial amount, respectively), as well as total and plant available forms of P (up to 11 % and 170 % of initial amount, respectively) and K (up to 70 % and 47 % of initial amount, respectively) in the upper 2 cm soil layer. The increase of organic matter was most prominent 3 d after the application of sewage sludge, after 3 months it was no longer significant. Contents of nutrients kept to be significantly higher in the sewage sludge treated soil till the end of experiment. Contents of some heavy metals (Zn, Cu, Pb) increased as well. The highest increase was found for Zn (up to 53 % of initial amount), however it was strongly bound to soil particles and its total content was kept below the maximum permissible limit for agricultural soil. Based on molecular fingerprinting of bacterial 16S rRNA gene and fungal ITS fragment on 3rd day and 3rd month after sewage sludge amendment, significant short term effects on bacterial and fungal communities were shown due to the sewage sludge. The effects were more pronounced and more long-term for bacterial than fungal communities. The mineralization of ^{14}Cglyphosate in the sewage sludge soil was 55.6 % higher than in the control which can be linked to (i) a higher glyphosate bioavailability in sewage sludge soil, which was triggered by the pre-sorption of phosphate originating from the sewage sludge and/or (ii) beneficial alterations of the sewage sludge to the physical– chemical characteristics of the soil.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information that sewage sludge amendment to soil has significantly increased the mineralization of ^{14}C-glyphosate	
Reliability	High
Endpoint	Total heavy metal content (mg/kg); daily degradation rates of ^{14}C-Glyphosate; amount of evolved $^{14}CO_2$; mineralization kinetics (a two component first-order kinetic model)
Protocol	Non-GLP
Test compound	^{14}C-glyphosate, ^{14}C-labelled on the phosphonomethyl group (purity >98.0 %), mixed with the commercial available product ''Round-up ready" (Monsanto, USA); Zn, Pb, Ni, Cr, Cu, Cd
Test system and conditions	Microcosm leaching experiment in columns: 1) Soil nutrient change by sewage sludge application, 2) Heavy metals contents, availability and mobility in soil, 3) Microbial community structure: Soil samples were taken from four depths at the 3rd day, the 3rd week and the 3rd month after sewage sludge application for further analysis. All treatments were performed in four replicates resulting in 24 columns for the whole experiment.; 4) Glyphosate mineralization: Biodegradation of ^{14}C-glyphosate was studied in a discontinuously aerated laboratory system; ^{14}C-glyphosate, ^{14}C-labelled on the phosphonomethyl group (purity >98.0 %), mixed with the commercial available product ''Round-up ready" (final specific radioactivity of 0.2 MBq/mg) and mixed with soil to a final glyphosate concentration of 7.3 µg/g, which corresponds to the recommended field concentration of 1.1 kg/ha; in the dark at 20 ± 1 °C; $^{14}CO_2$ from glyphosate mineralization was fixed in 0.1 M NaOH solution
Statistical design	4 replicates
Relevance	
Environmental relevance	Given; parameter influencing endpoints are measured and reported.

Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other studies. No negative evidence.

Yang et al. (2009)

Title: Real-time contaminant detection and classification in a drinking water pipe using conventional water quality sensors: Techniques and experimental results
Author: Y. Jeffrey Yang, Roy C. Haught, James A. Goodrich
Reference: Journal of Environmental Management 2009 Jun;90(8):2494-506
Year: 2009
Results and conclusion: Accurate detection and identification of natural or intentional contamination events in a drinking water pipe is critical to drinking water supply security and health risk management. To use conventional water quality sensors for the purpose, we have explored a real-time event adaptive detection, identification and warning (READiw) methodology and examined it using pilot-scale pipe flow experiments of 11 chemical and biological contaminants each at three concentration levels. The tested contaminants include pesticide and herbicides (aldicarb, glyphosate and dicamba), alkaloids (nicotine and colchicine), E. coli in terrific broth, biological growth media (nutrient broth, terrific broth, tryptic soy broth), and inorganic chemical compounds (mercuric chloride and potassium ferricyanide). First, through adaptive transformation of the sensor outputs, contaminant signals were enhanced and background noise was reduced in time-series plots leading to detection and identification of all simulated contamination events. The improved sensor detection threshold was 0.1 % of the background for pH and oxidation–reduction potential (ORP), 0.9 % for free chlorine, 1.6 % for total chlorine, and 0.9 % for chloride. Second, the relative changes calculated from adaptively transformed residual chlorine measurements were quantitatively related to contaminant-chlorine reactivity in drinking water. We have shown that based on these kinetic and chemical differences, the tested contaminants were distinguishable in forensic discrimination diagrams made of adaptively transformed sensor measurements.
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.
Type of information (critical, high/low weight, supporting, additional): Low weight

Reliability	
Endpoint	Concentrations, kinetics
Protocol	No standard protocol followed, none GLP-study
Test compound	Glyphosate (Roundup solution (Monsanto Corp., St. Louis) contained 18 % glyphosate), CAS-no: 1071-83-6
Test system and conditions	Pilot-scale pipe flow experiment
Statistical design	Not given in the publication
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Comparable studies are not known. Publication is plausible, and thus no negative evidence occurs.

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Detailed description of open literature – PEC in groundwater

de Paz and Rubio (2006)

Title: Application of a GIS–AF/RF model to assess the risk of herbicide leaching in a citrus-growing area of the Valencia Community, Spain	
Author: José M. de Paz, José L. Rubio	
Reference: Science of the Total Environment (article in press)	
Year: 2006	
Results and conclusion: The GIS–AF/RF system developed in the present study was used to compile herbicide pollution-risk maps for a citrus-growing area in eastern Spain. The GIS capabilities of the system enable it to identify areas of potential risk in terms of herbicide leaching. A ranking of the potential leaching risk of herbicides from the highest risk (terbumeton) to the lowest risk (diquat) was done. Sandy soils such as Arenosols were identified as having the highest herbicide leaching risk. The obtained ranking of the leaching potential of analysed herbicides were as follows, from highest to lowest risk: terbumeton > bromacil > simazine > terbuthylazine > diuron > linuron > glyphosate > diquat.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	PEC_{GW}
Protocol	Modelling study
Test compound	No test compounds used in the study
Test system and conditions	A one-dimensional model was developed to estimate the potential leaching of pesticides through the soil profile. The RF index is a measure of the time taken by a pesticide to leach throughout the root zone compared to the time taken by a non-adsorbed tracer. It is less complex than the standard FOCUS leaching models.
Statistical design	No information
Relevance	
Environmental relevance	The relevance is rather small since the Spanish conditions are different from conditions in the central zone. Furthermore the model was rather simple.
Weight of evidence	
"Positive"/"Negative" evidence	The trend is in line with results of standard FOCUS leaching models.

Lindahl and Bockstaller (2012)

Title: An indicator of pesticide leaching risk to groundwater	
Author: Anna M.L. Lindahl, Christian Bockstaller	
Reference: Ecological Indicators 23 (2012) 95–108	
Year: 2012	
Results and conclusion: The objective of this study is (i) to develop a new groundwater sub indicator for an existing indicator, I-Phy (former Ipest), that explicitly takes preferential flow into account, and (ii) to test the possibility of developing an indicator by means of data-mining methods using simulations of a mechanistic model. The groundwater sub indicator developed is in the form of decision trees based on fuzzy inference systems. It was derived through neuroadaptive learning on data sets from simulations running the process-based MACRO model. Unlike the previous version, the new indicator considers preferential flow, climatic differences and differences in soil texture with depth. Other benefits are less dependency on expert knowledge and the possibility to integrate a broad range of conditions.	
Proposed action: Consider as additional information. Recalculation of endpoints on degradation is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

References

de Paz J.M., Rubio, J.L. 2006. Application of a GIS–AF/RF model to assess the risk of herbicide leaching in a citrus-growing area of the Valencia Community, Spain. Science of the Total Environment (article in press)

Lindahl and Bockstaller 2012. An indicator of pesticide leaching risk to groundwater. Ecological Indicators 23 (2012) 95–108

Detailed description of open literature – PEC in surface water

Malaguerra et al. (2013)

Title: Assessment of the contamination of drinking water supply wells by pesticides from surface water resources using a finite element reactive transport model and global sensitivity analysis techniques	
Author: Flavio Malaguerra, Hans-Jørgen Albrechtsen, Philip John Binning	
Reference: Journal of Hydrology 476 (2013) 321–331	
Year: 2013	
Results and conclusion: A reactive transport model is employed to evaluate the potential for contamination of drinking water wells by surface water pollution. The model considers various geologic settings, includes sorption and degradation processes and is tested by comparison with data from a tracer experiment where fluorescein dye injected in a river is monitored at nearby drinking water wells. Three compounds were considered: an older pesticide MCPP (Mecoprop) which is mobile and relatively persistent, glyphosate (Roundup), a newer biodegradable and strongly sorbing pesticide, and its degradation product AMPA. Global sensitivity analysis using the Morris method is employed to identify the dominant model parameters. Results show that the characteristics of clay aquitards (degree of fracturing and thickness), pollutant properties and well depths are crucial factors when evaluating the risk of drinking water well contamination from surface water. This study suggests that it is unlikely that glyphosate and AMPA in streams can pose a threat to drinking water wells, while MCPP in surface water can represent a risk: MCPP concentration at the drinking water well can be up to 7 % of surface water concentration in confined aquifers and up to 10 % in unconfined aquifers. Thus, the presence of confining clay aquitards may not prevent contamination of drinking water wells by persistent compounds in surface water. Results are consistent with data on pesticide occurrence in Denmark where pesticides are found at higher concentrations at shallow depths and close to streams.	
Proposed action: Consider as additional information. The results of the modelling exercise are in line with the standard models.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	Quantification of the amount of pesticides that can leach from a stream into drinking water during water abstraction in a primary aquifer
Protocol	Modelling study
Test compound	No test compounds used in the study
Test system and conditions	In order to study the link between surface water and a nearby drinking water well, a generic model of contaminant transport from surface water into groundwater is established. The model is designed to quantify the amount of pesticides that can leach from a stream into drinking water during water abstraction in a primary aquifer. The conceptual model is illustrated in Fig. 2. A pumping well is placed at a distance d (m) from a stream and pumps water at a constant pumping rate Q (m^3/d) from a depth D (m). The geology is simplified to be a 3-layer system: a hyporheic layer separates the stream from an underlying sandy aquifer, below which a clay aquitard overlies a chalk aquifer; Ds, Dcl and Dch, respectively, are the thicknesses of the three layers, and Kcl is the hydraulic conductivity of the fractured clay till. The natural flow in the aquifer is driven by a regional groundwater gradient i (m/m) and to simplify the system, the hydraulic gradient is assumed to be the same in both aquifers. During pumping the well modifies the natural water flow, lowering the water head in the aquifer, so that surface water from the stream can seep into the groundwater and reach the pumping well. Pollutants in the stream may be retarded by sorption and degraded by microorganisms during their travel to the well. Both the sandy and chalk aquifer are considered to be strictly anaerobic, while the hyporheic zone can be aerobic.
Statistical design	Modelling study
Relevance	
Environmental relevance	Relevant

Weight of evidence	
"Positive"/"Negative" evidence	The results for glyphosate and AMPA confirm the risk assesmant according to standard approaches. No negative evidence.

Rousseau et al. (2012)

Title: A Hydrological Modeling Framework for Defining Achievable Performance Standards for Pesticides
Author: Alain N. Rousseau, Pierre Lafrance, Martin-Pierre Lavigne, Stéphane Savary, Brou Konan, Renaud Quilbé, Paul Jiapizian and Mohamed Amrani
Reference: J. Environ. Qual. 41:52–63 (2012)
Year: 2012
Results and conclusion: This paper proposes a hydrological modeling framework to define achievable performance standards (APSs) for pesticides that could be attained after implementation of recommended management actions, agricultural practices, and available technologies (i.e., beneficial management practices [BMPs]). An integrated hydrological modeling system, Gestion Intégrée des Bassins versants à l'aide d'un Système Informatisé, was used to quantify APSs for six Canadian watersheds for eight pesticides: atrazine, carbofuran, dicamba, glyphosate, MCPB, MCPA, metolachlor, and 2,4-D. Outputs from simulation runs to predict pesticide concentration under current conditions and in response to implementation of two types of beneficial management practices (reduced pesticide application rate and 1- to 10-m-wide edge offield and/or riparian buffer strips, implemented singly or in combination) showed that APS values for scenarios with BMPs were less than those for current conditions. Moreover, APS values at the outlet of watersheds were usually less than ecological thresholds of good condition, when available. Upstream river reaches were at greater risk of having concentrations above a given ecological thresholds because of limited stream flows and overland loads of pesticides. Our integrated approach of "hydrological modeling–APS estimation–ecotoxicological significance" provides the most effective interpretation possible, for management and education purposes, of the potential biological impact of predicted pesticide concentrations in rivers.
Proposed action: Consider as additional information.
Type of information (critical, high/low weight, supporting, additional): Additional

Reliability	
Endpoint	Not applicable, surface water entries are modelled to analyze the impact of different management practices
Protocol	Modelling study
Test compound	No test compounds used in the study
Test system and conditions	Development of a hydrological modeling framework for defining nonregulatory, watershedscale, agroenvironmental APSs for pesticides for six Canadian watersheds. Using an integrated modeling system consisting of hydrological, erosion, pesticide field transport, and water quality models, APSs for a specific pesticide in a given stream reach were defined as a statistical value of the cumulative frequency curve of simulated in-stream concentrations during the period of interest (e.g., summer) and simulation interval (e.g., 30 yr). For each watershed, simulations were run to predict pesticide concentration under current concentrations ("reference" scenario) and in response to implementation of two types of BMPs, applied singly or in combination: (i) reduced rate of pesticide application and (ii) implementation of an edge-of-field or riparian buffer strips (1–10 m wide, depending on the feasibility and the necessity for each watershed). The resulting APS for each pesticide and stream reach was then compared with ETs derived using dose–response curves or other approaches for determining aquatic life protection criteria.
Statistical design	Modelling study

Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

References

Malaguerra *et al.* 2013. Assessment of the contamination of drinking water supply wells by pesticides from surface water resources using a finite element reactive transport model and global sensitivity analysis techniques. Journal of Hydrology 476 (2013) 321–331.

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B.7.6.6.3 Monitoring data

Publications regarding off-site movement and surface water monitoring

Summary

Publications which are available in open literature mostly deal with glyphosate concentrations in runoff water rather than are derived from comprehensive monitoring programs of surface waters.

Out of the screened open literature, 19 citations deal with glyphosate and AMPA concentrations in runoff water within the EU, 11 are related to non-EU sites. They include analyses of storm water, urban runoff, roof and railways runoff as well as runoff from specific agriculturally used sites such as vineyards. Concentrations in rainwater, information on bulk atmospheric deposition (wet and dry) are published as well. Some of the publications present results obtained from field studies and thus do not present results from monitoring campaigns in their common sense.

20 citations show results on European surface water (e.g. streams, rivers, small creeks) monitoring, 13 give results on monitoring outside Europe. The design and also the presentation of the results obtained from monitoring campaigns are quite heterogeneous. Information is rather incomplete. Also, 2 different publications might deal with the same campaign (Ludvigsen and Ode, 2001; Ludvigsen and Ode, 2002) but publish slightly different information. Furthermore, a few references focus on modeling aspects, PEC determination, risk assessment using the monitoring data published elsewhere and risk mitigation. In one case, glyphosate is discussed only but without publication of decent data. In one case information was extracted from a presentation, and graphs only were given. Exact data were not obtained from the graphs.

Maximum glyphosate and AMPA concentrations in European surface waters as measured in comprehensive monitoring campaigns ([redacted], 2012; see point 8.6.3) range between 1.3-370 µg/L and 0.22-> 200 µg/L for glyphosate and AMPA, respectively. Compared to these findings, the maximum concentrations which were published in open literature are rather low, namely in the range of 0.21-7.2 µg/L and 0.2-13 µg/L for glyphosate and AMPA, respectively. Therefore, information published in open literature does not really modify the already existing assessment of glyphosate and AMPA occurrence in surface water (see B.8.6.3).

For reasons of completeness results extracted from the publications on off-site movement and surface water monitoring are listed in the following tables though information might be lacking.

Table B.7.6.–2: Results of the publications on off-site movement

Country/ Substance	Description of sample	Date	No. Sites	No. Samples	Detected (samples)		Samples ≥ 0.1 µg/L		Max. conc.	LOQ (LOD)
					No.	%	No.	%	µg/L	µg/L
France										
Glyphosate	Urban stormwater	2008-09	3	15	14	93	-	-	232	0.03
AMPA	Urban stormwater	2008-09	3	15	14	93	-	-	9.37	-
Glyphosate	Urban stormwater	2007-08	2	20	-	-	-	-	71	-
AMPA	Urban stormwater	2007-08	2	20	-	-	-	-	1.5	-
Glyphosate	Wet atmospheric deposition	2008-09	1	-	-	-	-	-	0.4	-
AMPA	Wet atmospheric deposition	2008-09	1	-	-	-	-	-	0.7	-
Glyphosate	Vineyard runoff	2003-06	1	303	303	100	-	100	86	0.1
AMPA	Vineyard runoff	2003-06	1	303	303	100	-	100	44	0.1
Glyphosate	Vineyard runoff	2009-10	1	48	-	-	-	-	3.9	0.1 (0.03)
AMPA	Vineyard runoff	2009-10	1	48	-	-	-	-	1.8	0.1 (0.03)
Glyphosate	Roof runoff (rural site)	2009-10	-	-	-	-	-	-	6	0.1
Glyphosate	Wet atmospheric deposition	2008-09	1	-	-	-	-	-	150	-
AMPA	Wet atmospheric deposition	2008-09	1	-	-	-	-	-	19	-
UK										
Glyphosate	road run-off	1997	1	2	-	-	-	-	51.81)	0.05 (0.01)
Glyphosate	Railway runoff	1999-2000	1	3	-	-	0	0	< 0.1	0.05 (0.01)
Glyphosate	surface water drains (storm drains)	2009	-	-	-	-	-	-	8.99	0.007 (0.002)
AMPA	surface water drains (storm drains)	2009	-	-	-	-	-	-	1.15	0.01 (0.003)

Table B.7.6.–2: Results of the publications on off-site movement

Belgium										
Glyphosate	rainwater	1997-2001	8	ca. 870 analyses	113	13	-	-	1.2	-
Glyphosate	storm drainage outflow	2013	-	-	-	-	-	-	6.1	-
AMPA	storm drainage outflow	2013	-	-	-	-	-	-	5.8	
Denmark										
Glyphosate	Stormwater runoff	2008-09	5	10	10	100	-	-	9.0	-
AMPA	Stormwater runoff	2008-09	5	10	10	100	-	-	1.0	-
Glyphosate	Landfill leachate	2003	10	-	2	20	-	-	27	-
AMPA	Landfill leachate	2003	10	-	2	20	-	-	4.3	-

[1)] Predicted - = no information

Table B.7.6.–3: Results of the publications on surface water monitoring

Country/ Substance	Description of sample	Date	No. Sites	No. Samples	Detected (samples)		Samples ≥0.1 µg/L		Max. conc	LOQ(LOD)
					No.	%	No.	%	µg/L	µg/L
Belgium										
Glyphosate	Rainwater	1997-2001	8	Ca. 870 analyses	-	13	-	-	1.2	-
Hungary										
Glyphosate	Rivers	2010-2011	13	24	9	-	6	-	0.68	0.05 to 0.12
Luxembourg										
Glyphosate	River	2008	1	14	-	-	-	-	6.220	0.001
AMPA	River	2008	1	14	-	-	-	-	1.118	0.001
France										
Glyphosate	Rivers	2007	3	104	-	-	-	-	1.0	0.1
AMPA	Rivers	2007	3	104	-	-	-	-	1.0	0.1
Glyphosate	Rivers	2007	1	5	-	-	-	-	0.12	-
AMPA	Rivers	2007	1	5	-	-	-	-	0.65	-
Norway										
Glyphosate	Streams, rivers	1996-2000	12	57	52	91	-	-	-	(0.01)
Glyphosate[2)]	Streams, rivers	1995-1999	12	49	42	86	-	-	0.92	(0.01)
AMPA[2)]	Streams, rivers	1995-1999	12	49	43	87	-	-	0.2	(0.01)
Netherlands										
Glyphosate	River	-	-	-	-	-	-	-	0.21	-
AMPA	River	-	-	-	-	-	-	-	2.28	-
Switzerland										
Glyphosate	Rivers	2005-2012	565	-	-		-	-	7.2	-

Table B.7.6.–3: Results of the publications on surface water monitoring

Germany										
Glyphosate	Local creek	1999	1	3	-	-	-	-	0.9	(0.04)
AMPA	Local creek	1999	1	3	-	-	-	-	0.4	(0.04)
Glyphosate	Rivers in Hessen	1995-2005	2	-	-	-	-	-	0.4	(0.05)
Glyphosate	Surface water in MV	2008	60	180	105	58	40	22	1.37	0.02
AMPA	Surface water in MV	2008	60	180	147	82	83	46	5.58	0.01
Glyphosate	Surface water in NRW	1996-2012	-	1899	-	-	225	12	0.93	-
AMPA	Surface water in NRW	1996-2012	-	1903	-	-	1377	72	13	-

[2)] Same campaign as first entry for Norway, other publication with partly differing numbers
- = no information

It has to be noted that there are a few results available from studies investigating the behaviour of glyphosate in railway systems in Switzerland (Brauchli-Theotokis, 2004). These investigations show that after the application of glyphosate in railway systems, concentrations up to 100 µg/L glyphosate are detected in the drainage water of the experimental set-ups. In general, it was shown that the higher the rainfall quantity, the higher the cumulative amount of glyphosate that was washed-off. During normal operation, glyphosate concentrations along railway tracks reached values up to 10 g/L in the drainage water. Similar concentrations were also detected in drainage ditches alongside railway lines in England. There, about 1800 g /ha glyphosate were applied experimentally and 12 µg/L glyphosate was measured in run-off water (Heather *et al.*, 1999). According to the representative GAP applications on railway facilities are not indented and therefore, not addressed in the risk assessment. Generally, the RMS considers that additional specific information in order to assess the potential contamination of surface water by runoff as well as the potential contamination of groundwater via run-off in surface water with subsequent bank filtration are required, if applications of glyphosate on railway facilities are intended at the national level.

Detailed description of open literature on off-site movement

Augustin and Seibel (2002)

Title: Herbicide treatment of urban areas – a possible source of surface water contamination
Author: Bernd Augustin and Helmut Seibel
Reference: GESUNDE PFLANZEN, 54. Jahrg., Heft 7
Year: 2002
Results and conclusion: A rough concrete surface with an inclination of 1-2 % for rainwater elimination was treated with Roundup Ultra® (Glyphosat), Basta® (Glufosinate) and Vorox G® (Glyphosat-Diuron). Run-off-water was collected after artificial rain (2 mm) given in different periods after herbicide application (1 and 24 h; 10 days). Chemical analysis showed that the run-off-water contained considerable quantities of Glyphosate and Glufosinate even 10 days after herbicide treatment and 17 mm of artificial and natural rainfall. The results are discussed considering recent detection of Glyphosate contamination of surface water in Germany.
Proposed action: Not to be considered in the summarizing table of the off-site movement paragraph as an artificial scenario (runoff from a concrete surface) was designed. Obtained data are not comparable to those given in the summarizing table.
Type of information (critical, high/low weight, supporting, additional): Low weight, may be additional information to already existing
Reliability Low

Endpoint	Concentration in leachate
Protocol	For chemical analysis: DFG-method 405, non-GLP. General set-up without specific protocol but research project.
Test compound	Roundup Ultra® (Glyphosat), Vorox G® (Glyphosat-Diuron), CAS-no.: 1071-83-6
Test system and conditions	A rough concrete surface with an inclination of 1-2 % for rainwater elimination was treated with Roundup Ultra® (Glyphosat), Basta® (Glufosinate) and Vorox G® (Glyphosat-Diuron). Run-off-water was collected after artificial rain (2 mm) given in different periods after herbicide application (1 and 24 h; 10 days).
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results support teh monitoring data. However, monitoring studies and campaigns are of more reliability and relevance. No negative evidence.

Augustin (2003)

Title: Urban areas-sources of pesticide-contamination of surface water?
Author: Augustin B.
Reference: Presentation at: Second International Symposium Plant Health in Urban Horticulture, Berlin, August 27-29, 2003
Year: 2003
Results and conclusion: In Rhineland-Palatinate numerous (14-days-mix) samples of surface water (Mosel, Nahe, Selz) were repeatedly controlled for pesticide pollution between 1997 and 1999. Investigations focused on 35 different active ingredients. Regularly present were Bentazon, Diuron, Dichlorprop, Ethofumesat, Glyphosat, IPU, MCPA, Mecoprop, Tebuconazol and Simazin. Especially Glyphosate and Bentazon were detected in all water sources and partly all over the year. An additional investigation of a sewage disposal plant ("Hahnheim"), which drains into river Selz clearly showed, that waste water was polluted by the same active ingredients. Pesticide concentration was about ten times as high as in the river water. Detectable pesticides mostly formed distinct peaks during time of investigation indicating a direct dependence on application period. This was not the case for Glyphosate. Up to now there are no indications for the presence of Glyphosate in drain-water of agricultural areas. Since the herbicide was detectable during the entire year, it is unlikely that it derived from application of farmland, vineyards or orchards. The fact that larger quantities are used on urban areas, let to the presumption, that there might also be runoff from sealed areas. In-depth worst-case investigations on Glyphosate concentrations in urban runoff (concrete runoff) showed concentrations between 0.03-17.9 mg/L depending on precipitation period and quantity.
Proposed action: Not to be considered as the publication is of low weight because of low reliability of data. No raw data are published.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing, summary from an oral presentation with several figures but without reliable results on concentrations.

Reliability	Low
Endpoint	Monitoring, concentrations in surface waters, sewage disposal plant and urban runoff
Protocol	No standard protocol, no further information on monitoring given
Test compound	Glyphosate (monitored, purity cannot be given, CAS-no: 1071-83-6)
Test system and conditions	Monitoring programme described briefly only without presentation of design of the campaigns, no LOD or LOQ given
Statistical design	Not known

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Birch et al. (2011)

Title: Micropollutants in stormwater runoff and combined sewer overflow in the Copenhagen area, Denmark	
Author: H. Birch, P. S. Mikkelsen, J. K. Jensen and H.-C. Holten Lützhøft	
Reference: Water Science & Technology; doi: 10.2166/wst.2011.687	
Year: 2011	
Results and conclusion: Stormwater runoff contains a broad range of micropollutants. In Europe a number of these substances are regulated through the Water Framework Directive, which establishes Environmental Quality Standards (EQSs) for surface waters. Results from a screening campaign including more than 30 substances at four stormwater discharge locations and one combined sewer overflow (CSO) in Copenhagen are reported here. Glyphosate was found in all samples. The results give a valuable background for designing further monitoring programs focusing on the chemical status of surface waters in urban areas.	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): High weight, additional information to already existing as the screening campaign was meant for designing further monitoring programs focusing on the chemical status of surface waters in urban areas. No data on Glyphosate concentrations published.	
Reliability	Low
Endpoint	Monitoring, concentrations in surface waters
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no. 1071-83-6) and AMPA (CAS-no.: 1066-51-9, monitored, purity cannot be given)
Test system and conditions	Substances for analysis primarily selected from the WFD list, but earlier Danish runoff studies and a risk assessment for one of the catchment areas were also considered. Sampling sites and regimes described. Total concentrations in the samples measured. No further information on analytical procedures, statistical treatment, quality assurance.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Botta et al. (2009)

Title: Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems	
Author: Fabrizio Botta, Gwenaëlle Lavison, Guillaume Couturier, Fabrice Alliot, Elodie Moreau-Guigon, Nils Fauchon, Bénédicte Guery, Marc Chevreuil, Hélène Blanchoud	
Reference: Chemosphere 77(1): 133-139 doi: 10.1016/j.chemosphere.2009.05.008	
Year: 2009	
Results and conclusion: A study of glyphosate and aminomethyl phosphonic acid (AMPA) transfer in the Orge watershed (France) was carried out during 2007 and 2008. Water samples were collected in surface water, wastewater sewer, storm sewer and wastewater treatment plant (WWTP). These two molecules a ppeared to be the most frequently detected ones in the rivers and usually exceeded the European quality standard concentrations of 0.1 µg/L for drinking water. The annual glyphosate estimated load was 1.9 kg year^{-1} upstream (agricultural zone) and 179.5 kg year^{-1} at the catchment outlet (urban zone). This result suggests that the contamination of this basin by glyphosate is essentially from urban origin (road and railway applications). Glyphosate reached surface water prevalently through storm sewer during rainfall event. Maximum concentrations were detected in storm sewer just after a rainfall event (75–90 µg/L). High concentrations of glyphosate in surface water during rainfall events reflected urban runoff impact. AMPA was always detected in the sewerage system. This molecule reached surface water mainly via WWTP effluent and also through storm sewer. Variations in concentrations of AMPA during hydrological episodes were minor compared to glyphosate variations. Our study highlights that AMPA and glyphosate origins in urban area are different. During dry period, detergent degradation seemed to be the major AMPA source in wastewater.	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information about non-agricultural application of glyphosate and the relevant contribution on the glyphosate annual load.	
Reliability	Medium
Endpoint	Concentration of glyphosate and AMPA
Protocol	For glyphosate and AMPA the analytical method was the DIN 38407-22 and international Norm NF ISO 21548
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Monitoring network: Sample campaigns were organized to gather data according four different levels: 1) at the basin scale to calculate the budget of glyphosate load in the Orge River, 2) at the urban area scale to verify the impact of sewage network on the river contamination, 3) at the network scale to study the transfer of glyphosate and its degradate by runoff in urban areas and 4) at the waste water treatment plant scale to verify the potential impact of urban wastes on surface waters.
Statistical design	No information
Relevance	
Environmental relevance	No reported data of environmental parameters.
Weight of evidence	
"Positive"/"Negative" evidence	Results supported by other publications; no negative evidence.

Bressy et al. (2012)

Title: Towards the determination of an optimal scale for stormwater quality management: Micropollutants in a small residential catchment	
Author: A. Bressy, M.-C. Gromaire, C. Lorgeoux, M. Saad, F. Leroy, G. Chebbo	
Reference: Water Research 46(20): 6799-6810. doi:10.1016/j.watres.2011.12.017	
Year: 2012	
Results and conclusion: Stormwater and atmospheric deposits were collected on a small residential urban catchment (0.8 ha) near Paris in order to determine the levels of certain micropollutants (using a preliminary scan of 69 contaminants, followed by a more detailed quantification of PAHs, PCBs, alkylphenols and metals). Atmospheric inputs accounted for only 10 % to 38 % of the stormwater contamination (except for PCBs), thus indicating substantial release within the catchment. On this small upstream catchment however, stormwater contamination is significantly lower than that observed downstream in storm sewers on larger adjacent urban catchments with similar land uses. These results likely stem from cross-contamination activity during transfers inside the sewer system and underscore the advantages of runoff management strategies at the source for controlling stormwater pollutant loads. Moreover, it has been shown that both contamination levels and contaminant speciation evolve with the scale of the catchment, in correlation with a large fraction of dissolved contaminants in upstream runoff, which differs from what has been traditionally assumed for stormwater. Consequently, the choice of treatment device/protocol must be adapted to the management scale as well as to the targeted type of contaminant.	
Proposed action: Not to be considered. The publication gives an overview of the stormwater and atmospheric deposits of 66 micropollutants, but not all raw data needed for the evaluation of a monitoring campaign are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	Medium
Endpoint	Total mass of contaminants in proportion to exposure time; flux
Protocol	Analyses were performed in a laboratory certified by a French Environment Ministry committee, i.e. COFRAC (French Accreditation Committee), in accordance with French (AFNOR) or International (ISO) standard methods to the extent of their availability.
Test compound	Glyphosate one of the compounds analyzed (CAS-no.: 1071-83-6)
Test system and conditions	1) the micropollutants present in runoff water were identified and quantified by encompassing a wide spectrum of substances over 3 rainfall events; 2) for a greater number of rain events over a one year period, the fluxes being conveyed were quantified for a selection of parameters
Statistical design	No information, no raw data
Relevance	
Environmental relevance	Environmental parameter are not reported.
Weight of evidence	
"Positive"/"Negative" evidence	Results are supported by other publications.

Baun et al. (2004)

Title: Xenobiotic organic compounds in leachates from ten Danish MSW landfills— chemical analysis and toxicity tests	
Author: A. Baun, A. Ledin, L.A. Reitzel, P.L. Bjerg, T.H. Christensen	
Reference: Water Research 38, 3845–3858	
Year: 2004	
Results and conclusion: Glyphosate and AMPA were found in the leachate of 2 (out of 10) Danish landfills at concentrations of 0.7 and 27 µg/L (Glyphosate) and 4.3 and 3.8 µg/L (AMPA). It furthermore was concluded to include degradation products in future monitoring programs as this may provide additional insight in the fate of chemicals in landfills. The present study provided several examples of concomitant presence of parent compounds and degradation products (Glyphosate/ AMPA).	
Proposed action: Supporting information which is presented in the summarising table and discussion.	
Type of information (critical, high/low weight, supporting, additional): High weight, supporting information to already existing.	
Reliability	Medium
Endpoint	Monitoring, concentration in landfill leachate.
Protocol	No standard protocol
Test compound	Glyphosate (CAS-no.: 1071-83-6) and AMPA (CAS-no: 1066-51-9) (monitored, purity cannot be given)
Test system and conditions	10 landfills tested comprehensive monitoring campaign for pesticides and several other organic compounds.
Statistical design	Not known
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other studies. No negative evidence.

Eriksson et al. (2007)

Title: Risk assessment of xenobiotics in stormwater discharged to Harrestrup Å, Denmark
Author: Eva Eriksson, Anders Baun, Peter Steen Mikkelsen, Anna Ledin
Reference: Desalination 215, 187–197
Year: 2007

Results and conclusion: Surface waters are highly manipulated in many cities in Europe, and the flow is largely impacted by discharges of stormwater and combined sewer overflow. Toxicity tests shown adverse effects in some of these recipients due to the presence of xenobiotic organic carbons (XOCs). Harrestrup Å, situated in the City of Copenhagen, is one of these recipients, where biotest using algae showed measurable toxicity in eight samples taken in 2003. Twenty-five different XOCs were quantified in the same samples. The present study aimed at identifying the most relevant XOCs out of these 25 to be selected for further analysis with respect to potential source control options. Fourteen XOCs (56 %) were identified to constitute a potential hazard based on the RICH evaluation (Ranking and Identification of Chemical Hazards), while 9 XOCs (36 %) were found to constitute a hazard towards the aquatic ecosystem based on an environmental-concentration/predicted-no-effect-concentration-quotient. The quantified levels did, however, fulfil the Danish and European surface water quality criteria (QC) and environmental quality standards (ESQ). Thus, although the QC and ESQ are met there is an actual risk due to stormwater-related pollutants. This clearly illustrates that there is a need for monitoring the stormwater quality in order to protect the ecosystems. It also shows that actions are needed to implement source control options and emission barriers. Twelve XOCs were selected for further evaluation of possible source control option to be implemented in order to improve the water quality. These are five pesticides (diuron, glyphosate, isoproturon, MCPA, Terbutylazin), 4 PAHs (acenaphthene, fluoranthene, fluorene, pyrene), 3 others (LAS, nonylphenol and dinitro-o-cresol.
Proposed action: Not to be considered as publication deals with hazard ranking based on monitoring and effect data and does not comprehensively report monitoring data.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.

Reliability	Low
Endpoint	Hazard ranking
Protocol	No protocol, no experimental design
Test compound	Glyphosate (CAS-no: 1071-83-6), no experimental design
Test system and conditions	Hazard ranking procedure, no experimental design but monitoring data cited from other sources
Statistical design	Not applicable
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Similar ranking approaches support the results. No negative evidence.

Eriksson et al. (2007)

Title: Selected stormwater priority pollutants – a European perspective
Author: E. Eriksson, A. Baun, L. Scholes, A. Ledin, S. Ahlman, M. Revitt, C. Noutsopoulos, P.S. Mikkelsen
Reference: Science of the Total Environment 383, 41–51
Year: 2007

Results and conclusion:
The chemical characteristics of stormwater are dependent on the nature of surfaces (roads, roofs etc.) with which it comes into contact during the runoff process as well as natural processes and anthropogenic activities in the catchments. The different types of pollutants may cause problems during utilisation, detention or discharge of stormwater to the environment and may pose specific demands to decentralised treatment. This paper proposes a scientifically justifiable list of selected stormwater priority pollutants (SSPP) to be used, e.g., for evaluation of the chemical risks occurring in different handling strategies. The SSPP-list consists of 25 pollutant parameters including eight of the priority pollutants currently identified in the European Water Framework Directive. It contains general water quality parameters (organic and suspended matter, nutrients and pH); metals (Cod, Cr, Cu, Ni, BP, Pt and Zn); PAH (naphthalene, preen and benzoic[a]preen); herbicides (pendimethalin, phenmedipham, glyphosate and terbutylazine); and other representative industrially derived compounds (nonylphenol ethoxylates, pentachlorophenol, di(2-ethylhexyl)phthalate, PCB-28 and methyl tert-butylether). Tools for flux modelling, enabling calculation of predicted environmental concentrations (PECs), and for ranking the susceptibility of a pollutant to removal within a range of structural stormwater treatment systems or best management practices (BMPs) have been developed, but further work is required to allow all SSPPs to be addressed in the development of future stormwater pollution control measures. In addition, the identified SSPPs should be considered for inclusion in stormwater related monitoring campaigns.

Proposed action:
Not to be considered as publication deals with hazard ranking and priority setting and does not comprehensively and in detail report monitoring data.

Type of information (critical, high/low weight, supporting, additional):
Low weight, additional information.

Reliability	Low
Endpoint	Hazard ranking and priority list
Protocol	No protocol, no experimental design
Test compound	Glyphosate (CAS-no: 1071-83-6), no experimental design
Test system and conditions	Hazard ranking procedure, no experimental design but monitoring data cited from other sources
Statistical design	Not applicable
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Similar ranking approaches support the results. No negative evidence.

Gregoire et al. (2010)

Title: Use and fate of 17 pesticides applied on a vineyard catchment

Author: Caroline Gregoire, Sylvain Payraudeau and Nicolas Domange

Reference: Intern. J. Environ. Anal. Chem. Vol. 90, Nos. 3–6, 15, 406–420

Year: 2010

Results and conclusion:
Flux of 17 pesticides from a small agricultural catchment was monitored. Some 78 % of the total pesticide applications in the catchment were herbicides and glyphosate was the most used herbicide with annual application ranging from 18 to 61 kg. The run-off coefficient was low (less than 2 %), but the frequency of determination was high for some pesticides such as the fungicide dimetomorph (72 %) and the herbicides diuron (98 %) and glyphosate (100 %). The pesticide export coefficients for Glyphosate ranged between 0.009 – 0.033 %. Every water sample exceeded the EU drinking water limit of 0.1 µg/L. In detail, concentrations were for Glyphosate: 7.5 µg/L (mean) and 86 mgL^{-1} (max), and for AMPA 2.9 µg/L (mean) and 44 mg/L (max).

Proposed action:
To be considered in the summarizing table and discussion.

Type of information (critical, high/low weight, supporting, additional): High weight, supporting information.	
Reliability	High
Endpoint	Glyphosate concentration in runoff water
Protocol	No standard protocol; for further details see under test system and conditions
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	The flux of 17 pesticides from a small (42.7 ha) agricultural (vineyard) catchment in the Alsatian piemont (France) was systematically monitored over 4 years (2003–2006) from June to September. A metrological station is located within the catchment area and run-off of 58 run-off events was monitored throughout. A water sample for pesticide analyses was collected every 8m^3 of run-off. Chemical analysis described. Calculations described
Statistical design	Frequency of determination calculated
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Huang et al. (2004)

Title: Herbicide Runoff along Highways. 1. Field Observations
Author: Xinjiang Huang , Theresa Pedersen, Michael Fischer, Richard White, and Thomas M. Young
Reference: Environ. Sci. Technol., 38, 3263-3271
Year: 2004
Results and conclusion: To determine whether herbicide runoff along highways threatens water quality, a field study was conducted at two sites in northern California for three rainy seasons. The herbicides oryzalin, isoxaben, diuron, glyphosate, and clopyralid were selected for study to include compounds with significant variation in physical/chemical properties. Concentrations of herbicides in runoff were monitored for up to 11 storms following herbicide application, and 24 samples were collected per storm, providing unprecedented temporal detail. Flow-weighted event mean concentrations were calculated for each herbicide in each storm and ranged from below detection limits to 43.13 µg/L for oryzalin. The least soluble compounds, isoxaben and oryzalin, were detected in all storms monitored while the more soluble compounds, diuron and clopyralid, declined to levels below detection limits before monitoring was concluded. Very small amounts of glyphosate were mobilized, but its transformation product aminomethylphosphonic acid was detected at higher concentrations, in more storm events, and at greater depth in the soil profile. A first order model successfully described the declining herbicide concentrations in spray zone soil and in surface run-off for all sites and herbicides. Fitted first-order coefficients were always higher for runoff than for soil, indicating that the herbicide that persists in the source zone becomes less available for runoff as the time since application increases. The percentage of the applied herbicide that was detected in surface runoff over a season ranged from 0.05 % to 43.5 %, and the most critical variables in controlling the variation were the solubility of the herbicide and the runoff volume. For a given herbicide and site, the most critical factors in determining seasonal herbicide loss to surface water were the timing and intensity of the first storm following application, affecting total seasonal runoff by up to 2 orders of magnitude. Minimizing runoff of herbicides along highways will thus require careful attention to the intrinsic mobility of the compound and the timing of its application.
Proposed action: Not to be considered as the study deals with a site outside the EU (USA)
Type of information (critical, high/low weight, supporting, additional): Low weight

Reliability	Medium
Endpoint	Variation in herbicide concentration over time, herbicide mass loading and runoff concentration; first-order Dissipation coefficients (k), pre-exponential factors (a), and fitting criteria (R 2) estimated from runoff, event mean concentration and herbicide concentrations
Protocol	Non-GLP
Test compound	Oryzalin, isoxaben, diuron, glyphosate (CAS-no.: 1071-83-6), clopyralid and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	The herbicides were applied to a 1.23 or 1.83 m wide strip along the highway shoulder using a truck sprayer. Application typically occurred after the first fall storm. Exact application rates were determined by analyzing herbicides recovered from deposit collection plates constructed of glass fibersample pad on corrugated cardboard that were located within the spray zone during herbicide application. At the monitoring location runoff flow rates were determined using a flume and an automatic sampling station, which included a rain gauge and a bubbler flow module, began taking samples when 0.01 mm of rain fell in 30 min and the flow level exceeded 3.0 mm. Each sampling event included up to 24 samples collected at intervals of between 20 and 120 min. Sampling times, rainfall volumes, and runoff flow rates were recorded by the automatic sampling system
Statistical design	-
Relevance	
Environmental relevance	Tables giving herbicide application rates and rainfall and runoff comparison at the two sampling sites and figures showing event first-flush analysis and vertical distribution of glyphosate and AMPA content within soil. This material is available free of charge via the Internet at http://pubs.acs.org.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Huang et al. (2004)

Title: Herbicide Runoff along Highways. 2. Sorption Control
Author: Xinjiang Huang , Theresa Pedersen , Michael Fischer, Richard White, and Thomas M. Young
Reference: Environ. Sci. Technol., 38, 3272-3278
Year: 2004
Results and conclusion: This study examines the sorption and desorption of five herbicides with a wide range of properties (isoxaben, oryzalin, diuron, clopyralid, and glyphosate) on soil samples from two roadsides in northern California and uses the results to examine field runoff data from multiple rainy seasons. Non-ideal sorption processes do not appear to be significant in determining herbicide runoff at the field sites because (i) sorption isotherms were linear or slightly nonlinear for all compounds but glyphosate, (ii) field runoff concentration ratios between isoxaben and oryzalin were consistent with linear partitioning predictions, (iii) runoff leaving the site appeared to be in equilibrium with local soil concentrations, and (iv) desorption distribution coefficients for aged herbicides on soil samples collected from the field site did not differ substantially from those obtained in short term laboratory adsorption experiments. Collectively, these findings indicate that linear equilibrium models are adequate for predicting the concentration of herbicides in runoff in these field settings and that more complicated non-ideal models do not need to be invoked. Vegetated slopes effectively reduced the herbicide loads, with average removals of 35-80 % occurring as runoff traversed a 3-m segment 1 m from the edge of the spray zone.
Proposed action: Not to be considered as the study deals with a site outside the EU (USA).

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information. This research has demonstrated that, although the herbicides display some evidence of non-ideal (i.e., nonlinear and incompletely reversible) sorption behaviours in the laboratory, ideal sorption models (i.e., linear isotherms and completely reversible) are likely to be sufficient for describing the sorption component of this process in the field.	
Reliability	High
Endpoint	Sorption isotherms; carbon-normalized distribution coefficients (K_{OC}), and regression R^2 values, K_d, K_f soil concentration
Protocol	Similar to OECD 106, non-GLP
Test compound	Oryzalin, isoxaben, diuron, glyphosate (CAS-no.: 1071-83-6), clopyralid and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	1) To measure herbicide attenuation as runoff moved down the grassy slope, six runoff collectors were installed at the sampling site at defined distances; vegetation samples along the slope were also collected and analyzed for herbicides; 2) Adsorption isotherms were determined for the five target herbicides and AMPA on surface soils from the field sites. To obtain greater than 50 % adsorption and final concentrations above method quantitation limits, soil: water ratios (g/mL) were determined. The time required to attain an apparent equilibrium was also determined. Batch adsorption experiments were conducted by combining herbicide solution in a 0.005 M $CaCl_2$ matrix with soil at the predetermined ratios in 45-mL Teflon centrifuge tubes. Six samples with varying initial herbicide concentrations (0-1000 µg/L) were mixed in triplicate at 23 +/- 1°C; 3) Combined herbicide adsorption and desorption study with isoxaben and oryzalin; 4) Desorption of herbicides from spray zone soils collected from the Tolay Creek field site at different times after herbicide application was determined.
Statistical design	Measurements in triplicate, Sorption isotherms: linear model and the Freundlich model
Relevance	
Environmental relevance	Given as influence by environmental parameter was tested.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Imfeld et al. (2012)

Title: Transport and attenuation of dissolved glyphosate and AMPA in a stormwater wetland
Author: Gwenaël Imfeld, Marie Lefrancq, Elodie Maillard and Sylvain Payraudeau
Reference: Chemosphere 90 (2013) 1333–1339
Year: 2012
Results and conclusion: Here we show that transport and attenuation of runoff-associated glyphosate and AMPA in a stormwater wetland differ and largely vary over time. Dissolved concentrations and loads of glyphosate and AMPA in a wetland receiving runoff from a vineyard catchment were assessed during three consecutive seasons of glyphosate use (March to June 2009, 2010 and 2011). The load removal of glyphosate and AMPA by the wetland gradually varied yearly from 75 % to 99 %. However, glyphosate and AMPA were not detected in the wetland sediment, which emphasises that sorption on the wetland vegetation, which increased over time, and biodegradation were prevailing attenuation processes. The relative load of AMPA as a percentage of total glyphosate increased in the wetland and ranged from 0 % to 100 %, which indicates the variability of glyphosate degradation via the AMPA pathway. Our results demonstrate that transport and degradation of glyphosate in stormwater wetlands can largely change over time, mainly depending on the characteristics of the runoff event and the wetland vegetation. We anticipate our results to be a starting point for considering degradation products of runoff-associated pesticides during their transfer in wetlands, in particular when using stormwater wetlands as a management practice targeting pesticide attenuation. Max. concentrations detected were 150 µg/L (glyphosate) and 19 µg/L (AMPA).

Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific targets, namely stormwater and wastewater, at a certain place and time.	
Reliability	Low
Endpoint	Monitoring, concentrations in surface waters (stormwater wetlands)
Protocol	Monitoring, for details see test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no.: 1066-51-9, monitored, purity cannot be given)
Test system and conditions	Runoff discharges entering and outflowing the wetland were continuously monitored from March 23 to June 30, 2009, 2010 and 2011.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Lamprea and Ruban (2010)

Title: Characterization of atmospheric deposition and runoff water in a small suburban catchment	
Author: Katerine Lamprea and Véronique Ruban	
Reference: Environmental Technology, Vol. 32, No. 10, 1141–1149	
Year: 2010	
Results and conclusion: A study has been carried out with the objective of characterizing atmospheric deposition, roof runoff and street runoff in a small (31 ha) suburban catchment in Nantes equipped with a separate sewer system. Street runoff: Glyphosate was not detected in winter, yet the summer glyphosate and AMPA concentrations were found to lie between 60 and 470 ng/L for glyphosate, and 50 and 770 ng/L for AMPA. Deposition: To the best of our knowledge, glyphosate and AMPA have rarely been identified in atmospheric deposition. Though their volatility remains low, these molecules are present in the atmosphere from having been transported via vaporization when applied to the catchment and in the neighbourhood. Glyphosate and AMPA were detected in roughly 10 % of samples. Roof-runoff: Glyphosate was detected in samples collected in September 2007 campaigns. The concentrations range from 50 to 980 ng/L. AMPA was detected in samples collected from slate roof in the June 2008 campaign, displaying a concentration of 120 ng/L. It is very likely, that atmospheric deposition constitutes the glyphosate and AMPA contributor to roof runoff.	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific targets, namely street runoff, roof-runoff and atmospheric deposition at a certain place and time.	
Reliability	Medium
Endpoint	Glyphosate concentrations in street-runoff, roof-runoff and atmospheric deposition.
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, monitoring

Test system and conditions	This study was conducted on the Pin Sec catchment in the eastern part of the French city of Nantes between the Loire and Erdre rivers. Sampling campaigns were performed on bulk atmospheric (both dry and wet) deposition, roof runoff and street runoff. Samples were analyzed for Glyphosate and AMPA, LOQ = 0.05 µg/L.
Statistical design	Mean values, standard deviations, Whisker blots.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Lamprea and Ruban (2011)

Title: Pollutant concentrations and fluxes in both stormwater and wastewater at the outlet of two urban watersheds in Nantes (France)	
Author: Katerine Lamprea and Véronique Ruban	
Reference: Urban Water Journal ,Vol. 8, No. 4, 219–231	
Year: 2011	
Results and conclusion: A two-year study of pollutants in both the stormwater and wastewater of urban watersheds has been conducted in Nantes (France). The present paper discusses the characteristics of pollutants transported by stormwater and wastewater collection networks in two urban watersheds. A physicochemical characterisation of the effluents was performed, along with an estimation of pollutant fluxes discharged into the Gohards River. Concentrations in stormwater: Glyphosate = 0.23-3.27 µg/L; AMPA = 0.1-0.46 µg/L. Concentrations in waste water during wet weather season: Glyphosate and AMPA = 0.3-49 µg/L.	
Proposed action: To be considered in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific targets, namely stormwater and wastewater at a certain place and time.	
Reliability	Medium
Endpoint	Glyphosate concentrations in stormwater and waste water.
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	So as to characterize quality and pollutant substances transported by stormwater, dry and wet weather conditions were studied in both stormwater and wastewater networks. The campaigns were carried out from September 2007 to October 2008 for stormwater, and from April 2007 to December 2008 for wastewater. Samples were analyzed for Glyphosate and AMPA, LOQ = 0.05 µg/L.
Statistical design	Mean values, standard deviations, Whisker blots
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Maillard et al. (2011)

Title: Removal of pesticide mixtures in a stormwater wetland collecting runoff from a vineyard catchment	
Author: Elodie Maillard, Sylvain Payraudeau, Etienne Faivre, Caroline Grégoire, Sophie Gangloff, Gwenaël Imfeld	
Reference: Science of the Total Environment 409, 2317–2324	
Year: 2011	
Results and conclusion: Here we show that stormwater wetlands can efficiently remove pesticides in runoff from vineyard catchments during the period of pesticide application, although flow and hydrochemical conditions of the wetland largely vary over time. During the entire agricultural season, the inflowing load of nine fungicides, six herbicides, one insecticide and four degradation products was 8.039 g whereas the outflowing load was 2.181 g. Removal rates of dissolved loads by the wetland ranged from 39 % (simazine) to 100 % (cymoxanil, gluphosinate, kresoxim methyl and terbuthylazine). Dimethomorph, diuron, glyphosate, metalaxyl and tetraconazole were more efficiently removed in spring than in summer. More than 88 % of the input mass of suspended solids was retained, underscoring the capability of the wetland to trap pesticide-laden particles via sedimentation. Only the insecticide flufenoxuron was frequently detected in the wetland sediments. Our results demonstrate that stormwater wetlands can efficiently remove pesticide mixtures in agricultural runoff during critical periods of pesticide application, although fluctuations in the runoff regime and hydrochemical characteristics can affect the removal rates of individual pesticides.	
Proposed action: Considered by listing in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information: stormwater wetlands have the potential to serve as a tool for urban and agricultural stormwater management practices, thus contributing to the improvement of water quality for receiving aquatic ecosystems.	
Reliability	High
Endpoint	Reduction of pesticide concentration, RC (%), was calculated for each runoff event as the reduction of mean concentrations at the outlet relatively to the mean concentrations at the inlet of the wetland. Removal rates of pesticide load RL (%) were calculated for each runoff event as the reduction of the load at the outlet relatively to the load at the inlet of the wetland.
Protocol	Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC)
Test compound	Azoxystrobin, Cymoxanil, Cyprodinil, Carbendazim, Dimethomorph, Diuron, DCPU, DCPMU, 3.4-dichloroaniline, Flufenoxuron, Gluphosinate, Glyphosate (CAS-no. 1071-83-6), AMPA (CAS-no.: 1066-51-9), Isoxaben, Kresoxim methyl, Metalaxyl, Pyrimethanil, Simazine, Terbuthylazine, Tetraconazole
Test system and conditions	The main objective of the present study was to assess the ability of a stormwater wetland to remove pesticides in runoff from a vineyard catchment during an entire period of pesticide application. Nine fungicides, six herbicides, one insecticide and four degradation products were selected for the present study. Samples were collected from the inlet, the sediment deposition zone, the gravel filter, and the outlet of the wetland from April 01 through September 29 2009, corresponding to the period of pesticide application. Runoff discharges were continuously monitored by measurements of water depth. Series of discrete water samples taken over a run-off event were combined in a single composite sample. In parallel, 10 sampling campaigns were performed every two weeks during quiescent period (i.e. in the period between two runoff events) to collect water and sediment samples within the wetland.
Statistical design	Detection and quantification limits, relative standard deviation (RSD) and recovery efficiencies

Relevance	
Environmental relevance	Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2011.01.057.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence

Maillard et al. (2011)

Title: Removal of dissolved pesticide mixtures by a stormwater wetland receiving runoff from a vineyard catchment: an interannual comparison	
Author: Elodie Maillard, Sylvain Payraudeau, Floro Ortiz and Gwenael Imfeld	
Reference: Intern. J. Environ. Anal. Chem., 1–16,	
Year: 2011	
Results and conclusion: We show here that stormwater wetlands that primarily serve for flood protection can also be effective tools for reducing concentrations and removing loads of runoff-related pesticides and some of their degradation products into downstream aquatic ecosystems. Dissolved concentrations and loads of seven fungicides, six herbicides and four degradation products in runoff from a vineyard catchment were continuously recorded at the inlet and the outlet of the stormwater wetland during two successive periods of pesticide application (April to June). Reduction of pesticide concentrations by the wetland ranged from 50 % (simazine) to 100 % (azoxystrobin, cymoxanil, cyprodinil, gluphosinate, terbuthylazine and tetraconazole). Removal rates of dissolved load ranged from 26 % for aminomethylphosphonic acid (AMPA) to 100 % (azoxystrobin, cymoxanil, cyprodinil, diuron, 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU), gluphosinate, kresoxym methyl, terbuthylazine and tetraconazole). More than 77 % of the input mass of total suspended solids was retained, underscoring the capability of the wetland to trap pesticide-laden particles via sedimentation. Inter-annual change in the removal of AMPA, isoxaben, kresoxim methyl and simazine was mainly linked to the larger vegetal cover in 2010. Our results demonstrate that stormwater wetlands can remove pesticide mixtures in agricultural runoff, although removal of individual pesticides can vary over time, depending on the characteristics of runoff events and the vegetation cover.	
Proposed action: Considered by listing in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information: Stormwater wetlands that primarily serve for flood protection can also be effective tools for reducing concentrations and removing loads of a wide range of runoff-related pesticides and some of their degradation products into downstream aquatic ecosystems	
Reliability	Medium
Endpoint	Reduction of pesticide concentration (RC (%)) was calculated as the relative decrease of mean concentration at the outlet with respect to that at the inlet. Removal rate of pesticide mass loading RL (%) was calculated as the relative decrease of mass loading at the outlet with respect to that at the inlet for each runoff event.
Protocol	Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC).
Test compound	Azoxystrobin, cymoxanil, cyprodinil, dimethomorph, diuron, gluphosinate, glyphosate (CAS-no.: 1071-83-6), isoxaben, kresoxim methyl, metalaxyl, simazine, terbuthylazine, tetraconazole, 3,4-dichlorophenyl urea (DCPU), DCPMU, 3,4-dichloroaniline and AMPA (CAS-no.: 1066-51-9)

Test system and conditions	Runoff is collected at the outlet of the vineyard catchment by a stormwater wetland and represents the main entry route of pesticides into the wetland. Runoff discharges entering and outflowing the wetland were continuously monitored by measuring waterdepth using bubbler flow modules. Water samples (300 mL) were collected in glass jars, stored in the dark at 4 °C after each runoff event. A series of discrete flow proportional water samples taken over a runoff event were combined in a single composite sample prior to analysis.
Statistical design	Paired nonparametric Wilcoxon Signed Rank test
Relevance	
Environmental relevance	
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Majewski et al. (2014)

Title: PESTICIDES IN MISSISSIPPI AIR AND RAIN: A COMPARISON BETWEEN 1995 AND 2007	
Author: M. S. MAJEWSKI, R. H. COUPE, W. T. FOREMAN and P. D. CAPEL	
Reference: Environmental Toxicology and Chemistry, Vol. 33, No. 6, pp. 1283–1293, 2014	
Year: 2014	
Results and conclusion: A variety of current-use pesticides were determined in weekly composite air and rain samples collected during the 1995 and 2007 growing seasons in the Mississippi Delta (MS, USA) agricultural region. Similar sampling and analytical methods allowed for direct comparison of results. Decreased overall pesticide use in 2007 relative to 1995 generally resulted in decreased detection frequencies in air and rain; observed concentration ranges were similar between years, however, even though the 1995 sampling site was 500m from active fields whereas the 2007 sampling site was within 3 m of a field. Mean concentrations of detections were sometimes greater in 2007 than in 1995, but the median values were often lower. Seven compounds in 1995 and 5 in 2007 were detected in ≥50 % of both air and rain samples. Atrazine, metolachlor, and propanil were detected in ≥50 % of the air and rain samples in both years. Glyphosate and its degradation product, aminomethyl-phosphonic acid (AMPA), were detected in ≥75 % of air and rain samples in 2007 but were not measured in 1995. The 1995 seasonal wet depositional flux was dominated by methyl parathion (88 %) and was >4.5 times the 2007 flux. Total herbicide flux in 2007 was slightly greater than in 1995 and was dominated by glyphosate. Malathion, methyl parathion, and degradation products made up most of the 2007 nonherbicide flux.	
Proposed action: Not to be considered as publication deals with air and rain monitoring outside EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as the articles deals with air and rain monitoring outside EU.	
Reliability	
Endpoint	Concentration of pesticides in air and rain samples
Protocol	Not given
Test compound	Pesticides (Glyphosate and AMPA (amongst others))

Test system and conditions	Weekly composite air and rainfall samples were collected during the growing season (March through September) in 1995 and 2007. The sampling sites were located in the Delta area of the lower Mississippi River watershed in west-central Mississippi. The 1995 sampling site was located at the center of a catfish farm pond complex near the town of Rolling Fork, Mississippi, and was approximately 500m from the nearest agricultural fields. This site was selected to minimize the influence of pesticide applications on nearby cotton, corn, alfalfa, and soybean fields. The 2007 sampling site was located near Pace, Mississippi, approximately 100 km north of the 1995 site and within approximately 3 m of a soybean field in an area surrounded by both soybean and rice fields. The analytical methods used in 1995 and 2007 were very similar overall, but there were a few differences, primarily in the number of target analytes and in the applied reporting levels.
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Martin et al. (2011)

Title: Seasonal Changes of Macroinvertebrate Communities in a Stormwater Wetland Collecting Pesticide Runoff From a Vineyard Catchment (Alsace, France)	
Author: Sylvain Martin, Aurélie Bertaux, Florence Le Ber, Elodie Maillard, Gwenael Imfeld	
Reference: Arch Environ Contam Toxicol (2012) 62:29–41	
Year: 2011	
Results and conclusion: Agricultural land use may influence macroinvertebrate communities by way of pesticide contamination associated with agricultural runoff. However, information about the relation between runoff-related pesticides and communities of benthic macroinvertebrates in stormwater wetland that receive agricultural runoff does not currently exist. Here we show changes in macroinvertebrates communities of a stormwater wetland that collects pesticide contaminated runoff from a vineyard catchment. Sixteen runoff-associated pesticides, including the insecticide flufenoxuron, were continuously quantified at the inlet of the stormwater wetland from April to September (period of pesticide application). In parallel, benthic macroinvertebrate communities, pesticide concentrations, and physicochemical parameters in the wetland were assessed twice a month. Twenty-eight contaminated runoffs ranging from 1.1 to 114 m^3 entered the wetland during the study period. Flufenoxuron concentrations in runoff-suspended solids ranged from 1.5 to 18.5 lg/kg and reached 6 lg/kg in the wetland sediments. However, flufenoxuron could not be detected in water. The density, diversity, and abundance of macroinvertebrates largely varied over time. Redundancy and formal concept analyses showed that concentrations of flufenoxuron, vegetation cover, and flow conditions significantly determine the community structures of stormwater wetland macroinvertebrates. This study shows that flow conditions, vegetation cover, and runoff-related pesticides jointly affect communities of benthic macroinvertebrates in stormwater wetlands.	
Proposed action: Not to be considered for listing in the summarizing table as pesticide concentrations are presented in graphs only.	
Type of information (critical, high/low weight, supporting, additional):	
Low weight, additional information	
Reliability	Medium
Endpoint	Pesticide inputs into stormwater wetlands
Protocol	Pesticide analysis was performed according to the NF XPT 90–210 French standards at the Pasteur Institute of Lille (France), which is a service of pesticide residues analysis accredited by the French National Accreditation Authority (COFRAC).

Test compound	Azoxystrobin, cymoxanil, cyprodinil, dimethomorph, diuron, gluphosinate, glyphosate (CAS-no.: 1071-83-6), isoxaben, kresoxim methyl, metalaxyl, simazine, terbuthylazine, tetraconazole, 3,4-dichlorophenyl urea (DCPU), DCPMU, 3,4-dichloroaniline and AMPA (CAS-no.: 1066-51-9).
Test system and conditions	The stormwater wetland has a surface area of 319 m^2 and a total volume of 1500 m^3 and was constructed in 2002 to control flood in the downstream urban area. Water samples for hydrochemical and pesticide analyses were collected at the inlet and in the sediment-deposition zone of the wetland from April 6 through September 29, 2009. Runoff discharges were continuously monitored by water-depth measurements using bubbler flow modules (Hydrologic; Sainte-Foy, Que´bec, Canada) combined to a Venturi channel. Water samples were collected every 6 m^3 at the inlet of the wetland using a 6712FR ISCO Teledyne automatic sampler (ISCO, Lincoln, NE, USA). Water samples (100 mL) were collected in jars and were stored in the dark at 4°C. The water samples systematically were collected after each runoff event and placed on ice during transportation to the laboratory.
Statistical design	Pesticide loads at the inlet of the wetland that correspond to a single runoff event were obtained by multiplying mean pesticide concentrations by the corresponding runoff volume. Loads at the inlet of the wetland were calculated from the integral sum of all event loads between two sampling campaigns.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Messing et al. (2011)

Title: Predicting wetland contamination from atmospheric deposition measurements of pesticides in the Canadian Prairie Pothole region
Author: Paul G. Messing, Annemieke Farenhorst, Don T. Waite, D.A. Ross McQueen, James F. Sproull, David A. Humphries, Laura L. Thompson
Reference: Atmospheric Environment 45: 7227-7234
Year: 2011
Results and conclusion: This is the first field study to compare the masses of pesticides entering wetlands by atmospheric deposition with those concentrations of pesticides detected in the water-column of prairie wetlands. Weekly air and bulk deposition samples were collected from May 26th to Sept. 15th, 2008 at the Manitoba Zero Tillage Research Association (MZTRA) Farm, Brandon, Manitoba, with four on-site wetlands (approximate sizes 0.15-0.45 ha) monitored every second week. Twelve pesticides were detected in the air, with MCPA (one of the three pesticides applied on the farm in 2008 in addition to clopyralid and glyphosate), trial late, and g-HCH being detected every week. Calculations were performed to predict wetland pesticide concentrations based on bulk deposits alone for those pesticides that had detectable concentrations in the bulk deposition samples (in order of the highest total seasonal deposition mass to the lowest): MCPA, glyphosate, 2,4-D, clopyralid, bromoxynil, atrazine, dicamba, metolachlor, and mecoprop. The estimated concentrations were closest to actual concentrations for MCPA (Pearson correlation coefficient's ¼ 0.91 to 0.98; p-values < 0.001) and predictions were also reasonable for a range of other herbicides, but a source other than atmospheric deposition was clearly relevant to detections of clopyralid in the wetland water-column. Although the types and levels of pesticides detected in the wetlands of the current study suggest that regional pesticide applications can contribute to pesticide surface water contamination following atmospheric transport and deposition, the greater frequency and concentrations of clopyralid, MCPA, and glyphosate detections in wetlands confirm that on-farm pesticide applications have a greater impact on on-site water quality. Beneficial management practices that reduce application drift, as well as rainfall or snowmelt runoff, will be important measures in reducing pesticide loading into wetlands situated in agricultural fields of the Prairie Pothole Region of North America.

Proposed action: Not to be considered for listing in the summarizing table as raw data are insufficient.	
Type of information (critical, high/low weight, supporting, additional): Medium weight; additional information	
Reliability	High
Endpoint	Maximum weekly averaged atmospheric concentrations of herbicides; maximum weekly bulk (wet + dry) deposition; maximum wetland grab sample concentrations.
Protocol	QA/QC protocols were followed including using field blanks, surrogate solutions as a check on extraction efficiency, matrix spikes, and blanks.
Test compound	Glyphosate (CAS-no.: 1071-83-6), MCPA, glufosinate, Bromoxynil, 2,4-D, Ethalfluralin, Trifluralin, Dicamba, Clopyralid, Atrazine, Triallate, Mecoprop, Metolachlor, AMPA (CAS-no.: 1066-51-9), a-HCH, g-HCH.
Test system and conditions	Air was sampled continuously; water samples were taken (grab samples) from the four wetlands every second week for a total of 9 sampling events.
Statistical design	No information
Relevance	
Environmental relevance	Given in supplementary material at doi:doi:10.1016/j.atmosenv.2011.08.074.
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Queiroz et al. (2011)

Title: GLYPHOSATE TRANSPORT IN RUNOFF AND LEACHING WATERS IN AGRICULTURAL SOIL	
Author: Gabriela Marina Pompeo Queiroz, Marcos Rivail da Silva, Renata Joaquim Ferraz Bianco, Adilson Pinheiro, Vander Kaufmann	
Reference: Quim. Nova, Vol. 34, No. 2, 190-195	
Year: 2011	
Results and conclusion: Glyphosate was determined in runoff and leaching waters in agricultural soil that received an application of active ingredient and was exposed to simulated intensive rain conditions. The concentrations decreased during the simulation period and the concentrations of the runoff were higher than those achieved in the samples of leaching waters. The concentrations were lower than the pattern in the Brazilian Regulation MS N. 518/2004 for drinking water. The transported load of the applied active ingredient by the leaching was of 15.4 % (w/w) and for the runoff was of 1.7 % (w/w).	
Proposed action: Not to be considered as the data are obtained from a site outside the EU (Brasil).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additonal information on Glyphosate concentrations in runoff and leachate waters in Brasil.	
Reliability	Low
Endpoint	Concentrations in leaching and runoff waters after application of Glyphosate
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6; purity 99.7 %)
Test system and conditions	Outdoor trials, 1m^2 plots, lysimeters
Statistical design	? (english summary only, text Spanish)
Relevance	
Environmental relevance	? (english summary only, text Spanish)

Weight of evidence	
"Positive"/"Negative" evidence	? (english summary only, text Spanish)

Ramwell, C.T. et al. (2014)

Title: Contribution of household herbicide usage to glyphosate and its degradate aminomethylphosphonic acid in surface water drains

Author: C.T. Ramwell, M. Kah, P. D. Johnson

Reference: Pest Manag Sci (2014), Published online in Wiley Online Library: (wileyonlinelibrary.com) DOI 10.1002/ps.3724

Year: 2014

Results and conclusion:
The aim of this study was to quantify the widely used herbicide glyphosate and its degradation product aminomethylphosphonic acid (AMPA) in surface water drains (storm drains) that could be attributed to amateur, non-professional usage alone.
Results: Maximum glyphosate and AMPA concentrations in surface water drains were 8.99 and 1.15 μL^{-1} respectively after the first rain event following the main application period, but concentrations rapidly declined to < 1.5 and < 0.5 μL^{-1}. The AMPA: glyphosate ratio was typically 0.35. Less than 1 % of the applied glyphosate was recovered in drain water.
In detail:
Of 148 houses in the catchment, 82 separate households were interviewed and, of these, 34 agreed to participate in the study. The study area was typical of a middle-class housing estate in the United Kingdom.
The majority of applications occurred within the first 2 weeks of the study, with a notable 53 g of glyphosate being applied on a single day. More than half of this application could be attributed to a single person who applied 5 L (and therefore 36 g of Roundup) over a period of 2 days primarily to an area of ~10m^2 that had a high weed infestation rate of >50 % for weeds that were ~ 10 cm high. It clearly states on the label that 5 L of Roundup can treat an area of 150m^2; thus, even when accounting for errors in the estimation of the area, over application was considerable. A total of 76.5 g of glyphosate was applied within the catchment during a period of approximately 1 month.
The first rain event after the main application period occurred on 3 July 2009 (2 weeks after the first recorded application), and three further events were monitored. The highest concentrations of glyphosate (8.99 μgL^{-1}) and AMPA (1.15 µg/L) occurred during this first rain event, although the concentrations rapidly declined within the first hour to <2 µg/L, with the final sample taken containing <1 µg/L. A short rain event on the following day (4 July 2009) generated further samples (after a further 0.79 g of glyphosate had been applied in the catchment), with peak concentrations of 2.08 µg/L of glyphosate and 0.66 µg/L of AMPA. Glyphosate concentrations in the last monitored rain event were <1 µg/L, in spite of more than 4 g of glyphosate being applied in the intervening dry period between sampling events. AMPA concentrations ranged from 0.17 to 0.54 µg/L in this last event.
These concentrations are the same order of magnitude as the initial 'background' samples. It should be noted that the glyphosate and AMPA concentrations reported here are those measured in the surface water drains, where there is relatively low discharge and therefore low dilution, and they are not representative of concentrations in surface water, where it would be expected that significant dilution would occur.
Although over 71 g of glyphosate was applied prior to the first monitored post-application rain event, less than 0.5 % of this glyphosate was detected in surface water drain flow, even when accounting for both the glyphosate +AMPA. Samples collected on the next day, the second rain event after application, added very little glyphosate and AMPA to the total loss, such that the accumulated loss as a percentage of amount applied was still <0.5 %.
Between 0.56 and 0.81 % (for the measured and extrapolated data respectively) of the applied glyphosate had been recovered in drain flow by the end of the sampling period. These findings highlight that only a very small percentage of the applied glyphosate is recovered in surface water drains, and it is assumed that the majority of the applied glyphosate is retained in the catchment and/or degraded. Glyphosate has previously been detected in the subsoil beneath and in the sand/soil in-between bricks after application, 15 confirming infiltration as a retention mechanism of glyphosate for bricked surfaces. The presence of glyphosate in the background sample indicated that more glyphosate was likely to have been applied than was accounted for, which means that the quantity of glyphosate recovered as a percentage of that applied (up to 0.81 %) would be lower in reality. Extrapolating the known usage from the households surveyed (76.5 g glyphosate used by 34 out of 82 households) to the total number of households in the catchment (n=148) would give a total of 138 g of glyphosate applied. The quantity of glyphosate detected in the drains would then equate to 0.31 or 0.45 % of the amount

applied using the measured and extrapolated sampling data respectively.
Conclusion: Glyphosate and AMPA losses from urban areas that arise solely from amateur usage have been quantified. In spite of overdosing occurring, glyphosate concentrations in drain flow were lower than concentrations reported elsewhere from professional use in urban areas.

Proposed action:
Consider as additional information.

Type of information (critical, high/low weight, supporting, additional):
Additional information due to the fact that the representativeness of the study for other EU Member States conditions is called into question.

Reliability

Endpoint	Glyphosate and AMPA concentrations in surface water drains, % glyphosate recovered in drain flow, AMPA:glyphosate ratio
Protocol	For details see under test system and conditions, non-GLP
Test compound	Plant protection products containing glyphosate (e.g. Fast Action Roundup Ready-To-Use weedkiller and Pathclear)
Test system and conditions	**Study site** A small, residential catchment (5.16 ha) where the houses had separate foul sewers and surface water drains was identified in York, England. There were 148 houses, detached or semi-detached, in the catchment. All the houses had front and back gardens which were, typically, very well maintained and neat. The surface water drains fed into a single collection point, enabling monitoring of the entire catchment. The areas of different land use ('soft' and 'hard' surfaces) were measured from aerial maps and groundtruthing. These were compared with existing data to ascertain the representativeness of the catchment. **Water sampling** Two ISCO 6172 automatic water samplers were installed to sample water (120 mL) from the final drain every 5min, with the water from three consecutive samples being directed to a single bottle, giving one composite sample (360 mL) every 15 min. One sampler was triggered when rainfall exceeded 0.4 mm within 2 h; the other was triggered when the water level in the drain was >0.01 m. This approach was taken to minimise missing a sampling event because of equipment failure. Rainfall was monitored using a tipping-bucket rain gauge (resolution 0.1 mm) sited on top of one of the boxes used to house the water samplers. Discharge was measured using an ISCO 750 area/velocity flow module. The study was undertaken in early summer (June–July 2009) when herbicide applications in private gardens are common in response to the favourable weather conditions for weed growth. Samples were taken during the first rain event (15 June 2009) after the equipment was installed (22 May 2009) and prior to the survey of the residents in order to monitor any 'background' levels of glyphosate. After that, samples were collected in response to all rain events until the end of July 2009. Samples were collected within 24 h. Samples were decanted from the glass collection bottles into high-density polyethylene (HDPE) bottles on return to the laboratory and stored in the freezer until dispatched for analysis. **Glyphosate usage in the catchment** The inputs of glyphosate into the catchment were established by means of a questionnaire. All houses in the catchment were approached by door-to-door visits over a period of 5 days during the day, in the evening and at the weekends. Houses were approached 4 times before they were excluded from the study owing to lack of contact. The aim of the study was explained to the participants in more detail, and they were asked questions regarding the types of pesticide used, timing and frequency of use and application method. The participants were requested to keep a note of pesticide usage on a pro forma, recording details of when and where a product was used, the nature of the surface type and the level of weed infestation

	as defined by a picture card with examples of weed infestation classes. No other instructions were given. When the pro formas were collected, participants were then questioned with regard to storage, disposal, safety precautions and the ease of use of the products. Fast Action Roundup Ready-To-Use (RTU) weedkiller (glyphosate 7.2 g/L MAPP 14481) in either a 1 L trigger sprayer or a 5 L 'pump and spray' container was supplied to those participants who requested it, or participants used products that they already had (n=2; Tesco's own-brand glyphosate and Path-clear – containing glyphosate, oxadiazon + diflufenican). The 1 L bottles were weighed before and after use in order to quantify the amount used. This was not possible with the 5 L RTUs as these were too heavy for field-portable scales. For the 5 L containers and where other products were used, the glyphosate usage was calculated from knowledge of the weed density, the size of the area treated and the intended rate of application (i.e. 33 mL treats 1 square metre). It was necessary to estimate the amounts applied for 39 % of the residents. Similarly, only the total quantity of glyphosate used per household was known, so the amount used per application was calculated from knowledge of the weed density and area treated, as indicated on their pro forma, in order to distribute the total amount of glyphosate spray solution used between each application date. The local authority was contacted in order to ascertain the nature of weed control on the roads within the catchment, which was ordinarily a subcontractor using glyphosate. The subcontractors postponed any treatment until the study was completed. Data analysis Measurements of concentration and discharge were used to calculate the total mass of glyphosate leaving the catchment. Discharge measurements were collected every minute, whereas bulk drain water samples were collected every 15 min. It was therefore necessary to extrapolate the chemical data. It was assumed that there was a linear increase or decrease in concentration between successive samples, enabling a concentration per minute to be estimated. In addition, two total masses per rainfall event were calculated. The first was the total load between the first and last measured concentration. However, as this was not always the very first or very last sample generated, because some samples had insufficient volume for analysis, a second calculation was made where a concentration of zero was assumed as soon as the water sampler was triggered, and concentrations up to the first analysed sample were calculated by linear extrapolation as described above. Similarly, towards the end of the sampling event, a concentration of zero was assumed when the discharge became constant. It is recognised that this approach has limitations as it is probable that, while glyphosate concentrations are likely to decrease, they will not be zero. This second extrapolated calculation of total mass is to account for the absence of samples at either end of the event and to avoid missing high discharges, with a potentially substantial influence on load, at the start of an event. These total masses are referred to as 'measured' and 'extrapolated' respectively. The final total glyphosate loss per event was calculated from the sum of the loads for glyphosate + AMPA, where the final mass of AMPA was calculated from initial mass of AMPA × (molecular weight of glyphosate/molecular weight of AMPA). **Calculation of predicted stream concentrations from measured loads** The volume of water (L) in a stream available for dilution during a rain event is calculated from [Root square of catchment area (m) × water depth × (0.3 m) × 1000] + volume of run-off (L). This formula was used to estimate a glyphosate concentration in an urban stream by dividing the measured loads by the calculated stream volume (full details in HardSPEC model).
Statistical design	See under test system and conditions

Relevance	
Environmental relevance	Given.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Ruel et al. (2010)

Title: On-site evaluation of the efficiency of conventional and advanced secondary processes for the removal of 60 organic micropollutants	
Author: S. Martin Ruel, M. Esperanza, J.-M. Choubert, I. Valor, H. Budzinski and M. Coquery	
Reference: Water Science & Technology, 2.12/2010	
Year: 2010	
Results and conclusion: The next challenge of wastewater treatment is to reliably remove micropollutants at the microgram per litre range in order to reduce the discharge for priority substances and to meet the environmental quality standards set by the European Water Framework Directive. The present work assessed the occurrence of 60 organic substances (priority substances and other relevant pollutants) in municipal wastewater and sludge. Their fate in the treatment processes and their removal efficiencies were quantified. Thorough on-site mass balances were carried out at 8 municipal wastewater treatment plants chosen among conventional and advanced secondary processes. It was found that 70 % of the substances were quantified in raw wastewater and 50 % in effluent, with a transfer without a limited degradation for most of them. Low loaded activated sludge (AS) process reduced the emission of more than half of micropollutants. At low sludge retention time (AS under high load), lower removal efficiencies were measured compared to low loaded AS. No influence of temperature of the biological reactor was shown. The membrane bioreactor process increased the removal efficiencies for one third of the substances that were partially removed with AS. Still, five substances were measured at concentrations exceeding the environmental quality standards at the outlet of the studied plants. In addition to efforts for source-reduction, complementary treatments need to be set-up. Glyphosate was quantified in more than 70 % of the samples. Three substances with concentration higher than 0.1 mg/L in raw wastewater were removed to less than 30 % (glyphosate, AMPA, diuron), thus passing through the process almost unaffected.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information	
Reliability	Medium
Endpoint	No endpoint was determined
Protocol	Non-GLP
Test compound	Monitoring
Test system and conditions	Eight WWTP were studied, representative of various sizes, types of sewer networks (rural vs. urban, combined vs. separate) and types of treatment processes. They included 7 activated sludge lines with different operating conditions (F/M ratio, sludge retention time, temperature) and 1 membrane bioreactor (MBR) process (Ultrafor) equipped with 4 Zenon ZW500d modules (hollow-fiber) for a total membrane surface of 10,000m^2. Daily average composite samples were collected on the influent (inlet), effluent (outlet), waste sludge and return of sludge dewatering during 2 or 3 successive 24 h period and under dry weather flow conditions.

Statistical design	Mass balances were performed to determine the fluxes of micropollutants at the inlet and outlet of the WWTP to calculate their removal efficiencies. Calculation is complex due to the variability of concentrations in raw wastewater, to the different WWTP that are considered and to analytical uncertainties. In order to obtain robust data, the following rules were elaborated: Two confidence levels (high/low) were defined for each substance and each type of matrix (raw wastewater, treated water and sludge) with respect to the LoQ. Low confidence level was for concentrations between LoQ and 5–10 times the LoQ. High confidence level was for concentrations higher than 5–10 times the LoQ. From analytical practice, at low confidence level a 50–100 % analytical uncertainty is a regular value for most substances whereas an analytical uncertainty below 30 % is usual for high confidence level; When both inlet and outlet concentrations were included within the low level, or if they were lower than the LoQ, the removal efficiency value was not calculated to address the fact that the analyticaluncertainty gets higher for values close to the limit of quantification; When a concentration among inlet or outlet concentrations was lower than the LoQ, a value equal to half of the LoQ was adopted and the removal efficiency was calculated. The quantification frequency (QF) of each substance, defined as number of times that a substance is quantified divided by the number of samples, was calculated for raw wastewater, treated effluent and treated sludge.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence. The results are supported by other publications.

Ruel et al. (2011)

Title: On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications
Author: S. Martin Ruel, J. M. Choubert, M. Esperanza, C. Miège, P. Navalón Madrigal, H. Budzinski, K. Le Ménach, V. Lazarova and M. Coquery
Reference: Water Science & Technology, 63.11/2011
Year: 2011
Results and conclusion: The next challenge of wastewater treatment is to reliably remove micro-pollutants at the microgram per litre range in order to meet reuse applications and contribute to reach the good status of the water bodies. A hundred priority and relevant emerging substances were measured to evaluate at full-scale the removal efficiencies of seven advanced treatment lines (one membrane bioreactor process and six tertiary treatment lines) that were designed for reuse applications. To reliably compare the processes, specific procedures for micro-pollutants were applied for sampling, analysis and calculation of removal efficiencies. The membrane bioreactor process allowed to upgrade the removal efficiencies of about 20 % of the substances measured, especially those that were partially degraded during conventional processes. Conventional tertiary processes like high rate clarification, sand filtration and polishing pond achieved significant removal for some micro-pollutants, especially for adsorbable substances. Advanced tertiary processes, like ozonation, activated carbon and reverse osmosis were all very efficient to complete the removal of polar pesticides and pharmaceuticals; metals and less polar substances were better retained by reverse osmosis.
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.

Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information	
Reliability	Medium
Endpoint	No endpoint was determined
Protocol	Non-GLP
Test compound	Monitoring
Test system and conditions	Seven WWTP of various sizes were studied, which included various types of treatment: one full-scale MBR; five full-scale conventional tertiary treatments, including high rate clarification, sand filtration or polishing pond; two advanced tertiary treatments at full-scale (ozonation and micro-filtration (MF) þ reverse osmosis (RO)) and two advanced tertiary treatments at pilot-scale (activated carbon filtration and silex filtration þ ultrafiltration þ RO). The upstream treatment stages achieved both carbon and nitrogen removal to meet regulatory requirements. Influent and effluents of the studied processes were collected under dry weather flow conditions during two successive 24 h or 2 h periods
Statistical design	Mass balances were performed based on wastewater flow and micro-pollutant concentration data at the inlet and at the outlet of the studied processes. The removal efficiencies (R) were calculated with the following rules to obtain robust information: High and low levels of concentration were defined for each substance with respect to the LoQ. Low confidence level was for concentrations between LoQ and 2.5–5 times the LoQ (depending on the substance). High confidence level was for concentrations higher than 2.5–5 times the LoQ, depending on the substance. From analytical practice, at low confidence level, an analytical uncertainty in the range of 50–100 % is a regular value for most substances whereas an analytical uncertainty below 30 % is usual a high confidence level. When both inlet and outlet concentrations were lower than the LoQ or within the low level, the removal efficiency value was not calculated. When only one concentration, either inlet or outlet concentration, was lower than the LoQ, a value equal to half of the LoQ was adopted and the removal efficiency was calculated. In addition to these criteria, removal efficiency data was displayed as a removal range (<30 %, 30–70 % and >70 %), since the analytical uncertainty and the variability of the concentrations related to micro-pollutants in wastewater do not allow to certify precise values.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence. The results are supported by other publications.

Ruel et al. (2012)

Title: Occurrence and fate of relevant substances in wastewater treatment plants regarding Water Framework Directive and future legislations
Author: S. Martin Ruel, J.-M. Choubert, H. Budzinski, C. Miège, M. Esperanza and M. Coquery
Reference: Water Science & Technology, 65.7/2012
Year: 2012

Results and conclusion:
The next challenge of wastewater treatment is to reliably remove micropollutants at the microgram per litre range. During the present work more than 100 substances were analysed through on-site mass balances over 19 municipal wastewater treatment lines. The most relevant substances according to their occurrence in raw wastewater, in treated wastewater and in sludge were identified, and their fate in wastewater treatment processes was assessed. About half of priority substances of WFD were found at concentrations higher than 0.1 µg/L in wastewater. For 26 substances, potential non-compliance with Environmental Quality Standard of Water Framework Directive has been identified in treated wastewater, depending on river flow. Main concerns are for Cd, DEHP, diuron, alkylphenols, and chloroform. Emerging substances of particular concern are by-products, organic chemicals (e.g. triclosan, benzothiazole) and pharmaceuticals (e.g. ketoprofen, diclofenac, sulfamethoxazole, carbamazepine). About 80 % of the load of micropollutants was removed by conventional activated sludge plants, but about two-thirds of removed substances were mainly transferred to sludge.

Proposed action:
To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.

Type of information (critical, high/low weight, supporting, additional):
Medium weight, additional information

Reliability	Medium
Endpoint	No endpoint was determined
Protocol	Non-GLP
Test compound	Monitoring
Test system and conditions	Overall, 19 WWTP treatment lines were studied, chosen as representative of various sizes (100 to 1,000,000 PE and of various types of treatment processes. Sampling was performed in the influent and effluent during two or three successive 24 h-periods under dry weather flow conditions, with refrigerated samplers equipped with Teflon pipes and glass containers. Grab samples were collected for treated sludge. Strict procedures of cleaning, sampling, and field blanks were carried out.
Statistical design	The results were described using: The frequency of quantification (Fq) and total concentration in influents, effluents and sludges. The specific daily average load received at WWTP (g/d/ PE), calculated for each substance. The removal rate for different processes, with some calculation rules to take into account the variability of concentrations in raw wastewater and the analytical uncertainties associated with low concentrations of substances in complex matrices. If inlet concentration was not higher than 10 times the limit of quantification, removal efficiency was not calculated. Additionally, results were displayed as a removal efficiency range: 0–30 %, 30–70 % or 70–100 %.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence. The results are supported by other publications.

Screpanti et al. (2005)

Title: Glyphosate and glufosinate-ammonium runoff from a corn-growing area in Italy	
Author: Claudio Screpanti, Cesare Accinelli, Alberto Vicari, Pietro Catizone	
Reference: Agron. Sustain. Dev. 25, 407-412	
Year: 2005	
Results and conclusion: The main objective of this experiment was to estimate field-scale runoff losses of glyphosate and glufosinate-ammonium under natural rainfall conditions. Glyphosate and glufosinate-ammonium were applied as pre-emergence herbicides on 350 m^2 field plots characterized by a uniform slope of 15 %. Field plots were cultivated with corn. The persistence and sorption isotherms of the two herbicides were also determined. During the 3-year experimental period low runoff volumes were observed. More specifically, annual runoff volumes did not exceed 4.7 mm. Glyphosate and glufosinate-ammonium concentrations in collected runoff samples rapidly declined with time. The highest glyphosate and glufosinate-ammonium concentrations were 16 and 24 µg/L, respectively. These peaks were observed in a runoff event occurring 1 day after herbicide treatment. The total maximum amounts of glyphosate and glufosinate-ammonium losses were 0.031 and 0.064 ‰ of the applied active ingredients, respectively. On the basis of the obtained results, both glyphosate and glufosinate-ammonium showed low potential to contaminate surface water resources. These results were supported by their estimated short persistence and strong sorption in soil. The half-lives of glyphosate and glufosinate-ammonium were 17.5 and 6.4 days, respectively, and their distribution coefficients (K_d) were 746.6 and 23.4 mL/g, respectively.	
Proposed action: Not to be considered in the summarizing table and discussion as results are shown in a figure only without listing exact data on concentrations, i.e. results are not sufficiently precisely reported.	
Type of information (critical, high/low weight, supporting, additional): Medium weight; additional information of degradation and sorption isotherms	
Reliability	Low
Endpoint	Outflow coefficient, herbicide half-life; distribution coefficient K_d, r^2
Protocol	Non-GLP; similar to OECD 106
Test compound	Roundup Bioflow (isopropylamine salt of glyphosate, 360 g a.i./L formulation; CAS-no.: 40465-66-5) Basta (ammonium salt of glufosinate-ammonium, 120 g a.i./L formulation)
Test system and conditions	1) Field plot management: 8 plots were cultivated with corn. Glyphosate and glufosinate-ammonium were applied shortly after seeding. 2) Laboratory investigations: a) Herbicide persistence: 5 g of soil were treated with glyphosate or glufosinate-ammonium (10 µg a.i./g soil) and were incubated at 25±0.5°C for 21 days. Triplicate samples were removed at 0, 1, 7, 14 and 21 days. b) Sorption isotherms: Sorption isotherms were determined using triplicate samples at 5 initial herbicide concentrations (0.05 to 10 µg/mL). Samples were shaken for 12 hours.
Statistical design	3-4 replicates, first-order kinetics, regression analyses (Statitica ver. 6.1); analysis of variance
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence; results supported by other publications

Shipitalo et al. (2008)

Title: Impact of Glyphosate-Tolerant Soybean and Glufosinate-Tolerant Corn Production on Herbicide Losses in Surface Runoff
Author: Martin J. Shipitalo, Robert W. Malone, and Lloyd B. Owens
Reference: Journal of Environmental Quality 37:401–408
Year: 2008
Results and conclusion: Residual herbicides used in the production of soybean and corn are often detected in surface runoff at concentrations exceeding their maximum contaminant levels (MCL) or health advisory levels (HAL). With the advent of transgenic, glyphosate-tolerant soybean and glufosinate-tolerant corn this concern might be reduced by replacing some of the residual herbicides with short half-life, strongly sorbed, contact herbicides. We applied both herbicide types to two chiseled and two no-till watersheds in a 2-yr corn-soybean rotation and at half rates to three disked watersheds in a 3-yr corn/soybean/wheat-red clover rotation and monitored herbicide losses in runoff water for four crop years. In soybean years, average glyphosate loss (0.07 %) was ~1/7 that of metribuzin (0.48 %) and about one-half that of alachlor (0.12 %), residual herbicides it can replace. Maximum, annual, flow-weighted concentration of glyphosate (9.2 µg/L) was well below its 700 µg/L MCL and metribuzin (9.5 µg/L) was well below its 200 µg/L HAL, whereas alachlor (44.5 µg/L) was well above its 2 µg/L MCL. In corn years, average glufosinate loss (0.10 %) was similar to losses of alachlor (0.07 %) and linuron (0.15 %), but about one-fourth that of atrazine (0.37 %). Maximum, annual, flow-weighted concentration of glufosinate (no MCL) was 3.5 µg/L, whereas atrazine (31.5 µg/L) and alachlor (9.8 µg/L) substantially exceeded their MCLs of 3 and 2 µg/L, respectively. Regardless of tillage system, flow-weighted atrazine and alachlor concentrations exceeded their MCLs in at least one crop year. The glyphosate and glufosinate concentrations never exceeded their established or proposed standards either on an annual flow-weighted basis or for an individual event, even when runoff occurred within 1 d after application. Thus, by growing transgenic, glyphosate-tolerant soybean and glufosinate-tolerant corn and by completely or partially replacing these residual herbicides with glyphosate or glufosinate the environmental impact of herbicide losses in runoff resulting from production of these crops should be reduced.
Proposed action: Not to be considered as study deals with a site outside the EU (USA).
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information: that by growing transgenic, glyphosate-tolerant soybean and glufosinate-tolerant corn and by completely or partially replacing these residual herbicides with glyphosate or glufosinate the environmental impact of herbicide losses in runoff resulting from production of these crops should be reduced.

Reliability	Medium
Endpoint	Flow-weighted average concentrations for each runoff event were computed using the concentrations measured in individual samples and runoff volumes obtained from the hydrographs. These values were then used to determine annual flow weighted concentrations.
Protocol	Non-GLP
Test compound	Alachlor, Atrazine, Atrazine, DEA, and DIA, Linuron, Metribuzin, Glyphosate (CAS-no.: 1071-83-6), AMPA (1066-51-9), Glufosinate
Test system and conditions	The transport of contact and residual herbicides was investigated for 4 crop years using seven small watersheds that were instrumented to automatically measure and sample surface runoff. Four watersheds were cropped in a 2-yr corn–soybean rotation, two receiving no tillage and the other two were chisel plowed each year. The three remaining watersheds were farmed in a 3-yr, corn/soybean/wheat-clover rotation with disking as the primary tillage operation during corn and soybean years. During runoff the samplers collected discrete samples (~300 mL) every 10 min for the first 100 min, every 20 min for the next 200 min, and every 60 min thereafter until the capacity of the samplers was reached or runoff ceased.
Statistical design	-
Relevance	
Environmental relevance	Environmental parameter partly reported and partly reported in other studies

Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence

Shipitalo and Owens (2011)

Title: Comparative Losses of Glyphosate and Selected Residual Herbicides in Surface Runoff from Conservation-tilled Watersheds Planted with Corn or Soybean
Author: Martin J. Shipitalo and Lloyd B. Owens
Reference: J. Environ. Qual. 40:1281–1289
Year: 2011
Results and conclusion: Residual herbicides regularly used in conjunction with conservation tillage to produce corn and soybean are often detected in surface water at concentrations that exceed their U.S. maximum contaminant levels (MCL) and ecological standards. These risks might be reduced by planting glyphosate-tolerant varieties of these crops and totally or partially replacing the residual herbicides alachlor, atrazine, linuron, and metribuzin with glyphosate, a contact herbicide that has a short half-life and is strongly sorbed to soil. Therefore, we applied both herbicide types at typical rates and times to two chisel-plowed and two no-till watersheds in a 2-yr corn/ soybean rotation and at half rates to three disked watersheds in a 3-yr corn/soybean/wheat–red clover rotation and monitored herbicide losses in surface run-off for three crop years. Average dissolved glyphosate loss for all tillage practices, as a percentage of the amount applied, was significantly less ($P \leq 0.05$) than the losses of atrazine (21.4 x), alachlor (3.5 x), and linuron (8.7 x) in corn-crop years. Annual, flow-weighted, concentration of atrazine was as high as 41.3 µg/L, much greater than its 3 µg/L MCL. Likewise, annual, flow weighted alachlor concentration (MCL = 2 µg/L) was as high as 11.2 and 4.9 µg/L in corn- and soybean-crop years, respectively. In only one runoff event during the 18 watershed-years it was applied did glyphosate concentration exceed its 700 µg/L MCL and the highest, annual, flow-weighted concentration was 3.9 µg/L. Planting glyphosate-tolerant corn and soybean and using glyphosate in lieu of some residual herbicides should reduce the impact of the production of these crops on surface water quality.
Proposed action: Not to be considered as study deals with a site outside the EU (USA).
Type of information (critical, high/low weight, supporting, additional): Medium weight; additional information: Annual, flow-weighted glyphosate concentrations were much less than its drinking water standard in each of the 18 watershed years it was applied. It is critical to maintain a diversity of weed management practices in the face of the evolution of glyphosate-resistant weeds.

Reliability	Medium
Endpoint	Concentrations in individual samples were used to calculate flow-weighted average concentrations for each runoff event and annual flow weighted concentrations
Protocol	Non-GLP
Test compound	Alachlor, Atrazine, Atrazine, DEA, and DIA, Linuron, Metribuzin, Glyphosate (CAS-no.: 1071-83-6), AMPA (1066-51-9)
Test system and conditions	Losses of glyphosate in surface runoff were compared with losses of commonly used residual herbicides for three crop years, using seven small watersheds used to grow glyphosate-tolerant corn and soybean. Two watersheds were in 2-yr, no-till corn/soybean rotation and two watersheds were in the same rotation but were chisel plowed before planting. Cereal rye was drilled into these watersheds following soybean harvest and served as a cover crop. Three watersheds were in a 3-yr, corn/soybean/ wheat–clover rotation and were disked in the spring just before corn and soybean planting. Approximately 300-mL discrete samples for each event every 10 min for the first 100 min, every 20 min for the next 200 min, and then every 60 min until all bottles were full or runoff ended were collected.
Statistical design	1015 runoff events were sampled for the seven watersheds during the 3-yr period
Relevance	
Environmental relevance	Environmental parameter reported in other studies.

Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Starrett and Klein (2008)

Title: Glyphosate Runoff When Applied to Zoysiagrass under Golf Course Fairway Conditions	
Author: Steven K. Starrett and Jamie Klein	
Reference: Chapter 14, pp 237-253, in: The Fate of Nutrients and Pesticides in the Urban Environment, Nett MT *et al.* (eds). ACS Symposium Series, Volume 997. DOI: 10.1021/bk-2008-0997.ch014	
Year: 2008	
Results and conclusion: Glyphosate is the primary ingredient of Roundup®, which is the most widely used herbicide by volume in the United States. Glyphosate runoff is therefore an important subject for both existing and newly constructed courses. The objectives of the current research were: (1) to measure glyphosate runoff from zoysiagrass fairways on a golf course following the application of Roundup herbicide, (2) to determine glyphosate runoff concentrations and their resulting effect on the environment, and (3) to provide up-to-date data of research findings on pesticide transport when applied to turfgrass. Previous research on glyphosate runoff from turfgrass has been done on test plots and not full-scale watersheds. In addition, Roundup applications for this study were made by the Colbert Hills Golf Course staff and not prescribed specifically for research purposes. Water quality and quantity monitoring systems were set up on the 115-acre study watershed, which contains a 3-acre detention and irrigation pond. Over 600 water samples were taken from fairway drains, the inlet and outlet of the pond, and the pond itself throughout a three-year study period. Ten of the twenty-three tested samples contained detectable concentrations (>0.10 µg/L) of glyphosate. The maximum observed glyphosate concentration was 5.18 µg/L, which is well below the United States Environmental Protection Agency (USEPA) Drinking Water Maximum Contaminant Level (MCL) of 700 µg/L. These results suggest that Roundup applications made to turfgrass fairways do not cause hazardous levels of glyphosate in downstream surface water.	
Proposed action: Not to be considered. Publication deals with situation outside the EU (USA).	
Type of information (critical, high/low weight, supporting, additional): Low weight. Glyphosate concentrations found in tested runoff samples from the 115 acre study watershed, following annual applications of Roundup, were much lower than associated health standards.	
Reliability	Low
Endpoint	Concentration
Protocol	Non-GLP
Test compound	Glyphosate (CAS-no.: 1071-83-6), AMPA (1066-51-9)
Test system and conditions	The objectives of this study were: 1.) To measure glyphosate runoff from zoysiagrass fairways on a golf course following the application of Roundup herbicide. 2.) To determine glyphosate runoff concentrations and the resulting effect on the environment. 3.) To provide up-to-date data of research findings on pesticide fate and transport when applied to turfgrass.
Statistical design	211 runoff samples from the inlet, and 125 samples from the outlet; 61 samples taken from three separate fairway drains; 208 samples were taken from different locations and depths of the pond; no statistics
Relevance	
Environmental relevance	The influencing endpoints are not reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Tang., T. et al. (2014)

Title: Losses of glyphosate and AMPA via drainflow in a typical Belgian residential area	
Author: T. Tang, W. Boënne, A. v. Griensven, P. Seuntjens, J. Bronders, N. Desmet	
Reference: Geophysical Research Abstracts, Vol. 16, EGU2014-3627, 2014, EGU General Assembly 2014	
Year: 2014	
Results and conclusion: To obtain concurrent high-resolution data for a detailed investigation on the losses of pesticide runoff from hard surfaces, a monitoring campaign was performed in a typical Belgian residential area (9.5 ha) between 7 May and 7 August, 2013. The campaign yielded a concurrent dataset of rainfall (1-mm rainfall interval), discharge (1-min interval), glyphosate application by the residents and the occurrences of glyphosate and its major degradation product aminomethylphosphonic acid (AMPA) in the separated storm drainage outflow during 12 rainfall events. In addition, detailed information was obtained on the spatial characteristics of the study area. The resulting dataset allows us to investigate the relevance of catchment hydrology, urban surface properties and pesticide application to the transport and losses of glyphosate in a residential environment. During the campaign, glyphosate was only applied by local residents, mainly on their private driveways. As a result of their continuous use, both glyphosate and AMPA were detected in all analysed outflow samples, with maximum concentrations of 6.1 µg/L and 5.8 µg/L, respectively. Overall, the storm drainage system collected 0.43 % of the applied amount of glyphosate. However, this loss rate varied considerably among rainfall events, ranging from 0.04 % to 23.36 %. According to statistical analysis of the 12 rainfall events, the loss rate was significantly correlated with three factors: the application amount prior to a rainfall event ($p < 0.005$), rainfall amount during the event ($p < 0.02$) and the weighted lag time between glyphosate application and the start of the rainfall event (negatively, $p < 0.05$). A regression analysis showed that these three factors can explain more than 85 % of the variation in the loss rate of glyphosate. Furthermore, three types of glyphosate runoff were classified by a clustering analysis based on these factors: events dominated by runoff availability (runoff-limited), dominated by glyphosate availability (pesticide-limited) and controlled by both runoff and glyphosate availability. To sum up, proper management of the amount and timing of glyphosate application can greatly help to control its losses from urban impervious surfaces.	
Proposed action: Consider as additional information.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Losses of glyphosate and AMPA from hard surfaces
Protocol	No protocol
Test compound	Glyphosate
Test system and conditions	No exact description of the test system and condition is given, since this the publication by Tang *et al.* is only an abstract.
Statistical design	No information provided in the abstract
Relevance	
Environmental relevance	Not assessable, since e.g. parameters influencing the losses of glyphosate and AMPA from hard surfaces are not described in detail.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Tournebize et al. (2012)

Title: Co-Design of Constructed Wetlands to Mitigate Pesticide Pollution in a Drained Catch-Basin: A Solution to Improve Groundwater Quality	
Author: JULIEN TOURNEBIZE, CHRISTELLE GRAMAGLIA, FRANCOIS BIRMANT, SAMI BOUARFA, CEDRIC CHAUMONT AND BERNARD VINCENT	
Reference: Irrig. and Drain. 61 (Suppl. 1): 75–86 (2012)	
Year: 2012	
Results and conclusion: Numerous situations exist in France in which groundwater is imperfectly protected by a shallow impervious layer in the topsoil, meaning that sinkholes may connect the surface water and relay pollution directly to the aquifer. These impervious layers induce subsurface drainage demand and construction so that the effluent can be collected at outfalls of drainage systems, and be processed in constructed wetlands before falling down the sinkholes, thus decreasing net pollution. The Champigny aquifer corresponds to this description, and has been shown to be highly vulnerable. The mission of AQUI'Brie, a non-profit organisation, is to protect this groundwater, which is one of the water resources of Paris. Although well aware of the benefits of buffer zones, but at this time without incentives to implement them, AQUI'Brie started a co-construction process of constructed wetlands, involving all the stakeholders of a small catchment located upstream from a sinkhole. This paper describes the co-construction from the first meeting to the final structures in a context of high land use pressure. It shows that the number and area of constructed wetlands has diminished beyond the threshold for which the performance of depollution process may fail. In 2011, a performance assessment programme was set up to determine if the co-constructed wetlands comply with the new current regulations.	
Proposed action: Not to be considered for listing in the summarizing table as raw data are insufficient.	
Type of information (critical, high/low weight, supporting, additional): Medium weight, additional information.	
Reliability	High
Endpoint	Concentration at catchment outlet
Protocol	Non-GLP
Test compound	Monitoring
Test system and conditions	The experimental site is representative of the infiltration of drainage water through sinkholes down into the Champigny aquifer. The catchment is located in the city of Rampillon (0303′37.300 E, 4832′16.700 N, 70 km south-east of Paris, France) and the total drained area is 355 ha (according to drainage maps). The average annual air temperature is 10.5 °C, the annual mean rainfall is 689 mm and the annual mean potential evaporation is 679 mm. Most of the basin, covered with tableland loess up to 10m thick, is relatively flat and sub-horizontal. The soil of the catchment is mainly Luvisol. A measurement station was installed upstream from the sinkhole at the basin's outfall. Fifteenminute time-step discharge measurements were taken using a wooden controlled section adapted to natural ditch cross section (Birgand *et al.*, 2005) and a height and velocity Doppler sensor. Quality was measured using an automated water sampler set up at a 14-h constant time step. This provides both accurate monitoring during peak flows and a basic weekly restored flow-weight sample. Water samples were measured using LC-MS-MS or GC-MS methods providing a quantification threshold of 0.01 mg/L for the majority of compounds tested. Continuous monitoring was carried out from September 2007 to September 2009. In 2006–2007, 11 compounds were analysed, 6 out of the 72 compounds applied were detected. In 2007–2008, 68 compounds were applied, 49 analysed and 24 detected. Weather data were provided by a National Weather Broadcast network station, located 6 km from the experimental site.
Statistical design	Not reported

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other studies. No negative evidence.

Vialle et al. (2013)

Title: Pesticides in roof runoff: Study of a rural site and a suburban site
Author: C. Vialle, C. Sablayrolles, J. Silvestre, L. Monier, S. Jacob, M.-C. Huau, M. Montrejaud-Vignoles
Reference: Journal of Environmental Management 120 (2013) 48-54
Year: 2013
Results and conclusion: The quality of stored roof runoff in terms of pesticide pollution was assessed over a one-year period. Two tanks, located at a rural and suburban site, respectively, were sampled monthly. The two studied collection surface were respectively a tile slope roof and a bituminous flat roof. Four hundred and five compounds and metabolites were screened using liquid and gas chromatography coupled with various detection systems. Principal Component Analysis was applied to the data sets to elucidate patterns. At the rural site, two groups of compounds associated with two different types of agriculture, vineyard and crops, were distinguished. The most frequently detected compound was glyphosate (83 %) which is the most commonly used herbicide in French vineyards. At the suburban site, quantified compounds were linked to agriculture rather than urban practices. In addition, all samples were contaminated with mecoprop which is a roof-protecting agent. Its presence was attributed to the nature of roofing material used for rainwater collection. For both sites, the highest number and concentrations of compounds and metabolites were recorded at the end of spring and through summer. These results are consistent with treatment periods and higher temperatures. In detail: At the rural site, the most frequently detected compounds were glyphosate (83 %), DNOC (75 %), AMPA (58 %), metolachlor (58 %), carbendazim (50 %), and 2,4-MCPA (50 %). Analysis revealed that the highest concentrations measured were for glyphosate (6 µg/L). In addition, concentrations of several hundreds of ng/L were measured for AMPA, metolachlor, DNOC and metaldehyde in order of decreasing concentrations. As a reference, limit values in potable water are 0.1 mg/L per pesticide and 0.5 mg/L for the sum, according to French regulation.
Proposed action: To be considered in the summarizing table.
Type of information (critical, high/low weight, supporting, additional): High weight, additional information to already existing.

Reliability	
Endpoint	Pesticide concentrations (e.g. glyphosate, AMPA) measured in a roof runoff tank
Protocol	Not given
Test compound	Four hundred and five compounds (e.g. glyphosate, AMPA)

Test system and conditions	Two sites in south-western France were selected to install commercially available domestic rainwater collection systems (Sotralentz Habitat). Rainwater is first collected from the roof area and then channelled via gutters through pipes to an underground PEHD storage tank in order to be reused later. Prior to entering the tank, the water is passed through a screen rake. The first site was a private house surrounded by cultivated fields. This site was located near a rural village 40 km north-west of Toulouse. The annual average rainfall in this region is 760 mm, and the average temperatures range from 7.9 to 18.3 ºC. Agriculture in this area is characterised by the vineyards of Gaillac and crops such as wheat, maize and colza. The second site was the research building of an engineering school located in the suburban area of Toulouse, which has an urban population of around 860 000 inhabitants. This site is 12 km from the city centre. The annual average rainfall is 668 mm, with average temperature ranging from 8.6 to 18.1 ºC. The area is near a well-travelled road and 70 ha of experimental cultivation fields. Stored roof runoff sampling was carried out monthly from January 2009 to December 2009 for site 1 and between November 2009 and October 2010 for site 2. Analysis was performed by La Drôme Laboratoires. Water samples were screened for 405 compounds. Liquide-liquid extractions with a dichloromethane/ethyl acetate mix (80/20, v:v) at various pH levels were conducted for each sample. Extracts were simultaneously analysed by liquid chromatography (HPLC) and gas chromatography (GC) with systematic multidetection: with diode array detector (HPLC-DAD), coupled with tandem mass spectrometry (HPLC-MS-MS), with an electron capture detector and a nitrogen phosphorus detector (GC-ECD-NPD), or coupled with mass spectrometry (GC-MS). Other sample aliquots were analysed by HPLC after a derivation, or by headspace with GC-MS. Some compounds were quantified by direct injection and analysis by HPLC-MS-MS. Glyphosate and AMPA were analysed by FMOCCl derivation + HPLC fluorescence (LOQ = 0.100 µg/L). PCA was performed using the commercial software XL stat. A data matrix, with columns representing the different samplings (12 observations per site) and rows corresponding to the measured compounds (variables), was constructed for analysis with PCA. For standardization each variable was replaced by its value minus the average of the variable and dividing by the standard deviation of the variable. Values less than the quantification limit were considered to be half of the quantification limit, and values less than the detection limit were considered to be zero. Pearson's correlations between different compounds were first obtained. Then components were determined, and the two first components corresponding to the greatest part of the total variance of the data set were retained.
Statistical design	See "Test system and conditions"
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive" "Negative" evidence	No negative evidence.

Yang et al. (2013a)

Title: Field evaluation of a new biphasic rain garden for stormwater flow management and pollutant removal	
Author: H. Yang, W. A. Dick, E. L. McCoy, P. L. Phelan, P. S. Grewal	
Reference: Ecological Engineering 54 (2013) 22-31	
Year: 2013	
Results and conclusion: Field-scale rain gardens were constructed using a novel biphasic concept, involving water movement from a saturated to an unsaturated zone in sequence, for increasing retention time of runoff and improving bioremediation. Hydraulic performance and removal efficiencies of the biphasic rain gardens were evaluated in natural and simulated runoff events. Influent and effluent of two replicate biphasic rain gardens from natural runoff events were monitored during a 2-yr study. Three agricultural runoff events with high concentrations of nutrients (i.e. nitrate and phosphate) and the herbicide, atrazine (6-chloro-N-ethyl-N`-(1-methylethyl)-1,3,5-triazine-2,4-diamine), were undertaken during the summer of 2008. Five urban runoff events with spiked concentrations of nutrients (i.e. nitrate and phosphate) and herbicides, glyphosate (N-(phosphonomethyl)glycine), dicamba (3,6-dichloro-2-methoxybenzoic acid), and 2,4-d (dichlorophenoxyacetic acid) were applied to the rain gardens during the summer of 2009. Both peak flow and runoff volume were reduced by holding runoff in the rain gardens (mainly in the water saturated zone) until the next runoff event. The created biphasic rain gardens in our study were highly effective in removing nitrate (~91 %), phosphate (~99 %), atrazine (~90 %), dicamba (~92 %), glyphosate (~99 %), and 2,4-d (~90 %) under high levels of pollution loading with simulated runoff events. Increased retention time of runoff pollutants and water-saturated conditions, as determined by design configuration and rainfall size, intensity, and interval, were found to significantly affect overall nutrient and herbicide removal in the biphasic rain gardens.	
Proposed action: Consider as additional information.	
Type of information (critical, high/low weight, supporting, additional): Additional information	

Reliability	
Endpoint	Removal of pollutants (glyphosate amongst others) in biphasic rain gardens
Protocol	Not given
Test compound	Glyphosate (amongst other herbicides)
Test system and conditions	Biphasic rain gardens: Two replicate biphasic rain gardens were constructed in the spring of 2008 at The Ohio State University's Wooster campus. The biphasic rain garden is a new type of design that consists of a biphasic (water saturated to unsaturated) sequence and a recharge zone. In these biphasic rain gardens, runoff is first directed through the water saturated (anaerobic) zone and then through the water unsaturated (aerobic) zone. The saturated zone is created by placing an impervious liner to capture the first flush of runoff and to increase retention time for more sustained water saturated conditions. Sediments are filtered and adsorption and/or biological treatment of pollutants occurs in the saturated zone. U-shaped reverse drainage pipes have only perforated portions at the bottom. This configuration maximizes the retention time of the first flush runoff in the saturated zone and allows only the treated overflow water to exit into the unsaturated zone. Effluent rate of the treated overflow water through the reverse drainage pipes is mainly controlled by the total head difference. The unsaturated zone is designed using an under drainage configuration to further retard flow for subsequent aerobic treatment. Impervious liner is placed at the bottom of the unsaturated zone to collect and discharge the treated water through a final discharge pipe. Finally, the treated water from the unsaturated zone is discharged into the recharge zone located at the bottom of the rain garden. The recharge zone filled with pea gravel is designed to facilitate groundwater recharge. Each rain garden was sized to handle 44.7 mm (1.76 in.) of runoff from a drainage area consisting of a 99 % impervious concrete surface pad with surface area of 69.5 m^2. This value was the precipitation of a 1-h intense rainfall with 10 year

	return frequency in Wooster, Ohio estimated by the National Oceanic and Atmospheric Administration Atlas 14 Model. The target water quality volume generated from 44.7 mm rainfall was estimated to be 3.1 m^3 based on the drainage area. The required size and infiltration rate of each zone in the rain garden was designed based on expected water volumes using the rational and mass continuity methods (Davis and McCuen, 2005). The surface area of the saturated zone was 6.8 m^2. The depth was 1.2 m made up of fine gravel (3.2-12.7 mm diam.; 0.15 m deep), soil medium (0.85 m deep), and 0.2 m free board space above the rain garden surface to permit ponding of water during runoff events. The saturated zone provides a storage volume of approximately 1.58 m^3 and a holding capacity of 25.4 mm runoff from the concrete surface pad. The storage volume in the unsaturated zone was 1.34 m^3 with a depth of 0.5 m (i.e. fine gravel of 0.15 m depth and soil medium of 0.35 m depth) subsequently. The soil medium used in this study was a mixture of sand, topsoil, and compost in a 6:2:2 volume ratio obtained from Kurtz Bros. Inc., Cleveland, OH. The soil medium consists of 90.6 % sand, 6.9 % silt, and 2.5 % clay with 0.7 % organic matter, 3.1 meq/100 g of cation exchange capacity (CEC), 12.0 cm/h of saturated conductivity, and pH of 7.2. Six native plant species were planted in 0.2 m^2 (2.25-ft^2) spacing intervals. These intervals were selected based on the growing habit of the selected plants. The saturated zone included boneset (Eupatorium perfoliatum), spiderwort (Tradescantia ohiensis), and culver's root (Veronicastrum virginicum), while purple love grass (Eragrostis spectabilis), Indian grass (Sorghastrum nutans), and purple coneflower (Echinacea purpurea) were planted in the unsaturated zone. Design, construction, soil medium properties, and hydraulic characteristics of the biphasic rain gardens have been described in detail previously (Yang *et al.*, 2009). Conditions: The experiments were performed under natural runoff conditions, simulated agricultural runoff conditions and simulated urban runoff conditions. Analytic method (given only for glyphosate): Agilent 6890 gas chromatography mass spectrometry (Limit of detection were <1.0 µg/L)
Statistical design	To examine changes in pollutant removal efficiency over the simulated urban runoff events, regression analysis was performed on mean removal efficiency of each pollutant using MINITAB v.15 (Minitab, Inc., State College, PA). Mean removal efficiency of target pollutants taken at the two replicate rain gardens was considered as a responding variable and the number of runoff events as a predictor. Regression slope was considered as an indicator of increase (positive slope) or decrease (negative slope) of removal efficiency over the events. When p-value for the regression slope was below 0.05, such changes were considered significant (regression slope is significantly different from zero).
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Detailled description of open literature on surface water monitoring

Aparicio et al. (2013)

Title: Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins	
Author: Virginia C. Aparicio, Eduardo De Gerónimo, Damián Marino, Jezabel Primost, Pedro Carriquiriborde and José L. Costa	
Reference: Chemosphere 93 (2013) 1866–1873	
Year: 2013	
Results and conclusion: The aim of this work is to study the environmental fate of glyphosate and its major degradation product, aminomethylphosphonic acid (AMPA), in surface water and soil of agricultural basins. In cultivated soils, glyphosate was detected in concentrations between 35 and 1502 lg g^{-1}, while AMPA concentration ranged from 299 to 2256 µg/kg. In the surface water studied, the presence of glyphosate and AMPA was detected in about 15 % and 12 % of the samples analyzed, respectively. In suspended particulate matter, glyphosate was found in 67 % while AMPA was present in 20 % of the samples. In streams sediment glyphosate and AMPA were also detected in 66 % and 88.5 % of the samples respectively. In the present study, it was demonstrated that glyphosate and AMPA are present in soils under agricultural activity. It was also found that in stream samples the presence of glyphosate and AMPA is relatively more frequent in suspended particulate matter and sediment than in water.	
Proposed action: Not to be considered. Monitoring data for a site outside the EU (Argentina) are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, concentrations in a non-EU country measured and compared to a developed model.	
Reliability	Medium
Endpoint	Concentrations in surface water, soil and sediment
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Monitoring
Test system and conditions	Sixteen agricultural sites and forty-four streams in the agricultural basins were sampled three times during 2012. The samples were analyzed by UPLC-MS/MS ESI (+/-).
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Bachor et al. (2008)

Title: Special report on detection of pesticides and pharmaceuticals in surface water and ground water in Mecklenburg-Vorpommern (Germany) in spring 2008
Author: Alexander Bachor, Gabriele Lemke, André Schumann
Reference: Report of the State Agency for the Environment, Nature Conservation and Geology Mecklenburg-Vorpommern (LUNG)
Year: 2008
Results and conclusion: During April 1st and 25.06.2008 samples from 60 surface water (river) and 83 groundwater measuring points in Mecklenburg-Vorpommern were analysed for different pesticides and pharmaceuticals. In 40 of 180 surface water samples glyphosate was found with concentrations > 0.1 µg/L and in 4 of 180 samples with > 1.0 µg/L. The maximum values for glyphosate were found in Sauter Bach/Wiepkenhagen with 1.37 µg/L. AMPA was found in 83 of 180 samples with concentrations > 0.1 µg/L and in 16 of 180 samples with values >1.0 µg/L (maximum 5.58 µg/L). Potential contamination pathway of surface water could be municipal sewage plants, because also pharmaceuticals were detected here in higher concentrations. Beside the intensive use of glyphosate in regional agriculture other origins could be washing/ cleaning agents and freezing agents.
Proposed action: To be considered in the summarizing table of surface water monitoring in spite of the short duration time. Obtained data are comparable to those given in the summarizing table.
Type of information (critical, high/low weight, supporting, additional): Low weight, may be used as additional information

Reliability	Low
Endpoint	Concentration in surface water and groundwater
Protocol	No information in the report on analysis methods
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given
Test system and conditions	No information in the report, only overview of results
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results support the long time monitoring data. However, monitoring studies and campaigns over more years and with more detailed information are of more reliability and relevance. No negative evidence (no clarification of contamination pathway).

Battaglin et al. (2014)

Title: Glyphosate and Its Degradation Product AMPA Occur Frequently and Widely in U.S. Soils, Surface Water, Groundwater, and Precipitation
Author: W.A. Battaglin, M.T. Meyer, K.M. Kuivila, and J.E. Dietze
Reference: JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION, Vol. 50, No. 2
Year: 2014

Results and conclusion:
Glyphosate use in the United States increased from less than 5,000 to more than 80,000 metric tons/yr between 1987 and 2007. Glyphosate is popular due to its ease of use on soybean, cotton, and corn crops that are genetically modified to tolerate it, utility in no-till farming practices, utility in urban areas, and the perception that it has low toxicity and little mobility in the environment. This compilation is the largest and most comprehensive assessment of the environmental occurrence of glyphosate and aminomethylphosphonic acid (AMPA) in the United States conducted to date, summarizing the results of 3,732 water and sediment and 1,018 quality assurance samples collected between 2001 and 2010 from 38 states. Results indicate that glyphosate and AMPA are usually detected together, mobile, and occur widely in the environment. Glyphosate was detected without AMPA in only 2.3 % of samples, whereas AMPA was detected without glyphosate in 17.9 % of samples. Glyphosate and AMPA were detected frequently in soils and sediment, ditches and drains, precipitation, rivers, and streams; and less frequently in lakes, ponds, and wetlands; soil water; and groundwater. Concentrations of glyphosate were below the levels of concern for humans or wildlife; however, pesticides are often detected in mixtures. Ecosystem effects of chronic low-level exposures to pesticide mixtures are uncertain. The environmental health risk of lowlevel detections of glyphosate, AMPA, and associated adjuvants and mixtures remain to be determined.

Proposed action:
Not to be considered as monitoring data outside the EU are reported.

Type of information (critical, high/low weight, supporting, additional):
Low weight, additional information to already existing, as monitoring was in USA, i.e. outside the EU

Reliability	High
Endpoint	Monitoring, concentrations in surface waters, soils, groundwater- and precipitation
Protocol	Monitoring, for details see under test system and conditions, quality assurance programme
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA(CAS-no: 1066-51-9), (monitored, purity cannot be given)
Test system and conditions	Monitoring campaign exactly described, i.e.: sampling sites and time, sampling procedures, analytical methods, quality assurance, laboratory reporting level (LRL)
Statistical design	Not known
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Battaglin et al. (2009)

Title: The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005–2006

Author: William A. Battaglin, Karen C. Rice, Michael J. Focazio, Sue Salmons, Robert X. Barry

Reference: Environ Monit Assess, 155:281–307

Year: 2009

Results and conclusion:
In this study, we investigated the occurrence of glyphosate, its primary degradation product aminomethyl-phosphonic acid (AMPA), and additional pesticides in vernal pools and adjacent flowing waters. Most sampling sites were chosen to be in areas where glyphosate was being used either in production agriculture or for non-indigenous plant control. The four site locations were in otherwise protected areas (e.g. in a National Park). When possible, water samples were collected both before and after glyphosate application in 2005 and 2006. Twenty-eight pesticides or pesticide degradation products were detected in the study, and as many as 11 were identified in individual samples. Glyphosate was measured at the highest concentration (328 µg/l) in a sample from Riley Spring Pond in Rock Creek National Park. This concentration exceeded the freshwater aquatic life standard for glyphosate of 65 µg/l. Aminomethylphosphonic acid (AMPA) was detected at concentrations greater than 3.0 µg/l.

Proposed action: Not to be considered as monitoring data outside the EU are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing, as monitoring was in USA, i.e. outside the EU	
Reliability	High
Endpoint	Monitoring, concentrations in surface waters
Protocol	Monitoring, for details see under test system and conditions, quality assurance programme
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA(CAS-no: 1066-51-9), (monitored, purity cannot be given)
Test system and conditions	Monitoring campaign exactly described, i.e.: sampling sites and time, sampling procedures, analytical methods, quality assurance, laboratory reporting level (LRL)
Statistical design	Not known
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Battaglin et al. (2005)

Title: GLYPHOSATE, OTHER HERBICIDES, AND TRANSFORMATION PRODUCTS IN MIDWESTERN STREAMS, 2002	
Author: William A. Battaglin, Dana W. Kolpin, Elizabeth A. Scribner, Kathryn M. Kuivila, and Mark W. Sandstrom	
Reference: Journal of the American Water Resources Association (JAWRA), 323-332	
Year: 2005	
Results and conclusion: The objective of this study was to document the occurrence of glyphosate and the transformation product aminomethylphosphonic acid (AMPA) in Midwestern streams and to compare their occurrence with that of more commonly measured herbicides such as acetochlor, atrazine, and metolachlor. The frequency of glyphosate and AMPA detection, range of concentrations in runoff samples, and ratios of AMPA to glyphosate concentrations did not vary throughout the growing season as substantially as for other herbicides like atrazine, probably because of different seasonal use patterns. Glyphosate was detected at or above 0.1 µg/l in 35 percent of pre-emergence, 40 percent of post-emergence, and 31 percent of harvest season samples, with a maximum concentration of 8.7 µg/l. AMPA was detected at or above 0.1 µg/l in 53 percent of pre-emergence, 83 percent of post-emergence, and 73 percent of harvest season samples, with a maximum concentration of 3.6 µg/l. Glyphosate was not detected at a concentration at or above the U.S. Environmental Protection Agency's maximum contamination level (MCL) of 700 µg/l in any sample.	
Proposed action: Not to be considered as monitoring data outside the EU are reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing as monitoring was in USA, i.e. outside the EU	
Reliability	High
Endpoint	Monitoring, concentrations in surface waters
Protocol	Monitoring, for details see under test system and conditions, quality assurance programme

Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA(CAS-no: 1066-51-9), (monitored, purity cannot be given)
Test system and conditions	Water samples were collected at sites on 51 streams in nine Mid-western states in 2002 during three runoff events: after the application of pre-emergence herbicides, after the application of post-emergence herbicides, and during harvest season. All samples were analyzed for glyphosate and 20 other herbicides using gas chromatography/mass spectrometry or high performance liquid chromatography/mass spectrometry. Quality assurance given, laboratory reporting level (LRL) given.
Statistical design	For all figures and statistics given in this report, non-detects were treated as zero. Box plots are used on some figures to show concentration distributions. Box plots are truncated at the MRL. The nonparametric Kruskal-Wallis test is used to test for differences in the distributions of either herbicide concentrations or transformation product to source herbicide concentration ratios from the three sample collection periods.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Bruchet et al (2012)

Title: Natural attenuation of priority and emerging contaminants during river bank filtration and artificial recharge	
Author: Auguste BRUCHET, Samuel ROBERT, Mar ESPERANZA, Marie-Laure JANEX-HABIBI, Cécile MIÈGE, Marina COQUERY, He´le`ne BUDZINSKI and Karine LEMENACH	
Reference: Eur. j. water qual. 42 (2011) 123–133	
Year: 2012	
Results and conclusion: The only pesticide or degradate found at a level exceeding 0.1 µg/L in the Seine river is glyphosate (on one occasion) and its degradate AMPA (systematically in the range 0.25– 0.65 µg/L). AMPA can also be present as a wastewater contaminant, from household detergent use. These two compounds are totally removed by bank filtration, in accordance with previous observations (Reemtsma and Jekel, 2002) and do not reappear in the aquifer.	
Proposed action: To be considered in the summarizing tables of surface water monitoring. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): No details are provided in the report about the finding localities and special causes. The information should be considered as additional.	
Reliability	
Endpoint	Concentrations of glyphosate and AMPA in surface water and groundwater
Protocol	Glyphosate and AMPA were determined by FMOC derivatization-HPLC-fluorescence.
Test compound	Glyphosate and AMPA

Test system and conditions	The fate of various emerging contaminants as well as priority pollutants from the European UnionWater Framework directive- was examined along a complex combination of natural and engineered processes used to produce drinking water downstream of a major metropolitan area. The sampling points examined omprised Seine riverwater downstream of the Paris area, water from a primary well after bank filtration, water from a secondary well influenced by an artificial recharge process and water from the mixture of secondary wells after drinkingwater treatment. More than 80 organic contaminants including drugs, polycyclic aromatic hydrocarbons, pesticides, oestrogenic hormones, polybrominated diphenyl ethers, chlorophenols, nonylphenols, were monitored during five campaigns. River bank filtration and to a lesser extent artificial recharge clearly decreased the variety of contaminants, in particular a variety of drugs detected in the river. On the other hand riverbank filtration was found to increase nonylphenols by anaerobic degradation of nonylphenolpolyethoxylate precursors. Traces of aspirin, nonylphenols and stimulants were occasionally detected in the finished drinking water above 0.1 µg/L.
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Busch and Reupert (LANUV) 2013

Title: Belastungsentwicklung von Oberflächengewässern und Grundwasser in NRW mit Glyphosat und AMPA
Author: Dieter Busch, Rolf Reupert (LANUV)
Reference: Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV)
Year: 2013
Results and conclusion: Results from a federal monitoring programme in North Rhine-Westphalia, Germany regarding concentrations of glyphosate and AMPA in surface water and groundwater are presented. The results are summarised in the following. 2001-2012: Concentration of glyphosate in surface water: Ruhr, 3 sites: max. 0.10-0.29 µg/L Concentration of AMPA in surface water: Ruhr, 3 sites: max. 0.86-2.02 µg/L 1996-2012: Concentration of glyphosate in surface water: NRW: 1899 samples, 225 samples > 0.1 µg/L (maximum 0.93 µg/L) 1996-2012: Concentration of AMPA in surface water: NRW: 1903 samples, 1377 samples > 0.1 µg/L (maximum 13 µg/L) 2006-2012: Concentration of glyphosate in groundwater: NRW: 245 samples, 0 samples > 0.1 µg/L (maximum 0.08 µg/L) 2006-2012: Concentration of AMPA in groundwater: NRW: 260 samples, 7 samples > 0.1 µg/L (maximum 0.45 µg/L)

Proposed action: To be considered in the summarizing tables of surface water and groundwater monitoring. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): No details are provided in the report about the finding localities and special causes. The information should be considered as additional.	
Reliability	
Endpoint	Concentrations of glyphosate and AMPA in surface water and groundwater
Protocol	In-house standard according to ISO 21458; DIN 38407-22
Test compound	Glyphosate and AMPA
Test system and conditions	Monitoring programme
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Busetto and Frattini (2010)

Title: Surveys of herbicide glyphosate and degradation product aminomethyl phosphonic acid in waterways of Monza-Brionza province	
Author: Busetto, M., Frattini, V.	
Reference: iL boLLettino 2010/4	
Year: 2010	
Results and conclusion: During the period 2006-2009 ARPA (the Lombardy Regional Environmental Agency) has been collecting analytical data concerning the presence and concentration of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in the water of Lambro, Seveso and Terrò rivers in the Brianza region. River flow-rate, COD, BOD5 and conductivity have also been measured in each sample. Both AMPA and glyphosate have been found in every sample, with AMPA concentration always higher than the glyphosate concentration. Larger amounts of herbicide have been observed in the water sampled in the autumnal season, while in the following months concentration decreases. Our data are in accordance with the available information about use and release of herbicide during the year.	
Proposed action: Not to be considered as the publication is written in Italian language.	
Type of information (critical, high/low weight, supporting, additional): Additional information.	
Reliability	
Endpoint	Concentrations of glyphosate and AMPA in surface water
Protocol	Not available (as the publication is written in Italian language)
Test compound	Glyphosate and AMPA
Test system and conditions	Monitoring programme
Statistical design	Not available (as the publication is written in Italian language)
Relevance	
Environmental relevance	Relevant

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Byer et al. (2008)

Title: Low Cost Monitoring of Glyphosate in Surface Waters Using the ELISA Method: An Evaluation	
Author: Byer J., Struger J., Klawunn P., Todd A. and Sverko E.	
Reference: Environ. Sci. Technol, 42, 6052–6057	
Year: 2008	
Results and conclusion: The goal of our study was to evaluate a cost effective method to measure glyphosate concentrations in surface waters. The reliability of enzyme-linked immune-sorbent assay (ELISA) results was evaluated against liquid chromatography tandem mass spectrometry, and linear regression results for 30 water samples from urban watersheds revealed a strong relationship (R^2 = 0.88). These results suggest that ELISA methods, used in conjunction with traditional methods, represent a cost-effective approach to enhance the spatial and temporal resolution of a water quality monitoring study. Additionally, we measured a total of 739 surface water samples from over 150 sampling locations throughout Ontario using ELISA from April to October 2007. Concentrations exceeded the method detection limit of 0.1 µg/L in 33 % of the samples, with a maximum concentration of 12.0 µg/L. Glyphosate showed a bimodal temporal distribution with peak concentrations occurring in late spring/early summer and fall, and did not exceed the Canadian Council of Ministers of the Environment (CCME) guideline for the protection of aquatic life (65 µg/L) in any of the samples.	
Proposed action: Not to be considered as publication deals with method development and monitoring data outside EU (Canada).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information to already existing as the articles deals with method development and monitoring data outside EU (Canada).	
Reliability	Medium
Endpoint	Monitoring, concentrations in surface waters
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6, monitored, purity cannot be given)
Test system and conditions	Sampling methodology, analytical methods described in detail. 30 surface water samples from three urban creeks in southern Ontario were analyzed via LC/MS/MS and ELISA. Quality control measures given, instrument variability considered.
Statistical design	Correlation between LC/MS/MS and ELISA-results by regression analysis
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supporting information by other monitoring programmes. No negative evidence.

Comoretto et al. (2007)

Title: Pesticides in the Rhône river delta (France): Basic data for a field-based exposure assessment	
Author: Laetitia Comoretto, Bruno Arfib, Serge Chiron	
Reference: Science of the Total Environment 380, 124–132	
Year: 2007	
Results and conclusion: The pesticide concentration levels flowing into paddy fields and surrounding lagoons of the Rhône river delta were investigated over a period of 6 months in 2004. The mean load of Glyphosate is assessed to be around 8 tons per year. However, Glyphosate was not found in any of the samples.	
Proposed action: Not to be considered as raw data are not sufficiently precisely reported.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Monitoring, concentrations in surface water
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) monitored, purity cannot be given
Test system and conditions	Pesticides were selected according to their occurrence in the Rhône river waters or their usage in rice farming. Water samples were collected at the outlets of the major ditches and in the lagoons in order to study the seasonal variation in pesticide concentrations and the spatial contamination profile. Twenty four pesticides were monitored, mainly herbicides and insecticides. Sampling sites described, sample preparation and chemical analysis described.
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Similar monitoring programmes not known. No negative evidence.

Coupe et al. (2012)

Title: Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins	
Author: Richard H Coupe, Stephen J Kalkhoff, Paul D Capel and Caroline Gregoire	
Reference: Pest Management Science 68(1): 16-30. DOI : 10.1002/ps.2212	
Year: 2012	
Results and conclusion: Results: Glyphosate and AMPA were frequently detected in the surface waters of four agricultural basins. The frequency and magnitude of detections varied across basins, and the load, as a percentage of use, ranged from 0.009 to 0.86 % and could be related to three general characteristics: source strength, rainfall runoff and flow route. Median concentrations were for Glyphosate: 0.1-380 µg/L, for AMPA: 0.1-26 µg/L. Conclusions: Glyphosate use in a watershed results in some occurrence in surface water; however, the watersheds most at risk for the offsite transport of glyphosate are those with high application rates, rainfall that results in overland runoff and a flow route that does not include transport through the soil.	
Proposed action: Not to be considered as highest reported median concentration of 380 µg/L were found in USA, and thus data are related to sites outside the EU data monitored in France are not sufficiently comprehensively reported.	

Type of information (critical, high/low weight, supporting, additional): Low weight, supporting information	
Reliability	High
Endpoint	Surface water concentrations, monitoring.
Protocol	Glyphosate and AMPA (USA): Water sample collection and processing followed USGS protocols. Online solid-phase extraction and analysis by HPLC/MS. Both compounds had a reporting level of 0.02 µg/L. Glyphosate and AMPA (France): filtered and analyzed using similar methods, with a reporting level of 0.1 µg/L.
Test compound	Glyphosate (CAS-no.: 1071-83-6), AMPA (CAS-no: 1066-51-9)
Test system and conditions	Surface water monitoring in 4 agricultural water basins in USA and France. The study conducted by the NAWQA Program of the USGS included four basins: the South Fork Iowa River, Iowa; Sugar Creek, Indiana; Bogue Phalia, Mississippi; Rouffach, France
Statistical design	6 field blanks, 11 replicates
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Daouk et al. (2013)

Title: Dynamics and environmental risk assessment of the herbicide glyphosate and its metabolite AMPA in a small vineyard river of the Lake Geneva catchment
Author: SILWAN DAOUK, PIERRE-JEAN COPIN, LUCA ROSSI, NATHALIE CHÈVRE and HANS-RUDOLF PFEIFER
Reference: Environmental Toxicology and Chemistry, Vol. 32, No. 9, pp. 2035–2044, 2013
Year: 2013
Results and conclusion: The use of pesticides may lead to environmental problems, such as surface water pollution, with a risk for aquatic organisms. In the present study, a typical vineyard river of western Switzerland was first monitored to measure discharged loads, identify sources, and assess the dynamic of the herbicide glyphosate and its metabolite aminomethylphosphonic acid (AMPA). Second, based on river concentrations, an associated environmental risk was calculated using laboratory tests and ecotoxicity data from the literature. Measured concentrations confirmed the mobility of these molecules with elevated peaks during flood events, up to 4970 ng/L. From April 2011 to September 2011, a total load of 7.1 kg was calculated, with 85 % coming from vineyards and minor urban sources and 15 % from arable crops. Compared with the existing literature, this load represents an important fraction (6–12 %) of the estimated amount applied because of the steep vineyard slopes (10 %). The associated risk of these compounds toward aquatic species was found to be negligible in the present study, as well as for other rivers in Switzerland. A growth stimulation was nevertheless observed for the algae Scenedesmus vacuolatus with low concentrations of glyphosate, which could indicate a risk of perturbation in aquatic ecosystems, such as eutrophication. The combination of field and ecotoxicity data allowed the performance of a realistic risk assessment for glyphosate and AMPA, which should be applied to other pesticide molecules.
Proposed action: Obtained data are comparable to those given in the summarizing table. Not be considered in the summarizing tables of surface water monitoring, as only the max. concentrations in the surface waters are reported.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.

Reliability	Medium
Endpoint	Monitoring, concentrations of glyphosate and AMPA in surface water
Protocol	Monitoring

Test compound	Glyphosate and AMPA, purity cannot be given, monitoring
Test system and conditions	The Lutrive River was sampled during the year 2011 upstream and downstream of the vineyard area. The automatic samplers were programmed for 2 types of sampling: a regular and an event-based sampling. One-third of the bottles were dedicated to the regular sampling and the other two-thirds to the event-based sampling. The first was done every 4 d and the second when the water level reached a predefined level, indicating the start of a rain event, and then every 2 h. Fifty samples for the downstream site and 20 for the upstream site were selected from April 2011 to October 2011 and analyzed. Twenty of them—15 downstream and 5 upstream— were regularly collected during dry periods to estimate background levels of glyphosate and AMPA. The herbicide glyphosate and its metabolite AMPA were quantified by ultraperformance liquid chromatography coupled to tandem mass spectrometry after their derivatization with 9-fluorenylmethyl chloroformate followed by solid-phase extraction.
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	The results support the long time monitoring data. However, monitoring studies and campaigns over more years and with more detailed information are of more reliability and relevance. No negative evidence.

Daouk et al. (2013a)

Title: The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part I: Method validation in different water matrices
Author: S. DAOUK, D. GRANDJEAN, N. CHEVRE, L. F. DE ALENCASTRO and H.- R.F. PFEIFER
Reference: Journal of Environmental Science and Health, Part B (2013) 48, 717-724
Year: 2013

Results and conclusion:
An analytical method for the quantification of the widely used herbicide, glyphosate, its main by-product, aminomethylphosphonic acid (AMPA) and the herbicide glufosinate at trace level was developed and tested in different aqueous matrices. Their derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) was done prior to their concentration and purification by solid phase extraction. The concentrated derivates were then analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Spiking tests at three different concentrations were realized in several water matrices: ultrapure water, Evian© mineral water, river water, soil solution and runoff water of a vineyard. Except for AMPA in runoff water, obtained regression curves for all matrices of interest showed no statistical differences of their slopes and intercepts, validating the method for the matrix effect correction in relevant environmental samples. The limits of detection and quantification of the method were as low as 5 and 10 ng/l respectively for the three compounds. Spiked Evian© and river water samples at two different concentrations (30 and 130 ng/l) showed mean recoveries between 86 and 109 %, and between 90 and 133 % respectively. Calibration curves established in spiked Evian© water samples between 10 and 1000 ng/l showed r^2 values above 0.989. Monitoring of a typical vineyard river showed peaks of pollution by glyphosate and AMPA during main rain events, sometimes above the legal threshold of 100 ng/l, suggesting the diffuse export of these compounds by surface runoff. The depth profile sampled in the adjacent lake near a waste water treatment plant outlet showed a concentration peak of AMPA at 25m depth, indicating its release with treated urban wastewater.

In detail: The validation of the method to quantify the herbicide glyphosate, its metabolite AMPA and the herbicide glufosinate at trace level in several types of natural waters was successful and allows following these potential hazardous molecules in the environment. Further investigations to better understand their behavior in soils after their application and their transport to surface water will be possible. Preliminary results of field studies show that river water samples exhibit a frequent pollution by the studied herbicides, which finally end up in Lake Geneva. Several samples showed concentrations above the legal threshold of 100 ng/l. This highlights the importance of monitoring these substances in the aquatic system.

Proposed action:
Consider as additional information as only method validation in different water matrices is described.

Type of information (critical, high/low weight, supporting, additional):
Additional information

Reliability	
Endpoint	Glyphosate and AMPA export to surface waters, method validation in different water matrices
Protocol	Not given
Test compound	Glyphosate (PESTANALR, 99.7 %), glufosinate-ammonium (PESTANALR, 99.2 %) and AMPA (99 %)
Test system and conditions	Environmental sampling: The Lutrive is a local river in the east of the city of Lausanne, at the western limit of the Lavaux vineyard area. Its small watershed (6.4 km^2) is characterized by different land uses: agricultural fields (45 %), of which 4.1 % are vineyards, urban and impervious surfaces (31 %) and forests (24 %). Grab samples were collected in the vineyard area during the growing season of 2010 and during both dry- and wet-weather conditions. Daily precipitations data of the meteorological station of Pully, located at 2 km west of the Lutrive River, were provided by MeteoSwiss. Lake Geneva was sampled during dry weather on the 1st of July 2010, in the Vidy Bay near the waste water treatment plant (WWTP)outlet at nine different depths:−2,−5,−10,−15, −18.5, −21, −23, −25 and −29 m. Corresponding real-time temperature and electrical conductivity data were obtained from Bonvin *et al.*[
Statistical design	Mean and maximum values, standard deviations
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Daouk et al. (2013b)

Title: The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part II: The role of infiltration and surface runoff	
Author: S. DAOUK, L. F. DE ALENCASTRO and H.-R. PFEIFER	
Reference: Journal of Environmental Science and Health, Part B (2013) 48, 725-736	
Year: 2013	
Results and conclusion: Two parcels of the Lavaux vineyard area, western Switzerland, were studied to assess to which extent the widely used herbicide, glyphosate, and its metabolite aminomethylphosphonic acid (AMPA) were retained in the soil or exported to surface waters. They were equipped at their bottom with porous ceramic cups and runoff collectors, which allowed retrieving water samples for the growing seasons 2010 and 2011. The role of slope, soil properties and rainfall regime in their export was examined and the surface runoff/throughflows ratio was determined with a mass balance. The results revealed elevated glyphosate and AMPA concentrations at 60 and 80 cm depth at parcel bottoms, suggesting their infiltration in the upper parts of the parcels and the presence of preferential flows in the studied parcels. Indeed, the succession of rainy days induced the gradual saturation of the soil porosity, leading to rapid infiltration through macropores, as well as surface runoff formation. Furthermore, the presence of more impervious weathered marls at 100 cm depth induced throughflows, the importance of which in the lateral transport of the herbicide molecules was determined by the slope steepness. Mobility of glyphosate and AMPA into the unsaturated zone was thus likely driven by precipitation regime and soil characteristics, such as slope, porosity structure and layer permeability discrepancy. Important rainfall events (>10 mm/day) were clearly exporting molecules from the soil top layer, as indicated by important concentrations in runoff samples. The mass balance showed that total loss (10–20 %) mainly occurred through surface runoff (96 %) and, to a minor extent, by throughflows in soils (4 %), with subsequent exfiltration to surface waters. Results in detail: The total amount of glyphosate and AMPA retrieved in both type of samples from parcel 2 (surface = 845 m^2), and likely to be exported from it, was 4.3 g in 2010 and 9.1 g in 2011. This represents respectively 10 and 20 % of the initial amount, which, despite the uncertainty of such kind of calculations, is in agreement with previous studies. The 80–90 % remaining were either retained and possibly as bound residues after some time, or degraded in the soil, as volatilization is not likely to happen due to their properties. The relative contribution of throughflows in the unsaturated zone versus surface runoff was 3–5 % versus 95–97 %.	
Proposed action: To be considered as additional information as it is pointed out that total loss (10–20 %) mainly occurred through surface runoff (96 %) and, to a minor extent, by throughflows in soils (4 %).	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Export of glyphosate and AMPA to surface waters, role of infiltration and surface runoff
Protocol	Not given
Test compound	Glyphosate
Test system and conditions	Study area and soil features: The Lavaux is a vineyard area located in western Switzerland of 830 ha including about 10,000 terraces on 40 km between Montreux and Lutry to the east of Lausanne. Based on a previous work dealing with risk assessment of pesticide transfer from vineyards to surface waters, two risky parcels were chosen close to the Lutrive River. The general character of the soils was obtained through drilling with an auger and digging two pits of a depth up to two meters at their bottom. Textures, colours and the presence of carbonates were determined in the field, whereas soil pH was measured in the laboratory on water extractions (Soil: H_2O = 1:2.5). More precise grain size analyses were obtained by laser diffractometry (Mastersizer 2000, Malvern, Worcestershire, UK) for samples of various depths at the bottom of both parcels, treated with HCl beforehand to remove carbonates. Soils of both parcels are colluvial calcosols, according to the French classification, with anthropogenic influences.

	The dominant colour, referring to the Munsell colour system, was medium to light brown (10YR4/3) with a grey tendency (2.5Y5/3) in the deepest horizons. Both soils showed a silt loam texture and light differences were observed between plots and depths. HCl reactivity at all depths revealed the carbonate nature of soils, which was confirmed by pH-values between 8 and 9. Organic carbon contents varied from the surface layer to deeper horizons between 1.7 and 0.7 % in parcel 1 and 2.2 and 0.8 % in parcel 2. Copper concentrations in top soils (0–30 cm) varied between 300 and 500 mg/kg in parcel 1 and 100 and 170 mg/kg in parcel 2. Sampling and analytical methods: In both parcels, the herbicide glyphosate was applied the same day and only under the rows, leaving a grass band in between them. It is mainly applied in spring time to avoid a nutrient and water competition between grapevines and weeds during the growing season. Application data were obtained from winegrowers. In previous years, the same amounts had been applied, but the authors assumed that all glyphosate and AMPA degrade from year to year according to their properties. Precipitation data were obtained from the closest meteorological station (Source: MeteoSwiss), located in Pully at 1.8 km from the two parcels. In order to sample the soil solution, both parcels were equipped at their bottom with porous ceramic suction cups (SPS200, Ø63 mm, porosity = 1 µm, SDEC, Reignac sur Indre, France) at four different depths: 20, 40, 60 and 80 cm (Fig. 1c). The applied tension was 0.6 bars and the recovery of samples was done every week or more frequently during intense rainfall periods. As the slope of parcel 2 is more representative of the Lavaux region, it was equipped with three runoff collectors. They were built inspired from previous studies and placed at the end of grapevine rows according to observed erosion paths. They comprised a bottle buried in the soil, a funnel with a sieve of 1 mm size to avoid macro fauna or large particles, a PVC conducting ramp placed just under the root zone and ending above the funnel, and a roof to avoid direct rain inputs. Samples were collected in 250 mL high density polyethylene bottles and transported to the laboratory in a cool box. They were placed in a freezer until their analysis, for which they were then gently de-frozen. Electrical conductivity and pH were determined in the field. The herbicide glyphosate and its metabolite AMPA were quantified by LC-MS/MS with a previously developed method, based on their precolumn derivatization with FMOC-Cl and their enrichment by solid phase extraction. The limit of quantification was 10 ng/L and it was tested successfully for the matrix effect that could occur by analyzing soil solution and runoff samples. Dissolved organic and inorganic carbon (DOC/DIC) concentrations were measured with a C-analyzer (LiquiTOC, Elementar, Hanau, Germany). Ion concentrations were obtained with an ion chromatography system (ICS-1100/2100, Dionex-Thermo Fischer, Olten, Switzerland). Copper analyzes were done by ICP-MS (ELANR® 6100 DRC, Perkin Elmer, Waltham, MA, USA) in some soil water samples, previously diluted, acidified with HNO_3 and treated with H_2O_2 to avoid possible interference with organic matter. A principal component analysis (PCA) was performed on the soil water samples using the R software to help interpreting all the analyses and discriminating the observations made in the two different parcels. Prior to it, each of the parameters was normalized to zero mean and unit variance, by subtracting the mean value of the variable and by dividing by the standard variation, allowing them to have the same influence in the PCA. In order to determine the surface runoff/throughflows ratio, a mass balance was done for both surface runoff and soil solution samples of parcel 2 (surface = 845 m^2). As glyphosate was applied only under the grapevine rows, the initial quantities correspond to half of the surface. The mass of glyphosate and AMPA were obtained by multiplying the concentrations with cumulated precipitations that fell on the parcel surface between two sampling events.
Statistical design	See “Test system and conditions”

Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

de Armas et al. (2007)

Title: SPATIAL-TEMPORAL DIAGNOSTIC OF HERBICIDE OCCURRENCE IN SURFACE WATERS AND SEDIMENTS OF CORUMBATAÍ RIVER AND MAIN AFFLUENTS
Author: Eduardo Dutra de Armas, Regina Teresa Rosim Monteiro, Paula Munhoz Antunes, Maria Alice Penna Firme dos Santos e Plinio Barbosa de Camargo
Reference: Quim. Nova, Vol. 30, No. 5, 1119-1127
Year: 2007
Results and conclusion: Residues of herbicides from sugarcane were monitored in waters and sediments of Corumbataí River and tributaries. Ametryne, atrazine, simazine, hexazinone, glyphosate, and clomazone were detected in water samples, with negligible levels of ametryne and glyphosate in sediment samples. The area of recharge of the Guarani aquifer presented the highest triazine and clomazone levels. The triazines were detected at higher levels, with atrazine above Brazil's potability and quality standards. Total herbicide levels at some sampling points were 13 times higher than the European Community potability limit.
Proposed action: Not to be considered as monitoring outside the EU (Brazil).
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.

Reliability	Low
Endpoint	Monitoring, concentrations in surface water and sediment
Protocol	Monitoring
Test compound	Glyphosate (CAS-no: 1071-83-6), purity cannot be given, monitoring
Test system and conditions	Sampling regime, analytical procedure, LOD given (in Spanish language)
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Similar monitoring programmes not known. No negative evidence.

Fooken and Stachel (2000)

Title: Selected organic trace impurities in the river Elbe and its tributaries during 1994-1999
Author: Cornelia Fooken, Burkhard Stachel,
Reference: Report of the Working Group for Prevention of ELBE Pollution (ARGE Elbe)
Year: Oktober 2000
Results and conclusion: 2-7 samples per year from 3-10 measuring points per year of Elbe and its tributaries were investigated. Glyphosate was detected in the range of 0.05-0.09 µg/l, AMPA with 0.2-1.0 µg/L. In the summer samples the concentrations of AMPA were 2-3 times higher than in winter samples.

Proposed action: Not to be considered in the summarizing table of surface water monitoring. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): Low weight, may be used as additional information	
Reliability	Low
Endpoint	Concentration in surface water (Elbe)
Protocol	Analysis: solid phase enrichment with cation exchange column, elution with hydrochloric acid two step derivatisation with hypochlorit and ophthaldialdehyd HPLC/FLD
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given
Test system and conditions	Both compounds were detected in surface water samples in 1998.
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
“Positive”/”Negative” evidence	The results support the long time monitoring data. However, monitoring studies and campaigns over more years and with more detailed information are of more reliability and relevance. No negative evidence.

Freire et al. (2012)

Title: Monitoring of toxic chemical in the basin of Maringá stream	
Author: Rosane Freire, Roselene Maria Schneider, Fabrício Hernandes de Freitas, Cássia Maria Bonifácio and Célia Regina Granhen Tavares	
Reference: Acta Scientiarum. Technology Maringá, v. 34, n. 3, p. 295-302, July-Sept., 2012	
Year: 2012	
Results and conclusion: This study aimed to track the spatial and temporal variations of toxic chemical compounds, such as the metals Al, Cd, Pb, Cu, Cr, Mn, Zn and the pesticide glyphosate, in Maringá stream and in a stretch of Pirapó river. The results pointed out that, in the case of metals, one of the possible sources of these elements is associated to agricultural activities. For glyphosate, were not found concentrations above those established by the Brazilian Water Quality Legislation (CONAMA 357/2005). Concerning this, we emphasized that the impact caused by the agrochemical on water quality should be evaluated considering the adverse effects to the environment caused by its degradation, that produces recalcitrant and surfactant compounds that may be even more toxic for humans and aquatic environment.	
Proposed action: Not to be considered as publication deals with monitoring outside the EU (Brazil).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in stream samples
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), purity not given, monitoring

Test system and conditions	Samples were collected at 9 sites distributed throughout the basin of the Maringá stream (monthly from July to December 2009). The concentration was determined by ion chromatography with suppressed conductivity detection. The climatological data relative to the study period were furnished by the Main Weather Station of the State University of Maringá.
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Glozier et al. (2011)

Title: Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007	
Author: Nancy E. Glozier, John Struger, Allan J. Cessna, Melissa Gledhill, Myriam Rondeau, William R. Ernst, Mark A. Sekela, Steve J. Cagampan, Ed Sverko, Clair Murphy, Janine L. Murray, David B. Donald	
Reference: Environ Sci Pollut Res (2012) 19:821–834	
Year: 2012	
Results and conclusion: A national survey was designed to monitor eight commonly used herbicides in urban rivers and streams across Canada. The herbicides 2,4-D, mecoprop, dicamba, glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) were most frequently detected. Maximum concentrations of glyphosate: 11,800 ng/L (glyphosate).	
Proposed action: Not to be considered as publication deals with a specific situation outside the EU (Canada).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in surface water
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Samples were collected monthly on one of two predetermined dates from April to September, 2007 from 19 sites within 16 watersheds, including 15 sites downstream of urban lands and two reference sites. Water samples were also collected approximately three times from each watershed during or after precipitation events. All samples were collected using a common sampling protocol and all were analyzed using the same analytical laboratories.
Statistical design	Nonparametric statistical tests were used. All statistical analyses (t tests, Mann–Whitney tests, twoway analysis of variance (ANOVA) analyses, regressions) were performed with Systat Version 11.
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Grunewald et al. (2001)

Title: Behaviour of Glyphosate and aminomethylphosphonic acid (AMPA) in soils and water of reservoir Radeburg II catchment (Saxony/Germany)	
Author: Grunewald K., Schmidt W., Unger C., Hanschmann G.	
Reference: J. Plant Nutr. Soil Sci, 164, 65-70	
Year: 2001	
Results and conclusion: The behaviour of Glyphosate and AMPA was investigated in soils and water in a well defined catchment of the reservoir Radeburg II near Dresden. Half-life of Glyphosate in soil ranged from 11 to 17 days. Glyphosate and AMPA completely disappeared from the soil after 5 months following application of "Roundup Ultra" and "Touchdown". The aquatic system in the test areas (surface water, soil solution, groundwater) was not significantly affected by the direct application of the compound. In general, there was a clear indication of strong sorption of the substances by soil particles. Settlement areas were recognized as possible sources of glyphosate and AMPA intake in aquatic systems.	
Proposed action: Considered by listing in the summarizing table and discussion.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in surface water, soil solution, groundwater and soil
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Study area located in the north/north-east sub-urban district of the town Radeburg near Dresden. Landscape has an undulating surface between 145 – 250 m a.s.l. Climatic conditions described. Soil sampling, groundwater sampling, runoffsampling and reservoir sampling described in detail. Glyphosate and AMPA were determined according to reference method No. 105 of Deutsche Forschungsgemeinschaft, 1991. LOD in soil and water is given.
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Houtman et al. (2013)

Title: A MULTICOMPONENT SNAPSHOT OF PHARMACEUTICALS AND PESTICIDES IN THE RIVER MEUSE BASIN
Author: C. J. HOUTMAN, R. T. BROEK, K. DE JONG, B. PIETERSE and J. KROESBERGEN
Reference: Environmental Toxicology and Chemistry, Vol. 32, No. 11, pp. 2449–2459, 2013
Year: 2013
Results and conclusion: The river Meuse serves as a drinking-water source for more than 6 million people in France, Belgium, and The Netherlands. Pharmaceuticals and pesticides, both designed to be biologically active, are important classes of contaminants present in this river. The variation in the presence of pharmaceuticals in time and space in the Dutch part of the Meuse was studied using a multicomponent analytical method for pharmaceuticals combined with univariate and multivariate statistical analyses of the results. Trends and variation in time in the presence of pharmaceuticals were investigated in a dead-end side stream of the Meuse that serves as an intake point for the production of drinking water, and 93 % of the selected compounds were detected. Highest concentrations were found for the antidiabetic metformin. Furthermore, a spatial snapshot of the presence of pharmaceuticals and pesticides was made along the river Meuse. Principal component analysis was successfully applied to reveal that wastewater-treatment plant effluent and water composition at the Belgian border were the main factors determining which compounds are found at different locations. The Dutch part of the river basin appeared responsible for approximately one-half of the loads of pharmaceuticals and pesticides discharged by the Meuse into the North Sea. The present study showed that multicomponent monitoring in combination with principal component analysis is a powerful tool to provide insight into contamination patterns in surface waters. In detail: Concentrations along the Meuse River basin: Nineteen detected pesticides belong to the class of herbicides. Among them were glyphosate and aminomethylphosphonic acid (its degradation product). They are notorious contaminants in the river Meuse. The main emission pathways to the Dutch part of the Meuse are runoff from pavements. Glyphosate is not well degraded in WWTPs. Degradation to aminomethylphosphonic acid takes place mainly in the environment. Glyphosate and aminomethylphosphonic acid were the only pesticides found in all samples. Concentrations detected in the 'snapshot' study: Glyphosate: max 0.21 µg/L AMPA: max 2.28 µg/L
Proposed action: To be considered in the summarizing tables of surface water monitoring. Obtained data are comparable to those given in the summarizing table.
Type of information (critical, high/low weight, supporting, additional): Supporting information

Reliability	
Endpoint	Concentrations of pharmaceuticals and pesticides (amongst others: glyphosate and AMPA) along the Meuse River basin
Protocol	Not given
Test compound	Glyphosate and AMPA (amongst others)
Test system and conditions	Analysis of pharmaceuticals with the ultra-HPLC/MS-MS multicomponent method Spatial snapshot of pharmaceuticals along the Meuse Analysis of general water-quality parameters Multicomponent analysis of pharmaceuticals and pesticides Statistical analysis Calculation of the loads discharged into the North Sea

Statistical design	The presence of pharmaceuticals in the Dutch part of the river Meuse was studied in time and space using a multicomponent analytical method combined with univariate and multivariate statistical analyses of the results. Box plot figures representing minimum, first quartile, median, third quartile, and maximum concentrations were made in Excel for pharmaceuticals that were detected in at least 5 samples (20 % of the samples). Concentrations less than the minimum reporting limit were artificially set at 25 % of the individual minimum reporting limit. The significance of longterm time trends and seasonal variation was tested using the statistical software package Trendanalist (AMO-Icastat). For this purpose, the obtained data set was complemented with archived monitoring results for those pharmaceuticals that had also been monitored with enough sensitivity with LC/MS and gas chromatography (GC)/MS methods at the same location from 2005 to 2010 (the test requires results of a period of at least 4.5 yr). Long-term time trends were tested with linear regression (in case of normally distributed data), and the Mann-Kendall test corrected for seasonal effects (if data were not normally distributed). Seasonal variation was tested with Kruskal-Wallis tests.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Kaiser (2011)

Title: Preliminary Study of Pesticide Drift into the Maya Mountain Protected Areas of Belize	
Author: Kristine Kaiser	
Reference: Bull. Environ. Contam. Toxicol. 86, 56–59	
Year: 2011	
Results and conclusion: In Belize, Central America, many farms surrounding the Protected Areas of the Maya Mountains rely heavily on the application of agrochemicals. The purpose of this study was to test whether orographic drift of glyphosate and organophosphates into the nearby Maya Mountain Protected Areas occurred by collecting phytotelmic water from seven sites over 3 years. Regardless of location within the Maya Mountain Protected Areas, glyphosate was present; organophosphates were more common at ridge sites. Although glyphosate concentrations were low (0.22 – 1.71 µg/L), due to the number of threatened species and the human use of stream water outside the Maya Mountain Protected Areas, better understanding of these effects is warranted.	
Proposed action: Not to be considered as publication deals with a specific situation outside the EU (Belize).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in groundwater, wells and waterworks
Protocol	for details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), purity not given, monitoring
Test system and conditions	A total of seven sites across the protected areas of the Maya Mountains were sampled during June–August, 2006–2007 the rainiest months of the year in this region. Based on self-reported use of agrochemicals glyphosate and common organophosphates and carbamate (OP/C) tests were implemented. Analyses by chemical specific test kits. Glyphosate ELISA-method applied.
Statistical design	Five samples were collected per site per year. Samples for all tests were run in duplicate. Statistical analysis was carried out using StataIC 10.0.
Environmental relevance	Given

Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Kolpin et al. (2006)

Title: Urban contributions of glyphosate and its degradate AMPA to streams in the United States	
Author: Dana W. Kolpin, E. Michael Thurman, Edward A. Lee, Michael T. Meyer, Edward T. Furlong, Susan T. Glassmeyer	
Reference: Science of the Total Environment 354, 191– 197	
Year: 2006	
Results and conclusion: In 2002, treated effluent samples were collected from 10 wastewater treatment plants (WWTPs) to study the occurrence of glyphosate and AMPA. Concentrations were generally low, although nine detections of AMPA (maximum concentration = 3.9 µg/L) and three detections of glyphosate (maximum concentration = 2.2 µg/L) exceeded 1 µg/L. The results document the apparent contribution of WWTP effluent to stream concentrations of glyphosate and AMPA, with roughly a two-fold increase in their frequencies of detection between stream samples collected upstream and those collected downstream of the WWTPs. Thus, urban use of glyphosate contributes to glyphosate and AMPA concentrations in streams in the United States. Overall, AMPA was detected much more frequently (67.5 %) compared to glyphosate (17.5 %).	
Proposed action: Not to be considered as publication deals with monitoring outside the EU (USA).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Glyphosate concentrations in stream samples upstream and down-stream of waste water treatment plants
Protocol	For details see under test system and conditions, non-GLP, all samples were sampled by the same personnel and according to special, consistent protocols.
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Stream samples were collected upstream and downstream of the 10 WWTPs. The network consisted of 40 sampling sites: eight up-stream samples, 11 WWTP effluents, 19 downstream samples. Two reference sites were sampled in areas anticipated to have little glyphosate use because of limited human activity. The 10 WWTP locations represent a variety of climatic conditions, population densities, stream sizes, and treatment practices. Sampling reported, analytical methods including reporting limit reported,
Statistical design	Significances for differences in concentrations tested by Kruskal–Wallis test.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Munz et al. (2012)

Title: Pestizidmessungen in Fliessgewässern-Schweizweite Auswertung	
Author: N. Munz, C. Leu, I. Wittmer	
Reference: AQUA & GAS No 11 | 2012	
Year: 2012	
Results and conclusion: A variety of current-use pesticides were analysed in river waters samples collected during the 2005 and 2012 at 565 sites in Switzerland. The results for glyphosate are summarised in the following: Max. Concentration: 7.2 µg/L 95. percentile: 0.6 µg/L Amounts above the limit of determination (LOD): 42 % Number of sites above LOD: 81 %	
Proposed action: To be considered in the summarizing tables of surface water monitoring. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): Supporting information	
Reliability	
Endpoint	Glyphosate concentrations in river waters
Protocol	Not given
Test compound	Pesticides (Glyphosate (amongst others))
Test system and conditions	- Sample taking (in river waters in Switzerland and determination of pesticides concentrations - Calculation of 95. percentiles, amounts above the limit of determination (LOD) and number of sites above LOD
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence

Ludvigsen and Lode (2001)

Title: RESULTS FROM "JOVÅ" THE AGRICULTURAL AND ENVIRONMENTAL MONITORING PROGRAM OF PESTICIDES IN NORWAY 1995 – 1999	
Author: Gro Hege Ludvigsen and Olav Lode	
Reference: Fresenius Environment Bulletin, Vol. 10 (5)	
Year: 2001	
Results and conclusion: In the monitoring program called "JOVÅ" we have the following types of investigations: streams and rivers, drainage water, groundwater, sediments and precipitations. In this paper only the results from 12 locations concerning streams and rivers are presented from the years 1995 – 1999. Results for glyphosate: 86 % positive findings, maximum concentration = 0.93 µg/L, average = 0.13 µg/L. Results for AMPA: 87 % positive findings, maximum concentration = 0.2 µg/L, average = 0.06 µg/L.	
Proposed action: Data are presented in the summarizing table and discussed in the summarizing paragraph.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication deals with specific objective, namely surface water concentrations at a certain place and time.	
Reliability	Medium
Endpoint	Glyphosate concentrations in surface waters

Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	In six of the drainage basins data on the use of pesticides have been collected. 851 samples have been collected during this period. Sampling and analyses described, LOD = 0.01 µg/L
Statistical design	Number analyzed: 49, average values, maximum values given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Ludvigsen and Lode (2002)

Title: TRENDS OF PESTICIDES IN NORWEGIAN STREAMS AND RIVERS (1996– 2000)	
Author: Gro Hege Ludvigsen and Olav Lode	
Reference: Intern. J. Environ. Anal. Chem., Vol. 82, No. 8–9, pp. 631–643	
Year: 2002	
Results and conclusion: In Norway twelve streams and medium size rivers have been monitored for pesticides in a four to six years period. Trend analyses have been done on the years 1996–2000 to gain information on whether there have been reductions in the retrieval of the pesticides. The situation in these streams has not changed much during this period, but there are indications towards a slight positive development. Trend analyses might therefore be useful together with careful interpretation. Results for glyphosate: 91 % positive findings, no further information given.	
Proposed action: Data are presented in the summarizing table and discussed in the summarizing paragraph.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication gives no explicit values.	
Reliability	Low
Endpoint	Glyphosate concentrations in surface waters
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), purity not given, monitoring
Test system and conditions	The basis for the monitoring programme is six rather small catchments that have continuous discharge measurements and water proportional samplings monitored 1995 till 2000. The catchments vary in size from 50 to 680 hectares and the total number of farms varies from 5 to 30. Sampling and analyses described, LOD = 0.01 µg/L.
Statistical design	Number analyzed: 57, no detailed values reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Major et al. (2003)

Title: Concentrations of Glyphosate and AMPA in Sediment Following Operational Applications of Rodeo® to Control Smooth Cordgrass in Willapa Bay, Washington, USA	
Author: W. W. Major III, C. E. Grue, S. C. Gardner, J. M. Grassley	
Reference: Bull. Environ. Contam. Toxicol. 71, 912-918	
Year: 2003	
Results and conclusion: Previous studies in the Bay have examined the fate and persistence of glyphosate and AMPA following an aerial spray of Rodeo® to Spartina clones at half the recommended application rate or repeated band applications. Here, we report concentrations of glyphosate and AMPA in sediment following operational, hand, and aerial applications of Rodeo® to Spartina in the Bay at maximum allowable rates. Sediment concentrations were for Glyphosate: 0.3-16.2 ppm (0 days after application, hand sprayed), < 1.8 ppm (360 days after appl., hand sprayed); 0.04-2.5 ppm (0 days after application, aerial spraying), < 1.8 ppm (360 days after appl., aerial spraying). For AMPA: 0.02-1.7 ppm (0 days after application, hand sprayed), < 0.5 ppm (360 days after appl., hand sprayed); 0.02-1.7 ppm (0 days after application, aerial spraying), < 0.5 ppm (360 days after appl., aerial spraying).	
Proposed action: Not to be considered. Publication deals with a specific event outside EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as publication gives information on concentrations in US sediments.	
Reliability	Low
Endpoint	Glyphosate concentrations in sediments
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate, isopropyl salt (CAS-no: 38641-94-0) and AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Sites being representative for hand spraying of clones were selected. Additionally, aerial spraying was performed. Aerial deposition (on filter paper) measured, sediment cores sampled and analyzed,
Statistical design	Recoveries from filter paper and sediment determined, relatively low recovery (86.7 % for Glyphosate, 78.5 % for AMPA) from sediment taken into account
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Results are valid for that particular place and time; no negative evidence.

Meyer et al. (2011)

Title: Concentrations of dissolved herbicides and pharmaceuticals in a small river in Luxembourg	
Author: Berenike Meyer, Jean-Yannick Pailler, Cédric Guignard, Lucien Hoffmann and Andreas Krein	
Reference: Environ Monit Assess (2011) 180:127–146	
Year: 2011	
Results and conclusion: The pollution is derived from leaching by subsurface flow, as well as wash-off and erosion caused by surface runoff. In the Luxembourgish Mess River catchment, the pharmaceutical and pesticide concentrations are comparable with those detected by other authors in different river systems worldwide. Some investigated pesticide concentrations infringe current regulations. Glyphosate (6,220 ng l^{-1}) and AMPA (1,118 ng l^{-1}) were among the pesticides found in the highest concentrations during flood events in the Mess River. The load of dissolved pesticides reaching the stream gauge is primarily determined by the amount applied to the surfaces within the catchment area. Storm water runoff from urban areas causes short-lived but high-pollutant concentrations and moderate loads, whereas moderate concentrations and high loads are representative for agricultural inputs to the drainage system. Glyphosate and AMPA were found in higher concentrations in urban basins, whereas terbutylazine, metolachlor, atrazine and DEA were prominent in rural zones.	
Proposed action: Data are presented in the summarizing table and discussed in the summarizing paragraph.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Concentrations in surface water
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Monitoring study
Test system and conditions	glyphosate and its main metabolite AMPA were analysed by LC-MS/MS.
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Peruzzo et al. (2008)

Title: Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina
Author: Pablo J. Peruzzo, Atilio A. Porta, Alicia E. Ronco
Reference: Environmental Pollution 156, 61-66
Year: 2008
Results and conclusion: Levels of glyphosate were determined in water, soil and sediment samples from a transgenic soybean cultivation area located near to tributaries streams of the Pergamino-Arrecifes system in the north of the Province of Buenos Aires, Argentina. In the field, levels of glyphosate in waters ranged from 0.10 to 0.70 mg/L, while in sediments and soils values were between 0.5 and 5.0 mg/Kg. Temporal variation of glyphosate levels depended directly on the time of application and the rain events. The results obtained from the application of the model are in accordance with the values found in the field.
Proposed action: Not to be considered. Monitoring data for a site outside the EU (Argentina) are reported.

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, concentrations in a non-EU country measured and compared to a developed model.	
Reliability	Medium
Endpoint	Concentrations in surface water, soil and sediment; model output
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given ("high purity")
Test system and conditions	a) Field work took into account both the pesticide application and the rains occurring after applications. The pesticide was analysed by HPLC-UV detection. b) In addition, SoilFug multimedia model was used to analyse the environmental distribution of the pesticides (suggested for the calculation of predicted environmental concentrations in water, since it generally produces acceptable results from a relatively small set of input data). This method assesses the degradation, evaporation, leaching and runoff of a pesticide applied to a surface soil and consequently its potential impact on nearby water bodies considering the properties of the system in study regarding soil, pesticide and characteristics of the application events (number of events, time of application, dose and rains). The model was loaded with adjusted parameters from runoff and leaching tests at laboratory scale conducted during the development of the research project. The scaling applied to the real situation and the rain and application events recorded specially for this case were considered for the application of this model to the field situation.
Statistical design	See under test system and conditions
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable, model development; no negative evidence.

Peschka et al. (2005)

Title: Trends in Pesticide Transport into the River Rhine
Author: M. Peschka, J.Müller, T. P. Knepper and P. Seel
Reference: Hdb Env Chem Vol. 5, Part L (2006): 155–175; DOI 10.1007/698_5_016
Year: 2005
Results and conclusion: The occurrence of relevant pesticides in the River Rhine and two of its tributaries is presented over a period of ten years. Trace determinations of 66 target pesticides and their metabolites in water from the River Main and the River Nidda were performed on continuously sampled wastewater and surface water utilizing different solid phase extraction protocols and detection by gas chromatography mass spectrometry, directly or after derivatization. The transport rates of pesticides in municipal wastewater treatment plant (WWTP) effluents and surface waters were determined from data obtained in 1994, and these show that WWTPs contribute significantly to the pesticide pollution in the surface water. A trial education program providing improved methodology, spraying equipment and support to farmers living close to a single WWTP lead to a drastic reduction (more than 90 %) in the total pesticide transport caused by this WWTP. During two extensive sampling campaigns in 1999 and 2000, mixed samples from a total of 106 (for 1999) and 35 (for 2000) WWTPs in agricultural used areas from Hesse (Germany) were investigated for selected priority pesticides and metabolites. In this case, the mitigation measures mentioned above were found to be unsuccessful overall, which is most likely attributable to less interaction with the pesticide users as compared to projects in small villages with high public attention.
Proposed action: To be considered by listing in the summarizing table and discussion.

Type of information (critical, high/low weight, supporting, additional): Medium weight; additional information of degradation and sorption isotherms	
Reliability	Medium
Endpoint	Concentration in surface water
Protocol	Not given
Test compound	Glyphosate (CAS no.:1071-83-6) and AMPA (CAS-no.: 1066-51-9)
Test system and conditions	Sampling and analyses in surface water
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence; results supported by other publications.

Robles-Molina et al. (2014)

Title: Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain
Author: J. Robles-Molina, B. Gilbert-López, J. F. García-Reyes, A. Molina-Díaz
Reference: Science of the Total Environment 479–480 (2014) 247–257
Year: 2014
Results and conclusion: The aim of this survey is to monitor a total number of 373 compounds belonging to different families (pesticides, PAHs, nitrosamines, drugs of abuse, pharmaceuticals and life-style compounds) in surface waters located at different points of the province of Jaén (Spain). Among these compounds some priority organic substances (regulated by the EU Directive 2008/105/EC) and pollutants of emerging concern (not regulated yet) can be found. A liquid chromatography electrospray time-off light mass spectrometry (LC–TOFMS) method covering 340 compounds was developed and applied, together with a gas chromatography triple–quadrupole mass spectrometry (GC–MS/MS) method which enabled the analysis of 63 organic contaminants (30 of these compounds are analyzed by LC–TOFMS as well). From April 2009 to November 2010 a total of 83 surface water samples were collected (rivers, reservoirs and wetlands). In this period numerous organic contaminants were detected, most of them at the ng/L level. The most frequently priority substances found were chlorpyrifos ethyl, diuron and hexachlorobenzene. Within the other groups, the most frequently detected compounds were: terbuthylazine, oxyfluorfen, desethyl terbuthylazine, diphenylamine (pesticide family); fluorene, phenanthrene, pyrene (PAHs group), codeine, paracetamol (pharmaceuticals compounds) and caffeine, nicotine (life-style compounds). As is could be expected, the total concentration of emerging contaminants is distinctly larger than that of priority pollutants, highlighting the importance of continuing with the study of their presence, fate and effects in aquatic environments. However, concentration levels (at the ng per liter level) are low in general for both kinds of contaminants which minimizes the possible harmful effect on the environment. Glyphosate concentrations in surface water are not provided in the study.
Proposed action: To be considered as additional information.

Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Concentration of pesticides, PAHs, nitrosamines, drugs of abuse, pharmaceuticals and life-style compounds in surface waters
Protocol	Not given

Test compound	373 compounds belonging to different families (pesticides, PAHs, nitrosamines, drugs of abuse, pharmaceuticals and life-style compounds)
Test system and conditions	A total of 19 sampling points were selected in surface waters located at different points of the province of Jaén (Spain). Representative samples of each point were collected in amber glass bottles with Teflon caps (1 L), and then were transported to the laboratory where they were stored at 4 °C for a maximum of 48 h before extraction. During a period of 20 months, 83 surface water samples were collected comprising 3 rivers, 5 reservoirs and 11wetlandswithin the province of Jaén. The analysis of the samples was carried out by using two different analytical methods depending on the vast array of different physicochemical features of the pollutants tested.
Statistical design	Not given
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Stewart et al. (2014)

Title: A survey of emerging contaminants in the estuarine receiving environment around Auckland, New Zealand

Author: M. Stewart, G. Olsen, C. W. Hickey, B. Ferreira, A. Jelić, M. Petrović, D. Barcelo

Reference: Science of the Total Environment 468–469 (2014) 202–210

Year: 2014

Results and conclusion:

Increasing urbanisation in the future will put mounting stresses on the receiving environments around those urban centres due to increased sedimentation and contaminant runoff. Emerging contaminants (ECs) are an extensive array of chemicals and many are not under regulatory action. Within New Zealand likely future pressures from ECs will be in both urban centres and rural areas due to intensive agriculture, although at present there is a lack of information on the state of the environment in both sectors. This study was initiated to gauge the distribution of ECs in the urban environment by measuring concentrations of flame retardants, plasticisers, alkylphenols, herbicides and pesticides, steroid oestrogens, pharmaceuticals and heavy metals in sediment from 13 estuarine sites around Auckland, New Zealand's biggest city. Total polybrominated diphenyl ether (PBDE) flame retardant concentrations (7ΣPBDE) ranged from 0.55 to 573 ng/g (dw). The phthalate plasticiser di(2-ethylhexyl)phthalate (DEHP) was measured at up to 11,500 ng/g from one site. Nonylphenol (NP) was found at up to 32,000 ng/g at one site adjacent to the city's major wastewater treatment plant (WWTP). However, median concentrations of NP were 153 ng/g, suggesting this site was not representative of the region. Nonylphenolmono- and di-ethoxylates (NPEO1,2) had highest concentrations (1600 ng/g) at a marina. Highest glyphosate concentrations (up to 950 ng/g) were observed at residential sites. Steroid oestrogens were detected at extremely low concentrations (maximum 2.2 ng/g), while all other pesticides or herbicides were not detected at any sites. Multi-residue analysis of 46 pharmaceuticals showed presence of 21 compounds at one or more sites, with average concentrations ranging from 0.16 to 7.66 ng/g. Generally, environmental concentrations of ECs were similar to those reported world-wide. However, comparisons for pharmaceuticals were problematic, due to very few studies on pharmaceutical concentrations in estuarine sediments, with most focussed on sewage and stream water phases.

Glyphosate and AMPA in estuarine sediments from Auckland, New Zealand: Glyphosate was detected at 8 of the 13 sites, ranging from 58 to 950 ng/g with a median value of 120 ng/g. AMPA was detected at 2 sites; Puketutu Island at 345 ng/g and Meola at 215 ng/g. Glufosinate was not detected at any site, with a LOQ of 20 ng/g. The sites with the highest glyphosate concentrations: Meola (950 ng/g); Whau (315 ng/g); and Motions (235 ng/g) are all established residential areas. Other potential inputs to these catchments include industrial (Whau) and sewage & landfill (Meola & Motions). These sites are not unique in their land-use and so it is difficult to assign land-use patterns to potential glyphosate sources. Other sites are residential areas and/or have industrial, sewage and landfill inputs but have much reduced glyphosate concentrations. Due to the high polarity and water solubility of glyphosate and AMPA, their environmental concentrations have been largely restricted to stream water samples (Botta *et al.*, 2009; Glozier *et al.*, 2012; Kolpin *et al.*, 2006; Scribner *et al.*, 2003), while those that do include sediment or soils are centred around the use of glyphosate resistant crops (Mamy *et al.*, 2010; Peruzzo *et al.*, 2008). Furthermore, little data appear to exist on the environmental occurrence of glyphosate and AMPA derived from the extensive urban use of glyphosate (Kolpin *et al.*, 2006). As such, it is difficult to make comparisons between the concentrations found in this study and those observed elsewhere.

Proposed action: Not to be considered as monitoring outside the EU (New Zealand).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	
Endpoint	Concentrations of emerging contaminants in sediment from 13 estuarine sites around Auckland, New Zealand
Protocol	Not given
Test compound	Glyphosate, glufosinate and AMPA amongst other emerging contaminants
Test system and conditions	The study was conducted in Auckland region, New Zealand. Thirteen sites were selected for sampling around the greater Auckland region. The contaminants chosen for analysis were based on information from a review on ECs of potential environmental concern in Auckland (Ahrens, 2008) and the logistics in finding analytical laboratories that provided robust analytical measurements of these contaminants. All analyses were undertaken between April and October 2008, with the exception of pharmaceuticals which were undertaken in May 2010. Sediments were analysed for 3 herbicides - glyphosate, aminomethylphosphonic acid (AMPA; primary breakdown product of glyphosate) and glufosinate amongst other emerging contaminants.
Statistical design	Replicate analyses were undertaken for glyphosate. A relative contamination ranking for each site was calculated based on contaminant concentrations.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Similar monitoring programmes not known. No negative evidence.

Detailed description of open literature – Groundwater monitoring

Bachor et al. (2008)

Title: Special report on detection of pesticides and pharmaceuticals in surface water and ground water in Mecklenburg-Vorpommern (Germany) in spring 2008	
Author: Alexander Bachor, Gabriele Lemke, André Schumann	
Reference: Report of the State Agency for the Environment, Nature Conservation and Geology Mecklenburg-Vorpommern (LUNG)	
Year: 2008	
Results and conclusion: During April 1st and 25.06.2008 samples from 60 surface water (river) and 83 groundwater measuring points in Mecklenburg-Vorpommern were analysed for different pesticides and pharmaceuticals. In 0 of 83 groundwater samples glyphosate was found with concentrations > 0.1 µg/L. AMPA was found in only 1 of 83 samples with concentrations > 0.1 µg/L (0.123 µg/L).	
Proposed action: To be considered in the summarizing table of groundwater monitoring in spite of the small time window. Obtained data are comparable to those given in the summarizing table.	
Type of information (critical, high/low weight, supporting, additional): Low weight (only small time window), may be used as additional information	
Reliability	Low

Endpoint	Concentration in groundwater
Protocol	No information in the report on analysis methods
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given
Test system and conditions	No information in the report, only overview of findings
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The results support the long time monitoring data. However, monitoring studies and campaigns over more years and with more detailed information are of more reliability and relevance. No negative evidence.

Busch and Reupert (LANUV) 2013

Title: Belastungsentwicklung von Oberflächengewässern und Grundwasser in NRW mit Glyphosat und AMPA

Author: Dieter Busch, Rolf Reupert (LANUV)

Reference: Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV)

Year: 2013

Results and conclusion:
Results from a federal monitoring programme in North Rhine-Westphalia, Germany regarding concentrations of glyphosate and AMPA in surface water and groundwater are presented. The results are summarised in the following.

2001-2012:
Concentration of glyphosate in surface water: Ruhr, 3 sites: max. 0.10-0.29 µg/L Concentration of AMPA in surface water: Ruhr, 3 sites: max. 0.86-2.02 µg/L

1996-2012:
Concentration of glyphosate in surface water:
NRW: 1899 samples, 225 samples > 0.1 µg/L (maximum 0.93 µg/L)

1996-2012:
Concentration of AMPA in surface water:
NRW: 1903 samples, 1377 samples > 0.1 µg/L (maximum 13 µg/L)

2006-2012:
Concentration of glyphosate in groundwater:
NRW: 245 samples, 0 samples > 0.1 µg/L (maximum 0.08 µg/L)

2006-2012:
Concentration of AMPA in groundwater:
NRW: 260 samples, 7 samples > 0.1 µg/L (maximum 0.45 µg/L)

Proposed action:
To be considered in the summarizing tables of surface water and groundwater monitoring. Obtained data are comparable to those given in the summarizing table.

Type of information (critical, high/low weight, supporting, additional):
No details are provided in the report about the finding localities and special causes. The information should be considered as additional.

Reliability	
Endpoint	Concentrations of glyphosate and AMPA in surface water and groundwater
Protocol	In-house standard according to ISO 21458; DIN 38407-22
Test compound	Glyphosate and AMPA
Test system and conditions	Monitoring programme
Statistical design	Not given
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

BVL (2010)

Title: Proceedings of the 25th meeting of the consulting committee of natural environment of BVL, 24./25.February 2010	
Author: Consulting committee of natural environment of BVL (Germany)	
Reference: Proceedings of the 25th meeting of the consulting committee of natural environment of BVL, 24./25.February 2010	
Year: 2010	
Results and conclusion: Discussion about glyphosate: Groundwater monitoring in the context of implementation of water framework directive (WRRL) requires improved cooperation with plant protection service. Harmonisation of the assessment approaches of different federal states in Germany regarding glyphosate findings in groundwater is preferable Considering latest research results regarding the pathway bank filtration of glyphosate a new runoff-mitigation measure (minimum: 5 m bare buffer zone) will be required for pesticides containing glyphosate. The discussion is ongoing.	
Proposed action: Not to be considered in the summarizing table of groundwater monitoring. The protocol only contains national management proposals.	
Type of information (critical, high/low weight, supporting, additional): Low weight (German approach), may be used as additional information	
Reliability	Low
Endpoint	None (management measures)
Protocol	Not relevant (proceedings)
Test compound	Not relevant (proceedings)
Test system and conditions	Not relevant (proceedings)
Statistical design	Not relevant (proceedings)
Relevance	
Environmental relevance	Relevant only for German authorities
Weight of evidence	
"Positive"/"Negative" evidence	Relevant only for German authorities

Crowe et al. (2011)

Title: Application of a glyphosate-based herbicide to *Phragmites australis*: Impact on groundwater and near-shore lake water at a beach on Georgian Bay	
Author: Allan S. Crowe, Natalie Leclerc, John Struger, Susan Brown	
Reference: Journal of Great Lakes Research 37, 616–624	
Year: 2011	
Results and conclusion: Groundwater and lake water were tested to determine if glyphosate enters the groundwater and lake at the beach and how long glyphosate will persist. Two days after application, the geometric mean concentration of glyphosate in the groundwater below the Phragmites was 0.060 µg/L with a maximum of 12.50 µg/L. Concentrations rapidly declined over the next two to three weeks to below minimum detection limits (0.020 µg/L). Glyphosate was also detected in the nearshore lake water with concentrations peaking at a geometric mean of 0.14 µg/L one week after application, and declining to 0.039 µg/L four weeks after application. An approximate half-life for the dissipation of glyphosate by degradation and dilution/flushing as groundwater flows toward the lake, assuming a first order kinetic reaction, yielded a half-life of 3.5 d during the 4 weeks after the herbicide was applied. The application of Roundup® resulted in a 90 % reduction in the size of the stand of Phragmites.	
Proposed action: Not to be considered as the monitoring was outside the EU (Canada).	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information.	
Reliability	Medium
Endpoint	Monitoring, concentrations in surface water and groundwater
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) AMPA (CAS-no: 1066-51-9) purity cannot be given, monitoring
Test system and conditions	Sampling campaign described, also clean-up, analytical methods, LOD
Statistical design	Not given
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Haarstad and Ludvigsen (2007)

Title: Ten Years of Pesticide Monitoring in Norwegian Ground Water	
Author: K. Haarstad and G. H. Ludvigsen.	
Reference: Ground Water Monitoring & Remediation 27, no. 3, pp. 75–89	
Year: 2007	
Results and conclusion: Pesticides in Norwegian groundwater have been monitored since 1995. Here, we report data including 2004. The monitoring has focused on shallow groundwater near agricultural fields (4 locations), on farm wells (22 locations), and on public waterworks (38 locations). 450 samples were analyzed for a total of 62 pesticide compounds and metabolites, and the result was 514 detections of single compounds. Though glyphosate has highest use in Norway (ca. 215 tons in 2004) it was detected in only one sample from waterworks, while the metabolite AMPA was detected in one farm well. Low detection is probably due to high adsorption in soils. Glyphosate have frequently been detected in streams in Norway.	
Proposed action: Considered by listing in the summarizing table and discussion. To be used as additional information.	

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	High
Endpoint	Glyphosate concentrations in groundwater, wells and waterworks
Protocol	for details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	Four different locations have been monitored, two of them for 10 years (S1 and S2). S1 and S2 sampling sites are intensively used for crop production and thus are prone for leaching. The mean groundwater depth at S1 is 1.0 m, with extreme values varying from 0.18 to 1.98 m below the surface, and the mean depth at S2 was 0.5 to 0.9 m, with extreme values varying from 0.15 to 1.7 m below the surface. A total of 22 farm wells were selected among volunteers after public advertising. Locations were chosen to represent different geology (rock and soil), well types, and depths, and to include wells with and without influence from point sources. Some wells were sampled only for a short period, while those with frequent detections have been followed over many years to evaluate trends. A total of 144 samples were taken. A total of 38 waterworks were sampled, located in most parts of Norway as far north as the county Nordland.
Statistical design	A Spearman's Rho nonparametric pair wise correlation analysis was carried out, between total pesticide concentrations and the well depths. The analysis included 19 farm wells and 5 wells sampling shallow groundwater. Only locations with at least five detections of pesticides were used, with a level of significance of 5 %. A linear regression trend analysis was also carried out on selected locations (Excel), testing if the angle of the straight regression line is significantly different from zero.
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Kjaer et al. (2009)

Title: The Danish Pesticide Leaching Assessment Programme
Author: Jeanne Kjær, Annette E. Rosenbom, Walter Brüsch, René K. Juhler, Lasse Gudmundsson, Finn Plauborg, Ruth Grant and Preben Olsen
Reference: Geological Survey of Denmark and Greenland
Year: 2011

Results and conclusion:
In 1998, the Danish Parliament initiated the Pesticide Leaching Assessment Programme (PLAP), an intensive monitoring programme aimed at evaluating the leaching risk of pesticides under field conditions. The objective of the PLAP is to improve the scientific foundation for decision-making in the Danish regulation of pesticides. The specific aim is to analyse whether pesticides applied in accordance with current regulations leach to groundwater in unacceptable concentrations. The programme currently evaluates the leaching risk of 42 pesticides and 41 degradation products at five agricultural sites ranging in size from 1.1 to 2.4 ha. The evaluation is based upon monitoring results representing detections in 1 meters depth (water collected via drains and suction cups) and detections in groundwater monitoring screens (1.5-4.5 meter below ground surface, hereafter m b.g.s.). This report presents the results for the entire monitoring period May 1999-June 2010. The results of the entire monitoring period 1999-2010 covering 42 pesticides, show that: The monitoring data indicate pronounced leaching of 14 of the applied pesticides and/or their degradation products. Glyphosate and its degradation product AMPA leached through the soil entering drains and suction cups (placed 1 m b.g.s) in average concentrations exceeding 0.1 µg/l. For glyphosate and AMPA, pronounced leaching is mainly confined to the depth of 1 meter, where pesticides were frequently found in samples collected from drains and suction cups, while a limited number of detections exceeding 0.1 µg/l were found in groundwater monitoring wells. In the following the maximum concentration (µg/l) from the groundwater monitoring screens are given:
Glyphosate Tylstrup:-Jyndevad: n.d. Silstrup: 0.031 µg/l
Estrup: 0.67 µg/l
Faardrup: 0.017 µg/l
AMPA
Tylstrup:-Jyndevad: 0.022 µg/l
Silstrup: 0.08 µg/l
Estrup: 0.07 µg/l
Faardrup: 0.029 µg/l
Numbers of glyphosate detections exceeding 0.1 µg/l in groundwater monitoring wells are very limited (only 3 samples).

Proposed action:
To be considered in the summarizing table of groundwater monitoring. Obtained data are comparable to those given in the summarizing table.

Type of information (critical, high/low weight, supporting, additional):
To be used as supporting information.

Reliability	
Endpoint	Concentration of glyphosate and AMPA in groundwater at agricultural sites
Protocol	No information
Test compound	Glyphosate and AMPA
Test system and conditions	Monitoring programme aimed at evaluating the leaching risk of pesticides under field conditions
Statistical design	Statistical analysis of the internal QA data: The statistical tool used is an analysis of variance (ANOVA) and encompasses all duplicate pesticide analyses, single analyses being excluded. The analysis can be divided into three stages: 1) Normality, 2) Between-day contribution, 3) Calculating standard deviations
Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Krause et al. (2009)

Title: Organic Trace Substances Relevant for Drinking Water – Assessing their Elimination through Bank Filtration	
Author: Björn Krause, Astrid Weigert, Stefan Heise, Norbert Litz	
Reference: in: Report of the 2nd experimental phase of the TRACE-project; Copyright 2009 by the Kompetenz Zentrum Wasser Berlin gGmbH.	
Year: 2009	
Results and conclusion:	
To estimate the occurrence of glyphosate and its main metabolite AMPA in the surroundings of Berlin samples from 22 surface water sites were analysed within this study. In 5 samples the glyphosate concentration was above the European threshold for herbicides of 0.1 µg/L in drinking water. Up to 70 % of Berlin's drinking water is produced via bank filtration and aquifer recharge characterized by comparatively low flow velocities (< 1 m/d), long contact times (3-6 months) and mainly anoxic redox conditions. Results of enclosures show that the breakthrough of glyphosate was retarded remarkably despite of the initially postulated low adsorption potential of the sandy filter substrate. Also a significant reduction, probably due to degradation was observed. However, adsorption and degradation parameters obtained in the laboratory and semi-technical experiments vary significantly due to the difficulty to imitate natural conditions in the laboratory.	
Proposed action: Not to be considered as publication deals with a specific situation. It furthermore addresses the laboratory to field extrapolation problems.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	High
Endpoint	Glyphosate concentrations in surface water around Berlin, in enclosure outlets, K_f-values, DT_{50}-values, break-through curves
Protocol	For details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), purity 98.7 %
Test system and conditions	a) surface water sampling around Berlin and analysis b) evaluation of the potential of bank filtration to protect the drinking water from glyphosate contaminations: Three enclosures dosed with three different concentration levels (average concentration: 0.7, 3.5 and 11.6 µg/L) over a time period of 14 days. The effluent was sampled daily for 34 days. Glyphosate and AMPA were analysed applying the HPLC method according to the German Standard DIN 38407-22/2001. c) laboratory column leaching (OECD 312), sorption (batch, OECD 106) and degradation (in sediment, similar to OECD 307, 8°C) studies
Statistical design	Freundlich isotherms, 1. order degradation kinetics, break-through curves,
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar experiments. However, results are valid for that particular place and time; no negative evidence.

Lindqvist et al. (2007)

Title: Om förekomst av bekämpningsmedelsrester i grundvatten: Erfarenheter från Simrishamn kommun 2002-2007 (Monitoring of pesticides in rawwater wells in Simrishamn)
Author: Bengt-Olov Lindqvist, Jan-Bertil Hansson, Christina Jönsson and Kenneth M. Persson
Reference: Vatten 63:159–163. Lund 2007
Year: 2007
Results and conclusion: In January 2007, all municipal water wells of Simrishamn, south Sweden, were sampled and analyzed for the presence of pesticide residuals. In total 34 wells were analyzed. The samples were analyzed at an accredited laboratory with respect to 77 different parameters of pesticides which either are used or have been used in the recharge area of the wells. The results from the investigation 2007 show that residuals of pesticides were detected in 12 different water wells distributed across the municipality. For virtually all analyses, the concentrations reported are very low, in the order of 0.01 µg/l, and only one or occasionally two parameters were found in each sample. The same investigation was undertaken in 2002. Compared with those results, an important finding is that more parameters were found in 2007 compared with 2002. The overall concentration of residuals has not changed since 2002. The concentrations are not higher but not lower either. The fundamental conclusion is that the work with water protection areas and the control of pesticide spread is still necessary and inevitable. Maximum glyphosate concentration reported: 0.08 µg/L
Proposed action: Not considered as the text written in Swedish language, thus the study cannot be fully evaluated. The results seem to be in the same range as other similar monitoring studies.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information

Reliability	High
Endpoint	Glyphosate concentrations in groundwater, wells and waterworks
Protocol	for details see under test system and conditions, non-GLP
Test compound	Glyphosate (CAS-no: 1071-83-6), AMPA (CAS-no: 1066-51-9), purity not given, monitoring
Test system and conditions	? (text in Swedish language)
Statistical design	? (text in Swedish language)
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Text written in Swedish language, thus the study cannot be fully evaluated. The results seem to be in the same range as other similar monitoring studies; no negative evidence.

Malaguerra et al. (2010)

Title: CONTAMINATION OF DRINKING WATER SUPPLY WELLS BY PESTICIDES FROM SURFACE WATER RESOURCES
Author: Flavio Malaguerra, Hans-Jørgen Albrechtsen and Philip J. Binning
Reference: Presentation at: XVIII International Conference on Water Resources, CMWR 2010, J. Carrera (Ed), ® CIMNE, Barcelona
Year: 2010

Results and conclusion: A reactive transport model is developed to evaluate the potential of contamination of drinking water wells by surface water pollution. The model is validated using data of a tracer experiment. The fate of MCPP, glyphosate and its degradation product AMPA is investigated. Global sensitivity analysis using the Morris method is used to identify model dominant parameters. Results show that the existence of a clay aquifer, pollutant properties and the well depth are the crucial factors when evaluating the risk of drinking water well contamination from surface water.	
Proposed action: Considered by listing in the summarizing table and discussion. To be used as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, model development	
Reliability	Not applicable
Endpoint	Model development, no experimental study
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, model development using published data
Test system and conditions	A conceptual model has been developed and applied using published experimental data. The sensitivity analysis is performed using Morris method. The Morris method is a global sensitivity analysis method, determining the sensitivity over the whole parameter space. The method determines elementary effects for each input. Parameters are varied one at a time, and for every change the model is evaluated: the elementary effect is then defined to be the output change divided by the input change. The distribution of elementary effects is evaluated for the parameter space and the mean and the standard deviation of the elementary effects are used as sensitivity measures.
Statistical design	See under test system and conditions
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable, model development; no negative evidence.

Malaguerra et al. (2012)

Title: Pesticides in water supply wells in Zealand, Denmark: A statistical analysis
Author: Flavio Malaguerra, Hans-Jørgen Albrechtsen, Lærke Thorling and Philip J. Binning
Reference: Science of The Total Environment 414: 433-444. doi:10.1016/j.scitotenv.2011.09.071
Year: 2012
Results and conclusion: Data from the Danish National Borehole Database are used to predict drinking water well vulnerability to contamination by pesticides, and to identify the dominant mechanisms leading to well pollution in Zealand, Denmark. The frequency of detection and concentrations of 4 herbicides and 3 herbicide metabolites are related to factors accounting for geology (thicknesses of sand, clay and chalk layers), geographical location (distance to surface water and distance to contaminated sites), redox conditions and well depth using logistic regression, the binomial test and Spearman correlation techniques. Parameters accounting for the hydraulic connection between the well and the surface (well depth and thickness of the clay confining layer) are often strongly related to well vulnerability. Results also show that wells close to surface water are more vulnerable to contamination, and that sandy layers provide better protection against the leaching of oxidizable pesticides than clay aquitards, because they are more likely to be aerobic The field data are used to create a set of probabilistic models to predict well vulnerability to contamination by pesticides.
Proposed action: Considered by listing in the summarizing table and discussion. To be used as additional information.

Type of information (critical, high/low weight, supporting, additional): Low weight, additional information, predictive model development	
Reliability	Not applicable
Endpoint	Predictive model development, no experimental study
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Glyphosate, (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), purity not given, model development using published data
Test system and conditions	The data base for Glyphosate was: 289 wells, 707 samples (Zealand), 44 wells, 108 samples (Jutland); for AMPA: 286 wells, 691 samples (Zealand), 44 wells, 108 samples (Jutland).
Statistical design	Spearman rank correlation was used to delineate trends between the parameters (D, Ds, Dcl, Dch, dsw, dcs) and pesticide concentrations. Spearman correlation was selected in order to consider non-linear responses and because the data were not normally distributed. Correlations were calculated for two separate datasets: to all drinking water wells and to the drinking water wells where the amount of pesticides detected was above the detection limit. This last dataset was used to avoid the bias caused by the points where the pesticide was detected but could not be quantified.
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable, model development; no negative evidence.

Mörtl et al. (2013)

Title: Determination of glyphosate residues in Hungarian water samples by immunoassay	
Author: Mária Mörtl, Gyöngyi Németh, Judit Juracsek, Béla Darvas, Lisa Kamp, Fernando Rubio and András Székács	
Reference: Microchemical Journal 107 (2013) 143–151	
Year: 2013	
Results and conclusion: An enzyme-linked immunosorbent assay (ELISA) for the detection of glyphosate was investigated for assay performance characteristics and was applied for determination of glyphosate contamination levels in selected surface and ground water resources in Hungary in 2010 and 2011. The method was applied for the analysis of 42 surface and ground water samples collected from Békés county in Hungary at 14 sampling sites in 2010 and 18 surface water samples collected from the Danube River and Lake Velencei in Hungary at 12 sampling sites in 2011. Exceedingly high glyphosate levels (nearly 1 ng/ml) were measured in 5 samples, and significant concentrations were determined in 16 cases (0.54–0.76 ng/ml) in 2010, while practically no contamination was found in 2011. The great contrast between the two sampling regimes is explained by differing agricultural locations, natural precipitation and, to a greater extent, catchment area characteristics, resulting in varying leaching or run-off of glyphosate to surface waters.	
Proposed action: Considered by listing in the summarizing table and discussion. To be used as additional information.	
Type of information (critical, high/low weight, supporting, additional):	
Low weight, additional information, model development	
Reliability	Medium
Endpoint	Glyphosate concentrations in groundwater and surface water
Protocol	Not applicable, see also under test system, non-GLP
Test compound	Monitoring study

Test system and conditions	In the scope of a national environmental survey, 42 water samples (6 surface water and 36 ground water samples) were obtained on September 7–8, 2010, from 14 sampling sites in Békés county, Hungary. In addition, 18 surface water samples were collected on October 1, 2011, from 11 sampling sites along the Danube River and one site at Lake Velencei, Hungary.
Statistical design	See under test system and conditions
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar studies. However, results are valid for that particular place and time; no negative evidence.

Riedl et al. (2005)

Title: Sickerwasserversuche an der Forschungsstation Wagna zur Untersuchung der Verlagerung des Herbizids Glyphosate in der ungesättigten Bodenzone	
Author: Hans-Erik Riedl and Heimo Stadlbauer	
Reference: Amt der Steiermärkischen Landesregierung Fachabteilung 17C Technische Umweltkontrolle und Sicherheitswesen, Dokumentation zum Thema Gewässerschutz	
Year: 2005	
Results and conclusion: (study is written in German language) Die Versuche beim Lysimeter in Wagna zeigten, dass unter den hydrometeorologischen Rahmenbedingungen, die dem langjährigen Mittel entsprechen (Sättigung des Bodens über die Wintermonate; intensive Frühjahrsniederschläge in April und Mai), eine noch raschere Verlagerung von Glyphosate und AMPA in höheren Konzentrationen bis in den Kiesbereich nicht ausgeschlossen werden kann. Dies vor allem dann, wenn der Wirkstoff kurz nach – oder noch schlechter, weil nicht vorhersehbar, kurz vor einem intensiven Niederschlagsereignis ausgebracht wird.	
Proposed action: Not considered for listing in the summarizing table and discussion. To be used as additional information.	
Type of information (critical, high/low weight, supporting, additional): High weight, additional information	
Reliability	Medium
Endpoint	Glyphosate concentrations in groundwater
Protocol	Non-GLP
Test compound	Roundup-Ultra
Test system and conditions	Die Verlagerung des Wirkstoffes Glyphosate bzw. dessen Metabolit AMPA wurde unter ortsüblichen Bewirtschaftungsweisen bei den hier herrschenden meteorologischen Rahmenbedingungen und den existenten Boden- und Fruchtfolgebedingungen detailliert untersucht und schlüssige Aussagen hinsichtlich einer potentiellen Gefährdung des Grundwassers der quartären Talfüllungen des Murtales durch den Einsatz des angeführten Herbizids abgeleitet.
Statistical design	Not reported
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Supported by other similar studies. However, results are valid for that particular place and time; no negative evidence.

Van Stempvoort et al. (2013)

Title: Residues of the herbicide glyphosate in riparian groundwater in urban catchments

Author: D.R. Van Stempvoort, J.W. Roy, S.J. Brown, G. Bickerton

Reference: Chemosphere 95 (2014) 455–463

Year: 2013

Results and conclusion:
The herbicide glyphosate and its putative metabolite aminomethylphosphonic acid (AMPA) have been found in urban streams, but limited information is available on their presence in urban riparian groundwater. Information is also lacking regarding the source of AMPA in these urban settings (glyphosate metabolite or wastewater), and whether, if present, glyphosate residues in urban riparian groundwater contribute significantly to urban streams. Glyphosate and AMPA were detected in shallow riparian groundwater at 4 of 5 stream sites in urban catchments in Canada and each were found in approximately 1 in 10 of the samples overall. Frequency of observations of glyphosate and AMPA varied substantially between sites, from no observations in a National Park near the Town of Jasper Alberta, to observations of both glyphosate and AMPA in more than half of the samples along two short reaches of streams in Burlington, Ontario. In these two catchments, AMPA was correlated with glyphosate, rather than the artificial sweetener acesulfame, suggesting that the AMPA is derived mainly from glyphosate degradation rather than from wastewater sources. Land use, localized dosage history, depth below ground and other factors likely control the occurrence of detectable glyphosate residues in groundwater.
Detections of glyphosate and AMPA in samples of riparian groundwater (2009):
All data:

Substance	Number of samples	With detections (%)	Maximum concentration [ng/L]
Glyphosate	281	37 (13.2 %)	42
AMPA		33 (11.7 %)	2870

Proposed action:
Not to be considered as the monitoring was outside the EU (Canada).

Type of information (critical, high/low weight, supporting, additional):
Low weight, additional information.

Reliability	
Endpoint	Glyphosate and AMPA concentrations in samples of riparian groundwater
Protocol	Not given
Test compound	Glyphosate and AMPA
Test system and conditions	Study sites: At Burlington there are many small streams that drain through urban areas into Lake Ontario. Groundwater was sampled in June 2009 along two of these streams: Tuck Creek and Shoreacres Creek. Groundwater samples were also collected in the catchments of two streams in the City of Barrie that drain into Lake Simcoe: along Dyment's Creek in September 2009 and along Hewitt's Creek in October 2009. Furthermore, groundwater was collected along two reaches of the Athabasca River in Jasper National Park in August 2009. Sampling and analysis: All of the groundwater samples from this study were collected at shallow depths within 2 m of the edge of the streams, usually within the streambed but occasionally on the shore. Groundwater samples were collected from depths of generally 0.25–1.0 m below the ground or streambed surface using a drive-point miniprofiler connected to a peristaltic pump. An ion chromatography electrospray ionization triple quadruple mass spectrometry (IC/MS/MS) was used to analyze glyphosate and aminomethylphosphonic acid (AMPA). The artificial sweetener acesulfame (as a wastewater indicator) was analyzed using an IC/MS/MS method.

Statistical design	In the study, the authors encountered many non-detections of both glyphosate and AMPA. For this reason, the statistical analyses were restricted to the Burlington datasets where the paired analytes were both present in the majority of the samples. For correlation analyses of these compounds, the authors used Minitab 16 (Minitab Inc., State College, PA, USA) to calculate Spearman rank correlation coefficients (q). The standard approach could be used because for each test, each analyte had a single detection (reporting) limit: all non-detections were given the same rank, and all non-quantifiable trace detections were ranked together immediately above the non-detections.
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

References

Off-side movement

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Detailed description of open literature – Other studies

Aisha et al. (2009)

Title: Kinetics of Reduction of Colloidal MnO_2 by Glyphosate in Aqueous and Micellar Media
Author: Aisha, U., Qamruzzaman, Rafiquee, M.Z.A.
Reference: International Journal of Inorganic Chemistry, Volume 2011
Year: 2011
Results and conclusion: The kinetics of the reduction of colloidal MnO_2 by glyphosate has been investigated spectrophotometrically in an aqueous and micellar (cetyltrimethylammonium bromide, sodium lauryl sulfate) media. The reaction follows first-order kinetics with respect to colloidal MnO_2 in both the aqueous and micellar media. The rate of oxidation increases with increase in [glyphosate] in the lower concentration range but becomes independent at its higher concentrations. The addition of both the anionic (NaLS) and cationic (CTAB) micelles increased the rate of reduction of colloidal MnO_2 by glyphosate while the nonionic TX-100 micelles did not influence the rate of reaction. In both aqueous and micellar media, the oxidation of glyphosate occurs through its adsorption over colloidal MnO_2 surface. The reaction in micellar media was treated by considering the pseudophase model. The values of reaction rates and binding constants in the presence of micelles were determined.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Non-Labelled glyphosate (CAS 38641-94-0)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Akamatsu et al. (2014)

Title: Evaluation of glyphosate application in regulating the reproduction of riparian black locust (Robinia pseudoacacia L.) after clear-cutting, and the possibility of leaching into soil	
Author: F. Akamatsu, M. Makishima, Y. Taya, S. Nakanishi, J. Miwa	
Reference: Landscape Ecol Eng (2014) 10:47–54, DOI 10.1007/s11355-013-0215-x	
Year: 2014	
Results and conclusion: Black locust (Robinia pseudoacacia L.)—an invasive alien species in riparian forests—is becoming more prevalent in many rivers of eastern Japan. Riparian black locust forests are typically cut down to maintain river-flow capacity. However, such forests often reproduce rapidly by stump sprouting and root suckering and regenerate by germination. Thus, more effective riparian forest management approaches are required. To regulate the reproduction of black locust forests after clear-cutting, we examined the regrowth-inhibiting effects of glyphosate herbicide application to stumps, in accordance with current river management protocol (i.e., winter logging operation). Further, we investigated the concentrations of glyphosate leaching into the soil at a depth of 30 cm in a riparian area of the Tenryu River. Our results showed that glyphosate application to stumps completely inhibited stump sprouting but not root suckering or seedling germination. The glyphosate concentration leaching into the soil reached a maximum (2.6 ± 0.7 mg/kg, mean ± standard error) on day 1 after the application, and subsequently declined to below the detection limit on day 2. Thus, the rapid degradation of glyphosate was confirmed, despite the fact that the herbicide leached into the soil after application to the stumps. The glyphosate application has limited effectiveness against root suckering and germination of riparian black locust forests after clear-cutting in winter, in accordance with the current river management protocol.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup Maxload, Nissan Chemical Industries Ltd., Tokyo, Japan)
Test system and conditions	Not applicable
Statistical design	Not applicable

Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Albrecht et al. (2012)

Title: RR soybean seed quality after application of glyphosate in different stages of crop development	
Author: Leandro Paiola Albrecht, André Prechlak Barbosa, André Felipe Moreira Silva, Matheus Akiyama Mendes, Alfredo Júnior Paiola Albrecht, Marizangela Rizzatti Ávila	
Reference: Revista Brasileira de Sementes (2012) Vol. 34 (3): 373-381	
Year: 2012	
Results and conclusion: This study was aimed at assessing physiological quality, as well as the seed health quality, of the transgenic soybean, cv. CD 219RR, produced under the use of glyphosate applied in different phenological stages of the soybean crop. The authors stated that the herbicide glyphosate can negatively affect the physiological quality of RR soybean seeds, cultivar 219RRCD, when applied in doses ranging from 1,440 to 2,880 g acid equivalent per hectare during the stage of vegetative development V6 and reproductive stage R2 of the soybean crop.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Alletto et al. (2009)

Title: Tillage management effects on pesticide fate in soils. A review	
Author: Lionel Alletto, Yves Coquet, Pierre Benoit, Djilali Heddadj, Enrique Barriuso	
Reference: Agron. Sustain. Dev. (2009) 10.1051/agro/2009018	
Year: 2009	
Results and conclusion: Major findings of the review were: Pesticide interception is enhanced under conservation tillage practices.Pesticide retention, which is generally positively correlated with organic carbon content, is increased in the topsoil layer under conservation tillage. Transport of pesticides is affected by tillage management and by its interactions with climatic conditions	

Proposed action: Consider as additional information. PEC_{GW} given as % of applied dose is reported but no comprehensive raw data are presented. Recalculation of endpoints on degradation or sorption is not necessary.	
Type of information (critical, high/low weight, supporting, additional): Additional	
Reliability	
Endpoint	PEC_{GW}, but given as % of applied dose
Protocol	Review article
Test compound	Non-Labelled glyphosate (CAS 38641-94-0) and AMPA (CAS: 74344-63-2)
Test system and conditions	No single test system
Statistical design	Not reported
Relevance	
Environmental relevance	Low relevance (concentrations are not reported)
Weight of evidence	
"Positive"/"Negative" evidence	The results cannot be compared with other studies as the endpoints were not calculated adequately.

Aslam et al. (2013)

Title: Adsorption and desorption behavior of selected pesticides as influenced by decomposition of maize mulch
Author: Sohaib Aslam, Patricia Garnier, Cornelia Rumpel, Serge E.Parent and Pierre Benoit
Reference: Chemosphere 91 (2013) 1447–1455
Year: 2013
Results and conclusion: Assessing pesticide fate in conservation agricultural systems requires adetailed understanding of their interaction with decomposing surface crop residues (mulch). Adsorption and desorption behavior of glyphosate, s-metolachlor and epoxiconazole was investigated on maize mulch residues decomposed under laboratory and field conditions. Our conceptual approach included characterization of chemical composition and hydrophobicity of mulch residues in order to generate parameters to predict sorption behavior. Adsorption of s-metolachlor and epoxiconazole greatly increased with mulch decomposition, whereas glyphosate adsorption was less affected but its desorption was increased. Mulch characteristics including aromaticity, hydrophobicity and polarity indices were strongly correlated to K_{OC} of the non-ionic pesticides. A predictive model based on compositional data (CoDa)analysis revealed that the sorption capacity of decomposing mulch can be predicted from descriptors such as aromatic and alkyl corresponding respectively to lignin and NDF biochemical fractions.The decomposition degree of mulch residues should be taken into account while predicting the fate of pesticides. Adsorption isotherms on mulch residues (0, 150 and 300 d) were described by the Freundlich model with $R^2 \geq 0.998$ for s-metolachlor and epoxiconazole and $R^2 \geq 0.989$ for glyphosate. All s-metolachlor and epoxiconazole isotherms were almost linear ($n \geq 0.9$) whereas glyphosate isotherms for initial mulch and mulch sampled after 150 dof field exposure were non-linear ($n < 0.9$). Adsorption coefficients ($K_{f/oc}$) were significantly different ($P < 0.01$) for the three molecules. Glyphosate was least adsorbed while epoxiconazole was most strongly adsorbed followed by S-metolachlor. Linear isotherms allowed estimating adsorption coefficients (K_d and K_{OC}) at single concentration of 0.75 mg/L for all mulch residues (Table 2). We did not observe a significant effect of mulch decomposition degree on the adsorption behavior of glyphosate and almost similar adsorption coefficients were recorded for all mulch residues ranging from 24 to 23 and 30 L/kg for maize residues decomposed under laboratory and field conditions respectively.
Proposed action: Not to be considered for the endpoint sorption and mobility. Raw data on mass balances and test item concentrations in the aqueous and solid phases are not reported. Though the study is plausible, the validity cannot be proven. Moreover, the adsorption on mulch is not a parameter considered relevant for the environmental risk assessment.
Type of information (critical, high/low weight, supporting, additional): Low weight, supportive information

Reliability	Low
Endpoint	K_f and K_{foc}
Protocol	Modified OECD 106, non-GLP
Test compound	^{14}C-labeled Glyphosate (purity not given)
Test system and conditions	Individual solutions of each pesticide were prepared in 0.01 M $CaCl_2$. All solutions were prepared by using both ^{14}C-labeled and unlabeled molecules to achieve the desired radioactivity. Adsorption isotherms with mulch decomposed for 0, 150 and 300 d were conducted with concentrations of 0.2, 0.4, 0.75, 1.5 and 3 mg/L for each molecule whereas intermediate concentration of 0.75 mg/L was selected to study adsorption on all other mulch samples. Centrifuge tubes with sorbents and pesticide solutions were rotated during 24 h with an end-over-head shaker and then centrifuged at 6000 g (Sorvall Evolution RC, Kendro) for 15 min. Radioactivity in the supernatants was measured by scintillation counting.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Positive evidence, no negative evidence.

Bai et al. (2014)

Title: Dissolved organic phosphorus use by the invasive freshwater diazotroph cyanobacterium, *Cylindrospermopsis raciborskii*	
Author: Fang Bai, Rui Liu, Yanjun Yang, Xiaofei Ran, Junqiong Shi, Zhongxing Wu	
Reference: Harmful Algae 39 (2014) 112–120	
Year: 2014	
Results and conclusion: This study examines the physiological responses of the diazotrophic cyanobacteria, *Cylin-drospermopsis raciborskii*, to different dissolved organic phosphorus (DOP) compounds to explore mechanisms of environmental acclimation in this invasive species. Our results show that the specific growth rates of *C. raciborskii* cells in media treated with b-glycerol phosphate, D-glucose-6-phosphate, and (2-aminoethyl)-phosphinic acid were significantly higher than those of cells grown in phosphorus free media. We observed that maximal net photosynthesis was highest when cells were cultured with D-glucose-6-phosphate and lowest when cells were cultured with glyphosate. Similarly, rates of photosynthetic activity (maximum quantum yield, maximum electron transport rate, and photosynthetic efficiency) were observed to be highest in media treated with D-glucose-6-phosphate. We report that rates of alkaline phosphatase activity to the different organophosphates tested changed markedly in response to the concentration of dissolved inorganic phosphorus (DIP); a result supported by the amount of green fluorescent products revealed by ELF197 phosphate dye (ELFP) and gene up-regulation for alkaline phosphatase (phoA). Our results indicate that *C. raciborskii* is able to use different organic phosphorus to support its growth when phosphorus is limited. In addition, we show that C. raciborskii has a higher availability to phosphate (C–O–P) than phosphonate (C–P). The results suggest that the strategic flexibility to environmental phosphorus might play an important role in the domination of *C. raciborskii*.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate purchased from Sigma–Aldrich (USA) (>99 %)

Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bailey et al. (2002)

Title: Glyphosate Interactions with Manganese	
Author: WILLIAM A. BAILEY, DANIEL H. POSTON, HENRY P. WILSON, and THOMAS E. HINES	
Reference: Weed Technology. Volume 16:792–799	
Year: 2002	
Results and conclusion: Field experiments were conducted on the Eastern Shore of Virginia from 1999 to 2001 to evaluate the effects of tank mixture applications of isopropylamine or trimethylsulfonium salts of glyphosate with two liquid formulations of manganese (Mn lignin or Mn chelate) on spray solution pH and weed control in glyphosate-resistant soybean. Additions of manganese to herbicide solutions resulted in a reduction in the acidifying effects of the herbicides as well as in the control of common lambsquarters, large crabgrass, morningglory spp., and smooth pigweed. Reduced control caused by manganese could be overcome with higher rates of the herbicides on some species, but reduced control of common lambsquarters was seen when manganese was included with any herbicide application rate. For most species, Mn chelate caused a greater reduction in control than did Mn lignin. Although manganese caused significant decreases in weed control, soybean yield was not influenced by glyphosate salt, application rate, or manganese. Reduced weed control caused by the addition of manganese to herbicide solutions may be due to the complexing of the herbicide formulations, which could result in the formation of insoluble salt complexes that are not readily absorbed through the plant cuticle, resulting in decreased glyphosate phytotoxicity.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (isopropylamine salt) was Roundup Ultra®, marketed by Monsanto Company, 800 N; CAS-no.: 38641-94-0. Trimethylsulfonium salt of glyphosate was Touchdown 5®, marketed by Zeneca Ag. Products, 1200 S.Glyphosate, CAS-no.: 81591-81-3
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Baker et al. (2014)

Title: THE DIRECT AND INDIRECT EFFECTS OF A GLYPHOSATE-BASED HERBICIDE AND NUTRIENTS ON CHIRONOMIDAE (DIPTERA) EMERGING FROM SMALL WETLANDS
Author: L. F. BAKER, J. F. MUDGE, J. E. HOULAHAN, D. G. THOMPSON and K. A. KIDD
Reference: Environmental Toxicology and Chemistry, Vol. 33, No. 9, pp. 2076–2085, 2014
Year: 2014
Results and conclusion: Laboratory and mesocosm experiments have demonstrated that some glyphosate-based herbicides can have negative effects on benthic invertebrate species. Although these herbicides are among the most widely used in agriculture, there have been few multiple-stressor, natural system–based investigations of the impacts of glyphosate-based herbicides in combination with fertilizers on the emergence patterns of chironomids from wetlands. Using a replicated, split-wetland experiment, the authors examined the effects of 2 nominal concentrations (2.88 mg acid equivalents/L and 0.21 mg acid equivalents/L) of the glyphosate herbicide Roundup WeatherMax, alone or in combination with nutrient additions, on the emergence of Chironomidae (Diptera) before and after herbicide-induced damage to macrophytes. There were no direct effects of treatment on the structure of the Chironomidae community or on the overall emergence rates. However, after macrophyte cover declined as a result of herbicide application, there were statistically significant increases in emergence in all but the highest herbicide treatment, which had also received no nutrients. There was a negative relationship between chironomid abundance and macrophyte cover on the treated sides of wetlands. Fertilizer application did not appear to compound the effects of the herbicide treatments. Although direct toxicity of Roundup WeatherMax was not apparent, the authors observed longer-term impacts, suggesting that the indirect effects of this herbicide deserve more consideration when assessing the ecological risk of using herbicides in proximity to wetlands.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup WeatherMax (glyphosate)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Balci (2009)

Title: Decontamination of Aqueous Glyphosate, (Aminomethyl) phosphonic Acid, and Glufosinate Solutions by Electro-Fenton-like Process with Mn^{2+} as the Catalyst
Author: BEYTÜL BALCI, MEHMET A. OTURAN, NIHAL OTURAN, AND IGNASI SIRÉS
Reference: J. Agric. Food Chem. 2009, 57, 4888–4894
Year: 2009

Results and conclusion: The ability of the modified electro-Fenton-like (EF-like) process to degrade aqueous solutions of glyphosate, which is the most widely used herbicide in the world, has been assessed with Mn^{2+} and other metal ions as catalysts to overcome the problems posed by some stable metal ion complexes of phosphonate herbicides. Bulk electrolyses with a carbon-felt cathode and Pt anode were performed in an undivided cell under galvanostatic conditions to study the effect of the applied current as well as Mn^{2+} and glyphosate concentrations. The herbicide was completely destroyed in all cases following a pseudo first-order kinetics, and the second-order rate constant for its reaction with •OH was determined. The decay trends obtained by high-performance liquid chromatography-fluorometric detection (HPLC-FL) and ion chromatography analysis were similar. AMPA [(aminomethyl)phosphonic acid] was the major reaction intermediate and showed slower pseudo first-order destruction kinetics. The high mineralization degree obtained for glyphosate solutions confirmed the great performance of the EF-like process with Mn^{2+}, which promotes the C-N cleavage by •OH attack as the first oxidation step and the C-P cleavage in a further step. High-level decontamination achieved for AMPA and glufosinate solutions corroborated the benefits of this oxidation process.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; glufosinate-ammonium, CAS-no.: 77182-82-2; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Balthazor and Hallas (1986)

Title: Glyphosate-Degrading Microorganisms from Industrial Activated Sludge	
Author: Balthazor, T.M. and Hallas, L.E.	
Reference: Applied and Environmental microbiology, Feb. 1986, Vol. 51, No. 2, 432-434	
Year: 1986	
Results and conclusion: A plating medium was developed to isolate N-phosphonomethylglycine (glyphosate)-degrading microorganisms, with glyphosate as the sole phosphorus source. Two industrial biosystems treating glyphosate wastes contained elevated microbial counts on the medium. One purified isolate metabolized glyphosate to aminomethylphosphonic acid, mineralizing this accumulating intermediate during log growth. This microorganism has been identified as a *Flavobacterium* species.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6

Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Basso et al. (2011)

Title: Foliar application of manganese in transgenic soybean tolerant to glyphosate	
Author: Claudir José Basso, Antônio Luis Santi, Fabiane Pinto Lamego, Eduardo Girotto	
Reference: Ciência Rural, v.41, n.10, p.1726-173	
Year: 2011	
Results and conclusion: The yellowing of Roundup Ready soybean after glyphosate application can be associated to a momentary manganese deficiency. Because of that, with the hypothesis that glyphosate tolerant soybean would need supplementary addition of manganese; the objective of this research was to evaluate different managements in the foliar application of manganese in some soybean parameters. It was developed two experiments, one at Taquaruçú do Sul and other at Boa Vista das Missões, RS in the year of 2009/2010. It was tested the following treatments: 1) without glyphosate application with manual weed control and without manganese foliar application (untreated check); 2) without glyphosate application with manual weed control and one manganese foliar application at 7 days after this manual weed control; 3) with glyphosate application and without manganese foliar application; 4) glyphosate application in mixture with manganese; 5) glyphosate application added of one manganese foliar application at 7 days after glyphosate application; 6) glyphosate application added of manganese foliar application split in two times, at 7 and 14 days after glyphosate application; 7) glyphosate application and one of manganese foliar application at 14 days after glyphosate application. The glyphosate application was realized in the V5 soybean stage, using 720 g/L i.e., while the used dose of Mn was 2.0 L/ha of a formulation with 14 % (m/v) of Mn. There were no significant difference among the treatments to plant height and height insertion of the first legume. The glyphosate application did not affect the absorption and the foliar amount of manganese and nitrogen in soybean crop. Even with the increase in foliar manganese amount, there was no increasing in soybean productivity. This shows that in soils with Mn levels above of the sufficient, it is not necessary foliar manganese addition in genetically modified soybean tolerant to glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bellaloui et al. (2009)

Title: Effect of Glyphosate-Boron Application on Seed Composition and Nitrogen Metabolism in Glyphosate-Resistant Soybean	
Author: NACER BELLALOUI, HAMED K. ABBAS, ANNE M. GILLEN, AND CRAIG A. ABEL	
Reference: J. Agric. Food Chem. 57, 9050–9056	
Year: 2009	
Results and conclusion: The objective of this study was to evaluate the effects of foliar application of glyphosate (Gly) alone, boron (B) alone, and Gly-B combined on seed composition and nitrogen metabolism in glyphosate-resistant soybean (Glycine max (L.) Merr.). No Gly and no B application plants were used as control (C). Results showed that Gly, Gly-B, or B applications increased protein, oleic acid, and total amino acid concentrations in seed. However, oil and linolenic acid concentrations decreased under those treatments compared with the nontreated control. Gly-B combined or B treatments increased B concentration in leaves and seed, nitrate reductase activity (NRA), and nitrogenase activity and resulted in a significant positive correlation between B concentration in leaves and NRA ($r = 0.54$; $P < 0.0001$) and B concentration in leaves and nitrogenase activity ($r = 0.35$; $P = 0.005$). The results suggest that Gly-B tank mixing may not antagonize B uptake and translocation to leaves and seeds, and the inhibitory effect of Gly on nutrient uptake and translocation may depend on the ion species and form of the nutrient mixed with Gly. These results demonstrate that Gly-B application alters seed composition, nitrogen metabolism, and B status in leaves and seed.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Flumetsulam, CAS-no.: 98967-40-9; metolachlor, CAS-no.: 51218-45-2; and paraquat CAS-no.: 1910-42-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bellaloui et al. (2009)

Title: Effects of Glyphosate Application on Seed Iron and Root Ferric (III) Reductase in Soybean Cultivars	
Author: NACER BELLALOUI, KRISHNA N. REDDY, ROBERT M. ZABLOTOWICZ, HAMED K. ABBAS, AND CRAIG A. ABEL	
Reference: J. Agric. Food Chem. 57, 9569–9574	
Year: 2009	

Results and conclusion: Previous research demonstrated that plant nutrient assimilation was reduced by glyphosate (Gly). A 2 year field experiment investigated the effects of Gly at drift rate (12.5 % of commercial use rate) on Fe concentrations in leaves and seeds of Gly-sensitive (GS) soy-bean, and a greenhouse experiment evaluated Gly effects on Fe assimilation using root in vivo ferric reductase activity (FRA) in two GS and one Gly-resistant (GR) soybean cultivars. Field studies showed that Gly drift rates resulted in a significant decrease in the Fe concentration in seeds and leaves compared to the non-treated plants. In greenhouse studies, leaf Fe and FRA were inhibited in GS cultivars Hutcheson and DP 5110 and the GR cultivar AG 4604RR and leaf Fe was positively correlated with root FRA ($p < 0.0001$). These results indicate that Gly can interfere with Fe assimilation in both GS and GR soybean. Understanding the implication of Gly on Fe nutrition in soybean seed would help soybean agronomists and breeders seeking to improve seed mineral nutrition qualities.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Flumetsulam, CAS-no.: 98967-40-9; metolachlor, CAS-no.: 51218-45-2; and paraquat CAS-no.: 1910-42-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bellaloui et al. (2006)

Title: Simulated Glyphosate Drift Influences Nitrate Assimilation and Nitrogen Fixation in Non-glyphosate-Resistant Soybean
Author: NACER BELLALOUI, KRISHNA N. REDDY, ROBERT M. ZABLOTOWICZ, ANDALEMU MENGISTU
Reference: J. Agric. Food Chem. 54, 3357-3364
Year: 2006

Results and conclusion: Non-target injury from glyphosate drift is a concern among growers using non-glyphosate-resistant (non-GR) cultivars. The effects of glyphosate drift on nitrate assimilation and nitrogen fixation potential, nodule mass, and yield of non-GR soybean were assessed in a field trial at Stoneville, MS. A non-GR soybean cultivar 'Delta Pine 4748S' was treated with glyphosate at 12.5 % of use rate of 0.84 kg of active ingredient/ha at 3 (V2), 6 (V7), and 8 (R2, full bloom) weeks after planting (WAP) soybean to simulate glyphosate drift. Untreated soybean was used as a control. Soybeans were sampled weekly for 2 weeks after each glyphosate treatment to assess nitrate assimilation and N_2 fixation potential. Nitrate assimilation was assessed using in vivo nitrate reductase assay in leaves, stems, roots, and nodules. Nitrogen fixation potential was assessed by measuring nitrogenase activity using the acetylene reduction assay (ARA). Nitrogen content of leaves, shoots, and seed and soybean yield were also determined. In the first sampling date (4 WAP); glyphosate drift caused a significant decrease in NRA in leaves (60 %), stems (77 %), and nodules (50 %), with no decrease in roots. At later growth stages, NRA in leaves was more sensitive to glyphosate drift than stems and roots. Nitrogenase activity was reduced 36-58 % by glyphosate treatment at 3 or 6 WAP. However, glyphosate treatment at 8 WAP had no effect on nitrogenase activity. Nitrogen content was affected by glyphosate application only in shoots after the first application. No yield, seed nitrogen, protein, or oil concentration differences were detected. These results suggest that nitrate assimilation and nitrogen fixation potential were significantly reduced by glyphosate drift, with the greatest sensitivity early in vegetative growth. Soybean has the ability to recover from the physiological stress caused by glyphosate drift.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Flumetsulam, CAS-no.: 98967-40-9; Metolachlor, CAS-no.: 51218-45-2; and Paraquat CAS-no.: 1910-42-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bellaloui et al. (2008)

Title: Nitrogen Metabolism and Seed Composition As Influenced by Glyphosate Application in Glyphosate-Resistant Soybean
Author: NACER BELLALOUI, ROBERT M. ZABLOTOWICZ, KRISHNA N. REDDY, AND CRAIG A. ABEL
Reference: J. Agric. Food Chem. 56, 2765–2772
Year: 2008

Results and conclusion: Previous research has demonstrated that glyphosate can affect nitrogen fixation or nitrogen assimilation in soybean. This 2-year field study investigated the effects of glyphosate ap-plication of 1.12 and 3.36 kg of ae ha^{-1} on nitrogen metabolism and seed composition in glyphosate-resistant (GR) soybean. There was no effect of glyphosate application on nitrogen fixation as measured by acetylene reduction assay, soybean yield, or seed nitrogen content. However, there were significant effects of glyphosate application on nitrogen assimilation, as measured by in vivo nitrate reductase activity (NRA) in leaves, roots, and nodules, especially at high rate. Transiently lower leaf nitrogen or ^{15}N natural abundance in high glyphosate application soybean supports the inhibition of NRA. With the higher glyphosate application level protein was significantly higher (10.3 %) in treated soybean compared to untreated soybean. Inversely, total oil and linolenic acid were lowest at the high glyphosate application rate, but oleic acid was greatest (22 %) in treated soybean. These results suggest that nitrate assimilation in GR soybean was more affected than nitrogen fixation by glyphosate application and that glyphosate application may alter nitrogen and carbon metabolism.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Metolachlor, CAS-no.: 51218-45-2; and Paraquat CAS-no.: 1910-42-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Beltrao et al. (2013)

Title: Changes in Soluble Manganese and Iron Concentrations of Tropical Wetland Soils as Influenced by Glyphosate Dosage
Author: DANIELLE S. BELTRAO, ALFREDO B. DE-CAMPOS, DANILLO B. MOURA AND RICARDO F. SOUSA
Reference: Communications in Soil Science and Plant Analysis, 44:1092–1096, 2013
Year: 2013
Results and conclusion: Glyphosate is largely used to control weeds in wetland soils of Brazil. We investigated changes in the chemistry of soluble manganese (Mn) and iron (Fe) in these soils as affected by glyphosate dosage. Triplicate samples of the A horizon of wetland soils with different organic-matter contents were incubated with deionized water (1:2) for 1, 3, and 30 days under flooding. Three different glyphosate doses (0, 0.048, and 0.096 g L^{-1} m^{-2}) were spiked on the flooded water at the beginning of the incubation periods. After incubation, pH was measured and samples of the supernatant were collected for determination of Mn/Fe concentrations by atomic absorption. Glyphosate application impacted Mn but had no effect on pH and Fe. Soluble Mn concentrations decreased as glyphosate dosage increased for the high organic-matter soil after 3 days of incubation. It indicated that glyphosate application can change the chemistry of soil metals. The intensity of these changes depends on the glyphosate dosage, evolved metal, incubation time, and soil properties.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional): No weight, not relevant for endpoints related to environmental fate.	
Reliability	
Endpoint	Dependence of Mn and Fe Concentrations from glyphosate dosage
Protocol	Not applicable
Test compound	Roundup-Nortox made by Monsanto Co., St. Louis, Mo
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bernards et al. (2005)

Title: Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation	
Author: Mark L. Bernards, Kurt D. Thelen, Donald Penner, Rajendra B. Muthukumaran, John L. McCracken	
Reference: Weed Science, 53:787–794	
Year: 2005	
Results and conclusion: It was hypothesized that Mn complexed with glyphosate in a similar manner to Ca^{2+}-forming salts that were not readily absorbed and, thereby, reducing glyphosate efficacy. This study was conducted to confirm the interaction of Mn^{2+} and glyphosate and to measure the effect of Mn^{2+} on glyphosate absorption and translocation in velvetleaf.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): No weight, not relevant for endpoints related to environmental fate.	
Reliability	
Endpoint	Glyphosate adsorption to Mn^{2+} in tank solution followed by adsorption and translocation in velvetleaf.
Protocol	Not applicable
Test compound	^{14}C-Glyphosate
Test system and conditions	Growth chamber bioassays were conducted to measure absorption and translocation of ^{14}C-labeled Glyphosate.
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bois et al. (2013)

Title: Herbicide mitigation in microcosms simulating stormwater basins subject to polluted water inputs	
Author: P. Bois, D. Huguenot, K. Je´ze´quel, M. Lollier, J.Y. Cornu and d, T. Lebeau	
Reference: Waterresearch 47 (2013) 1123-1135	
Year: 2013	
Results and conclusion: Non-point source pollution as a result of wine-growing activity is of high concern. Stormwater basins (SWB) found downstream of vineyard watersheds could show a potential for the mitigation of runoff water containing herbicides. In this study, mitigation of vinery-used herbicides was studied in microcosms with a very similar functioning to that recorded in SWB. Mitigation efficiency of glyphosate, diuron and 3,4-dichloroaniline (3,4-DCA) was investigated by taking into account hydraulic flow rate, mitigation duration, bioaugmentation and plant addition. Mitigation efficiency measured in water ranged from 63.0 % for diuron to 84.2 % for 3,4-DCA and to 99.8 % for glyphosate. Water-storage duration in the SWB and time between water supplies were shown to be the most influential factors on the mitigation efficiency. Six hours water-storage duration allowed an efficient sorption of herbicides and their degradation by indigenous microorganisms in 5 weeks. Neither bioaugmentation nor plant addition had a significant effect on herbicide mitigation. Our results show that this type of SWB are potentially relevant for the mitigation of these herbicides stemming from wine-growing activity, providing a long enough hydraulic retention time.	
Proposed action: Not to be considered; does not affect the fate relevant endpoints of the monograph.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	
Endpoint	Dissipation efficiency (in microcosms)
Protocol	No standard protocol, non-GLP
Test compound	Glyphosate
Test system and conditions	This work aimed at studying the effects of bioaugmentation by the mixed bacterial culture ‘106’, plants (Phragmites australis) and hydraulic regime on pollutant dissipation, in order to enhance glyphosate, diuronand 3,4-DCA removal in both runoff water (transiting through SWB) and sediment (accumulating into the basins). As pesticides are rarely applied alone, Cu was added to the mixture of glyphosate, diuron and 3,4-DCA supplied to the microcosms. Cu is indeed applied in vineyards until 120 years as Copper Bordeaux mixture to control powdery mildew. The study was performed in small-scale devices. These microcosms were by aspects (hydraulic regime, sandesediment mix) close to the aforementioned vineyard SWB.
Statistical design	Not applicable
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative” evidence	No negative evidence

Bott et al. (2011)

Title: Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilisation	
Author: Sebastian Bott, Tsehaye Tesfamariam, Angelika Kania, Birceyudum Eman, Nergiz Aslan, Volker Römheld, Günter Neumann	
Reference: Plant Soil, 342:249–263	
Year: 2011	
Results and conclusion: The results suggest that remobilization of glyphosate may represent an additional transfer pathway for glyphosate to non-target plants which is strongly influenced by soil characteristics such as P fixation potential, content of plant-available iron, pH, cation exchange capacity, sand content and soil organic matter.	
Proposed action: Not to be considered and included in the monograph; does not affect the fate relevant endpoints of the monograph.	
Type of information (critical, high/low weight, supporting, additional): Low weight; may be additional information on re-mobilisation	
Reliability	
Endpoint	Visual symptoms of glyphosate toxicity, plant biomass, intracellular shikimate accumulation as physiological indicator for glyphosate toxicity and the plant nutritional status were determined.
Protocol	No standard protocol, non-GLP
Test compound	glyphosate formulations (applied as Roundup Ultra-Max®)
Test system and conditions	In model experiments under greenhouse conditions, the potential for glyphosate re-mobilisation by P-fertiliser application was evaluated by bio-indication with soybean cultivated on five contrasting soils with or without glyphosate application at 10–35 days before sowing. Different levels of P-fertilisation (0, 20, 40, 80, 240 mg P/kg soil) were supplied at the date of sowing.
Statistical design	Experiments were conducted in a randomized block design with four replicates for each treatment. Analysis of variance and the Tukey test for detection of significant differences were performed using the SigmaStatsoftware.
Relevance	
Environmental relevance	Given, influence of environmental parameter investigated
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence

Bott et al. (2009)

Title: Evidence for glyphosate damage of winter wheat depending on waiting-times after pre-crop glyphosate application and density of desiccated weed plants under field and experimental conditions
Author: Bott, Sebastian, Lebender, Ulrike, Yoon, Duck-Joong, Tesfamariam, Tsehaye, Römheld, Volker, Neumann, Günter
Reference: The Proceedings of the International Plant Nutrition Colloquium XVI, http://escholarship.org/uc/item/25v599pr
Year: 2009

Results and conclusion: Previous model experiments under greenhouse conditions identified high weed density and short waiting times for sowing after glyphosate desiccation as potential risk factors, mediating glyphosate phytotoxicity to non-target crops. To evaluate these factors under field conditions, a set of three field trials with different waiting times after pre-crop glyphosate application was conducted in non-tillage winter wheat cropping systems in Southwest Germany. Additionally, model experiments with short waiting time (2 d) and a high density of target weeds were performed, using a track-spraying device to simulate conditions for field application. Both, in model experiments and under field conditions, short waiting times after pre-crop glyphosate application resulted in lower germination, delayed or arrested plant development, reduced shoot biomass production, partly impaired micronutrient acquisition as well as intracellular accumulation of shikimate as physiological indicator of glyphosate toxicity. Thus, it can be concluded that short waiting times and high density of target plants can be considered as relevant risk factors for phytotoxicity of glyphosate to non-target crops No-tillage cropping systems seem to be associated with a particularly high sensitivity to glyphosate-induced damage of crop plants. Recommendations of waiting times appropriate to the cropping system should be considered as promising strategy to avoid harvest losses due to phytotoxicity, impaired growth and micronutrient deficiency. Further elucidation of environmental risk factors promoting the expression of crop damage is necessary.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate as Roundup Ultra Max®
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Bott et al. (2008)

Title: Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (Glycine max L.)
Author: Sebastian Bott & Tsehaye Tesfamariam & Hande Candan & Ismail Cakmak & Volker Römheld & Günter Neumann
Reference: Plant Soil 312:185–194
Year: 2008

Results and conclusion:
This investigation demonstrated potential detrimental side effects of glyphosate on plant growth and micronutrient (Mn, Zn) status of a glyphosate-resistant (GR) soybean variety (Glycine max cv. Valiosa), which were found to be highly dependent on the selected growth conditions. In hydroponic experiments with sufficient Mn supply [0.5 µM]; the GR cv. Valiosa produced similar plant biomass, root length and number of lateral roots in the control treatment without glyphosate as compared to its non-GR parental line cv. Conquista. However, this was associated with 50 % lower Mn shoot concentrations in cv. Conquista, suggesting a higher Mn demand of the transgenic cv. Valiosa under the selected growth conditions. Glyphosate application significantly inhibited root biomass production, root elongation, and lateral root formation of the GR line, associated with a 50 % reduction of Mn shoot concentrations. Interestingly, no comparable effects were detectable at low Mn supply [0.1 µM]. This may indicate Mn-dependent differences in the intracellular transformation of glyphosate to the toxic metabolite aminomethylphosphonic acid (AMPA) in the two isolines. In soil culture experiments conducted on a calcareous loess sub-soil of a Luvisol (pH 7.6) and a highly weathered Arenosol (pH 4.5), shoot biomass production and Zn leaf concentrations of the GR-variety were affected by glyphosate applications on the Arenosol but not on the calcareous Loess sub-soil. Analysis of micronutrient levels in high and low molecular weight (LMW) fractions (80 % ethanol extracts) of young leaves revealed no indications for internal immobilization of micronutrients (Mn, Zn, Fe) by excessive complexation with glyphosate in the LMW phase.

Proposed action:
Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional):
Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate formulation Roundup® UltraMax (Monsanto Agrar, Düsseldorf, Germany) containing N-[phosphonomethyl]glycine isopropylamine salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cakmak et al. (2009)

Title: Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean	
Author: Ismail Cakmak, Atilla Yazici, Yusuf Tutus, Levent Ozturk	
Reference: Europ. J. Agronomy 31, 114–119	
Year: 2009	
Results and conclusion: Greenhouse experiments were conducted to study the effects of glyphosate drift on plant growth and concentrations of mineral nutrients in leaves and seeds of non-glyphosate resistant soybean plants (Glycine max, L.). Glyphosate was sprayed on plant shoots at increasing rates between 0.06 and 1.2 % of the recommended application rate for weed control. In an experiment with 3-week-old plants, increasing application of glyphosate on shoots significantly reduced chlorophyll concentration of the young leaves and shoots dry weight, particularly the young parts of plants. Concentration of shikimate due to increasing glyphosate rates was nearly 2-fold for older leaves and 16-fold for younger leaves compared to the control plants without glyphosate spray. Among the mineral nutrients analyzed, the leaf concentrations of potassium (K), phosphorus (P), copper (Cu) and zinc (Zn) were not affected, or even increased significantly in case of P and Cu in young leaves by glyphosate, while the concentrations of calcium (Ca), manganese (Mn) and magnesium (Mg) were reduced, particularly in young leaves. In the case of Fe, leaf concentrations showed a tendency to be reduced by glyphosate. In the second experiment harvested at the grain maturation, glyphosate application did not reduce the seed concentrations of nitrogen (N), K, P, Zn and Cu. Even, at the highest application rate of glyphosate, seed concentrations of N, K, Zn and Cu were increased by glyphosate. By contrast, the seed concentrations of Ca, Mg, Fe and Mn were significantly reduced by glyphosate. These results suggested that glyphosate may interfere with uptake and re-translocation of Ca, Mg, Fe and Mn, most probably by binding and thus immobilizing them. The decreases in seed concentration of Fe, Mn, Ca and Mg by glyphosate are very specific, and may affect seed quality.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salt of glyphosate (Roundup Ultra Herbicide, Monsanto Ltd., Adana, Turkey), CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cavalieri et al. (2012)

Title: Nutrient and Shoot Dry Matter Accumulation of Two GR Soybean Cultivars under the Effect of Glyphosate Formulations	
Author: Cavalieri, S.D., Velini, E.D., Silva, F.M.L., Sao Jose, A.R. and Andrade, G.J.M.	
Reference: Planta Daninha, Viçosa-MG, vol. 30 (2): 349-358	
Year: 2012	
Results and conclusion: The study of selectivity and secondary effects of herbicides on crops is extremely important to successful agriculture. This research aimed to evaluate the effect of glyphosate formulations on nutrient accumulation and dry matter production on the shoot of two glyphosateresistant (GR) soybean cultivars. The assay was carried out in a greenhouse and arranged in a randomized complete block design, replicated six times. The treatments were in a factorial arrangement including six glyphosate formulations (Roundup Original®, Roundup Ready®, Roundup Transorb®, Roundup WG®, Roundup Ultra® and Zapp Qi®), plus a control treatment, and two soybean cultivars (CD 225 RR and V Max RR). The herbicide applications were performed when the plants were at the V3 growth stage, using a dose of 960 g a.e. ha^{-1}. The macronutrient and micronutrient accumulation and dry matter production in the shoot of the soybean plants were greater in V Max RR cultivar than in CD 225 RR cultivar. The formulations Roundup Ready® and Roundup Ultra® did not promote nutrient accumulation reduction in the shoot of the cultivars. In addition, the formulations Roundup Original®, Roundup Transorb® and Roundup WG® caused the greatest damage to nutrient accumulation and dry matter production. It was concluded that nutrient accumulation and dry matter production in the shoots of the soybean plants are affected by glyphosate application, even for GR cultivars.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cerdeira and Duke (2006)

Title: The Current Status and Environmental Impacts of Glyphosate-Resistant Crops: A Review	
Author: Antonio L. Cerdeira and Stephen O. Duke	
Reference: J. Environ. Qual. 35:1633–1658	
Year: 2006	
Results and conclusion: Glyphosate [N-(phosphonomethyl) glycine]-resistant crops (GRCs), canola (Brassica napus L.), cotton (Gossypium hirsutum L.), maize (Zea mays L.), and soybean [Glycine max (L.) Merr.] have been commercialized and grown extensively in the Western Hemisphere and, to a lesser extent, elsewhere. Glyphosate-resistant cotton and soybean have become dominant in those countries where their planting is permitted. Effects of glyphosate on contamination of soil, water, and air are minimal, compared to some of the herbicides that they replace. No risks have been found with food or feed safety or nutritional value in products from currently available GRCs. Glyphosate-resistant crops have promoted the adoption of reduced- or no-tillage agriculture in the USA and Argentina, providing a substantial environmental benefit. Weed species in GRC fields have shifted to those that can more successfully withstand glyphosate and to those that avoid the time of its application. Three weed species have evolved resistance to glyphosate in GRCs. Glyphosate-resistant crops have greater potential to become problems as volunteer crops than do conventional crops. Glyphosate resistance transgenes have been found in fields of canola that are supposed to be non-transgenic. Under some circumstances, the largest risk of GRCs may be transgene flow (introgression) from GRCs to related species that might become problems in natural ecosystems. Glyphosate resistance transgenes themselves are highly unlikely to be a risk in wild plant populations, but when linked to transgenes that may impart fitness benefits outside of agriculture (e.g., insect resistance), natural ecosystems could be affected. The development and use of failsafe introgression barriers in crops with such linked genes is needed.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article: Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cerdeira and Duke (2010)

Title: Effects of glyphosate-resistant crop cultivation on soil and water quality	
Author: Antonio L. Cerdeira and Stephen O. Duke	
Reference: GM Crops 1:1, 1-9; January/February 2010; © 2010 Landes Bioscience	
Year: 2010	
Results and conclusion: Review article: Transgenic glyphosate-resistant crops (GRCs) have been commercialized and grown extensively in the western Hemisphere and, to a lesser extent, elsewhere. GRCs have generally become dominant in those countries where they have been approved for growing. Potential effects of glyphosate on soil and water are minimal, compared to the effects of the herbicides that are replaced when GRCs are adopted. Perhaps the most important indirect effect is that GRCs crops promote the adoption of reduced- or no-tillage agriculture, resulting in a significant reduction in soil erosion and water contamination. Glyphosate and its degradation product, aminomethylphosphonate (AMPA), residues are not usually detected in high levels in ground or surface water in areas where glyphosate is used extensively. Furthermore, both glyphosate and AMPA are considered to be much more toxicologically and environmentally benign than most of the herbicides replaced by glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article: Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Cerdeira et al. (2007)

Title: Review of potential environmental impacts of transgenic glyphosate-resistant soybean in Brazil
Author: ANTONIO L. CERDEIRA, DIONSIO L. P. GAZZIERO, STEPHEN O. DUKE, MARCUS B. MATALLO and CLAUDIO A. SPADOTTO
Reference: Journal of Environmental Science and Health Part B 42, 539–549
Year: 2007

Results and conclusion: Transgenic glyphosate-resistant soybeans (GRS) have been commercialized and grown extensively in the Western Hemisphere, including Brazil. Worldwide, several studies have shown that previous and potential effects of glyphosate on contamination of soil, water, and air are minimal, compared to those caused by the herbicides that they replace when GRS are adopted. In the USA and Argentina, the advent of glyphosate-resistant soybeans resulted in a significant shift to reduced- and no-tillage practices, thereby significantly reducing environmental degradation by agriculture. Similar shifts in tillage practiced with GRS might be expected in Brazil. Transgenes encoding glyphosate resistance in soybeans are highly unlikely to be a risk to wild plant species in Brazil. Soybean is almost completely self-pollinated and is a non-native species in Brazil, without wild relatives, making introgression of transgenes from GRS virtually impossible. Probably the highest agricultural risk in adopting GRS in Brazil is related to weed resistance. Weed species in GRS fields have shifted in Brazil to those that can more successfully withstand glyphosate or to those that avoid the time of its application. These include Chamaesyce hirta (erva-de-Santa-Luzia), Commelina benghalensis (trapoeraba), Spermacoce latifolia (erva-quente), Richardia brasiliensis (poaia-branca), and Ipomoea spp. (corda-de-viola). Four weed species, Conyza bonariensis, Conyza Canadensis (buva), Lolium multiflorum (azevem), and Euphorbia heterophylla (amendoim bravo), have evolved resistance to glyphosate in GRS in Brazil and have great potential to become problems.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review, no study
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Clua et al. (2012)

Title: The Effects of Glyphosate on the Growth of Birdsfoot Trefoil (Lotus corniculatus) and Its Interaction with Different Phosphorus Contents in Soil
Author: Clua, A., Conti, M. And Beltrano, J.
Reference: Journal of Agricultural Science; Vol. 4 (7): 208-218
Year: 2012
Results and conclusion: Glyphosate residues from applications or exuded by roots of treated crops and by senescing weeds could be absorbed by new crops. The aim of this work was to study the effect of glyphosate in soil on the growth of Lotus corniculatus and its interaction with phosphorus. A completely randomized 3 x 4 factorial design was used for the experiment, with 3 levels of phosphorus (0, 100, and 200 ppm) and 4 of glyphosate (0; 0.5; 1.0, and 2.0 times the recommended dosage, 4 L. ha^{-1}), amended to soil. Glyphosate residues decreased growth parameters, chlorophyll and protein contents, and membrane stability. Glyphosate effect was increased by the greater availability of phosphorus, so there was a significant interaction between glyphosate and phosphorus. The findings of this study provide evidence of the detrimental effect of glyphosate present in soil as well as its remobilization through the presence of additional phosphorus in soil.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review, no study
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Chang et al. (2011)

Title: OCCURRENCE AND FATE OF THE HERBICIDE GLYPHOSATE AND ITS DEGRADATE AMINOMETHYLPHOSPHONIC ACID IN THE ATMOSPHERE	
Author: FENG-CHIH CHANG, MATT F. SIMCIK, and PAUL D. CAPEL	
Reference: Environmental Toxicology and Chemistry, Vol. 30, No. 3, pp. 548–555	
Year: 2011	
Results and conclusion: This is the first report on the ambient levels of glyphosate, the most widely used herbicide in the United States, and its major degradation product, aminomethylphosphonic acid (AMPA), in air and rain. The frequency of glyphosate detection ranged from 60 to 100 % in both air and rain. The concentrations of glyphosate ranged from <0.01 to 9.1 ng/m^3 and from <0.1 to 2.5 mg/L in air and rain samples, respectively. The frequency of detection and median and maximum concentrations of glyphosate in air were similar or greater to those of the other high-use herbicides observed in the Mississippi River basin, whereas its concentration in rain was greater than the other herbicides. It is not known what percentage of the applied glyphosate is introduced into the air, but it was estimated that up to 0.7 % of application is removed from the air in rainfall. Glyphosate is efficiently removed from the air; it is estimated that an average of 97 % of the glyphosate in the air is removed by a weekly rainfall >30 mm.	
Proposed action: Not to be considered as publication deals with atmospheric concentrations outside EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information as the articles deals with ambient air monitoring outside EU.	
Reliability	Medium
Endpoint	Monitoring, concentrations in air and rain
Protocol	Monitoring, for details see under test system and conditions.
Test compound	Glyphosate (CAS-no: 1071-83-6) and AMPA (CAS-no: 1066-51-9), monitored, purity cannot be given
Test system and conditions	Concurrent, weekly integrated air particle and rain samples were collected during two growing seasons in agricultural areas in Mississippi and Iowa. Rain was also collected in Indiana in a preliminary phase of the study. Description of sampling sites, of field sampling, of analytical methods and quality assurance
Statistical design	Calculation of median, maximum, minimum, standard deviation, %D
Environmental relevance	Given

Weight of evidence	
"Positive"/"Negative" evidence	Similar monitoring programmes not known. No negative evidence.

Cornish and Burgin (2005)

Title: Residual Effects of Glyphosate Herbicide in Ecological Restoration	
Author: P. S. Cornish and S. Burgin	
Reference: Restoration Ecology Vol. 13, No. 4, pp. 695–702	
Year: 2005	
Results and conclusion: This study assesses the risks in ecological restoration arising from transplanting into soil containing glyphosate residues. Four Australian restoration species were grown for 60 days in non-adsorbing media treated continuously with glyphosate to establish threshold concentrations for damage. Visual signs of injury were observed in three species and severe effects on root growth in all species, at solution concentrations as low as 18 mg/L. Only the perennial grass Themeda sp. died at this concentration, with other species surviving at concentrations in the range 36–360 mg/L, beyond which all plants died. Fourteen days exposure followed by removal of glyphosate from root media produced similar effects. Field and glasshouse experiments with the relatively tolerant tree species Angophora costata showed that application rates in the range 10–50 L/ha of herbicide product (360 g/L) would be needed to sustain damage to young plants transplanted into soil typical of local restoration sites. The volume of spray delivered using a hand operated sprayer varied between operators by 5- and 10-fold to complete the same tasks, at the high end presenting a potential risk to the most tolerant species under field conditions, even when spray concentrations follow label instructions. For all but the most sensitive species, the risk of glyphosate residues in ecological restoration should be minimized by training operators of unregulated applicators to deliver controlled volumes of herbicide when spot spraying prior to transplanting.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup (Monsanto Australia Ltd., Melbourne, Australia), Nufarm Glyphosate 360 (Nufarm Ltd., Melbourne, Australia), Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Correia and Durigan (2009)

Title: Glyphosate and Foliar Fertilization Using Manganese in Transgenic Soybean Crop
Author: CORREIA, N.M., DURIGAN, J.C.
Reference: Planta Daninha, Viçosa-MG, v. 27, n. 4, p. 721-72
Year: 2009
Results and conclusion: Based on the hypothesis that glyphosate-tolerant transgenic soybean would need a manganese complementation due to alterations in the absorption and metabolism of this element by the plants, this work aimed to evaluate the interaction of transgenic soybean sprayed with glyphosate and manganese foliar fertilization. The experiment was carried out under field conditions in the agricultural year 2007/2008 on the UNESP Campus Teaching, Research and Production Farm in Jaboticabal, São Paulo, Brazil. An experiment was arranged in a randomized block design, in a factorial scheme (4 x 4), with four replications. Four weed controls [glyphosate (c.p. Roundup Ready) at 0.72 and 1.20 kg/ha of equivalent acid; fluazifop-p-butyl plus fomesafen (c.p. Fusiflex) at 0.25 plus 0.25 kg/ha and under mechanical control, without herbicide] and four manganese rates (0, 42, 84 and 126 g/ha) were applied on the soybean leaf. The treatments did not significantly affect grain yield, manganese concentration in the soil, height and dry matter of the soybean plants. Only the mixture fluazifop-p-butyl plus fomesafen caused visible injuries in the plants. However, the symptoms were restricted to the leaves that intercepted spraying. The herbicide treatments did not differ from the control for 100 grain mass, although the plants treated with glyphosate 0.72 kg/ha presented less grain mass. Manganese application did not influence element concentration in the plant treated with glyphosate and under mechanical control. Therefore, glyphosate did not impair manganese absorption or metabolism by the plant. Growth and development of the herbicide-treated plants were statistically similar to those of the plants not treated with herbicides.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (Roundup Ready), CAS-no.: 1071-83-6; fluazifop-p-butyl CAS-no.: 79241-46-6 plus fomesafen, CAS-no.: 72178-02-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Coutinho and Mazo (2005)

Title: METALLIC COMPLEXES WITH GLYPHOSATE: A REVIEW
Author: Cláudia F. B. Coutinho e Luiz Henrique Mazo
Reference: Quim. Nova, Vol. 28, No. 6, 1038-1045
Year: 2005
Results and conclusion: We present studies involving metallic ions and the herbicide glyphosate. The metallic complexes of Cu(II), Zn(II), Mn(II), Ni(II), Cd(II), Pb(II), Cr(III), Fe(III), Co(III), ammonium, sodium, Ag(I), alkaline earth metals and of some lanthanides ions are described. The complexes are discussed in terms of their synthesis, identification, stability and structural properties, based on data from the current literature.

Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Davis et al. (2011)

Title: Environmental impacts of irrigated sugarcane production: Herbicide run-off dynamics from farms and associated drainage systems	
Author: A.M. Davis, P.J. Thorburn, S.E. Lewis, Z.T. Bainbridge, S.J. Attard, R. Milla, J.E. Brodie	
Reference: Agric. Ecosyst. Environ. (2011), doi:10.1016/j.agee.2011.06.019	
Year: 2011	
Results and conclusion: This study determined the dynamics of off-site paddock-scale pesticide movement and subsequent concentrations in local receiving environments in fully irrigated sugarcane farming systems of the lower Burdekin floodplain region, the largest sugar producing area in Australia. Chemical movement (both mass and concentration) in paddock surface run-off followed a similar pattern across sites in the region for several of the commonly applied herbicides such as diuron, atrazine and ametryn. Highest losses (loads and event concentrations) occurred in the first irrigation run-off events following application, with subsequent irrigation losses tailing off rapidly. Significant losses could also occur during wet season rainfall run-off events from paddocks with recent pesticide applications. There was a strong seasonal signal evident in catchment monitoring results. Pesticide concentrations in nearby receiving creek systems were invariably an order of magnitude or more lower than values collected at paddock-scale, highlighting the considerable dilution that takes place over relatively short distances. While the concentrations found in receiving creek systems were considerably lower than direct paddock run-off, they regularly exceeded some ecological guidelines and results of pesticide risk modelling suggested concentrations, particularly under dry season conditions, posed considerable ecological risk to aquatic ecosystems.	
Proposed action: Not to be considered as modelling is presented but without data relevant for Glyphosate. Furthermore, the publication is related to a site outside the EU (Australia).	
Type of information (critical, high/low weight, supporting, additional): Low weight; modelling, no relevant data of glyphosate	
Reliability	Medium
Endpoint	Herbicide loads (active ingredient); box plots summarising herbicide concentration during wet-season flood events (B, D, and F) and low flow conditions
Protocol	Water samples were analyzed at the National Association of Testing Authorities accredited QHFSS laboratory; QHFSS method number 16315, QHFSS method number 16631

Test compound	Diuron, Atrazine, Ametryn, Hexazinone, 2,4-D, Glyphosate (CAS-no.: 1071-83-6), Paraquat
Test system and conditions	An emerging approach to predict ecosystem risk of a mixture of herbicides (as measured in monitoring) to freshwater ecosystems will be applied to lower Burdekin sub-catchment and catchment scale water quality monitoring results. Paddock scale data were collected from seven farms distributed across the lower Burdekin floodplain, spanning a wide range of soils, applied herbicides and application dates. The majority of paddock run-off monitoring effort focused on the initial irrigation events following herbicide application. At several instrumented sites, paddock run-off volumes were measured. To provide additional data on herbicide concentrations in irrigation tail water run-off, multiple discrete herbicide samples were also manually collected during irrigation events at a number of less intensively monitored sites lacking discharge monitoring capacity. A total of nine sampling sites were monitored. Grab samples were collected manually at all sites through the use of a sampling pole. A total of 275 samples were collected over the monitoring period, including 205 high flow event samples and 70 samples collected during low flow, dry season conditions. Modelling: dry season herbicide concentration data were analyzed with the Predict the Ecological Risk of Pesticides in freshwater ecosystems (PERPEST; Version 3.0).
Statistical design	BROLGA program; SPSS software package (SPSS 2007)
Relevance	
Environmental relevance	Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.agee.2011.06.019.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

de Andrade and Rosolem (2011)

Title: UPTAKE OF MANGANESE IN RR SOYBEAN UNDER GLIFOSATE APPLICATION	
Author: Gabriel José Massoni de Andrade & Ciro Antonio Rosolem	
Reference: R. Bras. Ci. Solo, 35:961-968, 2011	
Year: 2011	
Results and conclusion: It was hypothesized that Mn uptake efficiency and transport by transgenic, glifosate-resistant soybean would be affected by application of the herbicide. Two experiments were carried out to study manganese uptake, long-distance transport and absorption kinetics of genetically modified soybean as affected by glifosate application. Experiment 1: The treatments consisted of two near-isogenic soybean cultivars grown in nutrient solution (Conquista and Valiosa RR with or without application of glifosate). The Mn levels in the nutrient solution were 0, 0.085, 0.125, 0.250, 0.500 mg/L. Twenty-five days after emergence, part of the total transgenic soybean plants were sprayed with herbicide. Experiment 2: Plants were sprayed with glifosate on the 26th day of cultivation at rates of 0 (zero), 15 and 960 g/ha to study the Mn absorption kinetics of cultivar Valiosa RR. It was found that genetic resistance to glifosate did not affect manganese nutrition in soybean cultivar Valiosa RR. Despite reducing the root dry matter, glifosate does not hamper Mn absorption and transport in transgenic soybean plants. The Mn absorption kinetic parameters of Valiosa RR, *Km, Vmax and Cmin* are not altered by glifosate applied to leaves.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable

Test compound	Glyphosate isopropylammonium salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

De Souza et al. (2013)

Title: Degradation of the Commercial Herbicide Glyphosate by Photo-Fenton Process: Evaluation of Kinetic Parameters and Toxicity
Author: D. R. de Souza, A. G. Trovó, N. R. A. Filho, M. A. A. Silvac, A. E. H. Machadob
Reference: J. Braz. Chem. Soc., Vol. 24, No. 9, 1451-1460, 2013
Year: 2013
Results and conclusion: The aim of the present work was to evaluate at laboratory scale the influence of some parameters (use of Fe^{2+} and combination of Fe^{2+} and Fe^{3+} at a 1:1 molar ratio (Fe^{2+}/Fe^{3+}), addition of oxalate, concentration of H_2O_2 and oxalate) on the kinetics of mineralization and release of phosphate ion during the degradation of commercial glyphosate induced by photo-Fenton process. It was also monitored the degradation of glyphosate on a large scale under optimal experimental conditions, using a pilot plant and solar radiation in order to assess the possible commercial use of this technology. Parameters that influence the efficiency of the degradation of glyphosate (addition of Fe^{2+}, simultaneous addition of Fe^{2+} and Fe^{3+} at a 1:1 (Fe^{2+}/Fe^{3+}) molar ratio, addition of oxalate and of H_2O_2) were evaluated at lab-scale. Synergic effects on its degradation and release of phosphate were observed using Fe^{2+}/Fe^{3+}, as well as adding oxalate. On the other hand, the concentration increase of Fe^{2+}/Fe^{3+}, oxalate and H_2O_2 did not promote a linear increase of glyphosate mineralization and release of phosphate. Using high concentrations of these species, the efficiency of glyphosate mineralization and release of phosphate was constant or even decreased. Under optimized conditions (0.27 mmol/L of Fe^{2+}/Fe^{3+}, 1.13 mmol/L of oxalate and 10.3 mmol/L of H_2O_2), close results for mineralization and release of phosphate were obtained in lab-scale and using a solar pilot plant. A direct ratio between reducing the toxicity and glyphosate concentration was also observed.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered
Reliability

Endpoint	Parameters that influence the efficiency of the degradation of glyphosate
Protocol	Non-GLP
Test compound	Glyphosate

Test system and conditions	The photodegradation experiments were performed in a lab-scale using a 400 W high pressure mercury vapour lamp as irradiation source. The average irradiance in the UVA furnished by this kind of lamp was estimated as being equal to 1100 W m^{-2}, with a photonic flux of 3.3 × 10-6 einstein s^{-1} between 295 and 710 nm. These measurements were done using a Solar Light PMA 2100 photometer/radiometer, equipped with an UVA detector (320-400 nm) and a radiometric/photometric setup built. A total volume of 4 L of aqueous solution containing commercial glyphosate was recirculated by pumping at a flow rate of 2.37 L min^{-1} after the addition of iron solution, pH adjustment between 2.8 ± 0.2 and addition of H_2O_2. The lamp was only turned on when the reactor was filled with solution. The solution temperature was controlled using a thermostatic bath, keeping close to 40 ± 2 ºC. Aliquots (25 mL) of the solutions containing the photodegraded material were collected at 15 min intervals up to 60 min, and, at 30 min intervals up to 120 min. Using the lab-scale setup, the following parameters were evaluated: (i) the use of Fe^{2+} (0.27 mmol/L-15 mg/L) and Fe^{2+}/Fe^{3+} (0.27 and 0.135 mmol/L of each specie); (ii) total iron concentration (Fe^{2+}/Fe^{3+}): from 0.18 to 1.78 mmol/L (between 10.0 and 100.0 mg/L); (iii) oxalate concentration: 0.225 to 2.25 mmol/L (37.5 to 375.0 mg/L) and (iv) H_2O_2 concentration: 5.2 to 15.5 mmol/L (176.8 to 527.0 mg/L). Thus, kinetic experiments were carried out using 0.59 mmol/L (100 mg/L) glyphosate, 0.27 mmol/L Fe^{2+}/Fe^{3+}, 1.13 mmol/L oxalate (187.6 mg/L) and 10.3 mmol/L (350.2 mg/L) H_2O_2. After sampling and before analysis, a calculated volume of 2.0 mol/L Na_2SO_3 aqueous solution was added to the samples according to the stoichiometry between H_2O_2 and Na_2SO_3 and H_2O_2 concentration, ensuring the removal of the remaining H_2O_2, stopping the Fenton reaction. The experiments using solar radiation were carried in the winter under clear sky conditions, using a solar pilot plant. It consists of a compound parabolic collector (CPC) with an irradiated surface of 1.62 m^2 (irradiated volume of 12 L) and a reservoir with maximum capacity of 120 L. A volume of 50 L of glyphosate solution (0.59 mmol/L, 100 mg/L) circulates under turbulent flow into the CPC absorber tubes in a closed recirculating system. The solar irradiance was measured using the same radiometer applied in the lab-scale experiments, placed at the same angle of inclination of the reactor, being that an average solar irradiance of 40 ± 5 W m^{-2} was obtained. The photoreactor hydraulic circuit consists of a continuously stirred tank and a 0.50 HP centrifugal recirculation pump. At the beginning of the experiment, with the collectors covered, the same initial conditions defined for the reagents in kinetic experiments under lab-scale were used (0.27 mmol/L Fe^{2+}/Fe^{3+}, 1.13 mmol/L oxalate, 10.3 mmol/L H_2O_2 and pH 2.8 ± 0.2). The cover was then removed and the samples were collected at intervals of 100 kJ m^{-2} of UVA dose up to 800 kJ m^{-2} (30 min up to 240 min of irradiation). The same treatment using the Na_2SO_3 solution (previously described) was done to stop the Fenton reaction.
Statistical design	
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Duke et al. (2012)

Title: Effects of Glyphosate on the Mineral Content of Glyphosate-Resistant Soybeans (Glycine max)	
Author: Stephen O. Duke, Krishna N. Reddy, Kaixuan Bu and James V. Cizdziel	
Reference: J. Agric. Food Chem. 2012, 60, 6764−6771	
Year: 2012	
Results and conclusion: This article describes experiments designed to determine the effects of a recommended rate (0.86 kg ha^{-1}) of glyphosate applied once or twice on the mineral content of young and mature leaves, as well as in seeds produced by GR soybeans (Glycine max) in both the greenhouse and field using inductively coupled plasma mass spectrometry (ICP-MS). In the greenhouse, there were no effects of either one application (at 3 weeks after planting, WAP) or two applications (at 3 and 6 WAP) of glyphosate on Ca, Mg, Mn, Zn, Fe, Cu, Sr, Ba, Al, Cd, Cr, Co, or Ni content of young or old leaves sampled at 6, 9, and 12 WAP and in harvested seed. Se concentrations were too low for accurate detection in leaves, but there was also no effect of glyphosate applications on Se in the seeds. In the field study, there were no effects of two applications (at 3 and 6 WAP) of glyphosate on Ca, Mg, Mn, Zn, Fe, Cu, Sr, Ba, Al, Cd, Cr, Co, or Ni content of young or old leaves at either 9 or 12 WAP. There was also no effect on Se in the seeds. There was no difference in yield between control and glyphosate-treated GR soybeans in the field. The results indicate that glyphosate does not influence mineral nutrition of GR soybean at recommended rates for weed management in the field.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup WeatherMax, Monsanto Agricultural Co., St. Louis, MO, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Duke et al. (2012)

Title: Glyphosate Effects on Plant Mineral Nutrition, Crop Rhizosphere Microbiota, and Plant Disease in Glyphosate-Resistant Crops
Author: S. O. Duke, J. Lydon, W. C. Koskinen, T. B. Moorman, R. L. Chaney and R. Hammerschmidt
Reference: dx.doi.org/10.1021/jf302436u | J. Agric. Food Chem. 2012, 60, 10375−10397
Year: 2012

Results and conclusion: Claims have been made recently that glyphosate-resistant (GR) crops sometimes have mineral deficiencies and increased plant disease. This review evaluates the literature that is germane to these claims. Our conclusions are: (1) although there is conflicting literature on the effects of glyphosate on mineral nutrition on GR crops, most of the literature indicates that mineral nutrition in GR crops is not affected by either the GR trait or by application of glyphosate; (2) most of the available data support the view that neither the GR transgenes nor glyphosate use in GR crops increases crop disease; and (3) yield data on GR crops do not support the hypotheses that there are substantive mineral nutrition or disease problems that are specific to GR crops.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Duke and Powles (2008)

Title: Mini-review: Glyphosate: a once-in-a-century herbicide
Author: Stephen O Duke and Stephen B Powles
Reference: Pest Manag Sci 64:319–325
Year: 2008
Results and conclusion: Since its commercial introduction in 1974, glyphosate [N-(phosphonomethyl)glycine] has become the dominant herbicide worldwide. There are several reasons for its success. Glyphosate is a highly effective broad-spectrum herbicide, yet it is very toxicologically and environmentally safe. Glyphosate translocates well, and its action is slow enough to take advantage of this. Glyphosate is the only herbicide that targets 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), so there are no competing herbicide analogs or classes. Since glyphosate became a generic compound, its cost has dropped dramatically. Perhaps the most important aspect of the success of glyphosate has been the introduction of transgenic, glyphosate-resistant crops in 1996. Almost 90 % of all transgenic crops grown worldwide are glyphosate resistant, and the adoption of these crops is increasing at a steady pace. Glyphosate/glyphosate-resistant crop weed management offers significant environmental and other benefits over the technologies that it replaces. The use of this virtually ideal herbicide is now being threatened by the evolution of glyphosate-resistant weeds. Adoption of resistance management practices will be required to maintain the benefits of glyphosate technologies for future generations.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable

Protocol	Not applicable
Test compound	Review article: Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Eker et al. (2006)

Title: Foliar-Applied Glyphosate Substantially Reduced Uptake and Transport of Iron and Manganese in Sunflower (Helianthus annuus L.) Plants
Author: SELIM EKER, LEVENT OZTURK, ATILLA YAZICI, BULENT ERENOGLU, VOLKER ROMHELD, AND ISMAIL CAKMAK
Reference: J. Agric. Food Chem. 54, 119-125
Year: 2006
Results and conclusion: Evidence clearly shows that cationic micronutrients in spray solutions reduce the herbicidal effectiveness of glyphosate for weed control due to the formation of metal-glyphosate complexes. The formation of these glyphosate-metal complexes in plant tissue may also impair micronutrient nutrition of non-target plants when exposed to glyphosate drift or glyphosate residues in soil. In the present study, the effects of simulated glyphosate drift on plant growth and uptake, translocation, and accumulation (tissue concentration) of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were investigated in sunflower (Helian-thus annuus L.) plants grown in nutrient solution under controlled environmental conditions. Glyphosate was sprayed on plant shoots at different rates between 1.25 and 6.0 % of the recommended dosage (i.e. 0.39 and 1.89 mM glyphosate isopropylamine salt). Glyphosate applications significantly decreased root and shoot dry matter production and chlorophyll concentrations of young leaves and shoot tips. The basal parts of the youngest leaves and shoot tips were severely chlorotic. These effects became apparent within 48 h after the glyphosate spray. Glyphosate also caused substantial decreases in leaf concentration of Fe and Mn while the concentration of Zn and Cu was less affected. In short-term uptake experiments with radiolabelled Fe (^{59}Fe), Mn (^{54}Mn), and Zn (^{65}Zn), root uptake of ^{59}Fe and ^{54}Mn was significantly reduced in 12 and 24 h after application of 6 % of the recommended dosage of glyphosate, respectively. Glyphosate resulted in almost complete inhibition of root-to-shoot translocation of ^{59}Fe within 12 h and ^{54}Mn within 24 h after application. These results suggest that glyphosate residues or drift may result in severe impairments in Fe and Mn nutrition of nontarget plants, possibly due to the formation of poorly soluble glyphosate-metal complexes in plant tissues and/or rhizosphere interactions.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup Ultra [active ingredient N-[phosphonomethyl]glycine isopropylamine salt, Monsanto Co.], CAS-no.: 38641-91-0; radiolabelled Fe (59Fe), Mn (54Mn), and Zn (65Zn)
Test system and conditions	Not applicable
Statistical design	Not applicable

Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Ermakova et al. (2010)

Title: Bioremediation of glyphosate-contaminated soils	
Author: Inna T. Ermakova, Nina I. Kiseleva, Tatyana Shushkova, Mikhail Zharikov, Gennady A. Zharikov, Alexey A. Leontievsky	
Reference: Appl Microbiol Biotechnol 88:585–594	
Year: 2010	
Results and conclusion: Based on the results of laboratory and field experiments, we performed a comprehensive assessment of the bioremediation efficiency of glyphosate-contaminated soddypodzol soil. The selected bacterial strains Achromobacter sp. kg 16 (VKM B-2534D) and Ochrobactrum anthropi GPK 3 (VKM B-2554D) were used for the aerobic degradation of glyphosate. They demonstrated high viability in soil with the tenfold higher content of glyphosate than the recommended dose for the single in situ treatment of weeds. The strains provided a two-to threefold higher rate of glyphosate degradation as compared to indigenous soil microbial community. Within 1-2 weeks after the strain introduction, the glyphosate content of the treated soil decreased and integral toxicity and phytotoxicity diminished to values of non-contaminated soil. The decrease in the glyphosate content restored soil biological activity, as is evident from a more than twofold increase in the dehydrogenase activity of indigenous soil microorganisms and their biomass (1.2-fold and 1.6-fold for saprotrophic bacteria and fungi, respectively). The glyphosate-degrading strains used in this study are not pathogenic for mammals and do not exhibit integral toxicity and phytotoxicity. Therefore, these strains are suitable for the efficient, ecologically safe, and rapid bioremediation of glyphosate-contaminated soils.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine ammonium salt of glyphosate as a component of commercial Ground Bio herbicide, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Fan et al. (2012)

Title: Isolation, identification and characterization of a glyphosate-degrading bacterium, Bacillus cereus CB4, from soil	
Author: Jieyu Fan, Guoxia Yang, Haoyu Zhao, Guanying Shi, Yucong Geng, Taiping Hou, and Ke Tao	
Reference: J. Gen. Appl. Microbial., 58, 263-271 (2012)	
Year: 2012	
Results and conclusion: A bacterial strain named CB4, with highly effective glyphosate degradation capability, was isolated from soil after enrichment. On the basis of the Biolog omniLog Identification system (Biolog) and 165 ribosomal RNA (rRNA) gene sequencing methods, strain CB4 was identified as Bacillus cereus. Further experiments were carried out to optimize the growth of strain CB4 and the glyphosate degradation activity by high performance liquid chromatography (HPLC). The optimal conditions were found as follows: initial pH 6.0, incubation temperature 35°C, glyphosate concentration 6 g/L, inoculation amount 5 % and incubation time 5 days. Under the optimal conditions, stain CB4 utilized 94.47 % of glyphosate. This is the first report on B. cereus with a capacity to utilize herbicide glyphosate, and it can degrade glyphosate concentrations up to 12 g/L. Metabolization of glyphosate by strain B. cereus CB4 was studied. Results indicated that two concurrent pathways were capable of degrading glyphosate to AMPA, glyoxylate, sarcosine, glycine and formaldehyde as products. Glyphosate breakdown in B. cereus CB4 was achieved by the Cop lyase activity and the glyphosate oxidoreductase activity.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	No test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Fobbe et al. (2006)

Title: Polar Herbicides and Metabolites	
Author: Rita Fobbe, Birgit Kuhlmann, Jürgen Nolte, Gudrun Preuß, Christian Skark, and Ninette Zullei-Seibert	
Reference: Organic Pollutants in the Water Cycle. T. Reemtsma and M. Jekel (Eds.). Copyright © 2006 WILEY-VCH Verlag GmbH & Co. kgaA, Weinheim ISBN: 3-527-31297-8	
Year: 2006	
Results and conclusion: Overview article: Polar herbicides are widely used man-made substances. Because of their chemical diversity, different analytical methods are required for monitoring purpose. As a consequence, only a small fraction of all the herbicides in use is analyzed in groundwater, surface water, and drinking water, or in research work. The few comprehensive investigations show as a rule that the occurrence of polar herbicides in water bodies is strictly related to the amount applied as well as to failures in good application practice. Thus, positive findings mostly concern atrazine, simazine, terbutylazine and its metabolites, bentazone, isoproturon, diuron, chlorotoluron, MCPP, 2,4-D or glyphosate, and rarely sulfonylureas. Even if raw water used for drinking water production is contaminated by polar herbicides, the drinking water itself is usually not affected. Natural as well as technical filtration and oxidation steps or combined purification techniques are able to solve the problems. Therefore, in Europe only minor cases of exceeding the drinking water standard of 0.1 µg/L are reported. Globally, cases of contamination of drinking water always involve areas where the use of herbicides is intensive and the water treatment is inadequate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	No test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Franco et al. (2012)

Title: Evaluation of Glyphosate Application on Transgenic Soybean and its Relationship with Shikimic Acid	
Author: FRANCO, D.A.S., ALMEIDA, S.D.B., CERDEIRA, A.L., DUKE, S.O., MORAES, R.M., LACERDA, A.L.S. and MATALLO, M.B.	
Reference: Planta Daninha, Viçosa-MG, v. 30, n. 3, p. 659-666, 2012	
Year: 2012	

Results and conclusion: A field experiment was conducted at Engenheiro Coelho-SP, Brazil, during the agricultural year 2007/2008 to evaluate the effect of glyphosate on the growth, development, and seed quality of GRC soybean variety BRS Valiosa RR. A randomized block design was used with four replications. Glyphosate was applied at 720 and 960 g a.e. ha^{-1} (acid equivalent) and in sequence at the doses 720/720, 960/720, and 960/720/720 g a.e. ha^{-1} (acid equivalent). To evaluate transfer from GRC soybean to non GRC soybean cultivated in nutrient solution, a pot experiment was conducted at Instituto Biológico, SP, Brazil. Glyphosate was applied on the GRC soybean (M8045RR) at 2,400 g a.e. ha^{-1}. Both GRC soybean and non GRC soybean were sown in the same box with nutrient solution. At 0, 1, 3, 7, and 10 days after application, shikimic acid was measured by HPLC and the glyphosate and aminomethylphosphonic acid (AMPA) levels in nutrient solution were determined by GC-MS. The results showed that yield, plant height, seed oil, and protein contents were not affected by glyphosate application. GRC soybean accumulated shikimic acid in the field. Glyphosate and AMPA were released through the roots of GRC soybean, and subsequently taken up by non-GRC soybean, exerting inhibitory effects on their shikimic pathway.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	No test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Geng et al. (2015)

Title: Modeling the release of organic contaminants during compost decomposition in soil
Author: C. Geng, C.-S. Haudin, Y. Zhang, G. Lashermes, S. Houot, P. Garnier
Reference: Chemosphere 119 (2015) 423–431
Year: 2015
Results and conclusion: Composts, incorporated in soils as amendments, may release organic contaminants during their decomposition. COP–Soil is presented here as a new model to simulate the interaction between organic contaminants and compost, using one module for organic matter and one for organic pollutants, with these modules being linked by several assumptions. Published results of laboratory soil incubations using labeled carbon pollutants from compost were used to test the model for one polycyclic aromatic hydrocarbon (PAH), two surfactants and one herbicide. Several simulation scenarios were tested using (i) the organic pollutant module either alone or coupled to the organic matter module, (ii) various methods to estimate the adsorption coefficients (K_d) of contaminants on organic matter and (iii) different degrading biomasses. The simulations were improved if the organic pollutant module was coupled with the organic matter module. Multiple linear regression model for K_d as a function of organic matter quality yielded the most accurate simulation results. The inclusion of specific biomass in the model made it possible to successfully predict the PAH mineralization.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate amongst others
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Gerhardt et al. (2012)

Title: Active in Situ Biomonitoring of Pesticide Pulses Using Gammarus spp. in Small Tributaries of Lake Constance	
Author: A. Gerhardt, M. Koster, F. Lang, V. Leib	
Reference: Journal of Environmental Protection, 2012, 3, 573-583, doi:10.4236/jep.2012.37069 Published Online July 2012 (http://www.SciRP.org/journal/jep)	
Year:	
Results and conclusion: Gammarids are important members of a stream's macrozoobenthos biocoenosis and food web. Moreover, they proved to be very sensitive towards different types of pollution. GamToxTM is a new in situ ecotoxicity test, based on survival and feeding behavior of caged gammarids for active monitoring of small streams in agricultural areas. GamToxTM has been applied in two streams with specific pollution problems in the catchment of Lake Constance. Ten organisms were exposed in 5 replicates in flow through test tubes containing one conditioned alder leaf, placed in baskets which were attached in the stream bottom and on the banks. Each week, the number of living animals was counted, the percentage of leaf skeletized estimated in semi-quantitative classes and a new elder leaf provided. Dead organisms were removed. Simultaneously, chemical analyses of pesticides and nutrients (N-compounds, P) were performed on cumulative water samples over one week. Moreover, macrozoobenthos was collected and determined according to the IBCH method, and the SPEAR index calculated. GamToxTM proved to be very sensitive to detect pesticides, copper as well as nutrients, both during acute pollution pulses and chronic exposures of up to 6 weeks. Survival turned out to be a more sensitive and less variable parameter than feeding. GamToxTM is easy to perform and directly provides a measure of ecotoxicological effects of toxicant/nutrient mixtures, which cannot be predicted by biological indices based on macrozoobenthos data such as IBCH and SPEAR-index. This study was co-financed by the InterReg IV project "Ökotoxikologischer Index im Bodenseeraum", no. 227 (2011-2013) supported by the EFRE.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable

Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Greenpeace International (2011)

Title: Herbicide tolerance and GM crops – Why the world should be Ready to Round Up glyphosate
Author: Greenpeace International
Reference: -
Year: 2011
Results and conclusion: Review – Regarding environmental fate the following statements are made: General: It is glyphosate's capacity to bind tightly to soil particles that prevents it from being highly mobile. Binding can immobilise it in the soil provided that there are sufficient suitable sites. This varies depending on the soil type and composition. Studies have found that binding of glyphosate is greater in soils with lower pH (i.e. more acidic) (Gimsing *et al.* 2004) and that phosphates (Simonsen *et al.* 2008) can compete for binding sites. Water: A report by the World Health Organisation (WHO 2005) confirmed that glyphosate is found in surface waters at levels between 0.5 µg/l and 1 µg/l and its environmental breakdown product, AMPA, was present at levels around 6 µg/l. The levels of glyphosate exceed the maximum allowed for pesticides in drinking water under EU law and would require water companies to undertake expensive filtration before the water could be supplied to the public. In streams in the Midwest US, glyphosate was detected during every season up to a maximum concentration of 8.7 µg/l. The maximum concentration of AMPA recorded in this study was 3.67 µg/l. In Alberta, Canada glyphosate was found in 8 out of 13 sites and in 22 % of samples taken in wetland and streams, with a peak concentration of 6.07 µg/l. In Denmark, glyphosate was detected to the depth of the drainage system. Furthermore, loamy soils were found to be more prone to leaching of glyphosate and AMPA than coarse sandy soils, where matrixes of aluminium and iron provide the right conditions for sorption and degradation. On loamy soils, autumn application resulted in detectable concentrations of glyphosate and AMPA in the drainage water in the upper metre of soil, often at concentrations exceeding the EU's maximum concentration for drinking water. The maximum concentrations of glyphosate recorded in drainage water at the two most vulnerable sites were found in 2009 (31 µg/l and 4.7 µg/l respectively). Average concentrations of glyphosate in drainage water following the first drainage after application were well above 0.1 µg/l for some crops, for instance maize in 2005 (4.04 µg/l) and peas in 2001 (0.54 µg/l), both following the application RoundUp. Detection of glyphosate and AMPA was mainly confined to drainage water although it was detected at three sites below the drainage system. At one site in the wet August of 2008, glyphosate was frequently detected in groundwater, with a maximum concentration of 0.67 µg/l. Small catchment studies in Sweden, France and Greece have confirmed that glyphosate can leach into drainage systems and surface waters. A study in France showed that glyphosate can enter watercourses more readily from urban areas via the sewerage system than in rural environments due to applications on roads and railways. High levels were linked to rainfall events. Glyphosate is banned from use on hard surfaces in Denmark and by half of Swedish municipalities. In general, the statements are supported by citations.

Proposed action: Consider as additional information due to the fact that the article is a review and data are cited only. Furthermore, some monitoring data reviewed are obtained at sites outside the EU.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Behaviour of glyphosate in soil and water as well as results from surface water and groundwater monitoring
Protocol	No detailed information in the report on analysed studies
Test compound	Glyphosate and AMPA
Test system and conditions	No information in the report about the analysed studies
Statistical design	Not provided
Relevance	
Environmental relevance	Partly, since some monitoring sites are outside the EU.
Weight of evidence	
"Positive"/"Negative" evidence	Detailed information on the analysed studies is not provided in the report. Therefore, the data in the report cannot be considered for endpoint derivation and/or further risk assessment.

Hadi et al. (2013)

Title: New bacterial strain of the genus Ochrobactrum with glyphosate-degrading activity	
Author: F. HADI, A. MOUSAVI, K. A. NOGHABI, H. G. TABAR and A. SALMANIAN	
Reference: Journal of Environmental Science and Health, Part B (2013) 48, 208–213	
Year: 2013	
Results and conclusion: Thirty bacterial strains with various abilities to utilize glyphosate as the sole phosphorus source were isolated from farm soils using the glyphosate enrichment cultivation technique. Among them, a strain showing a remarkable glyphosate-degrading activity was identified by biochemical features and 16S rRNA sequence analysis as Ochrobactrum sp. (GDQS). Herbicide (3 mM) degradation was induced by phosphate starvation, and was completed within 60 h. Aminomethylphosphonic acid was detected in the exhausted medium, suggesting glyphosate oxidoreductase as the enzyme responsible for herbicide breakdown. As it grew even in the presence of glyphosate concentrations as high as 200 mM, Ochrobactrum sp. could be used for bioremediation purposes and treatment of heavily contaminated soils.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Harris et al. (2012)

Title: Computer Simulation of the Interactions of Glyphosate with Metal Ions in Phloem	
Author: Wesley R. Harris,R. Douglas Sammons, Raymond C. Grabiak, Akbar Mehrsheikh and Marian S. Bleeke‡	
Reference: J. Agric. Food Chem. 2012, 60, 6077−6087	
Year: 2012	
Results and conclusion: Essential nutrients such as trace metal ions, amino acids, and sugars are transported in the phloem from leaves to other parts of the plant. The major chelating agents in phloem include nicotianamine, histidine, cysteine, glutamic acid, and citrate. A computer model for the speciation of metal ions in phloem has been used to assess the degree to which the widely used herbicide glyphosate binds to Fe^{3+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, and Mg^{2+} in this fluid over the pH range of 8 to 6.5. The calculations show that glyphosate is largely unable to compete effectively with the biological chelating agents in phloem. At a typical phloem pH of 8, 1.5 mM glyphosate binds 8.4 % of the total Fe^{3+}, 3.4 % of the total Mn^{2+}, and 2.3 % of the total Mg^{2+} but has almost no effect on the speciation of Ca^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{2+}. As the pH decreases to 6.5, there are some major shifts of the metal ions among the biological chelators, but only modest increases in glyphosate binding to 6 % for Fe^{2+} and 2 % for Zn^{2+}. The calculations also indicate that over 90 % of the glyphosate in phloem is not bound to any metal ion and that none of the metal−glyphosate complexes exceed their solubility limits.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Haudin et al. (2013)

Title: Fate of ^{14}C-organic pollutant residues in composted sludge after application to soil	
Author: C.-S. Haudin,Y. Zhang, V. Dumény, G. Lashermes, V. Bergheaud, E. Barriuso, S. Houot	
Reference: Chemosphere 92 (2013) 1280–1285	
Year: 2013	
Results and conclusion: Organic micropollutants may be present in biosolids, leading to soil contamination when they are recycled in agriculture. A sludge spiked with ^{14}C-labelled glyphosate (GLY), sodium linear dodecylbenzene sulphonate (LAS), fluoranthene (FLT) or 4-n-nonylphenol (NP) was composted with green waste and the fate of the ^{14}C-micropollutant residues remaining after composting was assessed after the compost application to the soil.	

^{14}C-residues were mineralised in the soil and represented after 140 d 20– 32 % of the initial activity for LAS, 16–25 % for GLY, 6–9 % for FLT and 4–7 % for NP. The ^{14}C-residues at the end of composting that could not be extracted with methanol or ammonia were minimally remobilised or even increased for FLT. After 140 d, non-extractable residues represented 38–52 % of all of the ^{14}C-residues remaining in the soil for FLT, 50–67 % for GLY, 91–92 % for NP and 94–97 % for LAS and in most cases, less than 1 % of the ^{14}C-residues were water soluble, suggesting a low direct availability for leaching and microbial or plant assimilation. FLT was identified as the main compound among the methanol-extractable ^{14}C-residues that may be potentially available. The fate of the ^{14}C-organic pollutant residues in composts after application to soil could be assessed through a sequential chemical extraction scheme and depended on the chemical nature of the pollutant. In detail: The fate of the ^{14}C-organic pollutant residues depended on the pollutant and its distribution between the available and non-available fractions in the composts. The residues of each pollutant mineralised to different extents (LAS > GLY > FLT = NP). The composting appeared to have an impact on the stabilisation of the ^{14}C-residues because the non-extractable residues were apparently poorly remobilisable at this time scale (140 d). Nevertheless a part of GLY and FLT residues remained potentially available.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Fate of ^{14}C-organic pollutant residues in composted sludge
Protocol	Non-GLP
Test compound	Four organic pollutants: ^{14}C-labelled glyphosate (GLY), sodium linear dodecylbenzene sulphonate (LAS), fluoranthene (FLT) or 4-n-nonylphenol (NP)
Test system and conditions	A sludge spiked with ^{14}C-labelled glyphosate (GLY), sodium linear dodecylbenzene sulphonate (LAS), fluoranthene (FLT) or 4-n-nonylphenol (NP) was composted with green waste and the fate of the ^{14}C-micropollutant residues remaining after composting was assessed after the compost application to the soil.
Statistical design	Not given in the paper
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Henry et al. (2011)

Title: Glyphosate's Effect Upon Mineral Accumulation in Soybean	
Author: Ryan S. Henry, Kiersten A. Wise, and William G. Johnson	
Reference: Crop Management doi:10.1094/CM-2011-1024-01-RS	
Year: 2011	
Results and conclusion: Glyphosate has been demonstrated to reduce the macronutrient and micronutrient content of glyphosate-susceptible (GS) and first generation glyphosate-resistant (GR) or Roundup Ready (RR) soybean, possibly by complexation of the herbicide molecule with the nutrient. The recent release of newer GR soybean cultivars, second generation Roundup Ready 2 Yield (RR2Y), provides growers with newer technology for weed management programs, but it is unclear how the nutrient content of these cultivars is affected by glyphosate in a field setting. The objective of this experiment was to identify the effect of glyphosate on the concentration of macronutrient and micronutrients in RR and RR2Y soybean when grown using standard agronomic practices in Indiana. The macronutrients analyzed were nitrogen, phosphorus, potassium, sulphur, magnesium, and calcium. The micronutrients analyzed were boron, zinc, manganese, iron, copper, and aluminium. Our results indicate that while differences in accumulation of macro and micronutrients exist between the two cultivars tested, there was no consistent effect due to glyphosate treatment. Glyphosate-induced deficiency symptoms observed in previous reports were not observed in this study. Growers should continue to monitor soil nutrient levels to identify and correct nutrient deficiencies.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Jolley et al. (2004)

Title: Nutritional and management related interactions with iron-deficiency stress response mechanism	
Author: V.D. Jolley, N.C. Hansen, A.K. Shiffler	
Reference: Soil Sci Plant Nutr, 50 (7), 973-981	
Year: 2004	
Results and conclusion: Review article: Iron (Fe) deficiency symptoms develop in many agricultural and horticultural settings and generally occur when susceptible genotypes are grown in calcareous soils where Fe availability is limited. I some situations, Fe deficiency develops as a result of biological interactions with factors other than limited available Fe. We review physiological explanations for some factors known to interact with iron-deficiency stress. The discussion includes interactions with macronutrients and micronutrients, management factors such as grazing and companion cropping, and symbiotic nitrogen fixation. We also refer to several field observed interactions with Fe deficiency in soybean, where physiological explanations are yet to be identified. These include interactions with seeding rate and application of the herbicide glyphosate on glyphosate tolerant varieties.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	

Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Junges et al. (2013)

Title: Effectiveness evaluation of glyphosate oxidation employing the H_2O_2/UVC process: Toxicity assays with Vibrio fischeri and Rhinella arenarum tadpoles
Author: C. M. JUNGES, E. E. VIDAL, A. S M. ATTADEMO, M. L. MARIANI, L. CARDELL, A. C. NEGRO, A. CASSANO, P. M. PELTZER, R. C. LAJMANOVICH and C. S. ZALAZAR
Reference: Journal of Environmental Science and Health, Part B (2013) 48, 163-170
Year: 2013
Results and conclusion: The H_2O_2/UVC process was applied to the photodegradation of a commercial formulation of glyphosate in water. Two organisms (Vibrio fischeri bacteria and Rhinella arenarum tadpoles) were used to investigate the toxicity of glyphosate in samples M_1, M_2, and M_3 following different photodegradation reaction times (120, 240 and 360 min, respectively) that had differing amounts of residual H_2O_2. Subsamples of M_1, M_2, and M_3 were then used to create samples $M_{1,E,}$ $M_{2,E}$ and$M_{3,E}$ in which the H_2O_2 had been removed. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were measured in tadpoles to determine possible sub-lethal effects. In V. fischeri, $M_{1,E,}$ which was collected early in the photodegradation process, caused 52 % inhibition, while $M_{3,E,}$ which was collected at the end of the photodegradation process, caused only 17 % inhibition. Survival of tadpoles was 100 % in samples $M_{2,}$ $M_{3,}$ and in $M_{1,E,}$ $M_{2,E}$ and $M_{3,E}$. The lowest percentages of enzymatic inhibition were observed in samples without removal of H_2O_2: 13.96 % (AChE) and 16 % (BChE) for M_2, and 24.12 % (AChE) and 13.83 % (BChE) for M_3. These results show the efficiency of the H_2O_2/UVC process in reducing the toxicity of water or wastewater polluted by commercial formulations of glyphosate. According to the ecotoxicity assays, the conditions corresponding to M_2 (11 ± 1 mg a.e./L glyphosate and 11 ± 1 mg/L H_2O_2) could be used as a final point for glyphosate treatment with the H_2O_2/UV process.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	
Endpoint	Glyphosate oxidation by the H_2O_2/UVC process and toxicity assays
Protocol	Not given
Test compound	(a) glyphosate (AccuStandard) as a standard chromatographic and (b) glyphosate as the commercial herbicide Eskoba®, 35.6 % (w/v) as acid or 48 % as a monoisopropylamine salt (MIPA)

Test system and conditions	The H_2O_2/UVC process was applied to the degradation of a commercial formulation of glyphosate in water. Two different bioassays were used for determining sample toxicity at different stages of mineralization. The bioassays used the luminescence bacterium Vibrio fischeri (a traditional assay for evaluating AOPs) and tadpoles of Rhinella arenarum, a common anuran that is frequently found in forests, wetlands, agricultural land and urban territories and has an extensive Neotropical distribution. Rhinella was used to assess acute toxicity. In addition, total AChE and BChE activities were evaluated as possible indicators of sub-lethal toxicity of both untreated wastewater and wastewater treated by the H_2O_2/UVC process.
Statistical design	In the R. arenarum survival tests, the percentage of mortality expressed as the mean ± standard error of measurement (SEM) was recorded. The differences in the mortality proportions were estimated using a Chi-Squared Goodness of Fit model (with Yates correction). Enzymatic activities were expressed as the mean ± SEM. The influence of treatments on the B-esterase enzyme activities were analyzed statistically using the non-parametric Kruskal-Wallis ANOVA. Pairwise comparisons between samples from the all treatment were tested by the Dunnett's test for post-hoc multiple comparisons. Statistical significance was held at $\alpha = 0.05$. Analyses were performed with GraphPad InStats®.
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kempenaar et al. (2007)

Title: Trade off between costs and environmental effects of weed control on pavements
Author: C. Kempenaar, L.A.P. Lotz, C.L.M. van der Horst, W.H.J. Beltman, K.J.M. Leemans, A.D. Bannink
Reference: Crop Protection 26, 430–435
Year: 2007
Results and conclusion: An actor-participative project on sustainable weed control on pavements was started in 2000 in the Netherlands. The aim of the project was to develop a new concept of weed management that provides cost-effective and environmentally sound weed control. Early in 2002, practical guidelines were drawn up in support of decision making by managers of pavements, and weed control contractors. The guidelines are focused mainly on reduction of herbicide use and emission thereof. The new concept was tested in 2002 and 2003 in nine Dutch municipalities on defined urban areas of 5-25 ha, which formed units from a construction, hydrology and management point of view. Use of herbicides (mainly glyphosate) was reduced by 11-66 % compared to standard practice. Levels of weed control remained good and ecological threshold concentrations in surface waters were not exceeded. Monitoring showed a glyphosate emission factor via the sewage water system of 2 % on average. Costs of weed control with the new concept were higher (10-25 %) compared to the standard practice control of weeds (using herbicides) on pavements, but much lower compared to alternative (non-herbicide) weed control systems. It is concluded that the new concept provides a useful framework for finding a good trade off between economical and ecological aspects of weed control on pavements.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable

Test compound	Glyphosate, CAS-no.: 1071-83-6; and AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kirk et al. (2013)

Title: Glyphosate and fungicide effects on Cercospora leaf spot in four glyphosate-resistant sugar beet (Beta vulgaris) varieties	
Author: W. W. Kirk, L. E. Hanson, C. L. Sprague	
Reference: Crop Protection 44 (2013) 38-43	
Year: 2013	
Results and conclusion: The potential for improved management of Cercospora leaf spot (CLS), caused by Cercospora beticola, using the herbicide glyphosate in glyphosate-resistant sugar beet varieties was investigated. Controlled field experiments were conducted in 2008 and 2009 to determine if glyphosate and glyphosate-fungicide combinations improved the management of CLS in four commercial varieties of glyphosate-resistant sugar beet. Variety and fungicide main effects were significant for CLS development. However, regardless of the herbicide program, glyphosate or a conventional herbicide program, CLS development was not affected. Therefore, results from of this research indicate that glyphosate and glyphosate-fungicide combinations do not significantly contribute to CLS management.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate and glyphosate-fungicide combinations
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kleter et al. (2008)

Title: Review: Comparison of herbicide regimes and the associated potential environmental effects of glyphosate-resistant crops versus what they replace in Europe	
Author: Gijs A Kleter, Caroline Harris, Gerry Stephenson, John Unsworth	
Reference: Pest Manag Sci 64:479–488	
Year: 2008	
Results and conclusion: Review: While cultivation of transgenic crops takes place in seven of the EU member states, this constitutes a relatively limited part of the total acreage planted to these crops worldwide. The only glyphosate-resistant (GR) crop grown commercially until recently has been soybean in Romania. In addition, large-scale experimental European data exist for GR sugar and fodder beets, and, to a lesser extent, GR oilseed rape. These GR crops are likely to have an impact both on the use of herbicides and on the environmental impact of the latter. From the data on these GR crops, it appears that quantities of herbicides applied to GR beets are decreased while those on GR soybean are slightly increased compared with their conventional counterparts. Depending on the parameters used for prediction or measurement of environmental impacts of GR crops, generally similar or less negative impacts were observed compared with conventional crops. Favourable environmental effects of the glyphosate-containing herbicide regimes on GR crops appear feasible, provided appropriate measures for maintaining biodiversity and prevention of volunteers and gene flow are applied.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable, no test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kremer et al. (2005)

Title: Glyphosate affects soybean root exudation and rhizosphere micro-organisms
Author: ROBERT J. KREMER, NATHAN E. MEANS and SUJUNG KIM
Reference: Intern. J. Environ. Anal. Chem. Vol. 85, No. 15, 1165–1174
Year: 2005
Results and conclusion: Glyphosate is a non-selective, broad-spectrum herbicide that kills plants by inhibiting the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), which is necessary for synthesis of aromatic amino acids. A secondary mode of action involves infection of roots of glyphosate-susceptible plants by soil-borne micro-organisms due to decreased production of plant protection compounds known as phytoalexins. Varieties of several crops, including glyphosate-resistant (GR) or Roundup Ready soybean, are genetically modified to resist the herbicidal effects of glyphosate and provide farmers with an effective weed-management tool. After glyphosate is applied to GR soybean, glyphosate that is not bound to glyphosate-resistant EPSPS is translocated throughout the plant and accumulates primarily in meristematic tissues. We previously reported that fungal colonization of GR soybean roots increased significantly after application of glyphosate but not after conventional postemergence herbicides. Because glyphosate may be released into soil from GR roots, we characterized the response of rhizosphere fungi and bacteria to root exudates from GR and non-GR (Williams 82; W82) cultivars treated with and without glyphosate at field application rates. Using an immunoassay technique, glyphosate at concentrations >1000 ng/plant were detected in exudates of hydroponically grown GR soybean at 16 days post-glyphosate application. Glyphosate also increased carbohydrate and amino acid contents in root exudates in both soybean cultivars. However, GR soybean released higher carbohydrate and amino acid contents in root exudates than W82 soybean without glyphosate treatment. In vitro bioassays showed that glyphosate in the exudates stimulated growth of selected rhizosphere fungi, possibly by providing a selective C and N source combined with the high levels of soluble carbohydrates and amino acids associated with glyphosate treatment of the soybean plants. Increased fungal populations that develop under glyphosate treatment of GR soybean may adversely affect plant growth and biological processes in the soil and rhizosphere.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup Ultra®: Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Kryuchkova et al. (2014)

Title: Isolation and characterization of a glyphosate-degrading rhizosphere strain, Enterobacter cloacae K7	
Author: Y. V. Kryuchkova, G. L. Burygin, N. E. Gogoleva, Y. V. Gogolev, M. P. Chernyshova, O. E. Makarov, E. E. Fedorov, O. V. Turkovskaya	
Reference: Microbiological Research 169 (2014) 99-105	
Year: 2014	
Results and conclusion: Plant-growth-promoting rhizobacteria exert beneficial effects on plants through their capacity for nitrogen fixation, phytohormone production, phosphate solubilization, and improvement of the water and mineral status of plants. We suggested that these bacteria may also have the potential to express degradative activity toward glyphosate, a commonly used organophosphorus herbicide. In this study, 10 strains resistant to a 10 mM concentration of glyphosate were isolated from the rhizoplane of various plants. Five of these strains – Alcaligenes sp. K1, Comamonas sp. K4, Azomonas sp. K5, Pseudomonas sp. K3, and Enterobacter cloacae K7 – possessed a number of associative traits, including fixation of atmospheric nitrogen, solubilization of phosphates, and synthesis of the phytohormone indole-3-acetic acid. One strain, E.cloacae K7, could utilize glyphosate as a source of P. Gas–liquid chromatography showed that E. Cloacae growth correlated with a decline in herbicide content in the culture medium (40 % of the initial 5 mM content), with no glyphosate accumulating inside the cells. Thin-layer chromatography analysis of the intermediate metabolites of glyphosate degradation found that E. cloacae K7 had a C–P lyase activity and degraded glyphosate to give sarcosine, which was then oxidized to glycine. In addition, strain K7 colonized the roots of common sunflower (Helianthus annuus L.) and sugar sorghum (Sorghum saccharatum Pers.), promoting the growth and development of sunflower seedlings. Our findings extend current knowledge of glyphosate-degrading rhizosphere bacteria and may be useful for developing a biotechnology for the cleanup and restoration of glyphosate-polluted soils.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	The source of glyphosate in the study was the commercial formulation Roundup produced and packed by the ZAO Avgust (Russia) under a license agreement with Monsanto Europe S.A. (Belgium).
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Laboski et al. (2012)

Title: Effect of soybean variety, glyphosate use, and manganese application on soybean yield	
Author: Carrie A.M. Laboski, Todd Andraski, Shawn Conley and John Gaska	
Reference: Proc. of the 2012 Wisconsin Crop Management Conference, Vol. 51	
Year: 2012	
Results and conclusion: Application of Mn in starter or as foliar at R1, R3, or R1 + R3 did not increase soybean yield at locations where Mn was expected to be a problem based on low soil test levels or at locations with optimum soil test levels. At all of these locations, R1 tissue Mn concentrations were considered low based on current UW plant analysis interpretation guidelines; however there were no visual Mn deficiency symptoms. It should be noted that some Mn treatments at some locations may have increased yield by a couple bushels, yield reductions with Mn application were also observed. At some tissue sampling times in Outagamie and Walworth, the non-glyphosate resistant variety had greater tissue Mn concentrations compared to the glyphosate resistant variety with either conventional herbicides or glyphosate. The opposite of this was true at Dodge and Jefferson. Overall, these data do not suggest that glyphosate resistant soybean varieties are more sensitive to Mn, or benefit from foliar applications after glyphosate application. These data suggest that a tissue Mn sufficiency concentration range of 54 to 300 ppm may be too high because all sites had R1 tissue Mn concentrations below this range but did not respond to Mn applications. These data also suggest that even on soils where Mn deficiency has the potential to be a problem (low Mn soil test or pH over 6.9 on soils with OM greater than 6.0 %), if no visual deficiency symptoms are apparent, then application of Mn is likely not economical.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

LALLF (2008)

Title: Ergebnisse und Empfehlungen zum Integrierten Pflanzenschutz im Ackerbau 2009	
Author: LALLF (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern)	
Reference: Report of the Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern, Rostock, Germany	
Year: November 2008	
Results and conclusion: Based on experiences and investigation results general recommendations for the use of plant protection products in agricultural practices are given in the report of the federal authorities in Mecklenburg-West Pomerania in Germany. In this context, the relevance of glyphosate containing plant protection products as non-selective herbicides are emphasized. Results from a federal monitoring programme regarding entries of plant protection products in surface water bodies are additionally summarized. Finally, glyphosate was found in 105 of 180 samples with concentrations > 0.1 µg/L in 2008. This finding rate gives evidence for a high frequency of glyphosate deposition into small and medium sized surface water bodies in intensive used agricultural areas.	
Proposed action: To be used as additional information.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information	
Reliability	Reliable
Endpoint	Monitoring, summary information on measured concentrations in surface water bodies in Germany
Protocol	No detailed information available
Test compound	Glyphosate
Test system and conditions	No information available
Statistical design	Not provided
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	The glyphosate findings in surface water bodies with concentrations >0.1 g/L in intensive used agricultural areas in Mecklenburg-West Pomerania in Germany 2008 are in line with the results from other monitoring programmes. However, no details are provided in the report about the measurements, the finding localities and special causes. The information should be considered as additional.

Laitinen et al. (2008)

Title: Effects of Soil Phosphorus Status on Environmental Risk Assessment of Glyphosate and Glufosinate-Ammonium
Author: Pirkko Laitinen, Katri Siimes, Sari Rämö and Lauri Jauhiainen, Liisa Eronen, Seija Oinonen, Helinä Hartikainen
Reference: J. Environ. Qual. 37:830–838
Year: 2008
Results and conclusion: The increased use of herbicides poses a risk to the aquatic environment. Easy and economical methods are needed to identify the fields where specific environment protection measures are needed. Phosphorus (P) and organophosphorus herbicides compete for the same adsorption sites in soil. In this study the relationship between P obtained in routine Finnish agronomic tests (acid ammonium acetate [PAC]) and adsorption of glyphosate and glufosinate-ammonium was investigated to determine whether PAC values could be used in the risk assessment. The adsorption of glyphosate ((N-(phosphonomethyl)glycine) and glufosinate-ammonium (2-amino-4-(hydroxymethylphosphinyl)butanoic acid) was studied in a clay and a sandy loam soil enriched with increasing amounts of P added as potassium dihydrogen phosphate. Desorption was also determined for some P-enriched soil samples. The adsorption of both herbicides diminished with increasing PAC value. The correlations between Freundlich adsorption coefficients obtained in the adsorption tests and PAC were nonlinear but significant ($r > 0.98$) in both soils. The exponential models of the relationship between soil PAC values and glyphosate adsorption were found to fit well to an independent Finnish soil data set ($P < 0.1$ for glyphosate and $P < 0.01$ for glufosinate-ammonium). The desorption results showed that glufosinate-ammonium sorption is not inversely related to soil P status, and the high correlation coefficients obtained in the test of the model were thus artefacts caused by an abnormal concentration of exchangeable potassium in soil. The solved equations are a useful tool in assessing the leaching risks of glyphosate, but their use for glufosinate-ammonium is questionable.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; Glufosinate-ammonium, CAS-no.: 77182-82-2
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Lin et al. (2011)

Title: Reducing Herbicides and Veterinary Antibiotics Losses from Agroecosystems Using Vegetative Buffers	
Author: Chung-Ho Lin, Robert N. Lerch, Keith W. Goyne, and Harold E. Garrett	
Reference: J. Environ. Qual. 40:791–799	
Year: 2011	
Results and conclusion: Multiple species vegetative buffer strips (VBSs) have been recommended as a cost-effective approach to mitigate agrochemical transport in surface runoff derived from agronomic operations, while at the same time offering a broader range of long-term ecological and environmental benefits. However, the effect of VBS designs and species composition on reducing herbicide and veterinary antibiotic transport has not been well documented. An experiment consisting of three VBS designs and one continuous cultivated fallow control replicated in triplicate was conducted to assess effectiveness in reducing herbicide and antibiotic transport for claypan soils. The three VBS designs include (i) tall fescue, (ii) tall fescue with a switchgrass hedge barrier, and (iii) native vegetation (largely eastern gamagrass). Rainfall simulation was used to create uniform antecedent soil moisture content in the plots and to generate runoff. Our results suggested that all VBS significantly reduced the transport of dissolved and sediment-bound atrazine, metolachlor, and glyphosate in surface runoff by 58 to 72 %. Four to 8 m of any tested VBS reduced dissolved sulfamethazine transport in the surface runoff by more than 70 %. The tall fescue VBS was overall most effective at reducing dissolved tylosin and enrofloxacin transport in the runoff (>75 %). The developed exponential regression models can be used to predict expected field-scale results and provide design criteria for effective field implementation of grass buffers. Our study has demonstrated that an optimized VBS design may achieve desired agrochemical reductions and minimize acreage removed from crop production.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; atrazine, CAS-no.: 1912-24-9; s-metolachlor, CAS-no.: 87392-12-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Machado et al. (2009)

Title: Absorption, Translocation and Radicular Glyphosate Exudation in Eucalyptus sp. Clones	
Author: MACHADO, A.F.L., FERREIRA, L.R., SANTOS, L.D.T., SANTOS, J.B., FERREIRA, F.A. and VIANA, R.G	
Reference: Planta Daninha, Viçosa-MG, v. 27, n. 3, p. 549-554	
Year: 2009	
Results and conclusion: To evaluate absorption, translocation and radicular glyphosate exudation in two Eucalyptus sp. clones (2277 and 531), ^{14}C-glyphosate at 1440 g/ha were distributed on the third and fourth leaf blade, under 0.030 µCi of radioactivity. Evaluations were performed 0, 2, 8, 32 and 72 hours after herbicide application-HAA. After 8 HAA, ^{14}C-glyphosate on the leaf was similar in both clones. However, considering the plant, it was higher in 2277, at any evaluation time. After washing the leaves, higher amount of ^{14}C-glyphosate was verified in the water of 531, indicating its smaller herbicide absorption. In the ground tissue and in the roots, ^{14}C-glyphosate was similar in both clones, at any application time though, showing higher concentrations in the roots. Between 0.78 and 1.16 % any of the applied herbicide was exuded into the nutritive solution, without showing difference on translocation and radicular exudation in both clones. The different absorption between the clones can be a likely explanation for the different tolerance among genotypes.	
Proposed action: Not to be considered as publication does not focuses on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	^{14}C-Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Mallmann et al. (2013)

Title: Effects of swine wastewater on glyphosate leaching by liquid chromatography	
Author: L. S. Mallmann, S. C. Sampaio, S. R. Machado Coelho, M. Sorace and L. H. Andrade	
Reference: Journal of Food, Agriculture & Environment Vol.11 (2):908-914. 2013	
Year: 2013	
Results and conclusion: Chemical application on crops comes as an alternative of development and expansion due to the increase in food demand and yield. An example is the use of glyphosate herbicide, applied mainly in soybeans. The West of Paraná State presents itself as a major producer of grain and pork, but the effluents generated in the activity of swine present themselves as potential environmental pollutants when improperly used. An alternative for this is the use of micro-organisms to degrade herbicide in soil. This study aimed at evaluating glyphosate behavior in soil, using swine wastewater as a source of micro-organisms and organic matter. There were four acrylic columns containing soil (sterile or not), with distilled water or wastewater and glyphosate solution (112.5 g/L). The columns were incubated for seven days in a control environmental at 23 °C, so that micro-organisms could adapt themselves to these conditions. Then, it was made the leaching test in order to analyze the samples at high performance liquid chromatography. The treatments showed similar behavior among themselves, with no peak which represented glyphosate. This suggests that it has been adsorbed or mineralized, but organic matter had no influence on the studied treatments. Based on the results found in the work, it can be concluded: Swine wastewater, in the amount used in this study, did not affect the dynamics of herbicide glyphosate in clayey Oxisol. Glyphosate does not have sufficient mobility to contaminate groundwater, in this type of soil, if soil profile has 30 cm or more.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Mamy et al. (2010)

Title: Comparative environmental impacts of glyphosate and conventional herbicides when used with glyphosate-tolerant and non-tolerant crops	
Author: Laure Mamy, Benoît Gabrielle, Enrique Barriuso	
Reference: Environmental Pollution 158, 3172-3178	
Year: 2010	
Results and conclusion: The introduction of glyphosate-tolerant (GT) crops is expected to mitigate the environmental contamination by herbicides because glyphosate is less persistent and toxic than the herbicides used on non-GT crops. Here, we compared the environmental balances of herbicide applications for both crop types in three French field trials. The dynamic of herbicides and their metabolites in soil, groundwater and air was simulated with PRZM model and compared to field measurements. The associated impacts were aggregated with toxicity potentials calculated with the fate and exposure model USES for several environmental endpoints. The impacts of GT systems were lower than those of non-GT systems, but the accumulation in soils of one glyphosate metabolite (aminomethylphosphonic acid) questions the sustainability of GT systems. The magnitude of the impacts depends on the rates and frequency of glyphosate application being highest for GT maize monoculture and lowest for combination of GT oilseed rape and non-GT sugar beet crops.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Sulcotrione Cas-no.:99105-77-8, Metamitron Cas-no.:41394-05-2, Trifluralin Cas-no.:1582-09-8, Metazachlor Cas-no.:67129-08-2, Glyphosate CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Massot et al. (2012)

Title: Biodegradation of phytosanitary products in biological wastewater treatment
Author: A. Massot, K. Estéve, P. Noilet, C. Méoule C. Poupot and M. Mietton-Peuchot
Reference: J. Environ. Qual. 33:816–824
Year: 2012
Results and conclusion: Agricultural activity generates two types of waste: firstly, biodegradable organic effluents generally treated by biological processes and, secondly, phytosanitary effluents which contain residues of plant protection products. The latter are collected and treated. Current technological solutions are essentially based on concentration or physicalechemical processes. However, recent improvements in the biodegradability of pesticides open the way to the consideration of alternative, biological, treatment using mixed liquor from wastewater plant activated sludge. The feasibility of the biological treatment of viticultural effluents has been evaluated by the application of pesticides to activated sludge. The necessity for selection of a pesticide-resistant biomass has been highlighted. The elimination of the phytosanitary products shows the potential of a resistant biomass in the treatment of pesticides. The aerated biological storage ponds at three wineries, followed by a sand or reed-bed filter, were used for the treatment of the total annual volume of the viticulture effluents and validate the laboratory experiments. The results show that the biological purification of pesticides by activated sludge is possible by allowing approximately 8 days for biomass adaptation. Stability of purification occurs between 20 and 30 days.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Mazzei and Piccolo (2012)

Title: Quantitative Evaluation of Noncovalent Interactions between Glyphosate and Dissolved Humic Substances by NMR Spectroscopy
Author: Pierluigi Mazzei and Alessandro Piccolo
Reference: Environ. Sci. Technol. 2012, 46, 5939−5946
Year: 2012
Results and conclusion: Interactions of glyphosate (N-phosphonomethylglycine) herbicide (GLY) with soluble fulvic acids (FAs) and humic acids (HAs) at pH 5.2 and 7 were studied by ^{1}H and ^{31}P NMR spectroscopy. Increasing concentrations of soluble humic matter determined broadening and chemical shift drifts of proton and phosphorus GLY signals, thereby indicating the occurrence of weak interactions between GLY and humic superstructures. Binding was larger for FAs and pH 5.2 than for HAs and pH 7, thus suggesting formation of hydrogen bonds between GLY carboxyl and phosphonate groups and protonated oxygen functions in humic matter. Changes in relaxation and correlation times of ^{1}H and ^{31}P signals and saturation transfer difference NMR experiments confirmed the noncovalent nature of GLY−humic interactions. Diffusion-ordered NMR spectra allowed calculation of the glyphosate fraction bound to humic superstructures and association constants (Ka) and Gibbs free energies of transfer for GLY−humic complex formation at both pH values. These values showed that noncovalent interactions occurred most effectively with FAs and at pH 5.2. Our findings indicated that glyphosate may spontaneously and significantly bind to soluble humic matter by noncovalent interactions at slightly acidic pH and, thus, potentially pollute natural water bodies by moving through soil profiles in complexes with dissolved humus.
Proposed action: Not to be considered for endpoint and PEC-assessment as sorption of glyphosate to fulvic and humic acids has been investigated.
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information only

Reliability	Low
Endpoint	Adsorption of glyphosate to fulvic and humic acids
Protocol	NMR method
Test compound	Glyphosate was added (no purity given); CAS-no.: 1071-83-6
Test system and conditions	^{1}H and ^{31}P NMR techniques were applied to study the occurrence and type of interactions between glyphosate and water-soluble humic substances and calculate the corresponding thermodynamic parameters. On the basis of previous approaches, the adopted NMR techniques consisted of measurements of relaxation times to extrapolate nuclear correlation times and of self-diffusion values to calculate the association constants for humic−glyphosate complexes. Moreover, the homonuclear proton saturation transfer difference (STD) technique was employed here for the first time to prove the formation of noncovalent host−guest complexes between relatively large humic associations and the small glyphosate ligand.
Statistical design	Not reported
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not directly comparable to publications dealing with Glyphosate sorption; no negative evidence.

Mbanaso et al. (2014)

Title: Potential microbial toxicity and non-target impact of different concentrations of glyphosate-containing herbicide (GCH) in a model pervious paving system	
Author: F.U. Mbanaso, S.J. Coupe, S.M. Charlesworth, E.O. Nnadi, A.O. Ifelebuegu	
Reference: Chemosphere 100 (2014) 34–41	
Year: 2014	
Results and conclusion: Pervious Pavement Systems are Sustainable Drainage devices that meet the three-fold SUDS functions of stormwater quantity reduction, quality improvement and amenity benefits. This paper reports on a study to determine the impact of different concentrations of glyphosate-containing herbicides on non-target microorganisms and on the pollutant retention performance of PPS. The experiment was conducted using 0.0484 m^2 test rigs based on a four-layered design. Previous studies have shown that PPS can trap up to 98.7 % of applied hydrocarbons, but results of this study show that application of glyphosate-containing herbicides affected this capability as 15 %, 9 % and 5 % of added hydrocarbons were released by high (7200 mg/L), medium (720 mg/L) and low (72 mg/L) glyphosate-containing herbicides concentrations respectively. The concentrations of nutrients released also indicate a potential for eutrophication if these effluents were to infiltrate into aquifers or be released into surface waters. The effect of glyphosate-containing herbicides application on the bacterial and fungal communities was slightly different; fungi exhibited a ‘‘top-down’’ trend as doses of 7200 mg/L glyphosate-containing herbicides yielded the highest fungal growth whilst those with a concentration of 720 mg/L glyphosate-containing herbicides applied yielded the highest bacterial growth. In the case of protists, doses of glyphosate-containing herbicides above 72 mg/L were fatal, but they survived at the lower concentration, especially the ciliates Colpoda cucullus and Colpoda steinii thus indicating potential for their use as biomarkers of herbicide-polluted environments. Data also showed that at the lowest concentration of glyphosate-containing herbicides (72 mg/L), biodegradation processes may not be affected as all trophic levels required for optimum biodegradation of contaminants were present.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate-containing herbicide (GCH)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Motavalli et al. (2004)

Title: Impact of Genetically Modified Crops and Their Management on Soil Microbially Mediated Plant Nutrient Transformations
Author: P. P. Motavalli, R. J. Kremer, M. Fang, and N. E. Means
Reference: J. Environ. Qual. 33:816–824
Year: 2004
Results and conclusion: One of the potential environmental effects of the recent rapid increase in the global agricultural area cultivated with transgenic crops is a change in soil microbial mediated processes and functions. Among the many essential functions of soil biota are soil organic matter decomposition, nutrient mineralization and immobilization, oxidation-reduction reactions, biological N fixation, and solubilisation. However, relatively little research has examined the direct and indirect effects of transgenic crops and their management on microbial mediated nutrient transformations in soils. The objectives of this paper are to review the available literature related to the environmental effects of transgenic crops and their management on soil microbial mediated nutrient transformations, and to consider soil properties and climatic factors that may affect the impact of transgenic crops on these processes. Targeted genetic traits for improved plant nutrition include greater plant tolerance to low Fe availability in alkaline soils, enhanced acquisition of soil inorganic and organic P, and increased assimilation of soil N. Among the potential direct effects of transgenic crops and their management are changes in soil microbial activity due to differences in the amount and composition of root exudates, changes in microbial functions resulting from gene transfer from the transgenic crop, and alteration in microbial populations because of the effects of management practices for transgenic crops, such as pesticide applications, tillage, and application of inorganic and organic fertilizer sources. Possible indirect effects of transgenic crops, including changes in the fate of transgenic crop residues and alterations in land use and rates of soil erosion, deserve further study. Despite widespread public concern, no conclusive evidence has yet been presented that currently released transgenic crops, including both herbicide and pest resistant crops, are causing significant direct effects on stimulating or suppressing soil nutrient transformations in field environments. Further consideration of the effects of a wide range of soil properties, including the amount of clay and its mineralogy, pH, soil structure, and soil organic matter, and variations in climatic conditions, under which transgenic crops may be grown, is needed in evaluating the impact of transgenic crops on soil nutrient transformations. Future environmental evaluation of the impact of the diverse transgenic crops under development could lead to an improved understanding of soil biological functions and processes.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Neumann et al. (2006)

Title: Relevance of glyphosate transfer to non-target plants via the rhizosphere	
Author: G. NEUMANN, S. KOHLS, E. LANDSBERG, K. STOCK-OLIVEIRA SOUZA, T. YAMADA, V. RÖMHELD	
Reference: Journal of Plant Diseases and Protection, Special Issue/Sonderheft XX, 963-969 (2006), ISSN 1861-4051	
Year: 2006	
Results and conclusion: There is a common understanding that the widely used herbicide glyphosate is easily degraded and adsorbed in soils and thus, harmless for use in agriculture. We can demonstrate, however, that this conclusion is wrong and dangerous for farmers because in former risk assessments the behaviour of glyphosate in the rhizosphere was not properly considered. In nutrient solution, rhizobox and pot experiments we can show that foliar applied glyphosate to target plants is released into the rhizosphere after a fast translocation from shoots to roots. In the rhizosphere glyphosate can obviously be stabilized long enough to achieve negative effects on non-target plants. Such a negative side effect is for example inhibited acquisition of micronutrients such as Mn, but also Zn, Fe and B, which are involved in plant own disease resistance mechanisms. From this glyphosate transfer from target to non-target plants (e.g. from weed to trees in orchards) we predict an increase in disease problems, particularly on soils with low micronutrient availability as already reported in the USA. In view of plant and soil health, we urgently call for a re-assessment of glyphosate as herbicide.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup-Ultra (Monsanto, St. Louis, USA)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Ndjeri et al. (2013)

Title: Degradation of glyphosate and AMPA (amino methylphosphonic acid) solutions by thin films of birnessite electrodeposited: A new design of material for remediation processes?
Author: M. Ndjeri, A. Pensel, S. Peulon, V. Haldys, B. Desmazières, A. Chaussé
Reference: Colloids and Surfaces A: Physicochem. Eng. Aspects 435 (2013) 154-169
Year: 2013
Results and conclusion: The paper focuses on the possibility to use birnessite thin films for remediating aqueous solutions containing glyphosate and AMPA, the most found pollutants in environment. Indeed, glyphosate is the pesticide the most used in the world, and AMPA is its main metabolite, more toxic and more persistent than its parent. However, AMPA can also mainly come from the degradation of phosphonic acids present in detergents. We show that birnessite, electrodeposited as thin films onto a cheap transparent semiconductor substrate (SnO_2), can significantly degrade and mineralise glyphosate and AMPA. Glyphosate is spontaneously degraded with simultaneous production of AMPA, formaldehyde, phosphate ions, nitrate ions and ammonium ions, without macroscopic modification of birnessite. The last four by-products are also obtained during the degradation of AMPA by birnessite. Various experimental parameters such as temperature, concentration of pollutant, stirring of solution, presence or not of oxygen were studied and a schematic summary of observed evidences was proposed. The good mineralisation yields obtained during glyphosate/birnessite and AMPA/birnessite interactions allow us to envisage a possible application of these thin films for remediation.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate and AMPA
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Nourouzi et al. (2011)

Title: Glyphosate Utilization as the Source of Carbon: Isolation and Identification of new Bacteria
Author: M. MOHSEN NOUROUZI, T.G. CHUAH, THOMAS S.Y. CHOONG and C.J. LIM
Reference: E-Journal of Chemistry 8(4), 1582-1587
Year: 2011
Results and conclusion: Mixed bacteria from oil palm plantation soil (OPS) were isolated to investigate their ability to utilize glyphosate as carbon source. Results showed that approximately all of the glyphosate was converted to aminomethylphosphonic acid (AMPA) (99.5 %). It is worthy to note that mixed bacteria were able to degrade only 2% of AMPA to further metabolites. Two bacterial strains i.e. Stenotrophomonas maltophilia and Providencia alcalifaciens were obtained from enrichment culture. Bacterial isolates were cultured individually on glyphosate as a sole carbon source. It was observed that both isolates were able to convert glyphosate to AMPA.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate-contaminated soil and Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Nourouzi et al. (2012)

Title: Modeling biodegradation and kinetics of glyphosate by artificial neural network	
Author: MOHSEN M. NOUROUZI, TEONG G. CHUAH, THOMAS S.Y. CHOONG and F. RABIEI	
Reference: Journal of Environmental Science and Health, Part B (2012) 47, 455–465	
Year: 2012	
Results and conclusion: The result showed that ANN model was able to accurately predict the experimental results. A low ratio of self-inhibition and half saturation constants of Haldane equations (<8) exhibited the inhibitory effect of glyphosate on bacteria growth. The value of Ki/Ks increased when the mixed inoculum size was increased from 104 to 106 bacteria/mL. It was found that the percentage of glyphosate degradation reached a maximum value of 99 % at an optimum pH 6-7 while for pH values higher than 9 or lower than 4, no degradation was observed.	
Proposed action: To be considered as additional information. Sufficient information on water treatment procedures is available. Furthermore, no fate related endpoint or any PEC-calculation is affected by the results of the article.	
Type of information (critical, high/low weight, supporting, additional): Low weight	
Reliability	Medium
Endpoint	Not applicable, model development study
Protocol	Not applicable, model development study
Test compound	Not applicable, model development study
Test system and conditions	An artificial neural network (ANN) model was developed to simulate the biodegradation of herbicide glyphosate [2-(Phosphonomethylamino) acetic acid] in a solution with varying parameters pH, inoculum size and initial glyphosate concentration. The predictive ability of ANN model was also compared with Monod model.
Statistical design	Not applicable, model development study
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable, model development study

Olesen and Cedergreen (2010)

Title: Glyphosate uncouples gas exchange and chlorophyll fluorescence	
Author: Charlotte F Olesen and Nina Cedergreen	
Reference: Pest Manag Sci 66: 536–542	
Year: 2010	
Results and conclusion: Background: Changes in chlorophyll fluorescence have often been advocated as a sensitive biomarker of plant stress, assuming that any kind of plant stress serious enough to affect plant growth will also affect photosynthesis. Glyphosate affects photosynthetic electron transport indirectly by inhibiting sink processes. The question is how fast this inhibition can be observed on CO_2 assimilation and ultimately on chlorophyll fluorescence? Results: Experiments measuring CO_2 assimilation, conductance and chlorophyll fluorescence using four Kautsky curve parameters on barley (Hordeum vulgare L.) exposed to increasing doses of glyphosate showed a total cessation of CO_2 fixation and conductance without significant changes in chlorophyll fluorescence. The decrease in CO_2 fixation and conductance was significant 1 day after spraying and corresponded well to the decrease in biomass 5–7 days after spraying. Conclusion: A total cessation of CO_2 assimilation can take place without affecting chlorophyll fluorescence. Hypotheses concerning what happens to the energy from the photosynthetic apparatus that is not used for CO_2 assimilation are discussed. The results question the use of chlorophyll fluorescence as a universal indicator of stress on photosynthetic processes. Also, they demonstrate that changes in gas-exchange parameters are more sensitive biomarkers for glyphosate toxicity compared with chlorophyll fluorescence.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; glyphosate (isopropylamine salt), CAS-no.:38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Oliver et al. (2014)

Title: Banded applications are highly effective in minimising herbicide migration from furrow-irrigated sugar cane
Author: D.P. Oliver, J. S. Anderson, A. Davis, S. Lewis, J. Brodie, R. Kookana
Reference: Science of the Total Environment 466–467 (2014) 841–848
Year: 2014
Results and conclusion: Runoff from farm fields is a common source of herbicide residues in surface waters in many agricultural industries around the world. In Queensland, Australia, the runoff of PSII inhibitor herbicides (in particular diuron and atrazine) is a major concern due to their potential impact on the Great Barrier Reef. This study compared the conventional practice of broadcast application of herbicides in sugarcane production across the whole field with the banded application of particular herbicides onto raised beds only using a shielded sprayer. This study found that the application of two moderately soluble herbicides, diuron and atrazine, to only the raised beds decreased the average total load of both herbicides moving off-site by N90 % compared with the conventional treatment. This was despite the area being covered with the herbicides by the banded application being only 60 % less than with the conventional treatment. The average total amount of atrazine in drainage water was 7.5 % of the active ingredient applied in the conventional treatment compared with 1.8 % of the active ingredient applied in the banded application treatment. Similarly, the average total amount of diuron in drainage water was 4.6 % of that applied in the conventional treatment compared with 0.9 % of that applied in the banded application treatment. This study demonstrates that the application of diuron and atrazine to raised beds only is a highly effective way of minimising migration of these herbicides in drainage water from furrow irrigated sugarcane. Furthermore, the study found that glyphosate concentrations in drainage water from the Banded treatment bays were below the detection limit suggesting that it would be a good alternative herbicide to atrazine and diuron for use in furrows. However, other studies have found detectable concentrations of glyphosate in tailwater draining from furrow-irrigated sugarcane (Davis, unpublished data), which would suggest that further work on different soil types is required before glyphosate can be fully endorsed as an alternative to diuron and atrazine in the furrows.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Additional information

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Atrazine, diuron, 2,4-D, paraquat, glyphosate
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Orcaray et al. (2012)

Title: Impairment of carbon metabolism induced by the herbicide glyphosate	
Author: Luis Orcaray, Amaia Zulet, Ana Zabalza, Mercedes Royuela	
Reference: Journal of Plant Physiology 169, 27– 33	
Year: 2012	
Results and conclusion: The herbicide glyphosate reduces plant growth and causes plant death by inhibiting the biosynthesis of aromatic amino acids. The objective of this work was to determine whether glyphosate-treated plants show a carbon metabolism pattern comparable to that of plants treated with herbicides that inhibit branched-chain amino acid biosynthesis. Glyphosate-treated plants showed impaired carbon metabolism with an accumulation of carbohydrates in the leaves and roots. The growth inhibition detected after glyphosate treatment suggested impaired metabolism that impedes the utilization of available carbohydrates or energy at the expected rate. These effects were common to both types of amino acid biosynthesis inhibitors. Under aerobic conditions, ethanolic fermentative metabolism was enhanced in the roots of glyphosate-treated plants. This fermentative response was not related to changes in the respiratory rate or to a limitation of the energy charge. This response, which was similar for both types of herbicides, might be considered a general response to stress conditions.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (commercial formula, Glyfos, BayerGarden, Valencia, Spain), CAS-no.: 1071-83-6; glufosinate (commercial formula, Finale, BayerCropscience, Valencia, Spain), CAS-no.: 51276-47-2
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Ozturk et al. (2007)

Title: Glyphosate inhibition of ferric reductase activity in iron deficient sunflower roots
Author: Levent Ozturk, Atilla Yazici, Selim Eker, Ozgur Gokmen, Volker Römheld and Ismail Cakmak
Reference: New Phytologist doi: 10.1111/j.1469-8137.2007.02340 x
Year: 2007

Results and conclusion: Iron (Fe) deficiency is increasingly being observed in cropping systems with frequent glyphosate applications. A likely reason for this is that glyphosate interferes with root uptake of Fe by inhibiting ferric reductase in roots required for Fe acquisition by dicot and nongrass species. This study investigated the role of drift rates of glyphosate (0.32, 0.95 or 1.89 mM glyphosate corresponding to 1, 3 and 6 % of the recommended herbicidal dose, respectively) on ferric reductase activity of sunflower (Helianthus annuus) roots grown under Fe deficiency conditions. Application of 1.89 mM glyphosate resulted in almost 50 % inhibition of ferric reductase within 6 h and complete inhibition 24 h after the treatment. Even at lower rates of glyphosate (e.g. 0.32 mM and 0.95 mM), ferric reductase was inhibited. Soluble sugar concentration and the NAD(P)H oxidizing capacity of apical roots were not decreased by the glyphosate applications. To our knowledge, this is the first study reporting the effects of glyphosate on ferric reductase activity. The nature of the inhibitory effect of glyphosate on ferric reductase could not be identified. Impaired ferric reductase could be a major reason for the increasingly observed Fe deficiency in cropping systems associated with widespread glyphosate usage.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate formulated as Roundup Ultra (active ingredient N-[phosphonomethyl]glycine isopropylamine salt; Monsanto Ltd, Adana, Turkey), CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Pesce et al. (2008)

Title: Longitudinal changes in microbial planktonic communities of a French river in relation to pesticide and nutrient inputs
Author: Stéphane Pesce, Céline Fajon, Corinne Bardot, Frédérique Bonnemoy, Christophe Portelli, Jacques Bohatier
Reference: Aquatic Toxicology 86, 352–360
Year: 2008

Results and conclusion: To determine the effects of anthropic activities on river planktonic microbial populations, monthly water samples were collected for 11 months from two sampling sites characterized by differing nutrient and pesticide levels. The difference in trophic level between the two stations was particularly pronounced from May to November. Total pesticide concentrations were notably higher at the downstream station from April to October with a clear predominance of herbicide residues, especially the glyphosate metabolite aminomethylphosphonic acid (AMPA). From spring, algal biomass and density were favored by the high orthophosphate concentrations recorded at the downstream location. However, isolated drops in algal biomass were recorded at this sampling station, suggesting an adverse effect of herbicides on algal communities. No major difference was observed in bacterial heterotrophic production, density, or activity (CTC reduction) between the two sampling stations. No major variation was detected using the fluorescent in situ hybridization (FISH) method, but shifts in bacterial community composition were recorded by PCR-TTGE analysis at the downstream station following high nutrient and pesticide inputs. However, outside the main anthropic pollution period, the water's chemical properties and planktonic microbial communities were very similar at the two sampling sites, suggesting a high recovery potential for this lotic system.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Pipke and Amrhein (1988)

Title: Isolation and Characterization of a Mutant of *Arthrobacter* sp. Strain GLP-1 Which Utilizes the Herbicide Glyphosate as Its Sole Source of Phosphorus and Nitrogen	
Author: Pipke, R. And Amrhein, N.	
Reference: Applied and Environmental microbiology, Nov. 1988, Vol. 54, No. 11, 2868-2870	
Year: 1988	
Results and conclusion: *Arthrobacter* sp. strain GLP-1, grown on glucose as a carbon source, utilizes the herbicide glyphosate [N-(phosphonomethyl)glycine] as its sole source of phosphorus as well as its sole source of nitrogen. The mutant strain GLP-1/Nit-1 utilizes glyphosate as its sole source of nitrogen as well. In strain GLP-1, Pi was a potent competitive inhibitor of glyphosate uptake (Ki, 24, uM), while the affinity of Pi for the uptake system of strain GLP-1/Nit-1 was reduced by 2 orders of magnitude (Ki, 2.3 mM). It is concluded that the inability of strain GLP-1 to utilize glyphosate as a source of nitrogen is due to the stringent control of glyphosate uptake by excess phosphate released during the degradation of the herbicide.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable

Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.:1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Puértolas et al. (2010)

Title: Evaluation of side-effects of glyphosate mediated control of giant reed (Arundo donax) on the structure and function of a nearby Mediterranean river ecosystem

Author: Laura Puértolas, Joana Damásio, Carlos Barata, Amadeu M.V.M. Soares, Narcís Prat

Reference: Environmental Research 110, 556–564

Year: 2010

Results and conclusion:
The aim of this study was to evaluate the effect of the application of the herbicide Herbolex (Aragonesas Agro, S.A., Madrid, Spain) to control giant reed (Arundo donax), which has glyphosate as active ingredient, on the structure and function of a nearby river ecosystem. Specifically, we assessed glyphosate environmental fate in the surrounding water and its effects on transplanted Daphniamagna, field collected caddisfly (Hydropsyche exocellata) and on benthic macroinvertebrate structure assemblages. Investigations were conducted in the industrialized and urbanized Mediterranean river Llobregat (NE Spain) before and after a terrestrial spray of glyphosate. Four locations were selected to include an upstream site and three affected ones. Measured glyphosate levels in river water following herbicide application were quite high (20-60 mg/l) with peak values of 137 mg/l after three days. After 12 days of its application, leaching of glyphosate from sprayed river banks was quite high in pore water (20-85 mg/l) but not in the river. Closely linked with the measured poor habitat and water physico-chemical conditions, macroinvertebrate communities were dominated by taxa tolerant to pollution and herbicide application did not affect the abundance or number of taxa in any location. Nevertheless, significant specific toxic effects on transplanted D magna and field collected H. exocellata were observed. Effects included D. magna feeding inhibition and oxidative stress related responses such as increased antioxidant enzyme activities related with the metabolism of glutathione and increased levels of lipid peroxidation. These results emphasize the importance of combined chemical, ecological and specific biological responses to identify ecological effects of pesticides in the field.

Proposed action:
Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional):
Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6; AMPA, CAS-no.:1066-51-9
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Qin et al. (2013)

Title: Can rainwater induce Fenton-driven degradation of herbicides in natural waters?	
Author: J. Qin, H. Li, C. Lin, G. Chen	
Reference: Chemosphere 92 (2013) 1048–1052	
Year: 2013	
Results and conclusion: Microcosm experiments were conducted to examine Fenton reaction-driven degradation of three common herbicides exposed to a variety of Fe^{2+}–H_2O_2 combinations that are likely to be encountered in natural water environments. The results show that these combinations had significant ($P < 0.05$) effects on removing the water-borne herbicides. This discovery sheds some light on the possible role of rainwater-borne H_2O_2 in inducing Fenton reaction in many natural waters such as lakes, streams, estuaries and tidal zones, fishponds and paddy fields that may contain ferrous ion at micromolar levels. The research findings obtained from this preliminary work provide a rationale for undertaking further study to confirm the presence of an overlooked naturally-occurring process that may lead to rapid dissipation of many herbicides and other organic pollutants in open water environments.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Effect of Fe^{2+}–H_2O_2 combinations on removing herbicides
Protocol	Not given
Test compound	Diuron, butachlor and glyphosate (purity 98 %, 98 % and 97 %)
Test system and conditions	A total of nine Fe^{2+}-H_2O_2 combinations were set for the herbicide degradation experiment with the concentration of both Fe^{2+} and H_2O_2 ranging from 5 to 50 μM. In addition, three concentration levels were also set for ''Fe^{2+} only'' and ''H_2O_2 only'' systems. The aqueous system without the added Fe^{2+} and H_2O_2 served as the control. Each experiment was performed in triplicate in a room with the temperature being controlled at 25 ± 1 °C. Centrifuge tubes (capacity: 10 mL) were used as batch reactors. For each reactor, 4 mL of a relevant herbicide stock solution with appropriate concentration were added into the tube, followed by simultaneous addition of 3 mL of an appropriate Fe^{2+} stock solution and 3 mL of an appropriate H_2O_2 stock solution. After addition of all the ingredients, the tube was capped, hand shaken for 30 s, and stood for 1 h before taking samples for determinations of residual herbicides. Glyphosate in the solution was determined using a DIONEX ICS-900 ion chromatography system.
Statistical design	The statistical significance of difference between the treatment means was determined by the Duncan's multiple range test.
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Rampoldi et al. (2011)

Title: The Fate of Glyphosate in Crop Residues	
Author: E. Ariel Rampoldi, Susana Hang, Enrique Barriuso	
Reference: Soil Sci. Soc. Am. J. 75, Number 2:553–559	
Year: 2011	
Results and conclusion: The environmental fate of glyphosate [N-(phosphonomethyl)glycine] was studied in six crop residue (CR) types, three from maize (Zea mays L.) (M1, M2, and M3) and three from soybean [Glycine max (L.) Merr.] (S1, S2, and S3). Glyphosate adsorption was characterized through isotherms. The glyphosate distribution in CRs was characterized through the balance of ^{14}C-glyphosate radioactivity among the mineralized fraction, the extractable fractions (water and NH_4OH), and the non-extractable fraction. Crop residues were characterized by elemental composition, organic C, total N, and biochemical parameters (soluble fraction, cellulose, hemicellulose, and lignin). Total microbial activity (TMA) was also assessed. Limited and reversible glyphosate adsorption on soybean and maize CRs was determined. The sorption coefficient K_f index range for maize CR was 1.5 to 8.3 L/kg and 2.6 to 7.4 L/kg for soybean CR. Organic C and hemicelluloses partially explained adsorption variability. The addition of mineralized and non-extractable fractions of the initial ^{14}C-glyphosate applied on the CRs averaged 56 %; however, differences were detected between soybean and maize CRs. Mineralization and non-extractable residues were 30.7 ± 11 and 32.5 ± 6 % (soybean CR) and 44.3 ± 12 and 17 ± 7 % (maize CR), respectively. We hypothesized that glyphosate molecules could be used initially by microorganisms as a labile C source. High variability in ^{14}C-glyphosate mineralization was observed in all crop residues, suggesting that the magnitude of the glyphosate mineralization process would be regulated by accessibility and the lability of other carbonate sources.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	^{14}C-Glyphosate and unlabeled glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Reddy et al. (2010)

Title: Glyphosate Effect on Shikimate, Nitrate Reductase Activity, Yield, and Seed Composition in Corn	
Author: KRISHNA N. REDDY, NACER BELLALOUI, AND ROBERT M. ZABLOTOWICZ	
Reference: J. Agric. Food Chem. 58, 3646–3650	
Year: 2010	
Results and conclusion: When glyphosate is applied to glyphosate-resistant (GR) crops, drift to non-glyphosate-resistant (non-GR) crops may cause significant injury and reduce yields. Tools are needed to quantify injury and predict crop losses. In this study, glyphosate drift was simulated by direct application at 12.5 % of the recommended label rate to non-GR corn (Zea mays L.) at 3 or 6 weeks after planting (WAP) during two field seasons in the Mississippi delta region of the south-eastern USA. Visual plant injury, shikimate accumulation, nitrate reductase activity, leaf nitrogen, yield, and seed composition were evaluated. Effects were also evaluated in GR corn and GR corn with stacked glufosinate-resistant gene at the recommended label rate at 3 and 6 WAP. Glyphosate at 105 g ae/ha was applied once at 3 or 6 weeks after planting to non-GR corn. Glyphosate at 840 (lower label limit) or 1260 (upper label limit) g ae/ha was applied twice at 3 and 6 WAP to transgenic corn. Glyphosate caused injury (45-55 %) and increased shikimate levels (24-86 %) in non-GR compared to non-treated corn. In non-GR corn, glyphosate drift did not affect starch content but increased seed protein 8-21 % while reducing leaf nitrogen reductase activity 46-64 %, leaf nitrogen 7-16 %, grain yield 49-54 %, and seed oil 18-23 %. In GR and GR stacked with glufosinate-resistant corn, glyphosate applied at label rates did not affect corn yield, leaf and seed nitrogen, or seed composition (protein, oil, and starch content). Yet, nitrate reductase activity was reduced 5-19 % with glyphosate at 840 + 840 g/ha rate and 8-42 % with glyphosate at 1260 + 1260 g/ha rate in both GR and GR stacked corn. These results demonstrate the potential for severe yield loss in non-GR corn exposed to glyphosate drift.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup Weathermax, Monsanto Agricultural Co., St. Louis,MO), CAS-no.: 40465-60-5
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Rodríguez-Rodríguez et al. (2013)

Title: On-farm biopurification systems: role of white rot fungi in depuration of pesticide-containing wastewaters	
Author: C. E. Rodríguez-Rodríguez, V. Castro-Gutiérrez, J. S. Chin-Pampillo & K. Ruiz-Hidalgo	
Reference: FEMS Microbiol Lett 345 (2013) 1–12	
Year: 2013	
Results and conclusion: Environmental contamination with pesticides is an undesired consequence of agricultural activities. Biopurification systems (BPS) comprise a novel strategy to degrade pesticides from contaminated wastewaters, consisting of a highly active biological mixture confined in a container or excavation. The design of BPS promotes microbial activity, in particular by white rot fungi (WRF). Due to their physiological features, specifically the production of highly unspecific ligninolytic enzymes and some intracellular enzymatic complexes, WRF show the ability to transform a wide range of organic pollutants. This minireview summarizes the potential participation of WRF in BPS. The first part presents the potential use of WRF in biodegradation of pollutants, particularly pesticides, and includes a brief description of the enzymatic systems involved in their oxidation. The second part presents an outline of BPS, focusing on the elements that influence the participation of WRF in their operation, and includes a summary of the studies regarding the fungal-mediated degradation of pesticides in BPS biomixtures and other solid-phase systems that mimic BPS.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Review article
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Rojano-Delgado et al. (2012)

Title: Limited uptake, translocation and enhanced metabolic degradation contribute to glyphosate tolerance in Mucuna pruriens var. utilis plants	
Author: Antonia María Rojano-Delgado, Hugo Cruz-Hipolito, Rafael De Prado, María Dolores Luque de Castro and Antonio Rodríguez Franco	
Reference: Phytochemistry 73 (2012) 34–41	
Year: 2012	
Results and conclusion: Velvet bean (Mucuna pruriens, Fabaceae) plants exhibits an innate, very high resistance (i.e., tolerance) to glyphosate similar to that of plants which have acquired resistance to this herbicide as a trait. We analyzed the uptake of [^{14}C]-glyphosate by leaves and its translocation to meristematic tissues, and used scanning electron micrographs to further analyze the cuticle and 3D capillary electrophoresis to investigate a putative metabolism capable of degrading the herbicide. Velvet bean exhibited limited uptake of glyphosate and impaired translocation of the compound to meristematic tissues. Also, for the first time in a higher plant, two concurrent pathways capable of degrading glyphosate to AMPA, Pi, glyoxylate, sarcosine and formaldehyde as end products were identified. Based on the results, the innate tolerance of velvet bean to glyphosate is possibly a result of the combined action of the previous three traits, namely: limited uptake, impaired translocation and enhanced degradation.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Rosolem et al. (2009)

Title: Manganese uptake and redistribution in soybeans as affected by glyphosate	
Author: Rosolem, C A, Andrade, Gabriel JM, Lisboa, Izaias P, Zoca, Samuel M	
Reference: The Proceedings of the International Plant Nutrition Colloquium XVI, Department of Plant Sciences, UC Davis, UC Davis http://escholarship.ucop.edu/uc/item/3f53794z	
Year: 2009	
Results and conclusion: A detrimental effect of glyphosate on soybean Mn nutrition has been reported, which could happen even when applying the herbicide to weeds infesting soybean crops. Three experiments were conducted to study the effect of glyphosate on Mn absorption kinetics, accumulation, distribution within the soybean plant and soybean response to Mn as affected by this herbicide. In a nutrient solution experiment, using the solution depletion technique, Mn uptake kinetics (V_{max}, K_m and C_{min}) were determined for a conventional and its near-isogenic glyphosate-resistant counterpart cultivar as affected by glyphosate applied to the nutrient solution. In another nutrient solution experiment, differential Mn accumulation and distribution were studied for the same cultivars. In the third experiment, with Mn-deficient soil in pots, the response of glyphosate-resistant soybean cultivars to Mn application was studied in the presence of the herbicide. A few days after herbicide treatment, soybean plants developed yellowish leaves, a symptom that, in the field, could be misinterpreted as Mn deficiency. But there was no evidence of deleterious effects of glyphosate on Mn absorption, accumulation, distribution in the plant and response by soybean cultivars.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Rosolem et al. (2010)

Title: MANGANESE UPTAKE AND REDISTRIBUTION IN SOYBEAN AS AFFECTED BY GLYPHOSATE
Author: Ciro Antonio Rosolem, Gabriel José Massoni de Andrade, Izaias Pinheiro Lisboa & Samuel Menegatti Zoca
Reference: R. Bras. Ci. Solo, 34:1915-1922
Year: 2010

Results and conclusion: Detrimental effects of glyphosate on plant mineral nutrition have been reported in the literature, particularly on Mn uptake and redistribution. However, in most of the experiments conducted so far glyphosate-susceptible plants were used. Effects of glyphosate on Mn absorption kinetics, accumulation, and distribution within the plant, as well as soybean response to Mn as affected by glyphosate were studied in three experiments. In the first experiment, in nutrient solution, the effect of glyphosate on soybean Mn uptake kinetic parameters (I_{max}, K_m and C_{min}) was determined. In a second experiment, also in nutrient solution, differential Mn accumulation and distribution were studied for a conventional soybean cultivar and its near-isogenic glyphosate-resistant counterpart as affected by glyphosate. In a third experiment, response of glyphosate-resistant soybean cultivars to Mn application was studied in the presence of glyphosate, in pots with Mn-deficient soil. Maximum Mn influx (I_{max}) was higher in the herbicide-resistant (GR) cultivar than in its conventional counterpart. Glyphosate applied to nutrient solution at low rates decreased K_m and C_{min}. A few days after herbicide treatment, RR soybean plants developed yellowish leaves, a symptom which, in the field, could be misinterpreted as Mn deficiency, but herbicide application had no effect on Mn uptake or distribution within the plant. In the soil experiment, soybean Mn uptake was increased by Mn application, with no effect of glyphosate. Under greenhouse conditions, there was no evidence of deleterious effects of glyphosate on Mn absorption, accumulation and distribution in the plant and on soybean cultivars response to Mn application.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Santos et al. (2007)

Title: Effects of Glyphosate Formulations on Transgenic Soybean
Author: SANTOS, J.B., FERREIRA, E.A., REIS, M.R., SILVA, A.A., FIALHO, C.M.T. e FREITAS, M.A.M.
Reference: Planta Daninha, Viçosa-MG, v. 25, n. 1, p. 165-17
Year: 2007
Results and conclusion: This study aimed to evaluate the effects of three glyphosate formulations (Roundup Ready® and R. Transorb®-both with isopropylamine salt and Zapp Qi®, formulated as potassium salt), on transgenic soybean. CD 219RR variety soybean plants displaying the CP4Epsps gene, tolerant to glyphosate, were cultivated. At 25 days after emergence (DAE), when plants showed the second trifolium completely expanded (stadiums V2-V3), formulations were applied at 2,000 g/ha. Plants intoxication was evaluated 15 days after application as well as the number and dry matter of leaflets, number of radicular nodules and foliar content of N, P, K, S, Ca, Mg, Fe, Cu, Zn, and Mn at flowering and grain yield at the end of the cycle. Soil basal respiration rate, microbial biomass carbon and metabolic quotient were evaluated through soil samples collected during soybean flowering. Isopropylamine salt, present in the Roundup Transorb formulation, was more harmful to the soybean plants, also providing a negative effect on the soil microbiota. Roundup Ready formulation, registered as transgenic soybean, should not be applied on this crop at a higher rate, since it could alter the content of some nutrients, such as N, Ca, Mg, Fe and Cu, besides causing intoxication in the plants.

Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup Ready® and R. Transorb®-both with isopropylamine salt CAS-no.: 38641-94-0 and Zapp Qi®, formulated as potassium salt Glyphosate, CAS-no.: 70901-12-1
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Sarigiannis et al. (2013)

Title: Inventory of pesticide emissions into the air in Europe

Author: D.A. Sarigiannis, P. Kontoroupis, E.S. Solomou, S. Nikolaki, A.J. Karabelas

Reference: Atmospheric Environment 75 (2013) 6-14

Year: 2013

Results and conclusion:
Creation of a reliable and comprehensive emission inventory of the pesticides used in Europe is a key step towards quantitatively assessing the link between actual pesticide exposure and adverse health effects. An inventory of pesticide emissions was generated at a 1 × 1 km grid, for the year 2000. The emission model comprises three components: estimates of active substance (AS) wind drift taking into account crop type, volatilization during pesticide application and volatilization from the crop canopy. Results show that atmospheric emission of pesticides varies significantly across Europe. Different pesticide families are emitted from different parts of Europe as a function of the main crop(s) cultivated, agro-climatic conditions and production intensity.

The pesticide emission inventory methodology developed herein is a valuable tool for assessing air quality in rural and peri-urban Europe, furnishing the necessary input for atmospheric modelling at different scales. Its estimates have been tested using global sensitivity and Monte Carlo analysis for uncertainty assessment and they have been validated against national and local surveys in four European countries; the results demonstrate the robustness and reliability of the inventory. The latter may therefore be readily used for exposure and health risk assessment studies targeting farmers, applicators, but also bystanders and the general population in Europe.

Results in deatil: Glyphosate emissions do not exceed 70 kg yr^{-1} km^2. The highest values are computed in parts of Portugal, Germany, the UK and Denmark.

Quantities used and emissions for 1,3-dichloropropene, mancozeb, chlorpyrifos and glyphosate in Europe (total):

1,3-dichloropropene		**Mancozeb**		**Chlorpyrifos**		**Glyphosate**	
Quantities in tons yr^{-1}	**Emissions in tons yr^{-1}**	**Quantities in tons yr^{-1}**	**Emissions in tons yr^{-1}**	**Quantities in tons yr^{-1}**	**Emissions in tons yr^{-1}**	**Quantities in tons yr^{-1}**	**Emissions in tons yr^{-1}**
939	520	6960	2505	253	84	13335	3393

Proposed action:
To be considered as additional information.

Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Pesticide emissions into the air in Europe
Protocol	Not given
Test compound	Pesticides, e.g. glyphosate (herbicide), chlorpyrifos (insecticide), mancozeb (fungicide)
Test system and conditions	Overview: The multi-step methodology developed to create the EU-wide pesticide emission inventory at a 1×1 km grid is as follows: (a) Starting from data on crop cultivated areas per grid cell, pesticide AS lists per crop and country are created, taking into account the current legislation. The final AS list encompasses thus the effect of use restrictions of hazardous AS. (b) Annual pesticide usage data at the country level are disaggregated to a fine 1×1 km resolution via a spatial allocation algorithm. (c) On that basis, an emission model is developed capturing the main physico-chemical processes that govern pesticide emission into the air. (d) The effects of model input to the estimated emission are investigated using global sensitivity methods. On the basis of the relative weight of the input parameters the spatially distributed consumption data are used as input to the emission model to deduce yearly air emission per crop in each grid cell. (e) Pesticide input data are validated against pesticide usage and sales data in locations in North and South Europe at different spatial resolutions. Corrective steps are taken when necessary to increase the robustness and accuracy of the final estimates. (f) Emission rate output is validated via Monte Carlo simulation using its inputs to a dispersion model to deduce concentration in a rural site for a number of pesticides found in the inventory.
Statistical design	Global sensitivity analysis according to the Sobol method (Sobol, 1993) was used to quantify the variation in emission due to model inputs such as ambient temperature, fraction of pesticide lost due to drift, fraction intercepted by the crop and lateral distance from the source. After sensitivity analysis, uncertainty was assessed via Monte Carlo Simulation (MCS). It involved a large number of samples (typically hundreds of thousands) from the distribution of the input parameters (i.e. crop fraction per cell, the fraction intercepted by crop) that were combined to obtain probability distributions for the emission rate output and thus statistically quantify the residual uncertainty.
Relevance	
Environmental relevance	Given
Weight of evidence	
“Positive”/”Negative”	No negative evidence.

Schönherr and Schreiber (2004)

Title: Interactions of Calcium Ions with Weakly Acidic Active Ingredients Slow Cuticular Penetration: A Case Study with Glyphosate
Author: JÖRG SCHÖNHERR AND LUKAS SCHREIBER
Reference: J. Agric. Food Chem. 52, 6546-6551
Year: 2004

Results and conclusion:
Potassium and calcium salts of glyphosate were obtained by titrating glyphosate acid with the respective bases to pH 4.0, and rates of penetration of these salts across isolated astomatous cuticular membranes (CMs) were measured at 20 °C and 70, 80, 90, and 100 % humidity. K-glyphosate exhibited first-order penetration kinetics, and rate constants (k) increased with increasing humidity. Ca-glyphosate penetrated only when the humidity above the salt residue was 100 %. At 90 % humidity and below, Ca-glyphosate formed a solid residue on the CMs and penetration was not measurable. With Ca-glyphosate, the k value at 100 % humidity decreased with time and the initial rates were lower than for K-glyphosate by a factor of 3.68. After equimolar concentrations of ammonium oxalate were added to Ca-glyphosate, high penetration rates close to those measured with K-glyphosate were measured at all humidities. Adding ammonium sulfate or potassium carbonate also increased rates between 70 and 100 % humidity, but they were not as high as with ammonium oxalate. The data indicate that at pH 4.0 one Ca^{2+} ion is bound to two glyphosate anions. This salt has its deliquescence point near 100 % humidity. Therefore, it is a solid at lower humidity and does not penetrate. Its molecular weight is 1.82 times larger than that of K-glyphosate, and this greatly slows down rates of penetration, even at 100 % humidity. The additives tested have low solubility products and form insoluble precipitates with Ca^{2+} ions, but only ammonium oxalate binds Ca^{2+} quantitatively. The resulting ammonium salt of glyphosate penetrates at 70-100 % humidity and at rates comparable to K-glyphosate. The results contribute to a better understanding of the hard water antagonism observed with glyphosate. It is argued that other pesticides and hormones with carboxyl functions are likely to respond to Ca^{2+} ions in a similar fashion. In all of these cases, ammonium oxalate is expected to overcome hard water antagonism.

Proposed action:
Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional):
Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	^{14}C-Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Schuette (1998)

Title: Environmental fate of glyphosate
Author: Jeff Schuette
Reference: Revised Report of the Environmental Monitoring & Pest Management Department of Pesticide Regulation Sacramento, CA 95824-5624, California, US
Year: November 1998
Results and conclusion: A summary of the physical-chemical properties, the environmental fate of glyphosate in air, in water and sediment, in soil and in biota and the toxicity of the active substance are provided based on different studies from regulatory context and/or open literature. An overview of results from eight studies conducted in the forest environment is additionally provided. The summarized studies indicate that glyphosate is adsorbed to mineral clays and organic matter and is excluded from these sites by inorganic phosphate. Glyphosate has limited preemergence herbicidal activity in most soils because of its tendency to adsorb strongly to soil. The K_{OC} values indicate that glyphosate will not move readily through soil, and under conditions of the summarized studies, glyphosate would not leach into non-target areas. Glyphosate is inactivated in soil and water by microbial degradation. When applied to foliage, glyphosate is readily absorbed and translocated to various parts of plant via the phloem.
Proposed action: To be used as supporting information.
Type of information (critical, high/low weight, supporting, additional): Low weight, supporting information, review report on environmental fate studies

Reliability	High, review report from a regulatory authority
Endpoint	Range of degradation and adsorption endpoints in soil, water, air
Protocol	No detailed information in the report on analysed studies
Test compound	Glyphosate
Test system and conditions	No information in the report about the analysed studies
Statistical design	Not provided
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	Detailed information on the analysed studies is not provided in the report. Therefore, the data in the report cannot be considered for endpoint derivation and/or further risk assessment.

Sebiomo et al. (2012)

Title: The Impact of Four Herbicides on Soil Minerals
Author: A. Sebiomo, V.W. Ogundero and S.A. Bankole
Reference: Research Journal of Environmental and Earth Sciences 4(6): 617-624, 2012
Year: 2012

Results and conclusion: The aim of this study was to investigate the interaction of atrazine, primextra, paraquat and glyphosate with soil minerals. The treatments were carried out for a period of 6 weeks; at company recommended rates of 4l/h (at 350 mL in 15 L sprayer) for paraquat, glyphosate and primeextra while recommended rate of 3 kg/h (atrazine powder) was used for atrazine treatment (soil treatments were carried out in triplicates). Soil moisture content was determined using Satorious Moisture content Analyser. Soil mineral concentration were then determined by injecting sample solutions (extract) and standard solution for each mineral into the atomic absorption spectrophotometer into sample fray and the mean signal response was recorded for each of the element at their respective wavelength. The concentrations of the minerals were then calculated. Herbicide treated soils showed reduction in the moisture content from the second to the sixth weeks of treatment. There was significant ($p<0.001$) reduction in Sodium ion (Na) and Calcium ion (Ca) concentration compared to the control. The potassium, Magnesium, Iron and Zinc (K, Mg, Fe and Zn) increased significantly ($p<0.001$) compared to the control. Ths study has elucidated the ability of herbicides to chelate with soil minerals thereby reducing their availability for uptake by plants. It has also been shown that soil minerals are utilised by plants and microbes during microbial degradation.	
Proposed action: Not to be considered as the study was performed under outdoor conditions in Nigeria; not representative for EU.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information on Glyphosate sorption to soil minerals	
Reliability	Low
Endpoint	Mineral contents of the soils
Protocol	Non-GLP
Test compound	Glysate, Nantong Ji Angshan Agrochemicals (glyphosate, CAS-no.: 1071-83-6)
Test system and conditions	Soil treatments: The treatments were carried out for a period of 6 weeks, at company recommended rates of 4 l/h (at 350 mL in 15 L sprayer) for paraquat, glyphosate and primeextra while recommended rate of 3 kg/h (atrazine powder) was used for atrazine treatment (soil treatments were carried out in triplicates). Soil sampling: Top soil up to 5 cm depth samples was collected from cassava farm in Ijebu-Ode (Ogun State, Nigeria) with no prior pesticide treatment. The soil samples were sieved through a 2.0 mm width mesh to remove stones and plant debris.
Statistical design	Not specified
Relevance	
Environmental relevance	Given
Weight of evidence	
"Positive"/"Negative" evidence	Other studies support the results; no negative evidence.

Serra et al. (2011)

Title: Glyphosate influence on nitrogen, manganese, iron, copper and zinc nutritional efficiency in glyphosate resistant soybean
Author: Ademar Pereira Serra, Marlene Estevão Marchetti, Ana Carina da Silva Candido, Ana Caroline Ribeiro Dias, Pedro Jacob Christoffoleti
Reference: Ciência Rural, v.41, n.1, p. 77-84
Year: 2011

Results and conclusion:
After development of glyphosate-resistant (GR) soybean, there is a considerable raise in the use of this herbicide, with three to four applications during the culture cycle. Thus, these applications may be influencing the mineral nutrition of the crop. So, the aim of this research was evaluate the glyphosate influence on uptake, translocation and use efficiency of N, Mn, Cu, Zn and Fe by (GR) soybean 'P98R31' cultivar. The experiment was conducted in the greenhouse at ESALQ/USP, Piracicaba, State of São Paulo, Brazil, in 2009. The experimental unit was formed by 11 kg/vase of soil (Rhodic Paleudult) with two plants in each vase. The treatments have been arranged in a factorial pathway 5X5, with five levels of the factor Mn (0, 20, 40, 60 and 80 mg/dm³) and five of glyphosate drifts (0; 0,648; 1,296; 1,944 e 2,592 kg e.a./ha) and the Mn was supplied by the manganese sulphate. The experimental design was randomized blocks, with four repetitions. There was no influence on response from plants concerning the levels of Mn used into the experiment. The application of glyphosate interfered on mineral nutrition of soybean and the total contents of N, Mn, Cu, Zn and Fe. The use of glyphosate has caused reduction of the nodules number and reduced the output of dry mass.

Proposed action:
Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional):
Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Shushkova et al. (2010)

Title: Glyphosate bioavailability in soil

Author: Tatyana Shushkova, Inna Ermakova, Alexey Leontievsky

Reference: Biodegradation 21:403–410

Year: 2010

Results and conclusion:
Biodegradation of glyphosate in sod-podzol soil by both the indigenous micro flora and the introduced strain Ochrobactrum anthropi GPK 3 was studied with respect to its sorption and mobility. The experiments were carried out in columns simulating the vertical soil profile. Soil samples studied were taken from soil horizons 0-10, 10-20, and 20-30 cm deep. It was found out that the most of the herbicide (up to 84 %) was adsorbed by soil during the first 24 h; the rest (16 %) remained in the soluble fraction. The adsorbed glyphosate was completely extractable by alkali. No irreversible binding of glyphosate was observed. By the end of the experiment (21st day), glyphosate was only found in extractable fractions. The comparison of the effect of the introduced O. anthropi GPK 3 and indigenous microbial community on the total toxicant content (both soluble and absorbed) in the upper 10 cm soil layer showed its reduction by 42 % (21 mg/kg soil) and 10-12 % (5 mg/kg soil), respectively. Simultaneously, 14-18 % glyphosate moved to a lower 10-20 cm layer. Watering (that simulated rainfall) resulted in a 20 % increase of its content at this depth; 6-8 % of herbicide was further washed down to the 20-30 cm layer. The glyphosate mobility down the soil profile reduced its density in the upper layer, where it was available for biodegradation, and resulted in its concentration in lower horizons characterized by the absence (or low level) of biodegradative processes. It was shown for the first time how the herbicide biodegradation in soil can be increased manifold by introduction of the selected strain O. anthropi GPK 3.

Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Herbicide Ground Bio (Tekhnoexport, Russia), containing GP as its isopropylamine salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Soltani et al. (2011)

Title: Influence of manganese on efficacy of glyphosate in glyphosate-resistant soybean
Author: Nader Soltani, Christy Shropshire, and Peter H. Sikkema
Reference: Can. J. Plant Sci. 91: 1061-1064
Year: 2011
Results and conclusion: Four field trials were conducted from 2007 to 2010 in Ontario to evaluate the effect of various manganese (Mn) formulations (Mn1, Ecoman 5 % Mn; Mn2, MangaMax 5.5 % Mn; Mn3, ManMax 5.5 % Mn; Mn4, Superman 5 % Mn; Mn5, Stoller This 5 % Mn; Mn6, Nortrace 6 % Mn-EDTA (ethylenediaminetetraacetate); Mn7, Nortrace 22 % Mn and Mn8, WolfTrax 33 % Mn) applied at 2.0 kg actual Mn/ha on glyphosate efficacy at 900 g a.e./ha in glyphosate-resistant soybean. The tank mix of glyphosate plus Mn4, Mn6 or Mn8 caused as much as 6, 17 and 4 % injury in soybean, respectively. There was minimal crop injury (0-1.4 %) with other Mn tank mixes. The addition of Mn4 or Mn6 to glyphosate did not antagonize glyphosate efficacy on the weeds evaluated (AMARE, AMBEL, CHEAL and SETVI). The other Mn formulations antagonized glyphosate efficacy for the control of AMARE, AMBEL, CHEAL or SETVI under some environments. The addition of Mn3 or Mn6 to glyphosate reduced soybean yield as much as 15 and 10 % compared with glyphosate alone, respectively. Based on these results, it is recommended that glyphosate and manganese applications be applied sequentially to avoid weed control antagonism and maximize soybean yield.

Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (WeatherMax)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Sorvari and Jaakkonen (2011)

Title: Environmental Risks Caused by Pesticides at Forest Nurseries in Finland	
Author: Jaana Sorvari and Satu Jaakkonen	
Reference: Human and Ecological Risk Assessment, 17: 431–466	
Year: 2011	
Results and conclusion: Previously, various persistent pesticides were used extensively in the production of seedlings at Finnish forest nurseries. The extent and magnitude of the risks arising from the consequent environmental contamination are largely unknown. Therefore, we selected two representative nurseries for which we conducted tiered health risk assessments (HRA) using risk-based benchmarks and two calculation tools (SSL and Risc-Human software). Ecological risk assessments (ERA) involved comparisons of environmental concentrations with ecotoxicological benchmarks. Site investigations revealed that the concentration of several pesticides exceeded the Finnish soil quality guidelines in some places. The compost pile for organic residues and the pond receiving runoffs contained traces of pesticides and the maximum concentration of atrazine and terbuthylazine in groundwater exceeded the corresponding guideline for household water. Hexachloro-benzene proved to pose the highest health risks, the maximum hazard quotient being around 10 (carcino-genity-based) in the residential land use scenario. Owing to the conservative assumptions, health risks are expected to remain insignificant, however. Risks to the local terrestrial ecosystem would also remain low, while only further studies will reveal the actual risks to the adjoining aquatic ecosystem. Both calculation tools showed shortcomings that generate uncertainty in the HRA, whereas the ERA was hampered particularly by the lack of benchmarks.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate; CAS-no.: 1071-83-6; AMPA, CAS-no.: 1066-51-9 and other 68 pesticides
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Sprague et al. (2012)

Title: Relating Management Practices and Nutrient Export in Agricultural Watersheds of the United States	
Author: L. A. Sprague and J. A. M. Gronberg	
Reference: J. Environ. Qual. 41, doi:10.2134/jeq2012.0073	
Year: 2012	
Results and conclusion: Relations between riverine export (load) of total nitrogen (N) and total phosphorus (P) from 133 large agricultural watersheds in the United States and factors affecting nutrient transport were evaluated using empirical regression models. After controlling for anthropogenic inputs and other landscape factors affecting nutrient transport- such as runoff, precipitation, slope, number of reservoirs, irrigated area, and area with subsurface tile drains-the relations between export and the area in the Conservation Reserve Program (CRP) (N) and conservation tillage (P) were positive. Additional interaction terms indicated that the relations between export and the area in conservation tillage (N) and the CRP (P) progressed from being clearly positive when soil erodibility was low or moderate, to being close to zero when soil erodibility was higher, to possibly being slightly negative only at the 90th to 95th percentile of soil erodibility values. Possible explanations for the increase in nutrient export with increased area in management practices include greater transport of soluble nutrients from areas in conservation tillage; lagged response of stream quality to implementation of management practices because of nitrogen transport in groundwater, time for vegetative cover to mature, and/or prior accumulation of P in soils; or limitations in the management practice and stream monitoring data sets. If lags are occurring, current nutrient export from agricultural watersheds may still be reflecting the influence of agricultural landuse practices that were in place before the implementation of these management practices.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Stefanello et al. (2011)

Title: Effect of glyphosate and manganese on nutrition and yield of transgenic glyphosate-resistant soybean	
Author: Fabio Fernando Stefanello; Marlene Estevão Marchetti; Eulene Francisco da Silva; Josemar Stefanello; Rafael Bonifácio Sabino Doreto; Jose Oscar Novelino	
Reference: Ciências Agrárias, Londrina, v. 32, n. 3, p. 1007-1014, jul/set. 2011	
Year: 2011	
Results and conclusion: Research suggests that the application of glyphosate on transgenic glyphosate-resistant soybean can cause induced deficiency of Mn. Thus, the aim of this work was to evaluate the application of glyphosate and manganese in post-emergence on different phenological growth stages of RR soybean and its effects on leaf nutrient contents and productivity of grains. The experiment was carried out at two farms in Rio Brilhante-MS, both with randomized block experimental design with six replications at Lages de Pedra farm and four replications at São Manoel farm. Treatments were established in 3 x 8 factorial schemes, where the factor A consisted of three treatments with glyphosate (without the application of glyphosate, application of 720 g i.a. in the growth stage V2 + 480 g a. in V4, and application of 1.200 g i.a. in V4 growth stage). The factor B consisted of eight treatments with foliar application of Mn being without application, and seven Mn application was sprayed the leaves with 332 g/ha, divided into different growth stages. The application of glyphosate on transgenic soybean did not have effect on leaf nutrient contents, including the absorbing of Mn. Yield and mass of 100 grains were not influenced by applying of glyphosate neither by leaf fertilization with Mn, and leaf applying of Mn influenced only the leaf contents of Mn and Fe.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup Ready®, isopropylammmonium salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Steinmann et al. (2013)

Title: Glyphosat – ein Herbizid in der Diskussion und die Suche nach dem „Notwendigen Maß“	
Author: H.-H. Steinmann	
Reference: Gesunde Pflanzen (2013) 65:47–56, DOI 10.1007/s10343-013-0297-2	
Year: 2013	
Results and conclusion: Glyphosate is the most widely used herbicidal active ingredient in the world. In Germany, the distributed amounts have been doubled during the last ten years. There is public concern and criticism on this extensive use and use limitations are claimed. However, also loss of efficacy as reported from countries with high use intensity of glyphosate reduces long term use of these herbicides. Recently, scientific studies aimed to quantify the economic benefits of glyphosate to estimate the costs of losing or banning this herbicide. In this text, possibilities of reductions of glyphosate use in arable farming are discussed to obtain a necessary extent. It is pointed out, that those uses are preferential, that enable minimum soil cultivation that minimize soil erosion. Post-harvest applications have the potential for use reductions, due to replacement techniques such as soil cultivation that are available. Pre-harvest applications, which are targeted for crop maturation only, should not be used as a routine. It is suggested to seek for best management practices of glyphosate use.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	-
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Swift and Bell (2011)

Title: What are the environmental consequences of using silviculturally effective forest vegetation management treatments?	
Author: Kathie I. Swift and F. Wayne Bell	
Reference: THE FORESTRY CHRONICLE, VOL. 87, NO.2	
Year: 2011	
Results and conclusion: In this paper, we present examples of stand-level consequences of using forest vegetation management treatments in boreal and temperate forest ecosystems in Canada. Specifically, we address several selected indicators: air and water quality, soils and nutrients, plant diversity, and wildlife habitat. For each of these, we discuss direct and indirect effects of five broad categories of treatments: (1) silviculture and harvesting systems and (2) physical, (3) thermal, (4) cultural, and (5) chemical/biological treatments. Our emphasis is on forest vegetation management treatments that are currently used in Canada to manage conifers. By applying regulations and best management practices, conducting landscape-level analyses and developing longer-term monitoring programs resource managers can minimize the effects of FVM treatments on the environmental indicators presented in this paper. Continued monitoring of abiotic and biotic indicators will be needed to reduce uncertainty related to the use of FVM treatments in Canadian forests is recommended. Although the literature available does point to limited short-term effects, an understanding of the cumulative effects of FVM treatments over multiple rotations is lacking. In addition, FVM treatments continue to evolve and uncertainties about the effects of new or modified treatments will arise. For example, demand for fibre as an energy source is on the rise in the boreal forests of Canada and will affect the volume of woody and structural material removed from boreal ecosystems.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Syversen (2005)

Title: Cold-climate vegetative buffer zones as pesticide-filters for surface runoff	
Author: N. Syversen	
Reference: Water Science & Technology Vol 51 No 3-4 pp 63–71	
Year: 2005	
Results and conclusion: Vegetative buffer zones adjacent to watercourses can be effective filters for diffuse pollution from agriculture. Several investigations, even during snowmelt season, have shown that retention of sediments and sediment-bound nutrients in runoff water has been high through buffer zones (BZ). It is likely that BZ also can be effective filters for sediment-bound pesticides. The retention of glyphosate, propiconazole, fenpropimorph and soil particles was studied in surface runoff experiments with 5 m wide buffer zones. Volume proportional samples were collected after each runoff episode (1999-2002). The distribution coefficient (K_d) shows moderate to high adsorption of the pesticides to the experimental soil. Results show average retention efficiency of about 51 %, 48 %, 85 % and 34 % for particles, glyphosate, propiconazole and fenpropimorph, respectively. The amount of AMPA (which is a degradation product of glyphosate), entering the BZ was high; approximately the same amount as for glyphosate. The retention efficiency through the BZ for AMPA was about 67 %. There were no significant differences in removal efficiency (in %) between winter with snowmelt and summer. This is possibly due to detachment of coarser aggregates during winter, which trap more easily in the BZ. The conclusion based on this study suggests BZ to be contributors to reduced pesticide input to surface waters.	
Proposed action: Not to be considered as publication deals with risk mitigation. Thus, no fate related endpoints or the monitoring chapter are affected.	
Type of information (critical, high/low weight, supporting, additional): Low weight, additional information about buffer zones could be contributors to reduce pesticide input to surface waters. Glyphosate and AMPA are trapped in the BZ, because they are adsorbed to soil particles.	
Reliability	Medium
Endpoint	Average removal efficiency in %
Protocol	Norwegian Standard 4733
Test compound	Glyphosate (CAS-no.: 1071-83-6), propiconazole and fenpropimorph
Test system and conditions	Four study plots with an upper supply area of 10 m x 45 m each with cereal production (barley), and a lower part with a buffer zone area of 10 m x 5 m (two plots) and no buffer zone (two reference plots), respectively, were used. The application rate represented a normal pesticide application in the area. Volume proportional mixed samples were taken after every runoff event or as frequently as 1-2 times a day during the snowmelt period. From the sampling tank, water samples were collected for laboratory analysis of glyphosate, fenpropimorph, propiconazole, suspended solids (SS), and soil texture.
Statistical design	Glyphosate results were adjusted according to R = -9,64 Ln(SS) + 113 and also corrected for recovery of the analysis itself. A simple linear regression model (Eq. 2) was used to correct for differences in runoff between the plots
Relevance	
Environmental relevance	Environmental parameters are measured and reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence; results supported by other publications.

Syversen and Bechmann (2004)

Title: Vegetative buffer zones as pesticide filters for simulated surface runoff
Author: Nina Syversen, Marianne Bechmann
Reference: Ecological Engineering 22, 175-184
Year: 2004
Results and conclusion: Losses of pesticides from agricultural land to surface waters can cause environmental harm to fish and other aquatic organisms. Vegetated buffer zones (BZ) between agricultural land and surface waters have proved to be effective filters for sediments and sediment-bound nutrients. It is therefore; likely that BZ also can be effective filters for pesticides, especially sediment-bound pesticides. The retention of glyphosate, fenpropimorph, propiconazole and soil particles was studied in short-term BZ experiments with simulated surface runoff. Runoff water containing pesticides and soil particles was added directly to the BZ. The BZ was 5 m wide and consisted of natural grass/herbaceous vegetation. To calculate retention efficiency of pesticides and particles through the BZ, surface runoff was collected before entering and after passing the BZ. The average removal efficiency was 39, 71, 63 and 62 % for glyphosate, fenpropimorph, propiconazole and soil particles, respectively. Aminomethylphosphonic acid (AMPA), which is a degradation product of glyphosate, constituted only a small part of glyphosate (about 10 %) in this short-term experiment. Based on this study BZ can serve as contributors to reduce pesticide input to surface waters. .
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, Round UpEco; fenpropimorph and propiconazole, Tilt-Top
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Tang et al. (2010)

Title: Study on the Degradation Characteristics of Yeast Strain S-2 on Glyphosate Herbicide
Author: Tang *et al.*
Reference: Journal of Anhui Agri. Sci. 2010, 38 (4): 1992-1994
Year: 2010
Results and conclusion: Study is written in Chinese language.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Not applicable, study is written in Chinese language.

Reliability	Not applicable
Endpoint	Not applicable

Protocol	Not applicable
Test compound	Not applicable
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Tesfamariam et al. (2009)

Title: Fate of glyphosate stored in weed residues and the potential of phytotoxicity for following crops
Author: Tesfamariam, T., Bott, S., Roemheld, V., Neumann, G.
Reference: The Proceedings of the International Plant Nutrition Colloquium XVI, Department of Plant Sciences, UC Davis, UC Davis
Year: 2009
Results and conclusion: Glyphosate, a broad spectrum, non-selective herbicide, is the world's most important and widely used herbicide. The globally increasing adoption of no-till or reduced tillage systems is becoming a driving force for enhanced glyphosate use. In such systems, glyphosate is applied pre-sowing for weed control and glyphosate may remain in root and shoot residues. To evaluate potential risks associated with glyphosate residues, a pot experiment was conducted under controlled greenhouse conditions with two contrasting soils: weakly buffered acidic Arenosol and highly buffered Luvisol. Glyphosate was supplied as glyphosate enriched rye grass straw (1.2 g DM/kg soil) prior to sowing sunflower as a non-target plant. Several physiological parameters, such as intracellular shikimate accumulation as a metabolic indicator for glyphosate toxicity, biomass production and micronutrient status were analyzed. Detrimental effects on sunflower plants linked to glyphosate toxicity were observed only in the Arenosol but not in the Luvisol. This is most probably related to the difference in soil properties. The detoxification capacity of the fine-textured Luvisol, with a high clay content, was high enough for an adequate immobilization and inactivation of glyphosate. On the sandy Arenosol, the level of glyphosate supply exceeded the detoxification capacity. In addition to the difference in detoxification capacity, differences in nutrient bio-availability might also have aggravated the observed inhibition of nutrient acquisition. Thus, the findings suggest the importance of weed residues in transferring glyphosate from target to nontarget plants, particularly in no-till or reduced tillage systems, with the consequence of detrimental effects on following crop plants.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Additional information

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Todorovic et al. (2010)

Title: Dispersion of glyphosate in soils through erosion	
Author: Gorana Rampazzo Todorovic, Axel Mentler, Nicola Rampazzo, Winfried E.H. Blum, Alexander Eder, Peter Strauss	
Reference: Environmental Quality 4, 125-138	
Year: 2010	
Results and conclusion: For a better understanding of the influence of erosion processes on glyphosate behaviour and dispersion under rainy conditions after application in the practice, two rain simulation experiments were conducted on two different locations in Austria with complete different soil types in September 2008. The results of the experiments showed that under normal practical conditions, the potential adsorption capacity of the Kirchberg soil is confirmed compared to the low adsorption Chernosem soil (about 8,000 ppm pedogenic Fe-oxides). Considering the enormous differences in the run-off amounts between the two sites Pixendorf and Kirchberg it can be concluded how important the soil surface conditions and vegetation cover of the agricultural fields for erosion risk and pollution risk of surface water are. In the rainfall simulation experiments under comparable simulation conditions, the amount of run-off at Kirchberg was app. 10 times higher than at the Pixendorf site, due to its better infiltration rate. Moreover, the total loss of glyphosate (NT+CT) through run-off was more than double on the Kirchberg site, which confirms the higher risk of pesticide pollution for surface waters on the agricultural fields with higher erosion intensity.	
Proposed action: Not to be considered, no data for recalculation of endpoint, furthermore, no raw data are reported with sufficient precision.	
Type of information (critical, high/low weight, supporting, additional): Low weight, only information of the importance of the soil surface conditions and vegetation cover of the agricultural fields for erosion risk and pollution risk of surface water are presented.	
Reliability	Low
Endpoint	Figures of time dependent glyphosate concentration
Protocol	Non-GLP
Test compound	Round up (450 g glyphosate /L)
Test system and conditions	The rain simulation experiments took place in 3 field replications of the Conventional Tillage (CT)- and No Tillage (NT)-plots. Before starting the rain simulation, erosion plots were installed in each field repetition in a dimension of 2 m x 2 m. The culture type at time of the experiments was different in both sites (Pixendorf had a green cover after the wheat yield of July whereas Kirchberg stood immediately after the maize yield). For the rain simulation experiments a 2 % herbicide solution was sprayed homogeneously by hand pump in the same concentration and amounts as in practice (180 mg glyphosate/m^2). Immediately after application the rain simulation started (60 minutes, 30 mm). Run-off-fractions and soil samples were collected.
Statistical design	No data
Relevance	
Environmental relevance	No environmental are data reported.
Weight of evidence	
"Positive"/"Negative" evidence	No negative evidence.

Trinelli et al. (2013)

Title: Co-biosorption of copper and glyphosate by Ulva lactuca

Author: M. A. Trinelli, M. M. Areco, M. dos Santos Afonso

Reference: Colloids and Surfaces B: Biointerfaces 105 (2013) 251-258

Year: 2013

Results and conclusion:
This study investigated the adsorption of glyphosate (PMG) onto the green algae Ulva lactuca. PMG was not adsorbed by U. lactuca but PMG was adsorbed when the process was mediated by Cu(II) with molar ratios Cu(II):PMG ≥1.5:1.

U. lactuca was characterized by water adsorption surface area, FTIR, SEM and EDS. The Langmuir and Freundlich models were applied. Results showed that the biosorption processes for copper and PMG in the presence of copper were described by the Langmuir model (qmax = 0.85 ± 0.09 mmol g^{-1}, KL = 0.55 ± 0.14 l $mmol^{-1}$ and qmax = 3.65 ± 0.46 mmol g^{-1}, KL = 0.103 ± 0.03 l $mmol^{-1}$, respectively). Copper adsorption was greater in the presence of PMG than in the absence of the pesticide and the adsorption can only be represented by the Freundlich model (KF = 0.08 ± 0.01, $1/n = 1.86 \pm 0.07$).

In all cases studied, the maximum metal uptake (qmax) increased with increasing pH. Surface complexes with a stoichiometry ranging from $\equiv$Cu-PMG-Cu to $\equiv$Cu-PMG-Cu_3 are suggested as reaction products of the process. Due to the increasing amounts of PMG applied in Argentina, natural reservoirs present considerable amounts of this herbicide. The value of this work resides in using U. lactuca, a marine seaweed commonly found along coastlines all over the world, as a biosorbent for PMG.

Proposed action:
Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional):
Additional information

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate (PMG)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Tsui et al. (2005)

Title: Influence of glyphosate and its formulation (Roundup®) on the toxicity and bioavailability of metals to Ceriodaphnia dubia	
Author: Martin T.K. Tsui, Wen-Xiong Wang, L.M. Chu	
Reference: Environmental Pollution 138, 59-68	
Year: 2005	
Results and conclusion: This study examined the toxicological interaction between glyphosate (or its formulation, Roundup®) and several heavy metals to a freshwater cladoceran, Ceriodaphnia dubia. We demonstrated that all binary combinations of Roundup® and metals (Cd, Cu, Cr, Ni, Pb, Se and Zn) exhibited ''less than additive'' mixture toxicity, with 48-h LC50 toxic unit>1. Addition of glyphosate alone could significantly reduce the acute toxicity of Ag, Cd, Cr, Cu, Ni, Pb and Zn (but not Hg and Se). The ratio between glyphosate and metal ions was important in determining the mitigation of metal toxicity by glyphosate. A bioaccumulation study showed that in the presence of glyphosate the uptake of some metals (e.g. Ag) was halted but that of others (e.g. Hg) was increased significantly. Therefore, our study strongly suggests that glyphosate and its commercial formulations can control the toxicity as well as the bioavailability of heavy metals in aquatic ecosystems where both groups of chemicals can co-occur.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Roundup (41 % active ingredient and 10-20 % POEA) Glyphosate, CAS-no.: 1071-83-6 and isopropylamine (IPA) salt of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Tuffi Santos et al. (2005)

Title: Root Exudation of Glyphosate by Brachiaria decumbens and its Effects on Eucalypt Plants and Microbial Soil Respiration	
Author: TUFFI SANTOS, L.D., FERREIRA, F.A., BARROS, N.F., SIQUEIRA, C.H., SANTOS, I.C., MACHADO, A.F.L.	
Reference: Planta Daninha, Viçosa-MG, v. 23, n. 1, p. 143-152	
Year: 2005	
Results and conclusion: This study aimed to evaluate root exudation of the herbicide glyphosate by Brachiaria decumbens and its effects on eucalypt cultivated in soil and in nutritive solution; and to quantify microbial respiration in soil under different managements. One Eucalyptus grandis and four Brachiaria decumbens seedlings were planted in pots with lids with five perforations holding 8.0 L of the nutritive solution. A randomized block design in six replications was used, each pot being considered an experimental plot. The eucalypt and brachiaria plants were interplanted in a hydroponic solution for 30 days. Fifteen days after the transplant, the brachiaria plants were pruned to stimulate tillering. After this period, glyphosate treatments of 0, 720, 1440, 2160, and 2880 g a.e./ha were applied to the brachiaria plants. In the soil experiment, E. grandis seedlings were planted in 72 10-liter pots, half containing sandy soil and half clayey soil. The experiment was set up in a randomized block design with six replications, in a 2 x 6 factorial scheme (two soil types and six management combinations). Following the eucalypt seedlings, five Brachiaria decumbens seedlings per pot were planted in 48 pots (24 of each soil), and interplanted with a eucalypt seedling. The remaining eucalypt pots were cultivated in monoculture. The tested treatments were: 1- interplanted eucalypt and brachiaria (control); 2- Eucalypt without brachiaria + 1440 g a.e./ha of glyphosate applied in the soil; 3- interplanted eucalypt and brachiaria cut after spray with 1440 g/ha glyphosate; 4, 5 and 6- interplanted eucalypt and sprayed brachiaria with 720, 1440, and 2880 g a.e/ha glyphosate, respectively. In treatments 4, 5, and 6 the eucalypt plants were protected from contact with the herbicide applied to the brachiaria plants. In treatment 2, glyphosate was applied directly to soil. The eucalypt plants in treatment 3 were treated with the brachiaria plant cut shoots, seven days after the latter had been sprayed with 1440 g/ha glyphosate. All the tested rates controlled over 95 % of the grass species in both assays, and no toxicity symptoms were verified in the eucalypt plants. The microbial activity was greater in the sandy soil, mainly with the increase of the glyphosate rates applied to the brachiaria plants.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate isopropylammonium salt, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/“Negative” evidence	Not applicable

Tuffi Santos et al. (2009)

Title: Leaf anatomy and morphometry in three eucalypt clones treated with glyphosate	
Author: Tuffi Santos, LD., Sant'Anna-Santos, BF., Meira, RMSA., Ferreira, FA., Tiburcio, RAS. and Machado, AFL.	
Reference: Braz. J. Biol., 69(1): 129-136	
Year: 2009	
Results and conclusion: This work aimed to evaluate the effects of simulated drift of glyphosate on the morphoanatomy of three eucalypt clones and to correlate the intoxication symptoms on a microscopic scale with those observed in this visual analysis. The effects of glyphosate drift were proportional to the five doses tested, with Eucalyptus urophylla being more tolerant to the herbicide than E. grandis and urograndis hybrid. The symptoms of intoxication which were similar for the different clones at 7 and 15 days after application were characterized by leaf wilting, chlorosis and curling and, at the highest rates, by necrosis, leaf senescence and death. Anatomically glyphosate doses higher than 86.4 g/ha caused cellular plasmolysis, hypertrophy and hyperplasia, formation of the cicatrization tissue and dead cells on the adaxial epidermis. The spongy parenchyma had a decrease, and the palisade parenchyma and leaf blade thickness had an increase. The increased thickness in leaf blade and palisade parenchyma may be related to the plant response to glyphosate action, as a form of recovering the photosynthetically active area reduced by necroses and leaf senescence caused by the herbicide.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate: Commercial brand name Roundup SC, Monsanto of Brazil Ltd,
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Tuffi Santos et al. (2008)

Title: Radicular Exudation of Glyphosate by Brachiaria decumbens and Its Effects on Eucalypt Plant
Author: TUFFI SANTOS, L.D., SANTOS, J.B., FERREIRA, F.A., OLIVEIRA, J.A., BENTIVENHA, S., e MACHADO, A.F.L.
Reference: Planta Daninha, Viçosa-MG, v. 26, n. 2, p. 369-374
Year: 2008

Results and conclusion: Eucalypt plants commonly present symptoms of intoxication in areas where glyphosate is used. One possible way of contamination is through radicular exudation of glyphosate by the treated weed and later, plant absorption. This study aimed to evaluate glyphosate exudation by Brachiaria decumbens and its effects on eucalypt plants when ^{14}C-glyphosate, mixed to the solution of the commercial product Scout® was applied. Seedlings of two eucalypt clones (UFV05 and UFV06) were cultivated in pots, intercropped with Brachiaria decumbens, on two types of soil (clayey and sandy). At 35 days after transplantation, 50 µL of the mixture was applied on brachiaria by using a precision micro-syringe. After application, 2, 8, 16 and 24 days, samples of eucalypt plants were collected and fractioned in the primary apices, secondary apices, leaves and roots, following the usual methodology to determine radioactivity. Symptoms of intoxication were not observed in any eucalypt plant evaluation. However, ^{14}C-glyphosate was found in all plants, regardless of the soil type, clone or evaluation time, with the highest concentration being found in the sandy soil. Results show radicular exudation of glyphosate by B. decumbens and its absorption by eucalypt plants through roots. However, concentrations lower than necessary may cause crop intoxication.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	^{14}C-Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
“Positive”/”Negative” evidence	Not applicable

Veiga et al. (2001)

Title: Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain
Author: F. Veiga, J.M. Zapata, M.L. Fernandez Marcos, E. Alvarez
Reference: The Science of the Total Environment 271(2001)135-144
Year: 2001
Results and conclusion: Only glyphosate was quantified in the solid phase. Only a semi-quantitative estimation of AMPA could be carried out. Determined glyphosate concentrations in the soil solid phase ranged from 0 to 6.9 µg g^{-1}, averaging 0.85 µg g^{-1}. The glyphosate concentrations show a trend to decrease along the monitoring period, being very low from 1 month after the treatment, both in solid and liquid phases of the forest soil studied. The AMPA concentrations, after increasing during the first fortnight, while the glyphosate decomposed, decreased until the end of the experiment. Despite its adsorption onto soil components, glyphosate and AMPA quickly reached a 30 cm depth in soil solution. At this depth they are degraded more slowly than in the surface layer 0-20 cm, as a consequence of lower biological activity. Glyphosate concentrations in the soil are lower in the lower slope position as a consequence of its distribution in a thicker soil layer. The higher dose of herbicide 8 l/ha did not result in significantly higher concentrations in soil.
Proposed action: Consider as additional information. Refers to soil monitoring and stability in soil. However, the authors did not calculate any DT$_{50}$ values according to the recommended FOCUS procedure.

Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Analysis of processes involved in microbial glyphosate degradation
Protocol	Non-GLP study
Test compound	Non-labelled glyphosate (CAS 1071-83-6), non-labelled AMPA (CAS 1066-51-9)
Test system and conditions	The soil used in the study is an umbric Regosol, according to FAO classificationŽFAO 1990, developed on green shales. It is located in the range of MeiraŽLugo, north-west Spain, at 625 m altitude, in a humid, temperate climate; the slope is approximately 20 %. Previously to herbicide application, two soil depths 0-20 and 20-35 cm. were sampled and analysed for general properties. In spring 1996 *Eucalyptus nitens* seedlings were planted in rows along the maximum slope. Each row, consisting of 18 trees, was considered an experimental unit. After planting, two different Roundup doses (5 and 8 l/ha) were randomly applied to tree rows, with three replicates and leaving three control rows.
Statistical design	Three replicates
Relevance	
Environmental relevance	There is relevance for this specific type of application in railway tracks. However, the authors did not calculate any DT_{50} values according to the recommended FOCUS procedure.
Weight of evidence	
"Positive"/"Negative" evidence	The monitoring principally supports the results of mobility and degradation studies performed with glyphosate.

Wang et al. (2005)

Title: Influence of sediment on the fate and toxicity of a polyethoxylated tallowamine surfactant system (MON 0818) in aquatic microcosms
Author: Ning Wang, John M. Besser, Denny R. Buckler, Joy L. Honegger, Chris G. Ingersoll, B.T. Johnson, Mitchell L. Kurtzweil, Jon MacGregor, Michael J. McKee
Reference: Chemosphere 59, 545-551
Year: 2005
Results and conclusion: The fate and toxicity of a polyethoxylated tallowamine (POEA) surfactant system, MON 0818, was evaluated in water-sediment microcosms during a 4-d laboratory study. A surfactant solution of 8 mg/l nominal concentration was added to each of nine 72 l aquaria with or without a 3 cm layer of one of two natural sediments (total organic carbon (TOC) 1.5 % or 3.0 %). Control well water was added to each of nine additional 72-l aquaria with or without sediment. Water samples were collected from the microcosms after 2, 6, 24, 48, 72, and 96 h of aging to conduct 48-h toxicity tests with Daphnia magna and to determine surfactant concentrations. Elevated mortality of D. magna (43-83 %) was observed in overlying water sampled from water-only microcosms throughout the 96-h aging period, whereas elevated mortality (23-97 %) was only observed in overlying water sampled from water-sediment microcosms during the first 24 h of aging. Measured concentrations of MON 0818 in water-only microcosms remained relatively constant (4-6 mg/l) during the 96-h period, whereas the concentrations in overlying water from microcosms containing either of the two types of sediment dissipated rapidly, with half-lives of 13 h in the 3.0 % TOC sediment and 18 h in the 1.5 % TOC sediment. Both toxicity and the concentration of MON 0818 in overlying water decreased more rapidly in microcosms containing sediment with the higher percent TOC and clay and with a higher microbial biomass. Mortality of D. magna was significantly correlated with surfactant concentrations in the overlying water. These results indicate that the toxicity of the POEA surfactant in water rapidly declines in the presence of sediment due to a reduction in the surfactant concentration in the overlying water above the sediment.

Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	MON 0818 contains a complex polyethoxylated tallowamine (POEA) surfactant mixture used in Roundup
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Wang et al. (2009)

Title: The Inhibition of the Combined Pollution of Copper and Glyphosate to the Seed Germination and Root Elongation of Wheat	
Author: Wang Mi-dao, Cheng Feng-xia, Si You-bin	
Reference: Asian Journal of Ecotoxicology Vol. 4, No. 4, 591-596	
Year: 2009	
Results and conclusion: Interaction of pollutions is one of the research focuses in current environmental sciences. The combined toxicity of copper and glyphosate to the inhibition rates of wheat germination, sprout length and root elongation was studied. Results indicated that Cu^{2+} had no obvious effect on the wheat germination but could inhibit the root elongation and sprout length significantly. Glyphosate had obvious inhibition effect on the wheat germination, sprout length and root elongation. When Cu^{2+} and glyphosate were combined, the presence of Cu^{2+} decreased the inhibition of glyphosate to wheat germination and sprout length. But for the root elongation, the Cu^{2+} increased the toxicity of glyphosate when glyphosate was at low concentrations and decreased the toxicity of glyphosate to root elongation when glyphosate was at high concentrations. The possible reason of Cu^{2+} decreased the ecotoxicity of glyphosate is the complexation reaction of Cu^{2+} and glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Warnemuende et al. (2007)

Title: Effects of tilling no-till soil on losses of atrazine and glyphosate to runoff water under variable intensity simulated rainfall
Author: Elizabeth A. Warnemuende, Judodine P. Patterson, Douglas R. Smith, Chi-hua Huang
Reference: Soil & Tillage Research 95 (2007) 19-26
Year: 2007
Results and conclusion: This study focuses on the viability of glyphosate tolerant cropping systems as an alternative to atrazine-based systems, and the impact of tilling historically no-till ground on the runoff pollution potential of these systems. Variable intensity field rainfall simulations were performed on 2 m long x 1 m wide plots within a field in first-year disk and harrow following no-till (CT), and within a long-term no-tilled (NT) field, both treated with atrazine and glyphosate according to label. Rainfall sequence was: 50 mm/h for 30 min followed by 75 mm/h for 15 min, 25 mm/h for 15 min, and 100 mm/h for 15 min. Runoff was collected at regular time intervals during two simulated rainfall events and analyzed for herbicide concentration, sediment content, and volume. Maximum glyphosate concentration in runoff was 233 mg/L for NT and 180 mg/L for CT (approximately 33 % and 26 % of the maximum contaminant limit (MCL) for glyphosate (700 mg/L), respectively, while maximum atrazine concentrations in runoff was 303 mg/L for NT and 79 mg/L for CT (approximately 100 times and 26 times the atrazine MCL (3 mg/L)). Atrazine concentration and loading were significantly higher in runoff from NT plots than from CT plots, whereas glyphosate concentration and loading were impacted by tillage treatment to a much lesser degree. Results suggest that glyphosate-based weed management may represent a lower drinking water risk than atrazine-based weed management, especially in NT systems.
Proposed action: Not to be considered as publication deals with risk mitigation. Thus, no fate related endpoints or off-site monitoring are affected.
Type of information (critical, high/low weight, supporting, additional): Low weight, only additional information about tillage or no-tillage management

Reliability	Medium
Endpoint	Average runoff rates; Runoff herbicide concentrations are given as a function of time
Protocol	EPA Drinking Water Method for Chemical Contaminants #547; modified EPA method 525.2; soil samples: glyphosate (method: Monsanto RES-014-91) and atrazine (method:FAO PAM 302/SPE/NPD)
Test compound	Bicep II Magnum (33 % atrazine); Roundup Ultra Max (41 % glyphosate, CAS-no.: 1071-83-6)
Test system and conditions	The experimental design was a randomized complete block. Each block contained three plots, 2 m long and 1 m wide, representing three replications of CT and NT treatments. All plots were planted in glyphosate-tolerant corn, in annual rotation with soybeans. Herbicides were applied by a certified pesticide applicator to all plots 24 h prior to the first rainfall event according to label: Bicep II Magnum (33 % atrazine) at a rate 1621 g atrazine/ha and Roundup Ultra Max (41 % glyphosate) at a rate of 709 g glyphosate/ha. Immediately before herbicide application and immediately prior to the rainfall, soil was sampled and analyzed for herbicide levels to establish initial soil concentrations and confirm uniform and precise application. Two rainfall events were performed (rainfall simulator) on each plot, at 1 day and 8 days after herbicide application. Runoff samples were collected at 5 min intervals from the onset of runoff to 50 min and 3 min intervals from 53 min to 95 min.

Statistical design	Statistical analyses were performed using SAS 9.1, and SigmaPlot V. 6.0; runoff volumes, and concentrations and mass losses of sediment, glyphosate, and atrazine were determined using PROCGLM with $P \leq 0.05$. Regression analyses were performed using linear and logarithmic functions in SigmaPlot V. 6.0
Relevance	
Environmental relevance	Given; influencing endpoints are measured and partly reported.
Weight of evidence	
"Positive"/"Negative" evidence	The results are supported by other publications.

Watts (2009)

Title: Glyphosate	
Author: Meriel Watts (Pesticide Action Network Asia and the Pacific (PAN AP))	
Reference: -	
Year: 2009	
Results and conclusion: Review-Regarding environmental fate the following statements are made: Soils: Glyphosate is relatively persistent in soil, with residues still found up to 3 years later in cold climates. It is less persistent in warmer climates, with a half-life between 4 and 180 days. It is bound onto soil particles, and this was once thought to mean that glyphosate is not biologically active within soil, nor will it leach to groundwater. However it is now known that it can easily become unbound again, be taken up by plants or leach out, indicating a greater risk of groundwater contamination. It can reduce nitrogen and phosphate fertility of soils. Water: Glyphosate is soluble in water, and slowly dissipates from water into sediment or suspended particles. Although it does break down by photolysis and microbial degradation, it can be persistent for some time in the aquatic environment, with a half-life of up to nearly 5 months, and still be present in the sediment of a pond after 1 year. Residues of glyphosate have been found in a wide range of drains, streams, rivers and lakes, in many countries including Canada, China, France, Netherlands, Norway, USA, and the UK. Urban use on road and rail sides is contributing significantly to this contamination, with residues being found in sewage sludge and wastewater treatment plants. Contamination of 'vernal pools' – pools that are shallow and disappear in dry weather – are a concern for amphibia, for which these water sources are critical. Residues have also been found in groundwater in Canada, Denmark, the Netherlands, and USA. They have been detected in the marine environment off the Atlantic Coast of France; and in the rain in Belgium and Canada. In general, the statements are supported by citations.	
Proposed action: Consider as additional information due to the fact that the article is a review and data are cited only.	
Type of information (critical, high/low weight, supporting, additional): Additional information	
Reliability	
Endpoint	Degradation in soil and water, groundwater contamination, findings in of drains, streams, rivers and lakes, residues being found in sewage sludge and wastewater treatment plants
Protocol	No detailed information in the report on analysed studies
Test compound	Glyphosate
Test system and conditions	No information in the report about the analysed studies
Statistical design	Not provided

Relevance	
Environmental relevance	Relevant
Weight of evidence	
"Positive"/"Negative" evidence	Detailed information on the analysed studies is not provided in the report. Therefore, the data in the report cannot be considered for endpoint derivation and/or further risk assessment.

Zabaloy et al. (2011)

Title: Herbicides in the Soil Environment: Linkage Between Bioavailability and Microbial Ecology	
Author: M. Celina Zabaloy, Graciela P. Zanini, Virginia Bianchinotti, Marisa A. Gomez and Jay L. Garland	
Reference: Herbicides, Theory and Applications ISBN 978-953-307-975-2	
Year: 2011	
Results and conclusion: Although desorption has been considered a pre-requisite for biodegradation of soil-bound herbicides, there is increasing evidence that sorbed compounds may still be degraded by attached cells. However, there is still considerable work ahead for researchers to understand the mechanisms and populations intervening in these processes. Integrative approaches are essential to study physicochemical and biological factors that affect sorption, bioavailability and biodegradation of herbicides in soil. Development of new molecular methods coupling function and structure may improve our understanding of the role of microbial populations in herbicides degradation and how these compounds affect non-degrading members of the microbial community. Overall, a number of studies have shown that the herbicides 2,4-D, metsulfuron methyl and glyphosate at recommended rates have only transient impacts on soil microbial communities, being glyphosate the one with larger effects, while metsulfuron methyl may be toxic under certain soil conditions (e.g. high pH).	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional):	
Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	No test design
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zablotowicz and Reddy (2004)

Title: Impact of Glyphosate on the Bradyrhizobium japonicum Symbiosis with Glyphosate-Resistant Transgenic Soybean: A Minireview	
Author: Robert M. Zablotowicz and Krishna N. Reddy	
Reference: J. Environ. Qual. 33:825-831	
Year: 2004	
Results and conclusion: Glyphosate-resistant (GR) soybean [Glycine max (L.) Merr.] expressing an insensitive 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) gene has revolutionized weed control in soybean production. The soybean nitrogen fixing symbiont, Bradyrhizobium japonicum, possesses a glyphosate-sensitive enzyme and upon exposure to glyphosate accumulates shikimic acid and hydroxybenzoic acids such as protocatechuic acid (PCA), accompanied with B. japonicum growth inhibition and death at high concentrations. In a series of greenhouse and field experiments, glyphosate inhibited nodulation and nodule leghemoglobin content of GR soybean. Glyphosate accumulated in nodules of field-grown GR soybean, but its effect on nitrogenase activity of GR soybean was inconsistent in field studies. In greenhouse studies, nitrogenase activity of GR soybean following glyphosate application was transiently inhibited especially in early growth stages, with the greatest inhibition occurring under moisture stress. Studies using bacteroid preparations showed that the level of glyphosate inhibition of bacteroid nitrogenase activity was related to invitro glyphosate sensitivity of the B. japonicum strains. These studies indicate the potential for reduced nitrogen fixation in the GR soybean system; however, yield reductions due to this reduced N_2 fixation in early stages of growth have not been demonstrated.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Review article, no study design; Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans	
Author: L.H.S. Zobiole, R.J. Kremer, R.S. Oliveira Jr and J. Constantin	
Reference: Journal of Applied Microbiology 110, 118-127	
Year: 2010	
Results and conclusion: Aims: Glyphosate-resistant (GR) soybean production increases each year because of the efficacy of glyphosate for weed management. A new or 'second' generation of GR soybean (GR2) is now commercially available for farmers that is being promoted as higher yielding relative to the previous, 'first generation' (GR1) cultivars. Recent reports show that glyphosate affects the biology and ecology of rhizosphere micro-organisms in GR soybean that affect yield. The objective of this research was to evaluate the microbiological interactions in the rhizospheres of GR2 and GR1 soybean and the performance of the cultivars with different rates of glyphosate applied at different growth stages. Methods and Results: A greenhouse study was conducted using GR1 and GR2 soybean cultivars grown in a silt loam soil. Glyphosate was applied at V2, V4 and V6 growth stages at three rates. Plants harvested at R1 growth stage had high root colonization by Fusarium spp.; reduced rhizosphere fluorescent pseudomonads, Mn-reducing bacteria, and indoleacetic acid-producing rhizobacteria; and reduced shoot and root biomass. Conclusions: Glyphosate applied to GR soybean, regardless of cultivar, negatively impacts the complex interactions of microbial groups, biochemical activity and root growth that can have subsequent detrimental effects on plant growth and productivity. Significance and Impact of the Study: The information presented here will be crucial in developing strategies to overcome the potential detrimental effects of glyphosate in GR cropping systems.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup WeatherMax®; Monsanto, St Louis, MO), CAS-no.: 70901-12-1
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Glyphosate Affects Seed Composition in Glyphosate-Resistant Soybean	
Author: LUIZ H. S. ZOBIOLE, RUBEM S. OLIVEIRA JR., JESUI V. VISENTAINER, ROBERT J. KREMER, NACER BELLALOUI, AND TSUIOSHI YAMADA	
Reference: J. Agric. Food Chem. 58, 4517–4522	
Year: 2010	
Results and conclusion: The cultivation of glyphosate-resistant (GR) soybeans has continuously increased world-wide in recent years mainly due to the importance of glyphosate in current weed management systems. However, not much has been done to understand eventual effects of glyphosate application on GR soybean physiology, especially those related to seed composition with potential effects on human health. Two experiments were conducted to evaluate the effects of glyphosate application on GR soybeans compared with its near-isogenic non-GR parental lines. Results of the first experiment showed that glyphosate application resulted in significant decreases in shoot nutrient concentrations, photosynthetic parameters, and biomass production. Similar trends were observed for the second experiment, although glyphosate application significantly altered seed nutrient concentrations and polyunsaturated fatty acid percentages. Glyphosate resulted in significant decreases in polyunsaturated linoleic acid (18:2n-6) (2.3 % decrease) and linolenic acid (18:3n-3) (9.6 % decrease) and a significant increase in monounsaturated fatty acids 17:1n-7 (30.3 % increase) and 18:1n-7 (25 % increase). The combined observations of decreased photosynthetic parameters and low nutrient availability in glyphosate-treated plants may explain potential adverse effects of glyphosate in GR soybeans.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salts of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Glyphosate affects lignin content and amino acid production in glyphosate-resistant soybean	
Author: Luiz Henrique Saes Zobiole, Edicléia Aparecida Bonini, Rubem Silvério de Oliveira Jr., Robert John Kremer, Osvaldo Ferrarese-Filho	
Reference: Acta Physiol Plant 32:831-837	
Year: 2010	
Results and conclusion: Farmers report that some glyphosate-resistant soybean varieties are visually injured by glyphosate. Glyphosate is the main herbicide that directly affects the synthesis of secondary compounds. In this work, we evaluated the effect of increasing rates of glyphosate on lignin and amino acid content, photosynthetic parameters and dry biomass in the early maturity group cultivar BRS 242 GR soybean. Plants were grown in half-strength complete nutrient solution and subjected to various rates of glyphosate either as a single or in sequential applications. All parameters evaluated were affected by increasing glyphosate rates. The effects were more pronounced as glyphosate rates increased, and were more intense with a single total application than sequential applications at lower rates.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salts of glyphosate; CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans	
Author: Luiz Henrique Saes Zobiole, Rubem Silvério de Oliveira Jr, Don Morgan Huber, Jamil Constantin, César de Castro, Fábio Alvares de Oliveira, Adilson de Oliveira Jr.	
Reference: Plant Soil 328:57-69	
Year: 2010	
Results and conclusion: Although glyphosate-resistant (GR) technology is used in most countries producing soybeans (Glycine max L.), there are no particular fertilize recommendations for use of this technology, and not much has been reported on the influence of glyphosate on GR soybean nutrient status. An evaluation of different cultivar maturity groups on different soil types, revealed a significant decrease in macro and micronutrients in leaf tissues, and in photosynthetic parameters (chlorophyll, photosynthetic rate, transpiration and stomatal conductance) with glyphosate use (single or sequential application). Irrespective of glyphosate applications, concentrations of shoot macro- and micronutrients were found lower in the nearisogenic GR-cultivars compared to their respective non-GR parental lines Shoot and root dry biomass were reduced by glyphosate with all GR cultivars evaluated in both soils. The lower biomass in GR soybeans compared to their isogenic normal lines probably represents additive effects from the decreased photosynthetic parameters as well as lower availability of nutrients in tissues of the glyphosate treated plants.	

Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salt of glyphosate, CAS no.: 38641 94 0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative"	Not applicable

Zobiole et al. (2010)

Title: Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate	
Author: Luiz Henrique Saes Zobiole, Rubem Silvério de Oliveira Jr., Robert John Kremer, Jamil Constantin, Carlos Moacir Bonato, Antonio Saraiva Muniz	
Reference: Pesticide Biochemistry and Physiology , 97 182-193	
Year: 2010	
Results and conclusion: Previous studies comparing cultivars of different maturity groups in different soils demonstrated that early maturity group cultivars were more sensitive to glyphosate injury than those of other maturity groups. In this work, we evaluated the effect of increasing rates of glyphosate on water absorption and photosynthetic parameters in early maturity group cultivar BRS 242 GR soybean. Plants were grown in a complete nutrient solution and subjected to a range of glyphosate rates either as a single or sequential leaf application. Net photosynthesis, transpiration rate, stomatal conductance, sub-stomatal CO_2, carboxylation efficiency, fluorescence, maximal fluorescence and chlorophyll content were monitored right before and at different stages after herbicide application; water absorption was measured daily. All photosynthetic parameters were affected by glyphosate. Total water absorbed and biomass production by plants were also decreased as glyphosate rates increased, with the affect being more intense with a single full rate than half the rate applied in two sequential applications. Water use efficiency (WUE) was significantly reduced with increasing rates of glyphosate.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salt of glyphosate (Roundup Ready®, Monsanto Company), CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: NUTRIENT ACCUMULATION AND PHOTOSYNTHESIS IN GLYPHOSATE-RESISTANT SOYBEANS IS REDUCED UNDER GLYPHOSATE USE	
Author: Luiz Henrique Saes Zobiole, Rubem Silvério de Oliveira Junior, Robert John Kremer, Antônio Saraiva Muniz, and Adilson de Oliveira Junior	
Reference: Journal of Plant Nutrition, 33:1860-1873	
Year: 2010	
Results and conclusion: Global production of glyphosate-resistant (GR) soybean [Glycine max (L.) Merr.] continues to increase annually; however, there are no particular specific fertilizer recommendations for the transgenic varieties used in this system largely because reports of glyphosate effects on mineral nutrition of GR soybeans are lacking. Several metabolites or degradation products of glyphosate have been identified or postulated to cause undesirable effects on GR soybeans. In this work we used increasing glyphosate rates in different application on cv. 'BRS 242 GR' in order to evaluate photosynthetic parameters, macro- and micronutrient uptake and accumulation and shoot and root dry biomass production. Increasing glyphosate rates revealed a significant decrease in photosynthesis, macro and micronutrients accumulation in leaf tissues and also decreases in nutrient uptake. The reduced biomass in GR soybeans represents additive effects from the decreased photosynthetic parameters as well as lower availability of nutrients in tissues of the glyphosate treated plants.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salt of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Glyphosate affects photosynthesis in first and second generation of glyphosate-resistant soybeans	
Author: Luiz Henrique Saes Zobiole, Robert John Kremer, Rubem Silvério de Oliveira Jr, Jamil Constantin	
Reference: Plant Soil 336:251-265	
Year: 2010	
Results and conclusion: The crop area planted to conventional soybeans has decreased annually while that planted to glyphosate-resistant (RR) soybean has drastically increased mainly due to the wide adoption of glyphosate in current weed management systems. With the extensive use of glyphosate, many farmers have noted visual plant injury in RR soybean varieties after glyphosate application. A new generation designated as "second generation-RR2" has been recently developed and these RR2 cultivars already are commercially available for farmers and promoted as higher yielding relative to the previous RR cultivars. However, little information is currently available about the performance of RR2 soybean beyond commercial and farmer testimonial data. Thus, an evaluation of different glyphosate rates applied in different growth stages of the first and second generation of RR soybeans, revealed a significant decrease in photosynthesis. In general, increased glyphosate rate and late applications (V6) pronounced decrease photosynthetic parameters and consequently decreased in leaf area and shoot biomass production. In contrast, low rate and early applications were less damage for the RR soybean plants, suggesting that with early applications (V2), plants probably have more time to recover from glyphosate or its metabolites effects regarding late applications.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup Weather Max®, Monsanto Company), CAS-no.: 70901-12-1
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2011)

Title: Glyphosate affects chlorophyll, nodulation and nutrient accumulation of ''second generation'' glyphosate-resistant soybean (Glycine max L.)
Author: Luiz H.S. Zobiole, Robert J. Kremer, Rubem S. Oliveira Jr., Jamil Constantin
Reference: Pesticide Biochemistry and Physiology 99, 53-60
Year: 2011

Results and conclusion: The recently developed ''second generation'' of Roundup Readysoybean (RR2) cultivars commercially available for farmers in 2008 were promoted as higher yielding relative to the ''first generation'' RR cultivars (RR1). Previous studies showed that glyphosate reduced such yield components as photosynthesis, water absorption, nutrient uptake and symbiotic N_2 fixation in RR soybean cultivars; however, no data are available regarding the glyphosate effects on these physiological factors in RR2 soybean. Thus, the objective of this research was to evaluate the nutrient accumulation and nodulation of both generations of RR soybeans at different rates of glyphosate applied at various growth stages. In general, increased glyphosate rates and late applications decreased the nutrient accumulation, nodulation, and shoot and root biomass in both RR1 and RR2. All macro- and micronutrients, with exception of N and K, accumulated more in RR1 than RR2. Although this result may be an individual cultivar characteristic, it suggests that the RR2 cultivar was also inefficient in nutrient uptake and translocation or was unable to rapidly recover from potential chelating effects of glyphosate. These studies suggest that applying glyphosate at early growth stages using the lowest glyphosate rate might have less damage on growth and productivity of RR soybeans.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salt of glyphosate (Roundup Weather Max®, Monsanto Company), CAS-no.: 70901-12-1
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2011)

Title: Prevention of RR Soybean Injuries Caused by Exogenous Supply of Aminoacids
Author: ZOBIOLE, L.H.S., OLIVEIRA JR., R.S., CONSTANTIN, J., BIFFE, D.F.
Reference: Planta Daninha, Viçosa-MG, v. 29, n. 1, p. 195-205
Year: 2011
Results and conclusion: Glyphosate-resistant (RR) soybean crop areas have expanded every year. However, as a result of this expansion, the use of glyphosate has significantly increased, with the appearance of visual injuries in RR soybeans immediately after post-emergence application of the herbicide. Thus, two experiments were conducted in different years with different objectives. The first experiment aimed to evaluate the influence of glyphosate on photosynthetic variables and biomass production. The second experiment aimed to re-evaluate the same parameters affected in RR soybeans by glyphosate, as well as the use of various methods of amino acid application, as a form of a likely recovery of the soybean plants following these exogenous applications. The photosynthetic rate and SPAD index decreased as the glyphosate rate increased, with a pronounced decrease after a single herbicide application. Overall, due to a decrease in the photosynthetic rate and chlorophyll production, as well as to a likely immobilization of shoot nutrient concentration by glyphosate, a significant biomass decrease was verified in the treatments with glyphosate application. However, the use of exogenous amino acids may be a strategy to safeguard the undesirable effects of this herbicide on RR soybean.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.

Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylammmonium salt of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Use of Exogenous Amino Acid to Prevent Glyphosate Injury in Glyphosate-Resistant Soybean
Author: ZOBIOLE, L.H.S., OLIVEIRA JR., R.S., CONSTANTIN, J., BIFFE, D.F., KREMER, R.J.
Reference: Planta Daninha, Viçosa-MG, v. 28, n. 3, p. 643-653
Year: 2010
Results and conclusion: Cultivation of glyphosate-resistant (GR) soybeans has increased in Brazil as a result of the application of this technology in weed management systems developed for this crop. However, the expansion of GR soybean production has significantly increased the use of glyphosate and, in some cases, resulted in injury symptoms observed in GR soybean, known as "yellow flashing" or yellowing of the upper leaves. Thus, two experiments were conducted in different years. The first experiment aimed to evaluate the influence of glyphosate on GR soybeans regarding the photosynthetic variables, nodule parameters, and shoot and root dry biomass by comparing cultivar BRS 242 GR without glyphosate and BRS 242 RR + glyphosate at 1.200 g/ha at V4 growth stage, to the near isogenic non-GR parental line cv. Embrapa 58. The second experiment aimed to reassess the same parameters in GR soybeans at the V4 stage treated with glyphosate, plus the application of various amino acids, to evaluate the expected recovery of soybean growth under the exogenous use of supplemental amino acids. In general, the photosynthetic variables, nodulation parameters and shoot and root dry biomass were affected by glyphosate; however, the use of amino acids may be a strategy to prevent the undesirable effects of this herbicide on GR soybean.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Glyphosate, CAS-no.: 1071-83-6
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable

Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2010)

Title: Effect of glyphosate on symbiotic N_2 fixation and nickel concentration in glyphosate-resistant soybeans	
Author: L.H.S. Zobiole, R.S. Oliveira Jr., R.J. Kremer b, J. Constantin, T. Yamada, C. Castro, F.A. Oliveira, A. Oliveira Jr.	
Reference: Applied Soil Ecology 44, 176-180	
Year: 2010	
Results and conclusion: Decreased biological nitrogen fixation in glyphosate-resistant (GR) soybeans has been attributed directly to toxicity of glyphosate or its metabolites, to N_2-fixing microorganisms. As a strong metal chelator, glyphosate could influence symbiotic N_2 fixation by lowering the concentration of nickel (Ni) that is essential for the symbiotic microorganisms. Evaluation of different cultivars grown on different soil types at the State University of Maringá, PR, Brazil during the summer 2008 revealed, significant decreases in photosynthetic parameters (chlorophyll, photosynthetic rate, transpiration and stomatal conductance) and nickel content with glyphosate use (single or sequential application). This work demonstrated that glyphosate can influence the symbiotic N_2 fixation by lowering nickel content available to the symbiotic microorganisms.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	Not applicable
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salts of glyphosate, CAS-no.: 38641-94-0
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2012a)

Title: AMINO ACID APPLICATION CAN BE AN ALTERNATIVE TO PREVENT GLYPHOSATE INJURY IN GLYPHOSATE-RESISTANT SOYBEANS
Author: L. H. S. Zobiole, R. S. de Oliveira Jr., J. Constantin, R. J. Kremer and D. F. Biffe
Reference: Journal of Plant Nutrition, 35:268-287, 2012
Year: 2012
Results and conclusion: Glyphosate-resistant (GR) soybeans have continuously increased; however, this expansion significantly increased the use of glyphosate and therefore, in some cases, has resulted in injury symptoms observed in GR soybean, known as "yellow flashing". Previous reports of interference of glyphosate with nutrient availability and utilization by GR soybean may be linked to this injury symptom. Also, because glyphosate interferes with amino acid synthesis, supplementation with exogenous amino acids may help GR soybean recover from adverse effects of glyphosate. Therefore, an experiment was designed to evaluate different amino acid concentrations. Near-isogenic and GR soybean varieties were grown in the greenhouse in two soils with and without glyphosate at different rates and amino acids were foliarly applied with and without glyphosate. In general, the photosynthetic variables, nutrient contents, and shoot and root dry biomass parameters were affected by glyphosate, however, use of amino acid formulations suppressed harmful effects of glyphosate on these parameters.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Isopropylamine salts of glyphosate (480 g a.e. L^{-1})
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2012b)

Title: Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean	
Author: L. H. S. Zobiole, R. J. Kremer, R. S. de Oliveira Jr., and J. Constantin	
Reference: J. Plant Nutr. Soil Sci. 2012, 175, 319-330	
Year: 2012	
Results and conclusion: Previous greenhouse studies have demonstrated that photosynthesis in some cultivars of first-(GR1) and second-generation (GR2) glyphosate-resistant soybean was reduced by glyphosate. The reduction in photosynthesis that resulted from glyphosate might affect nutrient uptake and lead to lower plant biomass production and ultimately reduced grain yield. Therefore, a field study was conducted to determine if glyphosate-induced damage to soybean (Glycine max L. Merr. cv. Asgrow AG3539) plants observed under controlled greenhouse conditions might occur in the field environment. The present study evaluated photosynthetic rate, nutrient accumulation, nodulation, and biomass production of GR2 soybean receiving different rates of glyphosate (0, 800, 1200, 2400 g a.e. ha^{-1}) applied at V2, V4, and V6 growth stages. In general, plant damage observed in the field study was similar to that in previous greenhouse studies. Increasing glyphosate rates and applications at later growth stages decreased nutrient accumulation, nodulation, leaf area, and shoot biomass production. Thus, to reduce potential undesirable effects of glyphosate on plant growth, application of the lowest glyphosate rate for weedcontrol efficacy at early growth stages (V2 to V4) is suggested as an advantageous practice within current weed control in GR soybean for optimal crop productivity.	
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.	
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered	
Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	Potassium salts of glyphosate 540 g a.e. L^{-1} (Roundup Weather Max®, Monsanto Company)
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

Zobiole et al. (2012c)

Title: Nutrient Accumulation in Conventional and Glyphosate-Resistant Soybean under Different Types of Weed Control
Author: ZOBIOLE, L.H.S., OLIVEIRA JR., R.S., CONSTANTIN, J., OLIVEIRA JR., A., CASTRO, C., OLIVEIRA, F.A., KREMER, R.J., MOREIRA, A. and ROMAGNOLI, L.M
Reference: Planta Daninha, Viçosa-MG, v. 30, n. 1, p. 75-85, 2012
Year: 2012
Results and conclusion: The cultivation of soybean-Glycine max (Roundup Ready® – RR) has increased and little has been reported on the influence of glyphosate on the nutritional status of the plants. The aim of this work was to compare nutrient accumulation at different phenological stages between the cultivars BRS 184 (conventional) and BRS 243 RR (transgenic), with the same crop cycle, under different weed management systems (hand weed and herbicide). Nutrient accumulation and dry matter in conventional soybean was superior to that in the glyphosate-treated RR soybean, indicating that a higher level of nutrients might be required for the RR cultivars to achieve physiological efficiency and a new fertilizer recommendation for RR crops may be considered, due to the reduced nutritional efficiency imposed by glyphosate.
Proposed action: Not to be considered as publication does not focus on an environmental fate-related endpoint.
Type of information (critical, high/low weight, supporting, additional): Low weight, not to be considered

Reliability	
Endpoint	Not applicable
Protocol	Not applicable
Test compound	-
Test system and conditions	Not applicable
Statistical design	Not applicable
Relevance	
Environmental relevance	Not applicable
Weight of evidence	
"Positive"/"Negative" evidence	Not applicable

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