Glyphosate

DOCUMENT M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by one or more of the member companies of the European Glyphosate Renewal Group (GRG) with the members Bayer Agriculture BV, Barclay Chemicals Manufacturing Ltd., CIECH Sarzyna S.A., Albaugh Europe SARL, Nufarm GmbH & Co KG, SINON Corporation, Industrias Afrasa S.A., Syngenta Crop Protection AG and/or affiliated entities.

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

- From Bayer Agriculture BV or respective affiliate; or
- From Barclay Chemicals Manufacturing Ltd. or respective affiliate; or
- From CIECH Sarzyna S.A. or respective affiliate; or
- From Albaugh Europe SARL or respective affiliate; or
- From Nufarm GmbH & Co KG or respective affiliate; or
- From SINON Corporation or respective affiliate; or
- From Industrias Afrasa S.A. or respective affiliate; or
- From Syngenta Crop Protection AG or respective affiliate; or
- From other applicants once the period of data protection has expired.

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
22nd July 2020	CA 8.2.5.3 Update of *Chironomus riparius* report	Doc ID: 110054- MCA8-GRG_Rev 1 Jul_2020 Replaces the Doc ID 110054- MCA8-GRG_Jun_2020 – Changes are given in yellow

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

Table of Contents

CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE 6
CA 8.1 Effects on Birds and Other Terrestrial Vertebrates 8
CA 8.1.1 Effect on birds 8
CA 8.1.1.1 Acute oral toxicity to birds 8
CA 8.1.1.2 Short-term dietary toxicity to birds 39
CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds 39
CA 8.1.2 Effects on terrestrial vertebrates other than birds 56
CA 8.1.2.1 Acute oral toxicity to mammals 56
CA 8.1.2.2 Long-term and reproductive toxicity to mammals 57
CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals . 58
CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians) 58
CA 8.1.5 Endocrine disrupting properties 59
CA 8.2 Effects on Aquatic Organisms 59
CA 8.2.1 Acute toxicity to fish 60
CA 8.2.2 Long-term and chronic toxicity to fish 131
CA 8.2.2.1 Fish early life stage toxicity test 131
CA 8.2.2.2 Fish full life cycle test 162
CA 8.2.2.3 Bioconcentration in fish 167
CA 8.2.3 Endocrine disrupting properties 172
CA 8.2.4 Acute toxicity to aquatic invertebrates 190
CA 8.2.4.1 Acute toxicity to *Daphnia magna* 193
CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species 234
CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates 250
CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna* 252
CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species 287
CA 8.2.5.3 Development and emergence in *Chironomus riparius* 287
CA 8.2.5.4 Sediment dwelling organisms 292
CA 8.2.6 Effects on algal growth 292
CA 8.2.6.1 Effects on growth of green algae 292
CA 8.2.6.2 Effects on growth of an additional algal species 362
CA 8.2.7 Effects on aquatic macrophytes 402
CA 8.2.8 Further testing on aquatic organisms 455
CA 8.3 Effects on Arthropods 458

CA 8.3.1 Effects on bees ... 458
CA 8.3.1.1 Acute toxicity to bees ... 459
CA 8.3.1.1/1 Acute oral toxicity ... 461
CA 8.3.1.1/2 Acute contact toxicity ... 481
CA 8.3.1.2 Chronic toxicity to bees ... 507
CA 8.3.1.3 Effects on honeybee development and other honeybee life stages ... 512
CA 8.3.1.4 Sub-lethal effects ... 517
CA 8.3.2 Effects on non-target arthropods other than bees ... 523
CA 8.3.2.1 Effects on *Aphidius rhopalosiphi* ... 524
CA 8.3.2.2 Effects on *Typhlodromus pyri* ... 524
CA 8.4 Effects on Non-Target Soil Meso- and Macrofauna ... 524
CA 8.4.1 Earthworms – sub-lethal effects ... 524
CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms) 553
CA 8.4.2.1 Species level testing ... 553
CA 8.5 Effects on Nitrogen Transformation ... 577
CA 8.6 Effects on Terrestrial Non-Target Higher Plants ... 605
CA 8.6.1 Summary of screening data ... 605
CA 8.6.2 Testing on non-target plants ... 605
CA 8.7 Effects on Other Terrestrial Organisms (Flora and Fauna) ... 616
CA 8.8 Effects on Biological Methods for Sewage Treatment ... 617
CA 8.9 Monitoring Data ... 623
Annex M-CA 8-01: PUBLIC LITERATURE RAR 2015 ... 624
Annex M-CA 8-02: Endpoint Selection Considerations for Refinement of the Mammalian Risk Assessment ... 834

CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

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

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

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

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

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

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

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

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

[1] Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate in the framework of the renewal of the approval under Commission Regulation (EU) No 1141/2010; EFSA Journal 2015;13(11):4302, 107 pp; doi:10.2903/j.efsa.2015.4302.

[2] RAC Opinion proposing harmonised classification and labelling at EU level of glyphosate (ISO); N (phosphono-methyl)glycine. CLH-O-0000001412-86-149/F. Adopted 15 Mar 2017.

[3] COMMISSION IMPLEMENTING REGULATION (EU) 2017/2324.

[4] Due to the Bayer-Monsanto acquisition in 2018, the legal entity name Monsanto Europe S.A. / N.V. has been changed to Bayer Agriculture BVBA.

In the frame of the pre-submission meeting held between the GRG and the Assessment Group on Glyphosate (AGG) on 27th September 2019, the AGG provided a reference document to GRG on the process to be considered when summarizing studies from past submissions in the June 2020 renewal dossier[5].

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

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

A literature search for the active substance glyphosate and metabolites was performed in accordance with the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and according to the updated Appendix to this Guidance document[8]. The scientific literature review was performed for the period of 01st January 2010 until 31st December 2019, and total of 10 relevant and reliable articles were identified across sections of toxicology, ecotoxicology, residue and environmental fate. The identified relevant and reliable articles are presented as appendix E summaries in the specific M-CA sections. For further detailed information on the Literature Review Report (LRR) and the corresponding evaluation, please refer to M-CA Section 9 "Literature". In the frame of the pre-submission meeting held on 27th September 2019, the AGG provided a reference document to GRG on the process to be considered when presenting literature in the June 2020 submission dossier[9].

During the former EU processes, public literature data was evaluated, listed and reported by the RMS. An appendix, containing information about all previously submitted and/or included public literature articles from the former EU process is presented, for sake of completeness, as Annex to this M-CA section (See Annex M-CA 8-01).

Ecotoxicological studies have been carried out with the active substance glyphosate, glyphosate acid, glyphosate salts and its metabolites. All studies are presented in tabular form at the beginning of each relevant section and their full study summaries are provided for each organism groups. If reports were not available, short summaries to include endpoints are also provided. Endpoints from valid studies are

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

presented in endpoint tables for each section. Irrespective of the test item, all presented endpoints for glyphosate are given in glyphosate acid equivalents (i.e. recalculated to acid equivalents).

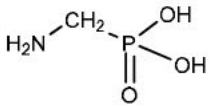
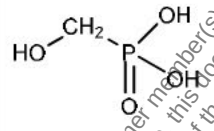
An overview of the batches used in ecotoxicological studies is provided in document J-CA (see Doc ID: 110054-JCA_GRG_Jun_2020).

Metabolites of the active substance

The metabolites which require ecotoxicological assessment according to the EFSA Guidance Documents are given in the following table.

The occurrence and risk from potentially ecotoxicological relevant metabolites has been considered and is discussed in M-CA Section 6 and 7 and M-CP Section 8 and 9. These major metabolites, to which non-target organisms could be exposed, are presented in the table below.

Table 8-1: Maximum occurrence of glyphosate and metabolites in relevant compartments

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments
AMPA (Aminomethylphosphonic acid)		111 g/mol	Soil: 63.0 % Water: 42.7 % Sediment: 18.7 %
HMPA (Hydroxymethylphosphonic acid)		112 g/mol	Water: 10 %

AMPA is ecotoxicologically relevant for the compartments soil, water and sediment.
HMPA is only ecotoxicologically relevant for the compartment water.

CA 8.1 Effects on Birds and Other Terrestrial Vertebrates

Studies on effects of the active substance glyphosate on birds and other terrestrial vertebrates to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

CA 8.1.1 Effect on birds

An extensive regulatory avian toxicology database has been summarised to evaluate acute and long-term toxicity of glyphosate, glyphosate salts and the glyphosate metabolite AMPA. The results of these studies demonstrate that glyphosate, glyphosate salts and AMPA are of low acute and long-term toxicity to birds.

CA 8.1.1.1 Acute oral toxicity to birds

Studies considering the acute toxicity to birds were assessed for their validity to current and relevant guidelines for glyphosate, glyphosate salts and the metabolite AMPA and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 8.1.1.1-1: Studies on acute oral toxicity of glyphosate and its metabolites to birds

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.1.1.1/001	[redacted] 2003	Acute oral	*Colinus virginianus*	Glyphosate K-salt (MON 78623)	Valid	-
CA 8.1.1.1/002	[redacted] 1997	Acute oral	*Colinus virginianus*	Glyphosate acid	Valid	-
CA 8.1.1.1/003	[redacted], 1991	Acute oral	*Colinus virginianus*	Glyphosate technical	Valid	-
CA 8.1.1.1/004	[redacted], 1999	Acute oral	*Coturnix coturnix japonica*	Glyphosate technical	Valid	non GLP
CA 8.1.1.1/005	[redacted], 1996	Acute oral	*Coturnix coturnix japonica*	Glyphosate technical	Valid	-
CA 8.1.1.1/006	[redacted], 1996	Acute oral	*Anas platyrhynchos*	Glyphosate technical	Valid	-
CA 8.1.1.1/007	[redacted] 1992	Acute oral	*Anas platyrhynchos*	Glyphosate technical	Valid	-
CA 8.1.1.1/008	[redacted], 1983	Acute oral	Pigeon	Glyphosate technical	Unknown	Study report not available, invalid in RAR (2015)
CA 8.1.1.1/009	[redacted] 1991	Acute oral	*Colinus virginianus*	AMPA	Valid	-

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on birds. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is available in Annex M-CA 8-01 to this document.

Endpoints of studies considered valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.1.1.1-2: Endpoints: Acute oral toxicity of glyphosate to birds

Reference	Test item	Species	Test design/ GLP	LD_{50} (mg a.e./kg bw)
[redacted] 2003CA 8.1.1.1/001	Glyphosate K- salt	*Colinus virginianus*	Acute oral	> 2241
[redacted] 1997 CA 8.1.1.1/002	Glyphosate acid	*Colinus virginianus*	Acute oral	> 2000
[redacted], 1991 CA 8.1.1.1/003	Glyphosate technical	*Colinus virginianus*	Acute oral	> 2000
[redacted], 1999 CA 8.1.1.1/004	Glyphosate technical	*Coturnix coturnix japonica*	Acute oral / non GLP	> 2000
[redacted], 1996 CA 8.1.1.1/005	Glyphosate technical	*Coturnix coturnix japonica*	Acute oral	> 2000
[redacted], 1996 CA 8.1.1.1/006	Glyphosate technical	*Anas platyrhynchos*	Acute oral	> 2000
[redacted] 1992 CA 8.1.1.1/007	Glyphosate technical	*Anas platyrhynchos*	Acute oral	> 2000
Proposed endpoint for risk assessment				
Extrapolated	Glyphosate acid	bird	Acute, 14 days ≥ 20 birds per limit/maximum dose group without effects	4334[1]

a.e.: acid equivalents
[1] Extrapolated with a factor of 2.167 as recommended by EFSA guidance document 1438/2009 and as described above.

A large number of acute studies in birds without any mortality at a limit dose/maximum dose of 2000 mg a.e./kg bw are submitted. EFSA Journal 7(12): 1438 (2009)[10] indicates that "it is permissible to extrapolate an LD_{50} value in cases where there is no mortality or a single mortality at a limit dose in an acute avian toxicity study". Therefore, an acute LD_{50} for risk assessment of 2000 × 2.167 = 4334 mg a.e./kg bw is proposed.

A study considering the acute toxicity of the metabolite AMPA to birds is available and reported in the following table. This study was assessed to be valid according to current and relevant guidelines and the corresponding study summary is available below. This acute study with the metabolite AMPA shows equally low acute toxicity as the parent, glyphosate.

[10] European Food Safety Authority (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal; 7(12): 1438, doi: 10.2903/j.efsa.2009.1438.

Table 8.1.1.1-3: Endpoints: Acute oral toxicity of AMPA to birds

Reference	Test item	Species	Test design/ GLP	LD_{50} (mg/kg bw)
[redacted] 1991 CA 8.1.1.1/009	AMPA	*Colinus virginianus*	Acute oral	>2250

Study summaries are provided below.

1. Information on the study

Data point	CA 8.1.1.1/001
Report author	[redacted]
Report year	2003
Report title	MON 78623: An acute oral toxicity study with the Northern Bobwhite
Report No	139-461
Document No	
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1. OPPTS 850.2100
Deviations from current test guideline	Deviation compared with OECD 223 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A laboratory study was performed to determine the acute oral toxicity of glyphosate K-salt (MON 78623) to bobwhite quail (*Colinus virginianus*). Ten quails (5 male, 5 female) per dose rate received nominal dietary doses of 291, 484, 807, 1344 and 2241 mg glyphosate acid equivalent/kg bw (mg a.e./kg bw) by oral gavage. The control group was administered an equivalent volume of the diluent (deionised water). Birds were individually observed for mortality, clinical signs of toxicity and abnormal behaviour twice daily for 8 days after study initiation. Body weights were measured at study initiation and after 3, 7 and 14 d. Food consumption for each cage of animals was measured per time interval covering day 0 – 3, 4 – 7 and 8 – 14.

No mortalities were observed at any dose tested and in control treatments. A number of birds showed a ruffled appearance at doses of 484 and higher. At 1344 and 2241 mg a.e./kg bw some birds were lethargic. A treatment related loss of body weight was observed at 2241 mg a.e./kg bw, while no effects on feed consumption were noted.

All validity criteria according to the current guideline OECD 223 were fulfilled.

The acute LD_{50} for Northern bobwhite exposed to glyphosate K-salt was determined to be > 2241 mg glyphosate acid equivalent/kg bw (nominal). The NOEC was determined to be 484 mg glyphosate acid equivalent/kg bw (nominal). This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 78623
Description:	Yellow liquid
Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7 % glyphosate acid

2. Vehicle and/or positive control: Vehicle: Deionised water
Positive control: None

3. Test organisms:

Species:	Northern bobwhite, Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, 30 weeks
Sex	5 male, 5 female per treatment/ control group
Weight	176 - 248 g (at test initiation)
Source:	[redacted]
Diet/Food:	Game bird ration, *ad libitum* during acclimation and during the test, 18 h fasting prior to test start. Birds were given water soluble antibiotic in their drinking water for seven days after arrival in the laboratory.
Acclimation period:	Approx. 4 months
Fasting	18 hours prior to dosing

4. Environmental conditions:

Temperature:	22.0 ± 0.2 °C
Relative humidity:	43 % ± 8 %
Photoperiod:	8 h light, 16 h dark

5. Dates of experimental work: 2002-10-15 to 2002-10-29

B. STUDY DESIGN

Experimental treatments

In an acute oral toxicity test, bobwhite quail were given nominal doses of 291, 484, 807, 1344 and 2241 mg glyphosate acid equivalent/kg bw by oral gavage and observed the following 14 d for mortality, clinical signs of toxicity, abnormal behaviour, body weight change and feed consumption. Ten quails (5 male, 5 female) were assessed per dose and control group. The control group was given diluent only.

Observations

After test initiation, birds were observed twice daily for mortality, clinical signs of toxicity and abnormal behaviour. Body weights were measured at study initiation and after 3, 7 and 14 d. Average feed consumption was determined by pen for each group for day 0 – 3, 4 – 7 and 8 – 14, by measuring the weight change of the presented feed.

Statistical calculations

Since the mortality was <50 %, no statistical calculation of LC_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LD_{50} and NOEL values are given below based on nominal doses:

Table 8.1.1.1-4: Endpoints

Endpoints	Glyphosate K-salt [mg a.e/kg bw]
LD_{50}	>2241
NOEL	484

Table 8.1.1.1-5: Effects of glyphosate K-salt on body weight, food consumption of Northern bobwhite quail

Glyphosate K-salt [mg a.e./kg bw]	Control	291	484	807	1344	2241
Mortality						
Day 14	0	0	0	0	0	0
Clinical signs						
Ruffled appearance	0	0	2[1]	4	5	2
Lethargy	0	0	0	0	1	1
Mean body weight [g] (male/female)						
Day 0	224/197	221/208	222/207	219/206	219/221	225/212
Day 14	221/201	224/209	223/210	221/209	221/226	223/216
Feed consumption [g] (male/female)						
Day 0 - 3	31/15	28/21	27/26	18/23	21/15	17/23
Day 4 - 7	28/21	29/22	23/26	20/24	23/23	25/28
Day 8 - 14	25/16	24/17	19/20	21/20	20/18	17/18

[1] Not considered to be treatment related due to the timing and isolated nature of the signs noted.

B. OBSERVATIONS

There was no treatment-related mortality observed. One control male suffered a leg injury during body weight procedures and lost weight afterwards.

Numerous birds developed foot injuries during the study, which were not treatment related. At 2241 mg a.e./kg bw one male received a foot injury. One male and one female in the 484 mg a.e./kg bw group got foot lesions with associated lameness and/or ruffled appearance. This was considered to be incidental to the treatment. At 807, 1344 and 2241 mg a.e./kg bw a number of birds showed a ruffled appearance. At 807 and 1344 mg a.e./kg groups all bird (except one male in 1344 mg a.e./kg group) had recovered by the morning of Day 11 of the test and were normal in appearance and behaviour for the remainder of the test. At the two highest test concentrations also lethargy was observed. No dose-response related increase of toxicity signs was noted.

When compared to the control group, no treatment related effects on body weight were noted except for the highest test concentration of 2241 mg a.e./kg bw. No treatment related effect on feed consumption was observed.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The acute LD_{50} for northern bobwhite exposed to glyphosate K-salt was determined to be > 2241 mg glyphosate acid equivalent/kg bw (nominal). The NOEC was determined to be 484 mg glyphosate acid equivalent/kg bw (nominal).

This study is considered valid and the acute oral LD_{50} for northern bobwhite exposed to glyphosate K-salt was determined to be > 2241 mg a.e./kg bw (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.1/002
Report author	[redacted]
Report year	1997
Report title	Glyphosate acid. Acute oral toxicity (LD_{50}) to Bobwhite quail
Report No	ISN 400963858
Document No	-
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1. Avian single dose LD_{50} test (1982)
Deviations from current test guideline	Deviation compared with OECD 223 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A laboratory study with the Bobwhite quail (*Colinus virginianus*) was conducted. After an acclimation period of 14 days, birds received a single dose of the test substance glyphosate acid diluted in methylcellulose (1% w/v) by oral gavage. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg a.s./kg bw. The control birds received a corresponding volume of methylcellulose only.

During the test all mortalities and health of the birds were observed daily. Body weights were measured individually 15 and 7 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Feed consumption was determined by cage of each dosage group and the control group 15, 8, 7 and 1 day(s) prior to test start and on days 1 to 7 and 8 to 14 of the test.

Post mortem examination was carried out on all ten control birds and all ten birds from the highest dose group.

There were no mortalities. All birds remained in good health following dosing, and no clinical signs of toxicity were observed. No treatment-related effects were recorded on body weight and food consumption. No abnormalities were detected in any birds during *post mortem* examination at termination of the study. All validity criteria according to the current guideline OECD 223 were fulfilled.
Under the conditions of this study, the acute oral LD_{50} of glyphosate acid to Bobwhite quail was found to be > 2000 mg a.s./kg. The NOEL in the study was determined to be 2000 mg a.s./kg. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White crystalline powder
Lot/Batch #:	P24
Purity:	95.6 %

2. Vehicle and/or positive control: Vehicle: Methylcellulose (1 % w/v)
Positive control: None

3. Test organisms:

Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, approximately 4 - 6 month old on arrival
Weight:	175 - 213 g (15 days prior to test initiation)
Source:	Commercial supplier ([redacted])
Diet/Food:	Standard HRC layer diet in pellet form obtained from Parker Brothers Ltd. (Lark Mills, Mildenhall, Suffolk, UK). Food was offered *ad libitum*, with the exception of an overnight starvation period of approximately 21 hours prior to dosing. Water was available at all times.
Acclimatisation:	15 days

4. Environmental conditions:

Temperature:	17 – 19 °C
Relative humidity:	68 %
Photoperiod:	10 hours light / 14 hours darkness

5. Dates of experimental work: 1996-12-17 to 1996-12-31

B. STUDY DESIGN

Experimental treatments

The dose level was based on existing toxicity data indicating that the test material is of low toxicity to birds. Young adult Bobwhite quails (5 males and 5 females per dosage) received a single dose of the test substance or vehicle by oral intubation using a disposable syringe and a Ch 10 Nelaton plastic catheter. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg a.s./kg bw (dosage concentrations: 5 %, 10 % and 20 % w/v). A constant dose volume of 10

mL/kg bodyweight was used for all treatment groups. The control birds received an equivalent volume of methylcellulose only.

Observations

During the test all mortalities, bird health and clinical signs of the birds were observed daily. Body weights were measured individually 15 and 7 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Feed consumption was determined by cage of each dosage group and the control group 15, 8, 7 and 1 day(s) prior to test start and on days 1 to 7 and 8 to 14 of the test.

Post mortem examination was carried out on all ten control birds and all ten birds from the highest dose group.

Statistical calculations

Since no mortality was reported, no statistical calculation of LD_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.1.1.1-6: Effects of glyphosate acid on body weight and food consumption of Bobwhite quail

Glyphosate acid [mg/kg bw]			Control	500	1000	2000
Average body weight per animal [g] (± SD)						
Body weight	Day -15	male	192 ± 5.9	195 ± 5.9	192 ± 3.7	195 ± 4.9
		female	191 ± 11.4	191 ± 15.6	191 ± 13.3	190 ± 8.9
	Day -7	male	196 ± 5.7	196 ± 6.5	194 ± 4.1	198 ± 5.6
		female	190 ± 10.2	190 ± 18.2	192 ± 7.8	189 ± 11.6
	Day 0	male	194 ± 4.7	197 ± 6.9	193 ± 4.8	198 ± 5.9
		female	190 ± 9.1	189 ± 17.1	192 ± 10.6	186 ± 10.5
	Day 7	male	198 ± 2.5	199 ± 6.1	196 ± 4.3	198 ± 8.8
		female	192 ± 13.0	192 ± 18.9	197 ± 13.3	191 ± 9.7
	Day 14	male	200 ± 2.3	199 ± 4.9	196 ± 3.8	196 ± 7.0
		female	192 ± 8.6	194 ± 17.0	198 ± 10.6	189 ± 9.5
Body weight change	Days 0-14	male	6.0 ± 2.4	2.0 ± 2.0	3.0 ± 1.0	-2.0± 1.1
		female	2.0 ± 0.5	5.0 ± 0.1	6.0 ± 0.0	3.0 ± 1.0
Mean food consumption per animal [g/bird/day]						
Food consumption	Day -15 to -8	male	13	13	12	13
		female	13	13	12	13
	Day -7 to -1	male	13	13	12	13
		female	13	13	13	13
	Day 1 to 7	male	14	15	14	13
		female	16	15	15	15
	Day 8 to 14	male	14	14	14	13
		female	15	13	14	14
Group mean	Day 1-14	male	14	14.5	14	13
		female	15.5	14	14.5	14.5

B. OBSERVATIONS

There were no mortalities observed in any treatment. All control and test birds remained in good health following dosing, and no clinical signs of toxicity were observed. Body weight changes were similar in all groups and there was no evidence of any treatment-related effects. Group mean food consumption was similar in all groups and there was no evidence of any treatment-related effects. No abnormalities were detected in any birds during *post mortem* examination at termination of the study.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The acute oral LD_{50} for Bobwhite quail exposed to glyphosate acid was determined to be > 2000 mg a.s./kg bw. The NOEL in the study was determined to be 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for Bobwhite quail exposed to glyphosate acid of > 2000 mg a.s./kg bw can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.1/003
Report author	[redacted]
Report year	1991
Report title	Glyphosate technical: Acute oral toxicity (LD_{50}) to the bobwhite quail
Report No	CHV 48/91266
Document No	-
Guidelines followed in study	FIFRA subdivision E, section 71-1
Deviations from current test guideline	Deviation compared with OECD 223 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
A laboratory study with the Bobwhite quail (*Colinus virginianus*) was conducted. After an acclimation period of 21 days, birds received a single dose of the test substance glyphosate diluted in methylcellulose (1 % w/v) by oral gavage. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg a.s./kg bw. The control birds received a corresponding volume of methylcellulose only.

During the test all mortalities, bird health and clinical signs of the birds were observed daily. Body weights were measured individually 21, 13, 6 and 0 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Feed consumption was determined by cage of each dosage group and the control group 21 to 14, 13 to 7, 6 to 1 day(s) prior to test start and on days 1 to 7 and 8 to 14 of the test.
Post mortem examination was carried out on all ten birds from the highest dose group.

There were no mortalities. All birds remained in good health following dosing, and no clinical signs of toxicity were observed groups. No treatment-related effects were recorded on body weight and food consumption. No abnormalities were detected in any birds during *post mortem* examination at termination of the study. All validity criteria according to the current guideline OECD 223 were fulfilled.
Under the conditions of this study the acute oral LD_{50} of glyphosate technical to bobwhite quail was found to be > 2000 mg a.s./kg bw. The NOEL in the study was 2000 mg a.s./kg bw. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	206-JAK-119-1
Purity:	97.5 %
Density:	Not stated
2. Vehicle and/or positive control:	Vehicle: Methylcellulose (1% w/v) in distilled water Positive control: None
3. Test organisms:	
Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, approximately 16 weeks old on arrival
Source:	Commercial supplier ([redacted])
Diet/Food:	Standard HRC layer diet in pellet form obtained from Parker Brothers Ltd. (Lark Mills, Mildenhall, Suffolk, UK). This diet, though not analysed for contaminants, was known to contain no added antibiotic or other growth promoter. Food was offered *ad libitum*, with the exception of an overnight starvation period of approximately 17 hours on day -7 and prior to dosing. The starvation on day -7 was carried out in anticipation of dosing birds the following day. Due to an inadequate formulation of test material, however, dosing was delayed for a further week. Water was available at all times.
Acclimatisation:	21 days
Body weight of the animals	180 g – 237 g at test start
4. Environmental conditions:	
Temperature:	14 - 17 °C
Relative humidity:	82 %
Photoperiod:	10 hours light / 14 hours darkness
5. Dates of experimental work:	November 29th, 1990 to January 3rd, 1991

B. STUDY DESIGN

Experimental treatments
The dose level was based on the results of a range-finding test where no mortalities occur at 2000 mg a.s./kg bw. Young bobwhite quail (5 adult males and 5 adult females per treatment) received a single dose of the

test substance or vehicle by oral intubation using a disposable syringe and a Ch 10 Nelaton plastic catheter. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg/kg bw (dosage concentrations: 5 %, 10 % and 20 % w/v). A constant dose volume of 10 mL/kg bodyweight was used for all treatment groups. The control birds received a corresponding volume of methylcellulose in distilled water only. For macroscopic *post mortem* examination the following tissues were examined: digestive tract, liver, kidneys, heart, spleen, muscle and subcutaneous fat.

Observations

During the test all mortalities, bird health and clinical signs of the birds were observed daily. Body weights were measured individually 21, 13, 6 and 0 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Feed consumption was determined by cage of each dosage group and the control group 21 to 14, 13 to 7, 6 to 1 day(s) prior to test start and on days 1 to 7 and 8 to 14 of the test. *Post mortem* examination was carried out on all ten birds from the highest dose group.

Statistical calculations

Since no mortality was reported, no statistical calculation of LD_{50} values was possible. The NOEL was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Determination of the glyphosate concentration in each of the dose formulations, physical stability and chemical stability of the 1% methylcellulose formulations were performed.

Table 8.1.1.1-7: Concentrations of glyphosate technical in dose formulations

Glyphosate technical [% w/v]	Analysed concentrations [% w/v]			Relative Mean Error [%]
	Analysis 1	Analysis 2	Mean	
0	ND	-	ND	-
5	4.80	5.37	5.09	+1.8
10	10.9	11.0	10.9	+9.0
20	20.7	19.3	20.0	+0.0

ND = Not detected (<0.015% w/v)

Table 8.1.1.1-8: Effects of glyphosate technical on body weight and food consumption of bobwhite quail

Glyphosate technical [mg/kg bw]			Control	500	1000	2000
Average body weight per animal [g]						
Body weight	Day 0	male	207	211	206	207
		female	187	186	189	182
	Day 7	male	212	219	210	213
		female	190	191	193	188
	Day 14	male	213	222	213	216
		female	191	194	195	191
Mean food consumption per animal [g/bird/day]						
Food consumption	Day 0-7	male	19	20	18	18
		female	17	18	17	18
	Day 7-14	male	19	19	18	18
		female	19	18	18	18

B. OBSERVATIONS

Analytical results: Mean results were within 9% of the nominal concentrations.
Clinical observations and mortalities: All birds remained in good health throughout the study and there were no mortalities observed.

Body weight and feed consumption: Body weight changes were variable in all groups and there was no evidence of any treatment-related effect. With the exception of reduced consumption in group 3 over days -21 and -17, food consumption was similar in all groups with no evidence of any treatment-related effect.

Macroscopic *post mortem* examination: No abnormalities were detected in any birds during *post mortem* examination at termination of the study.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The acute oral LD_{50} of glyphosate technical to bobwhite quail was determined to be > 2000 mg a.s./kg bw. The NOEL in the study was 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for bobwhite quail exposed to glyphosate technical of > 2000 mg a.e./kg bw can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.1/004
Report author	[redacted]
Report year	1999
Report title	Avian Single-Dose Acute Oral Toxicity Test in Japanese Quail with the chemical product Glifosate Técnico Nufarm
Report No	D8.1–382/99
Document No	-
Guidelines followed in study	Not stated
Deviations from current test guideline	Deviation compared with OECD 223 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No GLP stated in report
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A laboratory study was performed to determine the acute oral toxicity of glyphosate acid to Japanese quail (*Coturnix coturnix japonica*). Twenty animals were randomly allocated to two groups, one treatment item group and one control, each comprising five males and five females. On Day 0, a single oral dose of 2000 mg glyphosate acid/kg bw was administered enclosed in gelatin capsules. A control group received empty capsules.

Birds were observed for clinical signs of toxicity, behaviour, body weight effects, food consumption and mortality for 15 days after dosing. Birds were weighed at the beginning and at the end of test.

There were no mortalities observed in any treatment group and all birds remained in good health following dosing, with no clinical signs of toxicity were observed. All validity criteria according to the current guideline OECD 223 were fulfilled.

The acute oral LD_{50} for Japanese quail exposed to glyphosate acid was determined to be > 2000 mg a.s./kg bw. The NOEL in the study was determined to be 2000 mg a.s./kg bw. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Glyphosate acid
Description: powder
Lot/Batch #: 037-919-113
Purity: 95 % (nominal)
954.9 g/kg acid equivalent (measured)

2. Vehicle and/or positive control: Vehicle: Gelatin capsules
Positive control: None

3. Test organisms:

Species: Japanese Quail (*Coturnix coturnix japonica*)

Age: Young adults, at least 16 weeks old

Weight:	Males: 100 – 130 g at test start
	Females: 114 – 140 g at test start
Source:	Not stated
Diet/Food:	Commercial diet (GUABI ration) and water *ad libitum*.
Acclimatisation:	At least 15 days
4. Environmental conditions:	
Temperature:	25 – 28 °C
Relative humidity:	30 – 70 %
Photoperiod:	10 hours light / 14 hours dark
5. Dates of experimental work:	1999-10-05 to 1999-10-19

B. STUDY DESIGN

Experimental treatments

Young adult Japanese quails (5 males and 5 females per treatment) received a single limit dose of 2000 mg a.s./kg bw of the test substance, enclosed in gelatin capsules. A control group received empty capsules by oral gavage.

Observations

During the 15 days of the test, mortality, behaviour, clinical symptoms and anatomopathological alterations were observed daily. Birds were weighed at the beginning and at the end of test.

Statistical calculations

Since no mortality was reported, no statistical calculation of LD_{50} values was possible. The NOEL was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.1.1.1-9: Effects of glyphosate acid on body weight and food consumption of Japanese quail

Glyphosate acid [mg/kg bw]			Control	2000
Average body weight per animal [g] (± SD)				
Body weight	Day 0	male	109 ± 9.3	123 ± 5.3
		female	121 ± 5.8	122 ± 10.3
	Day 7	male	113 ± 11.1	119 ± 6.6
		female	122 ± 9.6	114 ± 9.9
	Day 14	male	119 ± 9.5	126 ± 6.9
		female	130 ± 9.6	124 ± 7.6
Body weight change	Days 0-14	male	10.2 ± 5.0	3.4 ± 5.5
		female	8.8 ± 7.4	1.8 ± 13.6

Table 8.1.1.1-9: Effects of glyphosate acid on body weight and food consumption of Japanese quail

Glyphosate acid [mg/kg bw]			Control	2000
Mean food consumption per animal [g/bird/day]				
Food consumption	Day 0-7		111.3	99.4
	Day 7-14		77.2	99.6
Group mean	Day 0-14	mean	94.25	99.5

B. OBSERVATIONS

There were no mortalities observed in any treatment. All control and test birds remained in good health following dosing, and no clinical signs of toxicity were observed. Body weight changes were similar in all groups and there was no evidence of any treatment-related effects. Group mean food consumption was similar in all groups and there was no evidence of any treatment-related effects. No abnormalities were detected in any birds during *post mortem* examination at termination of the study.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:

The acute oral LD_{50} for Japanese quail exposed to glyphosate acid was determined to be >2000 mg a.s./kg bw. The NOEL in the study was determined to be 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for Japanese quail exposed to glyphosate acid of >2000 mg a.s./kg bw can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.1/005
Report author	[redacted]
Report year	1996
Report title	Glyphosate: Acute Oral Toxicity to Japanese Quail
Report No	1413/4-1011
Document No	-
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1. Avian single dose LD_{50} test (1982)
Deviations from current test guideline	Deviation compared with OECD 223 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A study was performed to determine the acute oral toxicity of glyphosate acid to Japanese quail (*Coturnix coturnix japonica*). As no mortalities were observed in a range finder study at a maximum dose of 2000 mg a.s./kg bw, only this dose level was used for the definitive study. Twenty animals were randomly allocated to two groups, one treatment item group and one control, each comprising five males and five females. On Day 0, a single oral dose was administered by direct intubation of 2000 mg a.s./kg bw to the treatment item group. The control group was treated with vehicle only (0.5 % w/w CMC solution).

Birds were observed for clinical signs of toxicity, behaviour, body weight effects, food consumption and mortality for 14 days after dosing. Body weights were measured individually at test initiation (day 0), on day 3, 7 and 14 after test initiation. Food consumption for each cage of animals was measured per time interval covering day 0-7, and day 7-14.

No treatment related mortality was observed, except for one bird found dead due to trauma of reproductive tract. Furthermore, there were no effects observed on body weight or food intake, and no abnormal findings at necropsy. All validity criteria according to the current OECD guideline 223 were fulfilled.

The acute oral LD_{50} for Japanese quail exposed to technical glyphosate was determined to be > 2000 mg a.s./kg bw. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White/off-white crystalline powder
Lot/Batch #:	H95 D161A
Purity:	95.3%

2. Vehicle and/or positive control: Vehicle: 0.5% carboxymethyl cellulose (CMC)

Positive control: None

3. Test organisms:

Species:	Japanese quail (*Coturnix coturnix japonica*)
Age:	Young adults, approx. 23 weeks old
Weight:	202 - 300 g (at test initiation)
Source:	[redacted]
Diet/Food:	Proprietary avian food, *ad libitum*
Acclimation period:	5 weeks prior to dosing
Fasting	16 to 17 hours prior to dosing

4. Environmental conditions:

Temperature:	15 – 20°C
Humidity:	40 – 78%
Photoperiod:	8 hours light / 16 hours dark

5. Dates of experimental work: 1996-01-09 to 1996-01-23

B. STUDY DESIGN

Experimental treatments

Based on the results of a range finder study, an acute oral toxicity test was performed by administering a single limit dose of 2000 mg a.s./kg bw (glyphosate acid dissolved in 0.5 % carboxymethyl cellulose) by oral intubation to ten adult Japanese quails (5 males and 5 females) in one treatment group. In addition, one control group was administered an equivalent volume of the vehicle (CMC) only as the test groups, at a dose rate of 2 mL/kg bw. After dosing, birds where fed *ad libitum* throughout the study.

Observations

Birds were caged and observed continuously for signs of toxicity, abnormal behaviour and mortality for one hour after dosing, then at intervals throughout day 0 and twice daily thereafter. Food consumption was measured covering day 0-7, and day 7-14. Each animal was weighed at least on day 0, 3, 7 and 14. On day 14, all surviving animals were sacrificed, and a gross macroscopic examination was carried out. The necropsy comprised a general inspection of major visceral organs.

Statistical calculations

Since the mortality was <50 %, no statistical calculation of LC_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

No analytical verification was performed.

Table 8.1.1.1-10: Effects of glyphosate acid on body weight and food consumption of Japanese quail

Glyphosate acid [mg/kg bw]			Control	2000
Average body weight per animal [g] (± SD)				
Body weight	Day 0	male	249 ± 27.1	228 ± 22.3
		female	257 ± 15.3	260 ± 28.0
	Day 3	male	270 ± 31.4	231 ± 22.2
		female	268 ± 18.5	272 ± 36.1
	Day 7	male	275 ± 31.8	239 ± 17.3
		female	271 ± 18.5	271 ± 32.8
	Day 14	male	276 ± 33.5	243 ± 18.5
		female	276 ± 18.2	288 ± 28.7
Body weight change	Days 0-14	male	26 ± 12	15 ± 5.7
		female	19 ± 13.6	23 ± 3.0
Mean food consumption per animal [g/bird/day]				
Food consumption	Day 0-7	male	64.5	39.9
		female	56.1	60.9
	Day 7-14	male	50.0	41.8
		female	58.0	67.9
Group mean	Day 0-14	mean	57.2	52.0

B. OBSERVATIONS

There was no treatment-related mortality observed, except for one bird in treatment group found dead due to trauma of the reproductive tract. Furthermore, there were no adverse effects were observed on bodyweight or food intake. No findings at necropsy, considered to be treatment-related.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The acute oral LD_{50} for Japanese quail exposed to glyphosate acid was determined to be > 2000 mg a.s./kg bw. The NOEL in the study was determined to be 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for Japanese quail exposed to glyphosate acid of > 2000 mg a.s./kg bw can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.1/006
Report author	[redacted]
Report year	1996
Report title	Glyphosate: Acute Oral Toxicity to Mallard Duck
Report No	1413/5-1011
Document No	-
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1. Avian single dose LD_{50} test (1982)
Deviations from current test guideline	Deviation compared with OECD 223 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A study was performed to determine the acute oral toxicity of glyphosate technical to Mallard duck (*Anas platyrhynchos*). As no mortality was observed in a range finder study at a maximum dose of 2000 mg a.s./kg bw, only this dose level was used for the definitive study. Twenty animals were randomly allocated to two groups, one treatment item group and one control, each comprising five males and five females. On Day 0, a single oral dose of glyphosate technical was administered by direct intubation of 2000 mg a.s./kg bw to the treatment item group. The control group was treated with vehicle only (0.5 % w/w CMC solution).

Birds were observed for clinical signs of toxicity, behaviour, body weight effects, food consumption and mortality for 14 days after dosing. Body weights were measured individually at test initiation (day 0), and on day 5, 11 and 14 after test initiation. Food consumption for each cage of animals was measured per time interval, covering days 0-7, and days 7-14.

No mortalities and no post-dosing signs of toxicity were observed. Furthermore, the body weight was not affected by the treatment. There were equally no treatment-related effects on food consumption and no abnormalities were detected at necropsy of the animals 14 days after treatment.
All validity criteria according to the OECD guideline 223 were fulfilled.
The acute oral LD_{50} for Mallard duck exposed to technical glyphosate was determined to be >2000 mg a.s./kg bw. The NOEL was determined to be 2000 mg a.s./kg bw. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White / off-white crystalline powder
Lot/Batch #:	H95 D161A
Purity:	95.3 % w/w

2. Vehicle and/or positive control:	Vehicle: 0.5 % carboxymethyl cellulose (CMC) Positive control:
3. Test organisms:	
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	Young adults, approx. 23 weeks old
Sex	Males and females
Weight	903 – 1114 g (at test initiation)
Source:	[redacted]
Loading	Approx. 4.5 m^2 for 5 birds
Diet/Food:	Proprietary avian food, *ad libitum*
Acclimation period:	5 weeks prior to dosing
Fasting	16 to 17 hours prior to dosing
4. Environmental conditions:	
Temperature:	15 – 22 °C
Humidity:	42 – 74 %
Photoperiod:	14 hours light / 10 hours dark
5. Dates of experimental work:	December 14th, 1995 to February 18th, 1996

B. STUDY DESIGN

Experimental treatments

Based on the results of a range finding study, an acute oral toxicity test was performed as a limit test by administering a single limit dose of 2000 mg a.s./kg bw (technical glyphosate dissolved in 0.5 % carboxymethyl cellulose) by direct intubation to ten juvenile Mallard ducks (5 males and 5 females) in one treatment group. In addition, one control group comprising 5 males and 5 females was administered an equivalent volume of the vehicle (CMC) only, at a dose rate of 2 mL/kg bw. After dosing, birds where fed *ad libitum* throughout the study.

Observations

Birds were caged and observed for signs of toxicity, abnormal behaviour and mortality continuously for one hour after dosing, then at intervals throughout day 0 and twice daily thereafter. Food consumption was measured per time interval, covering day 0-7, and day 7-14. Each animal was weighed at least on day 0, 5, 11 and 14. On day 14, all surviving animals were sacrificed and a gross macroscopic examination was carried out. The necropsy comprised a general inspection of major visceral organs.

Statistical calculations

Since no mortality was reported, no statistical calculation of LD_{50} values was possible. The NOEL was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LD_{50} and NOEL values are given below based on nominal doses

Table 8.1.1.1-11: Endpoints

Endpoints	Glyphosate technical [mg a.s/kg bw]
LD_{50}	> 2000
NOEL	2000

Table 8.1.1.1-12: Effects of glyphosate technical on body weight and food consumption of Mallard duck

Glyphosate technical [mg a.s./kg bw]			Control	2000
Average body weight per animal [g] (± SD)				
Body weight	Day 0	male	1011 ± 41.5	1012 ± 76.4
		female	1072 ± 128.4	1018 ± 81.1
	Day 5	male	1101 ± 33.5	1048 ± 49.6
		female	1170 ± 160.0	1082 ± 60.8
	Day 11	male	1096 ± 54.8	1052 ± 69.6
		female	1191 ± 155.0	1175 ± 41.4
	Day 14	male	1104 ± 51.8	1053 ± 65.9
		female	1171 ± 122.6	1156 ± 66.5
Body weight change	Days 0-14	male	93 ± 30.9	42 ± 12.2
		female	99 ± 86.0	138 ± 110.8
Mean food consumption per animal [g/bird/day]				
Food consumption	Day 0-7	male	79	80
		female	131	121
	Day 7-14	male	72	76
		female	130	138

B. OBSERVATIONS

No mortalities and no post-dosing signs of toxicity were observed in any treatment and all animals remained in good health throughout the study. Furthermore, the body weight was not affected adversely by the treatment. There were equally no treatment-related effects on food consumption and no abnormalities were detected at necropsy of the animals 14 days after treatment.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
The acute oral LD_{50} for Mallard duck exposed to glyphosate technical was determined to be >2000 mg a.s./kg bw. The NOEL was determined to be 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for Mallard duck exposed to glyphosate technical was determined to be > 2000 mg a.e./kg bw and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.1/007
Report author	[redacted]
Report year	1992
Report title	Glyphosate technical: Acute oral toxicity (LD50) to mallard duck
Report No	CHV 49/91843
Document No	AVS94-00229
Guidelines followed in study	FIFRA subdivision E, section 71-1
Deviations from current test guideline	Deviation compared with OECD 223 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
An acute oral laboratory study with the mallard duck (*Anas platyrhynchos*) was conducted. After an acclimation period of 15 days, birds received a single dose of the test substance glyphosate technical diluted in methylcellulose (1% w/v) by oral gavage. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg a.s./kg bw body weight. The control birds received a corresponding volume of methylcellulose only.

During the test mortality, bird health and clinical signs of the birds were observed daily. Body weights were measured individually 15 and 7 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Feed consumption was determined by cage of each dosage group and the control group over days 15 to 8 and 7 to 1 prior to test start and on days 1 to 7 and 8 to 14 of after treatment. *Post mortem* examination was carried out on any bird which died during the study an on twenty birds from the highest dose groups in which there were survivors.

There were no mortalities, except of one male bird of one of the control groups. All birds remained in good health following dosing, and no clinical signs of toxicity were observed groups. No treatment-related effects were recorded on body weight and food consumption. No treatment-related abnormalities were detected in any birds during *post mortem* examination at termination of the study. All validity criteria according to the current guideline OECD 223 were fulfilled.
Under the conditions of this study the acute oral LD_{50} of glyphosate technical to mallard duck was found to be >2000 mg a.s./kg bw. The NOEL in the study was 2000 mg a.s./kg bw. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	206-JAK-119-1
Purity:	97.5 %
2. Vehicle and/or positive control:	Vehicle: Methylcellulose (1% w/v) in distilled water Positive control: None
3. Test organisms:	
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	Approximately 22 month old at test start
Source:	Commercial supplier ([redacted])
Diet/Food:	Standard HRC layer diet in pellet form obtained from Parker Brothers Ltd. (Lark Mills, Mildenhall, Suffolk, UK). Food was offered *ad libitum*, with the exception of an overnight starvation period of approximately 19 hours prior to dosing. Water was available at all times.
Acclimatisation:	15 days
Body weight of the animals	970 g – 1250 g
4. Environmental conditions:	
Temperature:	11 – 16 °C
Relative humidity:	92 %
Photoperiod:	10 hours light / 14 hours darkness
5. Experimental dates:	May 7th, 1991 to June 05th, 1991

B. STUDY DESIGN

Experimental treatments

The dose level was based on the results of a range-finding test. Mallard duck (5 males and 5 females per treatment) received a single dose of the test substance or vehicle by oral intubation using a disposable syringe and a Ch 10 Nelaton plastic catheter. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg a.s./kg bw (dosage concentrations: 10 %, 20 % and 40 % w/v glyphosate technical). A constant dose volume of 5 mL/kg bodyweight was used for all treatment groups. The control birds received a corresponding volume of methylcellulose in distilled water only.

Observations
Birds were observed daily during the study and at frequent intervals during the post-treatment period. Mortalities, bird health and clinical signs were recorded at each observation. Individual body weights were measured individually 15 and 7 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Group mean food consumption was determined over days 15 to 8 and 7 to 1 prior to test start and on days 1 to 7 and 8 to 14 days after treatment. *Post mortem* examination was carried out on any bird which died during the study an on twenty birds from the highest dose groups in which there were survivors.

Statistical calculations: Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

Determination of the glyphosate concentration in each of the dose formulations, physical stability and chemical stability of the 1 % methylcellulose formulations were performed.

Table 8.1.1.1-13: Concentrations of glyphosate technical in dose formulations

Glyphosate technical [% w/v]	Analysed concentrations [% w/v]			Relative Mean Error [%]
	Analysis 1	Analysis 2	Mean	
0	ND		ND	-
10	11.0	9.16	10.1	+1.0
20	25.4	18.5	22.0	+10.0
40	39.1	37.5	38.3	-4.3

ND = Not detected (<0.015% w/v)

Table 8.1.1.1-14: Effects of glyphosate technical on body weight and food consumption of mallard duck

Glyphosate technical [mg a.s./kg bw]			Control	500	1000	2000
Average body weight per animal [g]						
Body weight	Day 0	male	1036	1033	1066	1038
		female	1034	990	971	981
	Day 7	male	1098	1103	1142	1119
		female	1090	1079	1010	1042
	Day 14	male	1189	1129	1156	1132
		female	1092	1075	1012	1036
Mean food consumption per animal [g/bird/day]						
Food consumption	Day 1-7	male	88	91	103	117
		female	103	100	89	97
	Day 8-14	male	100	114	114	111
		female	91	80	86	89

B. OBSERVATIONS

Analytical results: Mean results were within 10% of the nominal concentrations.

Mortalities: On day 6 one male bird of one control group was found dead. This was possibly associated with the aggressive behaviour of one other male bird observed (as described below). There were no other mortalities in any treatment group.

Bird health and clinical observations: All birds remained in good health throughout the study and there were no clinical signs of toxicity. One male bird in one of the control groups became aggressive towards other birds in the group on day 7. This bird was removed from the pen and housed separately until the end of the study.

Body weight and feed consumption: Body weight changes were variable in all groups and there was no evidence of any treatment-related effect.

Macroscopic *post mortem* examination: One male bird of the highest treatment group (glyphosate technical: 2000 mg a.s./L) was found to have a fluid-filled body cavity, and one lobe of the liver was bulbous and had a fibrous coating. This was not considered to be treatment-related. No other abnormalities were detected in any other bird examined.

No non-incidental death was observed in the control groups. In contrast to guideline OECD 223, the total number of control birds used in the test was ten instead of five. Therefore, although one bird died incidentally, all validity criteria according to OECD 223 were fulfilled.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Under the conditions of this study the acute oral LD_{50} of glyphosate technical to mallard duck was found to be >2000 mg a.s./kg bw. The NOEL in the study was 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for mallard duck exposed to glyphosate technical was determined to be >2000 mg a.e./kg bw and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.1.1.1/008
Report author	[redacted]
Report year	1983
Report title	Report of the acute oral toxicity (MLD) to pigeon with glyphosate (tech) of [redacted]
Report No	AVS 95-00214
Document No	-
Guidelines followed in study	No information mentioned in the Monograph 2001.
GLP	No (information from the reference list of the Monograph 2001)
Previous evaluation	Not accepted in RAR (2015).

Short description of study design and observations	Acute oral toxicity of glyphosate (tech) to pigeon.
Short description of results	No information mentioned in the Monograph.
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available and no information mentioned in the Monograph 2001.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	CA 8.1.1.1/009
Report author	[redacted]
Report year	1991
Report title	AMPA: An Acute Oral Toxicity Study with the Northern Bobwhite
Report No	139-277
Document No	-
Guidelines followed in study	FIFRA Guideline 71-1
Deviations from current test guideline	Deviation compared with OECD 223 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In an acute oral toxicity study, AMPA was administered by oral gavage to fasted Northern bobwhite quail (*Colinus virginianus*). Ten birds (five males and five females) per dose received single oral nominal doses of AMPA of 0, 292, 486, 810, 1350 and 2250 mg/kg body weight at a dose volume of 6 mL/kg bw in corn oil.

Birds were observed for clinical signs of toxicity, behaviour, body weight effects, food consumption and mortality for 14 days after dosing. Body weights were measured individually at test initiation and by group on days 3, 7 and 14. Average estimated feed consumption was determined for each dosage group and the control for days 0-3, 4-7 and 8-14.

Results showed no mortalities at any of the dosages tested. In addition, birds were normal in appearance and behaviour throughout the test period, although one male at 810 mg/kg body weight was noted with foot lesions due to pen-wear on day 13 and 14.

When compared to the controls, there did not appear to be any notable effect on body weight or feed consumption at any of the dosages tested. All validity criteria according to the OECD guideline 223 were fulfilled.
The acute oral LD_{50} for northern bobwhite quail exposed to AMPA as a single oral dosage was >2250 mg/kg bw. The NOEL was 1350 mg/kg bw. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA
Description:	White powder
Lot/Batch #:	PIT-9008-2407T
Purity:	97 % (nominal), 87.8 % (measured)

2. Vehicle and/or positive control: Vehile: Corn oil (diluent)
Poitive control: None

3. Test organisms:

Species:	Northern bobwhite quail (*Colinus virginianus*)
Age:	18 weeks old
Sex:	Males and females
Weight:	164 – 220 g (at test initiation)
Source:	[redacted]
Loading:	Approx. 0.4 m^2 for 5 specimens
Diet/Food:	Game bird ration, *ad libitum* during acclimation and during the test.
Acclimation period:	16 days
Fasting:	At least 15 hours prior to dosing

4. Environmental conditions:

Temperature:	21 ± 1 °C
Humidity:	41 ± 15 %
Photoperiod:	8 hours light / 16 hours dark (approx.. 130 lux)

5. Experimental dates: October 19th, 1990 to November 2nd, 1990

B. STUDY DESIGN

Experimental treatments

After a fasting period of at least 15 hours, five male and five female quails were assigned separately to each of the treatment groups and the control group, i. e. there were five birds/pen and two pens/dose. The acute oral toxicity test was performed administering AMPA a geometric series of 5 nominal test doses, encompassing 292, 486, 810, 1350 and 2250 mg/kg bw, dissolved in corn oil by oral gavage. In addition, a control group was dosed with the diluent only.

Observations

After dosing, the birds were observed at least twice daily for 14 days for mortality, signs of toxicity, or abnormal behaviour. Body weights were measured individually at initiation of the test and by group on days 3, 7 and 14. Average estimated feed consumption was determined for each dosage group and the control for days 0-3, 4-7 and 8-14.

Statistical calculations: Descriptive statistics

II. RESULTS AND DISCUSSION

A. FINDINGS

No analytical verification was performed.

The LD_{50} and NOEL values are given below based on nominal doses.

Table 8.1.1.1-15: Endpoints

Endpoints	AMPA [mg/kg bw]
LD_{50}	> 2250
NOEL	1350

Table 8.1.1.1-16: Cumulative mortality and clinical signs of toxicity observed in Northern bobwhite quail exposed AMPA

AMPA [mg/kg bw]	Control		292		486		810		1350		2250	
	M	F	M	F	M	F	M	F	M	F	M	F
Mean cumulative mortality on day 14 [%]	0	0	0	0	0	0	0	0	0	0	0	0
Appeared normal [1]	5	5	5	5	5	5	5	5	5	5	0	3
Reduced reaction [1]	-	-	-	-	-	-	-	-	-	-	4	2
Ruffled appearance [1]			--		-	-	-	-	-	-	4	2
Lower limb weakness [1]	-	-	-	-	-	-	-	-	-	-	2	-

[1] Clinical signs of toxicity were only noted on day 0 only
M = male, F = females

Table 8.1.1.1-17: Effects of AMPA on body weight and food consumption of bobwhite quail

AMPA [mg/kg bw]			Control	292	486	810	1350	2250
Average body weight per animal [g]								
Body weight	Day 0	male	188	181	187	195	187	180
		female	181	184	181	173	182	188
	Day 7	male	197	188	194	204	191	187
		female	189	192	190	177	185	93
	Day 14	male	200	192	200	203	195	191
		female	192	197	196	183	190	201
Mean food consumption per animal [g/bird/day]								
Food consumption	Day 0-3	male	23	23	16	25	16	18
		female	18	16	17	16	16	19
	Day 4-7	male	25	26	19	23	23	20
		female	21	21	22	20	18	22
	Day 8-14	male	22	24	21	24	24	20
		female	21	19	24	22	18	19

B. OBSERVATIONS

There were no mortalities at any of the dosages tested. In addition, birds were normal in appearance and behaviour throughout the test period, although one male at 810 mg/kg bw was noted with foot lesions due to pen-wear on day 13 and 14.
At a dosage 2250 mg/kg bw, signs of toxicity were first noted approximately fifty-five minutes after dosing and persisted through the afternoon of day 0. By the morning of day 1, all birds were noted as normal in appearance and behaviour and remained so until study termination.
Signs of toxicity characteristic of intoxication with AMPA included lower limb weakness, a ruffled appearance, and reduced reaction to external stimuli (sound and movement). When compared to the controls, no notable effect on body weight or feed consumption was observed at any of the dosages tested. All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The acute oral LD_{50} for northern bobwhite quail exposed to AMPA as a single oral dosage was > 2250 mg/kg bw. The NOEL was 1350 mg/kg bw.

This study is considered valid and the acute oral LD_{50} for northern bobwhite quail exposed to AMPA as a single oral dosage was > 2250 mg/kg bw and can be used in risk assessment.

Assessment and conclusion by RMS:

CA 8.1.1.2 Short-term dietary toxicity to birds

The assessment of short term dietary toxicity data for birds is not considered to be necessary following the guidance document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12): 1438), in particular if there are no indications that the dietary LD_{50} will be lower than the LD_{50} based on an acute oral study.

During the previous EU evaluations of glyphosate, the assessment of dietary toxicity studies have indicated that the 5-day dietary toxicity studies with glyphosate and AMPA are both higher than the acute endpoints. Thus, the dietary studies are not considered in this assessment.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Studies considering the reproductive toxicity to birds were assessed for their validity to current and relevant guidelines for glyphosate and glyphosate salts are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 8.1.1.3-1: Studies on reproductive toxicity of glyphosate to birds

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.1.1.3/001	[redacted] 1999	Reproduction	*Colinus virginianus*	Glyphosate acid	Valid	Control mortality exceeds 10 %.
CA 8.1.1.3/002	[redacted] 2013	Position paper				Letter regarding control mortality in Frey *et al* study CA 8.1.1.3/001
CA 8.1.1.3/003	[redacted] 1978	Reproduction	*Colinus virginianus*	Glyphosate technical	Valid	-
CA 8.1.1.3/004	[redacted] 1999	Reproduction	*Anas platyrhynchos*	Glyphosate acid	Valid	-
CA 8.1.1.3/005	[redacted] 1978	Reproduction	*Anas platyrhynchos*	Glyphosate technical	Valid	-

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on birds. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01.

Endpoints of studies considered valid are shown in the table below.

Table 8.1.1.3-2: Endpoints: Reproductive toxicity of glyphosate to birds

Reference	Test item	Species	Test design	NOAEL (mg a.e./kg feed)	NOAEL (mg a.e./kg bw/d)
[redacted] 1999 CA 8.1.1.3/001	Glyphosate acid	*Colinus virginianus*	20 weeks reproduction	2250	201.0
[redacted], 1978 CA 8.1.1.3/003	Glyphosate technical	*Colinus virginianus*	17 weeks reproduction	1000	**96.3**
[redacted] 1999	Glyphosate	*Anas*	21 weeks	2250	300

Table 8.1.1.3-2: Endpoints: Reproductive toxicity of glyphosate to birds

Reference	Test item	Species	Test design	NOAEL (mg a.e./kg feed)	NOAEL (mg a.e./kg bw/d)
CA 8.1.1.3/004	acid	*platyrhynchos*	reproduction		
[redacted] 1978 CA 8.1.1.3/005	Glyphosate technical	*Anas platyrhynchos*	17 weeks reproduction	1000	125.3

a.e.: acid equivalents
Endpoint in **bold** is used for risk assessment.

Study summaries are provided below.

1. Information on the study

Data point:	CA 8.1.1.3/001
Report author	[redacted]
Report year	1999
Report title	Glyphosate Acid: A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*).
Report No	123-186
Document No	-
Guidelines followed in study	FIFRA Guideline 71-4 OECD Guideline 206
Deviations from current test guideline	OECD Guideline 206 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a reproductive toxicity study, glyphosate acid was fed for 20 weeks to Bobwhite quail (*Colinus virginianus*). Thirty-two adult quails (1 male and 1 female per pen and 16 pens per test dose and control) per dosage and control received nominal dietary doses of 500, 1000 and 2250 mg glyphosate acid/kg bw. Birds were allowed to lay eggs for approximately 10 weeks. Eggs were collected, incubated and allowed to hatch. During egg deposition period, incubation and post hatching period, eggs and hatchlings were observed for different reproductive parameters, encompassing total egg production, number of eggs cracked, eggshell thickness, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 days-old hatchlings and 14 day survivorship.
Results showed no treatment-related mortalities, overt symptoms of toxicity or treatment effects upon body weight or feed consumption at any of the dietary doses tested. In addition, no treatment-related effects upon any of the reproductive parameters measured at any of the test doses were observed. Some validity criteria according to the OECD guideline 206 were not fulfilled.

Based on the results of this study, the NOEL for Bobwhite quail exposed to glyphosate acid in a reproduction study was determined to be 2250 mg glyphosate acid/kg bw. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White powder
Lot/Batch #:	P24
Purity:	95.6 %

2. Vehicle and/or positive control:

Vehicle: None

Positve control: None

3. Test organisms:

Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, 30 weeks (at test initiation)
Sex	Males and females
Weight	196 to 250 g (at test initiation)
Source:	[redacted]
Loading	Approx. 0.138 m^2 for 2 birds (1 males and 1 female per pen)
Feed/Diet:	Game bird ration, *ad libitum*
Acclimation period:	0 weeks

4. Environmental conditions:

Temperature:	23.1 ± 1.8 °C (adults); 27.3 ± 1.2 °C (hatchling) 38°C (brooding compartment)
Humidity:	66 ± 12 % (adults); 40 ± 17 % (hatchling)
Photoperiod:	17 hours light / 7 hours dark, (approx. 265 lux)

5. Dates of experimental work:

1998-05-29 to 1998-11-23

B. STUDY DESIGN

Experimental treatments

A reproductive toxicity study was performed by feeding adult bobwhite quail *ad libitum* on a series of 3 nominal dietary doses, encompassing 500, 1000 and 2250 mg /kg feed. Sixteen replicates (1 male and 1 female per pen) were used for each treatment group and control. The birds were exposed to the treated diets for approximately 20 weeks and were evaluated for treatment-related effects upon bird health and reproduction. Eggs were collected daily and stored at 13.6 ± 0.6 °C and 82 ± 8 % relative humidity. All eggs laid within a week were considered as one lot and incubated in a Petersime Incubator. On day 21 of incubation, eggs were placed in a Petersime Hatcher and allowed to hatch. The hatchlings were maintained on untreated diet until 14 days of age. Homogeneity of the test substance in treated diets was evaluated by collecting 6 samples of each treatment group on day 0 of week 1. During weeks 2, 3, 4, 8, 12, 16 and 20 of the test, a single sample was collected from the control diet and an additional duplicate sample was collected from treatment group diet, to measure and/ or verify test concentrations.

Observations

Adult birds were observed daily for signs of toxicity and abnormal behaviour throughout the study. Adult body weight was measured at study initiation and termination, in addition to on weeks 2, 4, 6, and 8. For

each pen, food consumption was measured weekly throughout the study except for the last interval, where food consumption was measured over a 6 day period. At the end of each week, all collected eggs were counted and a single egg was randomly selected for eggshell thickness measurements. The remaining eggs were candled to detect egg shell cracks or abnormal eggs before incubation. During the incubation period, eggs were candled again on day 11 or 12 to evaluate embryo viability and on day 21 to determine embryo survival. During the study, total egg production, number of eggs cracked, eggshell thickness, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 day old hatchlings and survivorship of 14 day old hatchlings were determined.

Statistical calculations
An analysis of variance (ANOVA) was used to determine significant differences among the groups followed by Dunnett's multiple comparison procedure as the post-hoc test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.1.1.3-3: Effects of glyphosate acid on reproductive performance of bobwhite quail over 10 weeks.

Glyphosate acid [mg a.s./kg feed]	Control	500	1000	2250
Reproductive performance				
Number of eggs laid per female [mean]	47.7	42.2	39.0	44.0
Eggs laid/maximum laid [%]	70	62	57	65
Eggs cracked/egg laid [%]	5	4	11	5
Viable embryos/egg set [%]	81	91	94	92
Live 3-week embryos/viable embryos [%]	99	98	98	98
Hatchlings/live 3-week embryos [%]	95	95	95	97
14-day-old survivors/hatchlings [%]	93	96	95	96
Hatchlings/egg set [%]	75	85	88	88
14-day-old survivors/egg set [%]	70	82	83	85
Hatchlings/maximum set [%]	50	51	44	54
14-day-old survivors/ maximum set [%]	46	49	43	52
Eggshell thickness				
Mean shell thickness [mm]	0.220	0.228	0.222	0.216
Body weight of hatchling				
Mean body weight [g]	6	6	7	6
Body weight of 14-day old survivors				
Mean body weight [g]	26	28	28	27

Table 8.1.1.3-4: Effects of glyphosate acid on adult bodyweight and feed consumption of adult bobwhite quail.

Glyphosate acid [mg a.s./kg feed]		Control	500	1000	2250
Average body weight [g]					
Test initiation	M	215	223	216	214

Table 8.1.1.3-4: Effects of glyphosate acid on adult bodyweight and feed consumption of adult bobwhite quail.

Glyphosate acid [mg a.s./kg feed]		Control	500	1000	2250
	F	219	219	216	218
Test termination	M	219	229	219	215
	F	250	248	238	239
Body weight change (test start - test end)	M	4	6	3	2
	F	31	29	21	23
Average feed consumption [g/bird/day]					
Week 1	M + F	12	12	12	12
Week 5	M + F	12	12	12	13
Week 10	M + F	19	18	19	20
Week 15	M + F	26	26	26	28
Week 20	M + F	25	26	25	26

M = male, F = female

B. OBSERVATIONS

No treatment-related mortality of parental birds exposed to glyphosate acid was observed. No overt symptoms of toxicity or treatment related effects upon body weight or feed consumption were observed at any dietary dose tested. In addition, no treatment-related effects of reproductive parameters were observed at any dose tested.

Analysis of samples resulted in measured concentrations of 100 %, 99 % and 96 % of the nominal test doses of 500, 1000 and 2250 mg glyphosate acid/kg feed, respectively.

All validity criteria according to OECD 206 were not fulfilled, as the mortality of the control exceed 10 % at the end of the test (actual value: 6 of the 32 birds were found dead). But the average number of 14-day-old survivors per hen in the control was greater than 12. Also, the average egg shell thickness for the control group was greater than 0.19 and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:

The NOEL for bobwhite quail exposed to glyphosate acid in a reproduction study was determined to be 2250 mg glyphosate acid/kg feed (based on nominal doses).

The NOEL for bobwhite quail exposed to glyphosate acid in a reproduction study was determined to be 2250 mg/kg feed (201 mg kg bw/d) and can be used in risk assessment.

Although the control mortality exceeded 10% at the end of the test, the study is still considered valid. A letter (CA 8.1.1.3/002) from Wildlife International Ltd where this study was conducted, provides additional justification regarding the observed mortalities. It is indicated that a 'hysteria attack' occurred and the birds obtained serious injuries due to this and were not treatment related. The control performance from this study were compared with historical control data (from 21 studies) from the laboratory which shows that there was no significant difference.

The cage size used in this bobwhite study has been previously criticised and is also addressed in this letter from the laboratory. The cage size is acceptable based on the fact that the reproductive performance

of the studies are good and that both control and treated birds are housed in the same way without high mortality levels and therefore this is not a potential contributing factor for the control mortality observed in this study.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.3/002
Report author	[redacted]
Report year	2013
Report title	Letter concerning the study; Glyphosate Acid: A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*). Study report 123-186.
Report No	letter regarding 123-186
Document No	-
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	-
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes

Summary of a letter provided by study director at the performing laboratory concerning the study; Glyphosate Acid: A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*). Study report 123-186.

Although the control mortality exceeded 10% at the end of the test, the study is still considered valid. It is indicated that a 'hysteria attack' occurred and the birds obtained serious injuries due to this and were not treatment related. The control performance from this study were compared with historical control data (from 21 studies) from the laboratory which shows that there was no significant difference.

The cage size used in this bobwhite study has been previously criticised and is also addressed in this letter from the laboratory. The cage size is acceptable based on the fact that the reproductive performance of the studies are good and that both control and treated birds are housed in the same way without high mortality levels and therefore this is not a potential contributing factor for the control mortality observed in this study.

1. Information on the study

Data point	CA 8.1.1.3/003
Report author	[redacted]
Report year	1978
Report title	One-Generation Reproduction Study – Bobwhite Quail; Glyphosate Technical.
Report No	139-141
Document No	-
Guidelines followed in study	Non-stated
Deviations from current test guideline	OECD guideline 206 Major: - none Minor: - Parental mortality data was not reported.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a 17 week reproductive toxicity study, technical glyphosate was fed to Bobwhite quail (*Colinus virginianus*). Three adult quails per pen (1 male and 2 female) in 12 replicates treatment received three nominal dietary doses of 50, 200 and 1000 mg technical glyphosate/kg diet.

Birds were fed on the treated diet for 9 weeks prior to egg deposition and were allowed to lay eggs for 8 weeks. Eggs were collected, incubated and allowed to hatch. During the egg deposition period, incubation and post hatching period, eggs and hatchlings were observed for different parameters encompassing total egg production, number of eggs cracked, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 day-old hatchlings, 14 day survivorship, egg weight and eggshell thickness.

Results showed significant reduction in egg weight occurring at the highest test item dose of 1000 mg technical glyphosate/kg diet. However, no further effects on reproduction were observed at this dose level. Therefore, the reduction in egg weight was not considered to be biologically relevant. A high incidence of eggshell cracks was noted during the course of this reproduction study, which can be attributed to the fact that the specimens were inadvertently not debeaked prior to study initiation.

The validity of the present study according to OECD guideline 206 is questionable, since parental mortality data were not reported.

Based on the results of this study, the NOEL for the Bobwhite quail exposed to technical glyphosate in a one-generation reproduction study was determined to be 1000 mg technical glyphosate/kg diet. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White powder with a slight odour
Lot/Batch #:	XHI 162
Purity:	83 % (measured)
2. Vehicle and/or positive control:	Vehicle: Corn oil Positive control: None
3. Test organisms:	
Species:	Bobwhite quail (*Colinus virginianus*)
Age:	5 months old (young adults)
Sex	Males and females
Weight	Not stated
Source:	In-house production flock
Loading	1 males and 2 females per pen
Diet/Diet:	Game bird breeder ration, *ad libitum*
Acclimation period:	Not stated
4. Environmental conditions:	
Temperature:	21.1 – 26.7 °C (research facility) 15.6 °C (eggs storage), 37.4 - 37.6 °C (eggs incubation)
Humidity:	55 % (eggs storage)
Photoperiod:	9 hours light / 15 hours dark (first 6 weeks) 17 hours light / 7 hours dark (following 16 weeks)
5. Dates of experimental work:	1978-03-01 to 1978-08-01

B. STUDY DESIGN

Experimental treatments: A reproductive toxicity study was performed by feeding three adult Bobwhite quails (1 male and 2 females per pen) per replicate *ad libitum* on a series of 3 nominal dietary doses, encompassing 50, 200 and 1000 mg glyphosate acid/kg diet. The diet was prepared by incorporating appropriate concentrations of the test item and corn oil into the aliquots of basal diet. Twelve replicates were exposed per treatment group and control. The birds were exposed for nine weeks to the treated diet prior to egg deposition and for additional eight weeks during egg collection. Eggs were collected daily, stored at 15.6 °C and 55 % relative humidity and were cleaned weekly. The eggs were then incubated at 37.5 ± 0.06 °C. On day 19 of incubation, the eggs were placed in a Humidaire hatcher and allowed to hatch. All hatchlings were housed according to the appropriate parental grouping and maintained on control diet until 14 days of age.

Observations: Body weights were recorded at study initiation, 5 weeks after study initiation prior to onset of egg deposition and at termination of the study. Food consumption was recorded every second week throughout the study. All eggs were candled on day 0 of incubation for eggshell cracks, on day 14 to measure embryo viability, and on day 19 to measure embryo survival. Weekly throughout the egg deposition period, one egg of each pen in each group was randomly selected for egg weight and eggshell thickness measurement. During the study total egg production, number of eggs cracked, egg set, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 days-old hatchlings, 14 day survival, egg weight and eggshell thickness were determined.

Statistical calculations: To evaluate differences between reproductive parameters, Student's t-test was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

The NOEL value is given below based on nominal doses:

Table 8.1.1.3-5: Endpoints

Endpoints	Glyphosate acid [mg a.s./kg feed]
NOEL reproduction	**1000**

Table 8.1.1.3-6: Effects of glyphosate on reproductive parameters of bobwhite quail

Glyphosate acid [mg a.s./kg diet]	Control	50	200	1000
Reproductive success				
Number of eggs laid per hen in 8 weeks (mean)	31.9	28.0	28.0	32.5
Number of eggs cracked [%]	9.7	7.6	9.2	6.3
Viable embryos of egg set	91.3	80.7	91.7	87.0
Live 3-week embryos of viable embryos [%]	97.3	97.2	97.5	96.5
Hatchlings of live 3-week embryos [%]	81.5	70.3	73.4	74.4
14-day-old survivors of normal hatchlings [%]	95.5	93.1	95.7	93.5
14- day-old survivors per hen [1]	18.7	12.3	14.8	16.7
Egg weight				
Mean egg weight [g]	10.3	9.9	10.2	9.4 [2]
Eggshell thickness				
Mean eggshell thickness [mm]	0.214	0.204	0.211	0.224
Body weight of representative hatchling				
Mean body weight [g]	6.8	6.9	6.9	6.7
Body weight of representative 14-day old survivors				
Mean body weight [g]	22.0	22.2	22.6	22.0

[1] based on 24 hens
[2] Statistically significant compared to control (Student's t-test)

B. OBSERVATIONS

There were no statistically significant impacts on any reproductive parameters with one exception. A statistically significant reduction in egg weight occurred at the highest test dose of 1000 mg glyphosate acid/kg diet. Although there was a small reduction in egg weight at 1000 mg/kg feed there was not a significant impact on the biologically relevant endpoints that included initial hatchling body weight, 14 day hatchling body weight, egg shell thickness and hatchling survival. Egg weight is not a standard endpoint in guideline avian reproduction studies, it is not included in OECD 206, and was a carryover from poultry performance studies. A high incidence of eggshell cracks was noted during the course of the study. This can be attributed to the fact that the bobwhite quail utilized for this study were inadvertently not debeaked prior to study initiation. In fact, caged quail have a natural propensity to peck at their eggs causing cracks.

All current validity criteria were fulfilled, as the mortality of the control did not exceed 10 % at the end of the test and the average number of 14-day-old survivors per hen in the control was ≥ 14. Also, the average egg shell thickness for the control group was ≥ 0.34 mm and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Based on the overall results of this study, the NOEL for bobwhite quail exposed to glyphosate acid in a one-generation reproduction study was determined to be 1000 mg glyphosate acid/kg diet.

This study is considered valid and the NOEL for bobwhite quail exposed to glyphosate acid in a one-generation reproduction study was determined to be 1000 mg/kg diet (96.3 mg/kg bw/d) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.3/004
Report author	[redacted]
Report year	1999
Report title	Glyphosate Acid: A Reproduction Study with the Mallard (*Anas platyrhynchos*)
Report No	123-187
Document No	-
Guidelines followed in study	FIFRA Guideline 71-4 OECD Guideline 206
Deviations from current test guideline	OECD Guideline 206 - none
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a reproductive toxicity study, glyphosate acid was fed to Mallard duck (*Anas platyrhynchos*) for a total duration of 21 weeks. Thirty-two adult ducks (1 male and 1 female per pen and 16 pens per test dose and control) per dosage and control received nominal dietary doses of 500, 1000 and 2250 mg glyphosate acid/kg bw.

Birds fed on the treated diet were allowed to lay eggs for approximately 11 weeks. Eggs were collected, washed and incubated and allowed to hatch. During egg deposition period, incubation and post hatching period, eggs and hatchlings were observed for different reproductive parameters, encompassing the total egg production, number of eggs cracked, eggshell thickness, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 day old hatchlings and survivorship of 14 day-old hatchlings.

Results showed no treatment-related mortalities, overt symptoms of toxicity or treatment effects upon body weight or feed consumption at any of the dietary doses tested. In addition, no treatment-related effects upon any of the reproductive parameters measured at any of the test doses were observed. All validity criteria according to the OECD guideline 206 were fulfilled.

Based on the results of this study, the NOEL for Mallard duck exposed to glyphosate acid in a reproduction study was determined to be 2250 mg a.s./kg bw. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White powder
Lot/Batch #:	P24
Purity:	95.6 %

2. Vehicle and/or positive control: Vehicle: None
Positive control: None

3. Test organisms:

Species:	Mallard duck (*Anas platyrhynchos*)
Age:	21 weeks (at test initiation)
Sex:	Males and females
Weight:	868 to 1259 g (at test initiation)
Source:	[redacted]
Loading:	Approx. 0.675 m^2 for 2 birds (1 males and 1 female per pen)
Feed/Diet:	Game bird ration, ad libitum
Acclimation period:	6 weeks

4. Environmental conditions:

Temperature:	22.4 ± 0.9 °C (adults); 29 °C (hatchling); 38°C (brooding compartment)
Humidity:	69 ± 13 % (adults); 61 ± 15 % (hatchling)
Photoperiod:	17 hours light / 7 hours dark, (approx. 292 Lux)

5. Dates of experimental work: 1998-05-29 to 1998-12-03

B. STUDY DESIGN

Experimental treatments

A reproductive toxicity study was performed by feeding young adult mallard ducks *ad libitum* on a series

of 3 nominal dietary doses encompassing 500, 1000, and 2250 mg a.s./kg feed. Sixteen replicates (1 male and 1 female per pen, 16 pen per treatment group) were used for each treatment group and control. The birds were exposed to the treated diets for approximately 21 weeks, and were evaluated for treatment-related effects on bird health and reproduction. Eggs were collected daily, washed and stored in a cold room at 13.6 ± 0.6 °C and 82 ± 8 % relative humidity. All eggs laid within a week were considered as one lot and were incubated in a Petersime incubator. On day 24 of incubation, eggs were placed in a Petersime hatcher and were allowed to hatch. The hatchlings were maintained on untreated diet until 14 days of age. Homogeneity of the test substance in treated diet was evaluated by collecting 6 samples from each treatment group on day 0 of week 1. During weeks 2, 3, 4, 8, 12, 16 and 20 of the test, a single sample was collected from the control diet and an additional duplicate sample was collected from treatment group diet, to measure and/ or verify test concentrations.

Observations

Parental birds were observed daily throughout the study for signs of toxicity and abnormal behaviour. Adult body weights were measured at study initiation and termination in addition to on weeks 2, 4, 6, and 8 of the adult in-life period. For each pen, feed consumption was measured weekly. At the end of each week, all eggs collected were counted and selected by indiscriminate draw for eggshell thickness measurement. The remaining eggs were candled to detect egg shell cracks or abnormal eggs before incubation. During the incubation period, eggs were candled again on day 14 to investigate embryo viability and on day 21 to determine embryo survival.

During the study, total egg production, number of eggs cracked, eggshell thickness, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 day old hatchlings and survivorship of hatchlings after 14 days were determined.

Statistical calculations

An analysis of variance (ANOVA) was used to determine significant differences among the groups and Dunnett's multiple comparison procedure was used as post-hoc test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Verification of Glyphosate Acid concentrations in avian diets were performed. Glyphosate acid concentrations in control were < 20 ppm throughout the study.

Table 8.1.1.3-7: Concentrations of glyphosate acid in diets

Nominal concentration of glyphosate acid [mg a.s./kg feed]	500		1000		2250	
Day 0 (% of nominal)	520 (104)		1010 (101)		2250 (100)	
Day 2 (% of nominal)	481 (96)	476 (95)	927 (93)	945 (95)	1990 (88)	2210 (98)
Day 3 (% of nominal)	465 (93)	455 (91)	947 (95)	973 (97)	2040 (91)	2130 (95)
Day 4 (% of nominal)	465 (93)	473 (95)	935 (94)	957 (96)	1990 (88)	2220 (99)
Day 8 (% of nominal)	478 (96)	469 (94)	938 (94)	848 (85)	2010 (89)	2040 (91)
Day 12 (% of nominal)	523 (105)	568 (114)	1030 (103)	1040 (104)	2220 (98)	2230 (99)
Day 16 (% of nominal)	586 (117)	544 (109)	1090 (109)	1190 (119)	2510 (112)	2220 (99)
Day 20 (% of nominal)	523 (105)	512 (102)	1000 (100)	999 (100)	2190 (97)	2200 (98)

Table 8.1.1.3-8: Effects of glyphosate acid on reproductive performance of mallard duck[1]

Glyphosate acid [mg a.s./kg feed]	Control	500	1000	2250
Reproductive performance				
Number of eggs laid per female [mean]	43.6	40.1	40.2	44.3
Eggs laid/maximum laid [%]	61	56	56	62
Eggs cracked/eggs laid [%]	2	1	1	2
Viable embryo/egg set [%]	73	68	93	81
Live 3-week embryos/viable embryos [%]	98	99	99	99
Hatchlings/live 3-week embryos [%]	91	89	84	88
14-day-old survivors/hatchlings [%]	100	91	98	99
Hatchlings/egg set [%]	66	60	78	72
14-day-old survivors/egg set [%]	65	58	76	71
Hatchlings/maximum set [%]	34	31	43	42
14-day-old survivors/ maximum set [%]	34	30	42	42
Eggshell thickness				
Number of eggs measured	58	59	61	65
Mean shell thickness [mm]	0.388	0.374	0.373	0.376
Body weight of hatchling				
Number of juvenile ducks weighted	329	302	414	440
Mean body weight [g]	36	34	35	34

[1] values represent pen means for experimental groups

Table 8.1.1.3-9: Effects of glyphosate acid on adult bodyweight and feed consumption of adult mallard duck.

Glyphosate acid [mg a.s./kg feed]		Control	500	1000	2250
Average body weight [g]					
Test initiation	male	1091	1103	1106	1107
	female	1024	1021	1019	999
14-day	male	1075	1079	1097	1078
	female	1002	1011	998	983
Test termination	male	1161	1105	1134	1088
	female	1114	1104	1112	1080
Body weight change (test start - test end)	male	68	0	27	-19
	female	99	76	90	81
Average feed consumption [g/bird/day]					
Week 1		89	102	86	93
Week 5		95	93	92	101
Week 10		137	125	117	127
Week 15		193	193	168	198
Week 21		169	167	170	173

B. OBSERVATIONS

Analytical results: Analytical recovery of the test item ranged from 85 to 119 % throughout the study. Therefore, calculated endpoints will be based on nominal concentrations.

All validity criteria according to OECD 206 were fulfilled, as the mortality of the control group did not exceed 10 % at the end of the test and the average number of 14-day-old survivors per hen in the control was greater than 14. Also, the average egg shell thickness for the control group was greater than 0.34 and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

There were no treatment related mortalities at any of the concentrations. However, three incidental adult mortalities occurred during the course of the study. One incidental mortality occurred in the control group and in both the 500 and 1000 mg a.s./kg feed treatment groups. Except for incidental clinical findings, all birds appeared normal throughout the study. Clinical signs as lameness and wing droop were observed and frequently were associated with the incidental injuries.

There were no treatment related effects upon reproductive performance at any of the concentrations tested. However, offspring in the 2250 ppm treatment group did show a slight, but statistically significant ($p<0.05$) reduction in the mean body weight of 14-day old survivors when compared to the control. The mean body weight value for 14 day old survivors in the control group was 262 ± 32 g while mean values for the 500, 1000 and 2250 mg a.s./kg feed treatment groups were 236 ± 35 g, 260 g ± 16 g, 235 g ± 23 g, respectively. As especially the parameters concerning hatchling weight were affected at 2250 mg a.s./kg feed, it cannot be excluded that the observed changes in hatchling weight do not represent a population relevant adverse effect. Therefore, this endpoint will be considered as a NOAEL of 1000 mg a.s./kg feed, corresponding to 116 mg a.s./kg/bw/d.

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The NOEL for mallard duck exposed to glyphosate acid in a reproduction study was determined to be 2250 mg a.s./kg feed (based on nominal doses).

This study is considered valid and the NOEL for mallard duck exposed to glyphosate acid in a reproduction study was determined to be 2250 mg a.s./kg feed (300 mg a.s./kg bw/day), and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.1.1.3/005
Report author	[redacted]
Report year	1978
Report title	One-Generation Reproduction Study - Mallard Duck; Glyphosate technical.
Report No	139-143
Document No	
Guidelines followed in study	Non-stated
Deviations from current test guideline	OECD guideline 206 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
In a reproductive toxicity study, technical glyphosate was fed to Mallard ducks (*Anas platyrhynchos*) for 17 weeks. Five replicates per dose, containing seven adult ducks (2 males and 5 females per pen) each were treated with nominal dietary doses of 50, 200 and 1000 mg glyphosate technical/kg diet for nine weeks. Reproductive parameters were measured for a further eight weeks beginning at the onset of egg laying. Eggs were collected, incubated and allowed to hatch. During the egg deposition period, incubation and post hatching period, eggs and hatchlings were observed for different reproductive parameters, encompassing the total egg production, the number of egg cracked, embryos viability, embryos survival, number of hatchlings, body weight of representative new hatchlings, body weight of representative 14 days-old hatchlings, 14 day-old survivorship, egg weight and the eggshell thickness.

No symptoms of toxicity or behavioural abnormalities at any of the dietary doses tested and in control were observed for the entire test duration for the parental birds exposed to glyphosate. In addition, no mortality was observed in control and treatments groups, except at the highest test item concentration, where a single mortality was observed on week 12 after study initiation. This death was however considered incidental and not compound related. The evaluation of reproductive data and statistical analysis of the above mentioned reproductive parameters demonstrated that glyphosate caused no reproductive impairment at the dose levels tested. All validity criteria according to the OECD guideline 206 were fulfilled.

Based on the results of this study, the NOEL for the Mallard duck exposed to glyphosate technical in a one-generation reproduction study was determined to be 1000 mg a.s./kg diet. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder with a slight odour
Lot/Batch #:	XHI 162 (Assay of batch as of 6-16-78)
Purity:	83 % a.s.

2. Vehicle and/or positive control:	Vehicle: Corn oil Positive control: None
3. Test organisms:	
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	6 months old (adults, at test initiation)
Sex	Males and females
Weight	1047 - 1257 g (at test initiation)
Source:	In-house production flock
Loading	Approx. 8.2 m^2 for 7 specimens (2 males and 5 females per pen)
Diet/Diet:	Game bird breeder ration, *ad libitum*
4. Environmental conditions:	
Temperature:	37.4 – 37.6 °C (eggs incubation)
Humidity:	55 % (eggs storage)
Photoperiod:	outdoor (natural daylight/photoperiod)
5. Dates of experimental work:	1978-03-01 to 1978-08-01

B. STUDY DESIGN

Experimental treatments

A reproductive toxicity study was performed by feeding adult mallard ducks (2 males and 5 females per replicate) *ad libitum*, on a series of 3 nominal dietary doses of glyphosate technical encompassing 50, 200 and 1000 mg a.s./kg diet. The diet was prepared by incorporating appropriate concentrations of the test item and corn oil into the aliquots of basal diet. Five replicates were used for each treatment group and the control. The birds were exposed to the treated diet for 9 weeks prior to egg deposition and for additional 8 weeks during egg collection. Eggs were collected daily and stored at 15.6 °C and 55 % relative humidity and were cleaned weekly. The clean eggs were then incubated at 37.5 ± 0.06 °C. On day22 or 23 of incubation, the eggs were allowed to hatch. The hatchlings were housed according to the appropriate parental grouping and maintained on control diet until 14 days of age.

Observations

Body weights were recorded at study initiation, 5 weeks after study initiation, prior to the onset of egg deposition, and at termination of the study. Food consumption was recorded bi-weekly throughout the study. All eggs were candled on day 0 of incubation for eggshell cracks, on day 14 to measure embryo viability and to remove any *E coli*-contaminated eggs, and on day 21 to measure embryo survival. Weekly throughout egg deposition period, one egg from each pen in each experimental group and the controls was randomly selected for egg weight and eggshell thickness measurement. During the study, the total egg production, the number of eggs cracked, embryos viability, embryos survival, number of hatchlings, body weight of representative new hatchling, body weight of representative 14 days-old hatchlings, 14 day-old survivorship, egg weight and the eggshell thickness were determined.

Statistical calculations

To evaluate the differences between each of the above-mentioned reproductive parameters, Student's t-test was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

The NOEL value is given below based on nominal doses:

Table 8.1.1.3-10: Endpoints

Endpoints	Glyphosate technical [mg a.s./kg feed]
NOEL reproduction	1000

Table 8.1.1.3-11: Effects of glyphosate technical on reproductive parameters of Mallard duck

Glyphosate technical [mg a.s./kg diet]	Control	50	200	1000
Reproductive success				
Number of eggs laid per hen in 8 weeks	28	23	28	29
Number of eggs cracked [%]	3	5	5	6
Viable embryos of egg set	90	93	85	86
Live 3-week embryos of viable embryos [%]	96	93	95	95
Hatchlings of live 3-week embryos [%]	74	77	77	81
14-day-old survivors of normal hatchlings [%]	97	99	98	96
14- day-old survivors per hen [1]	16	14	15	16
Egg weight				
Number of eggs analysed	38	38	38	39
Mean egg weight[g]	57.5	58.3	56.3	58.9
Eggshell thickness				
Number of eggs analysed	38	38	38	39
Mean shell thickness [mm]	0.394	0.375	0.372	0.375
Body weight of representative hatchling				
Number of ducklings analysed	72	73	72	73
Mean body weight[g]	33	33	32	34
Body weight of representative 14-day old survivors				
Number of ducklings analysed	72	72	72	73
Mean body weight[g]	217	206	208	205

[1] based on 25 hens

B. OBSERVATIONS

For the parental birds exposed to glyphosate, there no symptoms of toxicity or behavioural abnormalities were recorded at any of the dietary doses tested or the control treatments for the entire test duration. In addition, no mortality was observed in control and treatments groups, except for the highest test dose, at which a single mortality was observed on week 12 after study initiation. This death was however considered incidental, and not compound related. The evaluation of the reproductive data and statistical analysis of above-mentioned reproductive parameters demonstrate that glyphosate caused no reproductive impairment at the dose levels tested. All validity criteria according to current guidelines were fulfilled, as the mortality of the control did not exceed 10 % at the end of the test and the average number of 14-day-old survivors per hen in the control was ≥ 14. Also, the average egg shell thickness for the control group was ≥ 0.34 mm and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Based on the results of this study, the NOEL for Mallard duck exposed to glyphosate technical in a one-generation reproduction study was determined to be 1000 mg a.s./kg diet.

This study is considered valid and the NOEL for Mallard duck exposed to glyphosate technical in a one-generation reproduction study was determined to be 1000 mg a.e./kg diet (125.3 mg a.e/kg bw/day) and can be used in risk assessment.

Assessment and conclusion by RMS:

CA 8.1.2 Effects on terrestrial vertebrates other than birds

An extensive regulatory toxicology database has been summarised to evaluate acute and long-term toxicity of glyphosate and relevant metabolites to mammals.

CA 8.1.2.1 Acute oral toxicity to mammals

Studies considering the acute toxicity to mammals were assessed for their validity to current and relevant guidelines for glyphosate and the metabolite AMPA and are summarised in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in document M-CA Section 5. A detailed evaluation is provided in Annex M-CA 8-02 to this document which outlines the selection of endpoints and the discussion surrounding those relevant to the environmental assessment.

Endpoints of studies considered valid are shown in the table below.

Table 8.1.2.1-1: Endpoints: Acute oral toxicity of glyphosate to mammals

Reference	Test item	Species	Test design/ GLP	LD_{50} (mg a.e./kg bw)
Lowest endpoint CA 5.2.1/001 to CA 5.2.1/039.	Glyphosate	Rat	Acute oral	>2000
21 relevant studies. CA 5.2.1/001 to CA 5.2.1/039.	Glyphosate	Rat	Acute oral	Geometric mean: 3578.9
Six relevant studies. CA 5.2.1/001 to CA 5.2.1/039	Glyphosate	Mice	Acute oral	Geometric mean: 3809.4
Proposed endpoint for risk assessment				
Extrapolated	Glyphosate acid	Rat/Mice	Acute, overall geometric mean	3694.1[1]

a.e.: acid equivalents
[1] Discussed in Annex M-CA 8-02 on this document.

A study considering the acute toxicity of the metabolite AMPA to mammals is available and reported in the following table. This study was assessed to be valid according to current and relevant guidelines and the corresponding study summary is presented in document M-CA Section 5. This acute study with the metabolite AMPA shows equally low acute toxicity as the parent, glyphosate.

Table 8.1.2.1-2: Endpoints: Acute oral toxicity of AMPA to mammals

Reference	Test item	Species	Test design	LD_{50} (mg/kg bw)
CA Section 5	AMPA	Mouse	Acute toxicity	> 5000

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on mammals. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

Studies considering long-term developmental and reproductive toxicity of glyphosate and AMPA to mammals, assessed for validity according to current and relevant test guidelines, are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries including validity assessments, for all studies are presented in document M-CA Section 5.

A detailed evaluation is provided in Annex M-CA 8-02 to this document which outlines the selection of endpoints and the discussion surrounding those relevant to the environmental assessment.

Endpoints of studies considered valid are shown in the table below.

Table 8.1.2.2-1: Endpoints: Reproductive toxicity of glyphosate to mammals

Reference	Test item	Species	Test design	NOAEL (mg a.e./kg bw/d)
CA Section 5	Glyphosate acid	Rabbit	Developmental toxicity (long-term)	Screening Step / Tier 1: 50
CA Section 5	Glyphosate acid	Rabbit	Developmental toxicity (long-term)	Tier 2: 100
CA Section 5	Glyphosate acid	Rat	Developmental toxicity (long-term)	Tier 3: 300
CA Section 5	AMPA	Rat	13 week oral	> 1000

a.e.: acid equivalents
Endpoint in **bold** is used for risk assessment.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

According to the data requirements for active substances (EU) No 283/2013, if a substance has a octanol water partition coefficient (Log Pow) that is greater than 3 and is stable (>90 % remaining after 24 hours via hydrolysis) then the bioconcentration of the substance shall be assessed.

In the bird and mammal guidance document (EFSA/2009/1438), it states for organic substances with a log Pow ≥3, indicates a potential for bioaccumulation. Where this is the case then the potential for dietary exposure of birds and mammals to these substances should be assessed considering bioaccumulation and food chain behavior. The EFSA /2009/1438 describes three issues that should be considered. These are a) Food chain from earthworm to earthworm – eating birds and mammals; b) Food chain from fish to fish – eating birds and mammals; and c) Biomagnification in terrestrial food chains.

Glyphosate acid is stable in water and does not rapidly hydrolyse. Glyphosate has a very low log P_{OW} value of <-3.2. Similarly, the main metabolite AMPA is also stable in water and also has a very low log P_{OW} value of -2.47. Therefore, as the log P_{OW} values for both glyphosate and AMPA are substantially lower than EFSA/2009/1438 trigger value (Log Pow ≥3) the potential for bioaccumulation is considered to be low to negligible. Further consideration of the bioaccumulation potential and food chain behaviour of glyphosate and AMPA is not therefore considered necessary.

This conclusion is supported by the results of a fish bioconcentration study, conducted with bluegill sunfish that achieved a bioconcentration factor (BCF) of 1.1 ± 0.61, which is far below the Annex VI BCF trigger value of 1000. Therefore, a study is not necessary to determine bioaccumulation in aquatic non-target organisms.

In accordance with the bioaccumulation criteria as stated in the Regulation (EC) No 1107/2009, glyphosate does not fulfill the criteria as the BCF is substantially lower than the criterion trigger BCF of 1000.

Bioconcentration factor (BCF)	BCF = 1.1 ± 0.61; steady state after 120 ± 59 d log P_{ow} of glyphosate acid and its metabolites was < 3, accumulation potential in aquatic non-target organisms is hence considered to be low
Annex VI Trigger for the bioconcentration factor	1000
Clearance time CT_{50} CT_{90}	Not relevant
Level of residues (%) in organisms after the 14-day depuration phase	Not relevant

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

A consideration of the potential effects of glyphosate and glyphosate products on amphibians was part of the previous Annex I renewal of glyphosate in the EU (Glyphosate RAR 11 Vol. 3 CA-CP_B9, 2015). This is discussed in document M-CP Section 10.1.3. An amphibian publication was identified as relevant and reliable and is presented in CA 8.2.8.

Furthermore, a report has been prepared to address the impact on biodiversity, namely 'Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment[11]'. The purpose of this report is two-fold: (1) provide a biodiversity assessment that principally informs on indirect effects through trophic interactions and (2) to inform risk assessors and managers on risk mitigation options that are protective of aquatic and terrestrial biodiversity. The outcome of the present biodiversity assessment for glyphosate is summarized for the different environmental compartments and taxa where appropriate in the document M-CP Section 10.

CA 8.1.5 Endocrine disrupting properties

According to the endocrine disrupting (ED) criteria laid down in Regulation (EU) 2018/605, endocrine mediated adversity as well as activity and the biological link between those two must be apparent to identify a substance as an endocrine disruptor. A detailed evaluation of endocrine disrupting properties has been made according to EFSA Journal 2018;16(6):5311 incorporating relevant regulatory studies and reliable literature articles. The results are summarised below, see report CA 5.8.3/010 for full details.

Concerning the ED assessment of mammals, potential effects of glyphosate on the HPT and HPG axis were addressed in several repeated dose toxicity studies of subacute to chronic exposure also considering different life stages (level 4 and 5 studies of the OECD conceptual framework). In addition, *in vitro* and *in silico* information are available and considered for the ED assessment of glyphosate. With regard to EATS-mediated adversity, a review of the available mammalian guideline studies in four species (dog, mouse, rabbit, rat), conducted with glyphosate over different exposure periods and considering different life stages (in rat), did not show carcinogenicity or any other EATS-mediated adverse effects based on a sufficient dataset as required in the ECHA/EFSA ED Guidance. Potential EATS-related activity was investigated in the male and female pubertal assay, where hormone measurements were performed, as well as the Uterotrophic and Hershberger Assay providing *in vivo* mechanistic data. Neither the described *in vivo* assays nor *in vitro and in silico* information provide any indication on EATS-related endocrine activity of glyphosate.

Hence, the ED criteria for glyphosate with regards to human health and mammals are not met, since neither EATS-mediated adversity nor endocrine activity has been observed.

In conclusion, glyphosate does not induce EATS-mediated adversity and no EATS-related endocrine activity was observed *in silico, in vitro,* and *in vivo* for humans and mammals as well as for non-target organisms. This conclusion is in concordance with the current Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate (EFSA Journal 2017; 15(9): 4979) as well as with the conclusion of EPA on the Endocrine Screening Program (EDSP) Tier l (US EPA, 2015).

Since glyphosate has not been shown to induce EATS-mediated adversity or endocrine activity, it is concluded that the ED criteria with regard to EATS-modalities in humans and mammals as well as non-target organisms are not met for glyphosate.

CA 8.2 Effects on Aquatic Organisms

Studies on the effects of the active substance glyphosate and its relevant metabolites on aquatic organisms to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

An extensive regulatory fish toxicology database has been summarised to evaluate acute and long-term toxicity of glyphosate, glyphosate salts and the metabolites AMPA and HMPA. The results of these studies demonstrate that glyphosate, glyphosate salts, AMPA and HMPA are of low acute and long-term toxicity to fish.

[11] [redacted] (2020) Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment (TRR0000305).

CA 8.2.1 Acute toxicity to fish

Studies considering the effects of glyphosate on fish were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 8.2.1-1: Studies on acute toxicity of glyphosate and metabolites to fish

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.1/001	[redacted] 2003	Acute / static	*Oncorhynchus mykiss*	Glyphosate K-salt	valid	-
CA 8.2.1/002	[redacted] 1995	Acute / static	*Oncorhynchus mykiss*	Glyphosate acid	valid	-
CA 8.2.1/003	[redacted], 1995	Acute / static	*Oncorhynchus mykiss*	Glyphosate technical	valid	-
CA 8.2.1/004	[redacted], 1993	Acute/ static	*Oncorhynchus mykiss*	Glyphosate IPA-salt	valid	-
CA 8.2.1/005	[redacted], 1990	Acute / static	*Oncorhynchus mykiss*	Glyphosate technical	valid	-
CA 8.2.1/006	[redacted] 1981	Acute / static	*Salmo gairdneri (Oncorhynchus mykiss)*	Glyphosate IPA-salt	supportive	No analytical test verifications, exposure cannot be confirmed
CA 8.2.1/007	[redacted] 1978	Acute / static	*Salmo gairdneri (Oncorhynchus mykiss)*	Glyphosate technical	supportive	No analytical test verifications, exposure cannot be confirmed
CA 8.2.1/008	[redacted] 1972	Acute / static	*Oncorhynchus mykiss* *Lepomis macrochirus*	Glyphosate acid (CP 65573)	invalid	Glyphosate acid is mentioned in the RAR. No information in the report.
CA 8.2.1/009	[redacted] 1995	Acute / static	*Lepomis macrochirus*	Glyphosate acid	valid	-
CA 8.2.1/010	[redacted] 1991	Acute / static	*Lepomis macrochirus*	Glyphosate technical	valid	-
CA 8.2.1/011	[redacted] 1981	Acute / static	*Lepomis macrochirus*	Glyphosate IPA-salt	invalid	No analytical test verifications, exposure cannot be confirmed and some validity criteria not met
CA 8.2.1/012	[redacted] 1978	Acute / static	*Lepomis macrochirus*	Glyphosate acid	supportive	No analytical test verifications, exposure cannot be confirmed

Table 8.2.1-1: Studies on acute toxicity of glyphosate and metabolites to fish

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.1/013	[redacted] 2006	Acute / semi-static	*Cyprinus carpio*	Glyphosate technical	valid	-
CA 8.2.1/014	[redacted] 1973	Acute / static	*Cyprinus carpio*	Glyphosate acid	valid	Error in the RAR on the Authors name
CA 8.2.1/015	[redacted], 2000	Acute / semi-static	*Brachydanio rerio* *(Danio rerio)*	Glyphosate technical	supportive	Insufficient analytical test verifications, exposure cannot be confirmed
CA 8.2.1/016	[redacted] 1993	Acute / static	*Leuciscus idus*	Glyphosate, IPA-salt	valid	-
CA 8.2.1/017	[redacted] 1998	Acute / static	*Oncorhynchus mykiss*	AMPA	valid	-
CA 8.2.1/018	Anonymous, 1994	Acute / static	*Oncorhynchus mykiss*	AMPA	invalid	The notifier has no access to this study report.
CA 8.2.1/019	[redacted] 1991	Acute / static	*Oncorhynchus mykiss*	AMPA	valid	32 mg/L based on report and RAR. This is based on irrelevant 3 h time point.
CA 8.2.1/020	[redacted] 1991	Acute / static	*Oncorhynchus mykiss*	AMPA	valid	-

Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the acute impact of glyphosate on fish are summarised in the table below. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to fish, please refer to document M-CP Section 10.2.

Table 8.2.1-2 Literature on acute toxicity of glyphosate and metabolites to fish

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.1/021	Antunes *et al*., 2017. Gender-specific histopathological response in guppies *Poecilia reticulata* exposed to glyphosate or its metabolite aminomethylphosphonic acid	Acute, fish	Glyphosate and AMPA	Reliable with restrictions.	The acute 96 hour-LC_{50} values for male and female guppies *P. reticulata* after exposure to glyphosate were 68.78 mg/L and 70.87 mg/L, respectively. The acute 96 hour-LC_{50} values for AMPA for male and female guppies were 180 mg/L and 164.3 mg/L, respectively.
CA 8.2.1/022 CA 8.2.1/023	Gholami *et al*., 2013. Toxicity evaluation of Malathion, Carbaryle and Glyphosate in common carp fingerlings (*Cyprinus carpio*, Linnaeus, 1758).	Acute, fish	glyphosate	Reliable with restrictions.	The acute 96 hours- LC_{50} for common carp fingerlings was determined to be 6.75 mg/L by static exposure to glyphosate at 5 test concentrations between 5.5 and 9.5 mg/L.

Endpoints of studies considered valid for glyphosate are shown in the table below. Studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate technical are automatically expressed as acid equivalent.

Table 8.2.1-3: Endpoints: Acute toxicity of glyphosate to fish

Reference	Test item	Species	Test design/ GLP	LC_{50} (mg a.e./L)	NOEC (mg a.e./L)
[redacted] 2003 CA 8.2.1/001	Glyphosate K-salt	*Oncorhynchus mykiss*	Acute, 96 h, static	> 1193 (nom)	149
[redacted] 1995 CA 8.2.1/002	Glyphosate acid	*Oncorhynchus mykiss*	Acute, 96 h, static	130 (nom)	32
[redacted] 1995 CA 8.2.1/003	Glyphosate technical	*Oncorhynchus mykiss*	Acute, 96 h, static	> 100 (nom)	≥ 100
[redacted] 1993 CA 8.2.1/004	Glyphosate IPA-salt	*Oncorhynchus mykiss*	Acute, 96 h, static	1001 (nom)	236
[redacted], 1990 CA 8.2.1/005	Glyphosate technical	*Oncorhynchus mykiss*	Acute, 96 h, static	87.7 - 135 (gm)	87.7
[redacted] 1995 CA 8.2.1/009	Glyphosate acid	*Lepomis macrochirus*	Acute, 96 h, static	**47 (nom)**	32
[redacted] 1991 CA 8.2.1/010	Glyphosate technical	*Lepomis macrochirus*	Acute, 96 h, static	119 - 173 (gm)	119

Table 8.2.1-3: Endpoints: Acute toxicity of glyphosate to fish

Reference	Test item	Species	Test design/ GLP	LC_{50} (mg a.e./L)	NOEC (mg a.e./L)
[redacted], 2006 CA 8.2.1/013	Glyphosate technical	*Cyprinus carpio*	Acute, 96 h, semi-static	> 100 (nom)	≥ 100
[redacted] 1973 CA 8.2.1/014	Glyphosate acid	*Cyprinus carpio*	Acute, 96 h, static	115	
[redacted], 1993 CA 8.2.1/016	Glyphosate IPA-salt	*Leuciscus idus*	Acute, 96 h, static	> 2282 (nom)	≥ 3080

a.e.: acid equivalents
nom: nominal, gm : geometric mean measured
Endpoint in **bold** is used for risk assessment

Endpoints of studies considered valid for AMPA are shown in the table below.

Table 8.2.1-4: Endpoints: Acute toxicity of AMPA to fish

Reference (Data owner)	Test item	Species	Test design/ GLP	LC_{50} (mg/L)	NOEC (mg/L)
[redacted], 1998 CA 8.2.1/017	AMPA	*Oncorhynchus mykiss*	Acute, 96 h, static	> 100 (nom)	≥ 100
[redacted] 1991 CA 8.2.1/019	AMPA	*Oncorhynchus mykiss*	Acute, 96 h, static	**520 (nom)**	100
[redacted] 1991 CA 8.2.1/020	AMPA	*Oncorhynchus mykiss*	Acute, 96 h, static	> 180 (nom)	18

nom: nominal
Endpoint in **bold** is used for risk assessment

Study summaries are provided below.

1. Information on the study

Data point	CA 8.2.1/001
Report author	[redacted]
Report year	2003
Report title	MON 78623: A 96-hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)
Report No	139A-310C
Document No	-
Guidelines followed in study	OECD Guideline 203 OPPTS 850.1075
Deviations from current test guideline	Deviation compared with OECD 203: Major: - none Minor: The temperature was lower than recommended (12.2 – 12.7 °C instead of the recommended 13 – 17 °C), since it has been found to be an acceptable temperature to maintain healthy rainbow trout.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The toxicity of glyphosate potassium (K) salt on rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour static (without media renewal) toxicity test conducted at nominal test concentrations of 156, 313, 625, 1250 and 2500 glyphosate K-salt/L, corresponding to 74.4, 149, 298, 596 and 1193 mg glyphosate acid/L (mg a.e./L). A negative control group (dilution water only) was also prepared. Duplicate vessels were prepared for the control and each test item level, with 10 fish added to each vessel.

Observations for sub-lethal effects and mortality were performed at 4, 24, 48, 72 and 96 hours after the start of the test (fish addition). The pH-value and oxygen saturation of the test solutions were measured at test initiation and at daily intervals. Temperature was measured at test initiation and termination. Samples of test media were taken at the start (before fish addition), and after 48 and 96 hours for the analysis of glyphosate K salt using an HPLC method of analysis. Overall mean measured glyphosate K-salt concentrations were 159, 329, 646, 1302 and 2573 mg a.s./L. Glyphosate K-salt was not detected in the control group. Measured concentrations ranged from 99.8 to 109 % of nominal concentrations. Toxicity evaluations were based on nominal concentrations.

There was no mortality in the control, 156, 313 and 625 mg a.s./L treatment groups. In the 1250 and 2573 mg a.s./L treatment groups, there was 5 and 15%, respectively, with significant sub-lethal effects (including erratic swimming, and loss of equilibrium) observed in the 625, 1250 and 2500 mg a.s./L treatment groups within 15 minutes of fish addition. Test media pH was negatively correlated with test concentration. All validity criteria according to the guideline OECD 203 were fulfilled.
The 96 hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate K-salt was determined to be > 2500 mg a.s./L, equivalent to >1193 mg a.e./L. The 96 hour NOEC was determined to be 313 mg a.s./L, equivalent to 149 mg a.e./L. This study is considered valid.

I. MATERIALS AND METHODS

1. Test material:

Test item:	MON 78623 (Glyphosate K-salt)
Description:	yellow liquid
Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7 %

2. Vehicle and/or positive control: Vehicle: dechlorinated and filtered tap water
Positive control: none

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juvenile
Size (mean standard length):	43 mm (38 – 56 mm)
Weight (mean wet weight):	0.94 g (0.59 – 1.3 g)
Loading:	0.47 g fish/L
Source:	[redacted]
Acclimation period:	5 weeks prior to the test initiation

4. Environmental conditions:

Temperature:	12.2 – 12.7 °C
Photoperiod:	16 h light, with a 30 min transition period
pH:	Control (start – 96 h): 8.2 – 8.0 156 mg/L (start – 96 h): 7.5 – 8.1 313 mg/L (start – 96 h): 7.1 – 8.0 625 mg/L (start – 96 h): 6.7 – 7.9 1250 mg/L(start – 96 h): 6.2 – 7.1 2500 mg/L (start – 96 h): 5.7 – 5.8
Dissolved oxygen:	≥ 7.3 mg/L (≥ 67 % saturation)
Conductivity:	280 µS/cm
Hardness:	144 mg $CaCO_3$/L.
Alkalinity:	184 mg $CaCO_3$/L

5. Dates of experimental work: 21th February to 25th February 2003

B. STUDY DESIGN

Experimental treatments: A definitive toxicity test was performed using nominal concentrations of 156, 313, 625, 1250 and 2500 mg a.s./L (mean measured: 159, 329, 646, 1302 and 2573 mg a.s./L) in a static test setup, based on the results of a range finding test,. A negative control group (dilution water only) was prepared in parallel. Duplicate vessels (38 L glass vessels containing 20-L control water or test medium) were prepared for the control and treatment groups, each containing ten fish (20 fish per treatment).

Observations: Observations for sub-lethal effects and mortality were performed at 4, 24, 48, 72 and 96 hours after test initiation (fish addition). The pH-value and oxygen saturation of the test solutions were measured at test initiation and on each observation date. Temperature was measured at test initiation and termination. Hardness, alkalinity and specific conductivity of the test water were measured at the start of the test only. Fish wet weights and total lengths were measured in the control. Samples of control or test media from all vessels was taken at 0 (before fish addition) 48 and 96 hours and analysed to determine the to measure glyphosate K salt concentration.

Statistical calculations: Since the mortality was <50 %, no statistical calculation of LC_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Chemical analyses were performed on samples of the test solutions to quantify glyphosate concentrations in the test solution. Measured concentrations were between 99.8 and 109 % of nominal confirming the stability of the test substance in the test system. The ecotoxicological endpoints are based on the nominal concentrations of 156, 313, 625, 1250 and 2500 mg glyphosate K-salt /L. The limit of quantitation (LOQ) was 10.5 mg/L (5.0 mg a.e./L).

Table 8.2.1-5: Analytical results

Nominal concentrations Glyphosate K-salt [mg a.s./L]	Mean measured concentration Glyphosate K-salt [mg a.s./L]	% of nominal	Mean measured concentration Glyphosate acid equivalent [mg a.e./L]
Control	< LOQ	-	-
156	159	102	74.4
313	329	105	149
625	646	103	298
1250	1302	104	596
2500	2573	103	1193

The 96 hour LC_{50} and NOEC values for rainbow trout (*Oncorhynchus mykiss*) exposure to glyphosate K-salt based on nominal concentrations are given below.

Table 8.2.1-6: Endpoints

Endpoints	Expressed as Glyphosate K-salt [mg a.s./L]	Expressed as Glyphosate acid [mg a.e./L]
96 h LC_{50}	> 2500	1193
96 h NOEC	313	149

B. OBSERVATIONS

There was no mortality or sub-lethal effects in the negative control and at the mean measured concentrations of 156 and 313 mg glyphosate K salt/L. At 1250, 625 and 2500 mg glyphosate K-salt/L, 0, 5 and 15 % mortality were observed respectively.

At the three highest test concentrations, sub-lethal effects were noted within 15 minutes after test initiation (including surfacing, laying on the bottom of test chamber, erratic swimming, loss of equilibrium).

The severity of effect generally increased with increasing concentration, which correlated to the concentration-responsive decrease in pH. The pH at 0 h decreased from 8.2 for the controls to 5.7 at the highest test concentration. All surviving fish in 625 and 1250 mg a.s./L appeared normal by 24 h and appeared normal for the remainder of the test. Effects were still evident in three of the 17 surviving fish in 2500 mg test item/L at test termination. The pH remained below 6 in the highest test concentration throughout the test.

The biological results achieved during the fish acute toxicity test are presented below:

Table 8.2.1-7: Lethal effects of glyphosate K-salt to rainbow trout (*Oncorhynchus mykiss*)

Glyphosate K-salt [mg a.s./L]	Glyphosate acid [mg a.e./L]	Number of dead fish / number of fish with intoxication symptoms and observed symptoms					
		0 h	4 h	24 h	48 h	72 h	96 h
Control		0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
156	**74.4**	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
313	**149**	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
625	**298**	0 / 0	0 / 20 A	0 / 11 A	0 / 0	0 / 0	0 / 0
1250	**596**	0 / 3 R / 17 E,N	1 / 17 A / 2R	1 / 0	1 / 0	1 / 0	1 / 0
2500	**1193**	0 / 8 R / 12 E,N	0 / 7 R / 13 A,E,N	0 / 6 R / 4 A / 2 E,N	3 / 0	3 / 3 R / 1 C	3 / 3 R

A = surfacing; R= laying at bottom of test chamber; E = erratic swimming, N = loss of equilibrium

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The 96 hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to the glyphosate K-salt was determined to be > 2500 mg a.s./L (nominal), corresponding to >1193 mg a.e./L. The 96 hour NOEC was determined to be 313 mg a.s./L, corresponding to 149 mg a.e./L.

This study is considered valid and the acute LC_{50} for for rainbow trout exposed to glyphosate K-salt was determined >1193 mg a.e./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/002
Report author	[redacted]
Report year	1995
Report title	Glyphosate acid: Acute Toxicity to rainbow trout (*Oncorhynchus mykiss*)
Report No	AB0503/D
Document No	
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 72-1.
Deviations from current test guideline	Deviations from the current OECD 203 guideline (2019): None.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute effects of glyphosate acid to rainbow trout (*Oncorhynchus mykiss*) was evaluated in a 96-hour static toxicity test conducted at nominal test concentrations of 32, 56, 100, 180, 320 and 560 mg glyphosate acid/L. A dilution water only control was also included in the test. Ten fish were exposed in the control and in each treatment. All fish were observed at daily intervals over the 96 hour study duration, with mortality and sub-lethal signs of toxicity recorded.

Dissolved oxygen, pH and temperature were measured daily in each test vessel. Samples of control and test media were analysed for glyphosate acid at 0 hours (before fish addition) and after 48 and 96 hours. Glyphosate acid was not detected in the control group. The overall mean measured concentrations of glyphosate acid in the treatment groups ranged from 91 to 100 % of nominal concentrations.

There were no fish mortalities or sublethal effects in the control group. At the 32, 56 and 100 mg a.s./L treatments, there were also no fish mortalities but there were transient sublethal effects including dark dicolouration and loss of balance, observed in the 56 and 100 mg a.s./L treatments. All fish in these three groups appeared normal at 96 hours, whilst in the 180, 320 and 560 mg a.s./L there was 100 % mortality. All validity criteria according to the guideline OECD 203 were fulfilled.

The 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid was determined to be 130 mg a.s./L (nominal) with a 95 % confidence interval of 100 to 180 mg a.s./L. The 96-hour NOEC value was 32 mg a.s./L. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Glyphosate acid
Description: White solid
Lot/Batch #: P24
Purity: 95.6 %

2. Vehicle and/or positive control:

Vehicle: dechlorinated and filtered tap water
Positive control: none

3. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)

Age:	Juvenile
Size:	Length: 40 – 71 mm (mean: 57 mm)
Body weight of the animals:	1.16 – 4.56 g/fish (mean: 2.68 g)
Loading:	0.89 g fish/L (10 fish per 30 litres of test medium)
Source:	[redacted]
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimation period:	32 days

4. Environmental conditions:

Temperature:	11.5 – 12.6 °C
Photoperiod:	16 hours
pH:	Control (start – 96 h): 7.7 - 7.9 32 mg/L (start – 96 h): 6.4 – 6.2 56 mg/L (start – 96 h): 5.9 – 6.0 100 mg/L (start – 96 h): 4.7 – 5.1 180 mg/L(start – 24 h): 3.5 320 mg/L (start – 24 h): 3.0 560 mg/L (start – 24 h): 2.8 – 2.7
Dissolved oxygen:	6.2 – 10.4 mg O_2/L
Conductivity:	281 µS/cm³ in the dilution water
Hardness:	56.3 mg $CaCO_3$/L

5. Dates of experimental work: September 11th to September 15th 1995

B. STUDY DESIGN

Experimental treatments: The toxicity test was performed at nominal concentrations of 32, 56, 100, 180, 320 and 560 mg a.s./L prepared using filtered and dechlorinated tap water treated with ultra violet steriliser. The test was conducted under static test conditions. A negative control (dilution water only) was also prepared. A single replicate vessel was prepared for the control and at each treatment level, each containing ten fish (added to 40 L glass aquariums containing 30 L test medium).

Observations: Fish in all vessels were observed for sublethal effects and mortality after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. At test termination, the ten fish from the dilution water control were weighed and measured. Analytical measurements were performed by HPLC analysis at test initiation and after 48 and 96 hours.

Analytical procedures: Samples were taken from the centre of the test solutions. Glyphosate acid concentrations in the test solutions were determined at 0, 48 and 96 hours by high performance liquid chromatography method using a fluorescence detector. The samples were quantified against standards of glyphosate acid. Prior to analysis, samples and standards were derivatised using flourenylmethyl chlorformate, to prepare a fluorescing derivate.

Statistical calculations: The LC_{50} values and their 95 % confidence intervals were calculated using non-linear interpolation. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The mean measured concentrations of glyphosate acid ranged from 91 to 100%. As the measured concentrations of glyphosate were between 80 and 120% of nominal, the ecotoxicological

endpoints were evaluated using nominal concentrations of the test item. The limit of detection was 0.004 mg/L.

Table 8.2.1-8: Analytical results

Nominal concentration Glyphosate acid [mg a.s./L]	Measured concentration Glyphosate acid at 48 hours [mg a.s./L]	Measured concentration Glyphosate acid at 96 hours [mg a.s./L]	% of nominal
Dilution water control	< 0.004	< 0.004	-
32	29	29	91
56	54[1]	55[1]	96
100	91	94	93
180	170	-	100
320	320	-	100
560	540	-	98

Not sampled, 100 % mortality on previous sampling occasion
[1] mean of triplicate analysis

The 96 hour LC_{50} and NOEC values are presented below.

Table 8.2.1-9: Endpoints

Endpoints	Glyphosate acid [mg a.s./L]
LC_{50} (95% C.L.) (96 h)	130 (100 – 180)
NOEC (96 h)	32

B. OBSERVATIONS

Until 100 mg a.s./L no mortality occurred, but all fish died at the test concentrations of 180 mg a.s./L and higher. Transient sublethal effects of dark discolouration and loss of balance were observed at 56 and 100 mg a.s./L respectively. All surviving fish in the study appeared normal at the end of test.

All measured water quality parameters were within the specifications recommended by the OECD 203 test guideline, except pH, where the levels of pH declined with increasing concentration of the test item. At 180 mg a.s./L, the pH was 3.5 and lower.

The biological observations recorded during the test are presented below.

Table 8.2.1-10: Effects of glyphosate acid to rainbow trout

Nominal concentration of glyphosate acid [mg a.s./L]	Number of dead fish / number of fish with intoxication symptoms[1] and observed symptoms			
	24 h	48 h	72 h	96 h
Control	0 / 0	0 / 0	0 / 0	0 / 0
32	0 / 0	0 / 0	0 / 0	0 / 0
56	0 / 0	0 / 0 DC	0 / 0 DC	0 / 0
100	0 / 0 DC	0 / 0 DC, LB	0 / 0	0 / 0
180	[2]	[2]	[2]	[2]
320	[2]	[2]	[2]	[2]
560	[2]	[2]	[2]	[2]

[1] Dead fish are added to the sum of fish with symptoms
[2] All fish dead
DC Dark colouration; LB: Loss of balance

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
The 96 hour LC_{50} value for rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate acid was calculated to be 130 mg a.s./L (nominal) with 95% confidence interval of 100 to 180 mg a.s./L. The NOEC after 96 h was 32 mg a.s./L.

This study is considered valid and the acute LC_{50} value for rainbow trout exposed to glyphosate acid was determined to be 130 mg a.s./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.1/003
Report author	[redacted]
Report year	1995
Report title	The acute toxicity of glyphosate to Rainbow trout (*Oncorhynchus mykiss*)
Report No	710/21
Document No	-
Guidelines followed in study	Information mentioned in the Monograph:

	The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Toxicity of technical glyphosate (purity >94 %) to aquatic organisms (*Oncorhynchus mykiss*) in a 96 hours static test
Short description of results:	LC_{50} >100 mg a.e./L and NOEC >100 mg a.e./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	The full study report is not available to the applicant. However these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR (2015).
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 8.2.1/004
Report author	[redacted]
Report year	1993
Report title	Acute Toxicity Testing in Fish Test Article: 'Glyphosate isopropylamine salt'
Report No	80-91-2328-03-93
Document No	
Guidelines followed in study	OECD Guideline 203; EEC Directive 92/69
Deviations from current test guideline	Deviations from the current OECD 203 guideline (2019): None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate isopropylamine salt on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. The toxicity test was performed using nominal concentrations of 107, 235, 517, 1136 and 2500 mg test item/L, corresponding to 65.9, 145, 318, 700 and 1540 mg glyphosate isopropylamine salt/L (mg a.s./L) or 48.8, 107, 236, 519 and 1141 mg glyphosate/L (mg a.e./L). Further a dechlorinated and deionised tap water control was tested. Ten fish were exposed to each treatment level.

Mortality was recorded after 2-4, 24, 48, 72 and 96 hours after the start of the test. Records on visible abnormalities were equally made. At termination of the test, all animals were weighed and measured.

At the nominal concentration of 1136 and 2500 mg test item/L, after 24 h of exposure the fish showed reduced activity and a tendency of staying at the bottom of the test vessels. In comparison to the control group, no obvious abnormal effects were seen at or below the concentration of 517 mg test item/L. All validity criteria according to the guideline OECD 203 were fulfilled.
In a static acute toxicity study of glyphosate isopropylamine salt, the LC_{50} (96 h) for rainbow trout exposed to glyphosate isopropylamine salt was determined to be 2192 mg test item/L, corresponding to 1350 mg a.s./L or 1001 mg a.e./L (nominal). The NOEC was determined to be 517 mg test item/L, corresponding to 318 mg a.s./L or 236 mg a.e./L (nominal). This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt
Description:	Viscous liquid
Lot/Batch #:	01/06/93
Purity:	61.6% Glyphosate isopropylamine salt
Density:	1.23 g/cm^3 at 20°C

2. Vehicle and/or positive control: Vehicle: dechlorinated and deionised tap water
Positive control: none

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Not stated
Size:	Length: 6.70 cm (mean of 10 representative individuals)
Loading:	10 L for 5 fish
Source:	Commercial supplier ([redacted])
Acclimation period:	≥ 48 h in a 250 L glass aquarium under general test conditions
Body weight of the animals:	1.92 g (mean body weight of 100 individuals)

4. Environmental conditions:

Temperature:	14.5 – 16.3 °C
Photoperiod:	16 hours light / 8 hours dark, 600 - 800 lux
pH:	7.5 – 8.5
Dissolved oxygen:	8.2 – 10.2 mg O_2/L)
Conductivity:	Not stated
Hardness:	14° dH (1dH= 10 mg CaO/L)

5. Dates of experimental work: 24th August to 04th September 1993

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, definitive toxicity test was performed using nominal concentrations of 107, 235, 517, 1136 and 2500 mg test item/L in a static test

setup. In addition a control group was exposed to dechlorinated and deionised tap water only. There were two vessels per treatment, each containing five fish (12 L glass containers containing 10 L test medium)

Observations: Assessment of effects and mortality of test fish after 2-4, 24, 48, 72 and 96 hours was conducted. Temperature, pH-value and oxygen saturation (% air saturation value [% ASV]) of the test solutions were measured on a daily basis. Hardness of the test water was measured at the start of the test.

Mortality was recorded on each observation date. Records on visible abnormalities were equally made. At start and termination of the test, all animals were weighed and measured.

Analytical control measurements of the actual concentrations of the test item were performed by mean of HPLC analysis. Glyphosate isopropylamine salt levels were determined based on the concentrations of glyphosate. Three representative concentrations (107, 517 and 2500 mg test item/L, corresponding to 65.9, 318 and 1540 mg a.s./L or 48.8, 236 and 1141 mg a.e./L) were analysed at 24 h intervals.

Statistical calculations: 24 h, 48 h, and 72 h LC_{50} values were determined directly from the raw data. The 96 h LC_{50} value was calculated by Probit analysis according to Finney (1971).

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} values are given below based on nominal concentrations.

Table 8.2.1-11: Endpoints

Endpoints (96 h)	Test item [mg/L]	Glyphosate isopropylamine salt [mg a.s./L]	Glyphosate [mg a.e./L]
LC_{50} (95% C.L.))	2192 (1501-19088)	1350	1001
NOEC	517	318	236
LOEC	1136	700	519

Analytical data: Analytical control measurements were performed on three representative concentration levels of glyphosate isopropylamine salt, at 107 mg test item/L, corresponding to 48.8 mg a.e./L, 517 mg test item/L, corresponding to 236 mg a.e./L and at the highest concentration tested, 2500 mg test item/L, corresponding to 1141 mg a.e./L. Before introduction of the fish 99.2 %, 102.7 % and 95.1 % of glyphosate were recovered at 107, 517 and 2500 mg test item/L, respectively. In the aged test media 95.2 %, 90.3 % and 85.1 % of the nominal concentration were recovered. Consequently, during the test period of 96 hours the fish were exposed to a mean concentration of 90.2% (average for test concentrations of 107, 517 and 2500 mg test item/L, respectively) of nominal concentration.

As the mean measured content of the test item always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 8.2.1-12: Analytical results

Nominal concentration of test item [mg/L]	Nominal concentration of glyphosate [mg a.e./L]	Time (hours)	Measured concentration of glyphosate [mg a.e./L]	% of nominal
107	48.8	0	48.44	99.2
		48	47.79	97.9
		96	46.50	95.2
517	236	0	242.45	102.7
		48	215.31	91.2
		96	213.09	90.3
2500	1141	0	1085.45	95.1
		48	1046.00	91.7
		96	971.45	85.1

B. OBSERVATIONS

Clinical observations:

At the nominal concentration of 1136 and 2500 mg test item/L, the fish showed reduced activity and showed a tendency of staying at the bottom of the test aquarium after 24 h.
In comparison to the control group, no abnormal effects were seen at or below the concentration of 517 mg test item/L.

Table 8.2.1-13: Lethal effects of glyphosate isopropylamine salt to rainbow trout

	Control					
Test item [mg/L]	**-**	**107**	**235**	**517**	**1136**	**2500**
Glyphosate isopropylamine salt [mg a.s./L]	**-**	**65.9**	**145**	**318**	**700**	**1540**
Glyphosate [mg a.e./L]	**-**	**48.8**	**107**	**236**	**519**	**1141**
Mortality (2-4 h) [%]	0	0	0	0	0	0
Mortality (24 h) [%]	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	20
Mortality (72 h) [%]	0	0	0	0	0	40
Mortality (96 h) [%]	0	0	0	0	10	60

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a static acute toxicity study of glyphosate isopropylamine salt, the LC_{50} (96 h) for rainbow trout exposed to glyphosate isopropylamine salt was determined to be 2192 mg test item/L, corresponding to 1350 mg glyphosate isopropylamine salt/L (mg a.s./L) or 1001 mg glyphosate/L (mg a.e./L) (nominal). The NOEC was determined to be 517 mg test item/L, corresponding to glyphosate isopropylamine salt/L (mg a.s./L) or 236 mg glyphosate/L (mg a.e./L) (nominal).

This study is considered valid and the acute LC_{50} value for rainbow trout exposed to glyphosate isopropylamine salt was determined to be >1001 mg a.e./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.1/005
Report author	[redacted]
Report year	1990
Report title	Glyphosate technical: 96-hour Acute Toxicity Study (LC_{50}) in the Rainbow Trout
Report No	271631
Document No	-
Guidelines followed in study	OECD Guideline 203 (1983)
Deviations from current test guideline	Deviation according to the current guideline OECD 203: None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate technical on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. Groups of ten fish each were exposed to glyphosate technical at concentrations of 95, 171, 309, 556, and 1000 mg a.s./L (nominal concentrations), corresponding to 87.7, 135, 188, 497 and 1019 mg a.s./L based on geometric mean measured concentrations. The number of surviving organisms and the occurrence of sub-lethal effects, as well as the measurement of dissolved oxygen, pH and water temperature were determined and recorded after 2, 24, 48, 72 and 96 hours after starting the exposure period. The concentrations of glyphosate in the test medium were determined at test initiation, and 2, 48 and 96 hours thereafter.

At test concentration of 87.7 mg a.s./L there was no fish mortality within the 96 hour duration of the study. Increasing the mean measured test concentration by a factor of about 1.5 to 135 mg a.s./L the mortality resulted in 100 % within the first 48 h of exposure. All validity criteria according to the guideline OECD 203 were fulfilled.

The 96-h LC_{50} for *Oncorhynchus mykiss* exposed to glyphosate technical was estimated to be between 87.7

and 135 mg a.s./L based on (geometric) mean measured concentration. The NOEC after 96 h was 87.7 mg a.s./L. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	Solid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9 %

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: none

3. Test organism:

Species:	Rainbow trout (*Salmo gairdneri*, currently known as *Oncorhynchus mykiss*)
Size of animal:	Weight 0.8 g (average) Length 42.4 mm (average)
Number of animals/dose level:	10 in each vessel
Mean loading rate (biomass per volume of test solution):	0.4-0.6 g/L
Supplier:	[redacted]

4. Environmental conditions:

Temperature:	11-12 °C
pH:	2.8-8.1
Dissolved oxygen:	10.8-12.3 mg O_2/L (continuously aerated during the test)
Conductivity:	Not reported
Hardness:	250 mg $CaCO_3$
Illumination:	16 hours light/8 hours dark, 500-1500 lux

5. Experimental dates of work: May 28th to June 1st 1990

B. STUDY DESIGN

Experimental treatments

The effects of glyphosate technical on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. Groups of ten fish each were exposed to glyphosate technical at nominal concentrations of 95, 171, 309, 556, and 1000 mg a.s./L. The test solutions were prepared by adding 1.425, 2.565, 4.635, 8.34, and 15 g test item to 15 L test medium (reconstituted water prepared according to the OECD Guideline) in the respective tanks. In addition fish were exposed to test medium without test substance (blank control).

Observations

The number of surviving organisms and the occurrence of sub-lethal effects, as well as the measurement of dissolved oxygen, pH and water temperature were determined and recorded after 2, 24, 48, 72 and 96 hours after starting the exposure period. The concentrations of glyphosate in the test medium were determined at

test initiation, and 2, 48 and 96 hours thereafter.

Statistical calculations
The Logit-Model could not be used to estimate the LC_{50} value since the mortality rose from 0 % to 100 % within two test concentrations.

II. RESULTS AND DISCUSSION

A. FINDINGS
At test initiation the concentrations of glyphosate in the test medium were in a range of 59.6 to 101.9% of nominal. At the end of the test, the concentration of glyphosate in the tank where all fish survived (95 mg a.s./L) was 104.6% of nominal. Therefore, the toxicity values are based on (geometric) mean measured concentrations. Analytical results are shown below.

Table 8.2.1-14: Analytical results

Nominal concentration of glyphosate technical [mg a.s./L]	Time (hours)	Mean concentration of Samples A and B [mg a.s./L]	% of nominal	Geometric mean measured concentrations [mg a.s./L]
95	0	75.82	79.8	87.7
95	2	77.32	81.4	
95	48	95.33	100.3	
95	96	99.33	104.6	
171	0	124.4	72.7	135
171	2	108.5	63.5	
171	48	182.8	106.9	
309	0	184.1	59.6	188
309	2	192.6	62.3	
556	0	528.2	95.0	497
556	2	470.3	84.6	
1000	0	1019	101.9	1019
1000	2	1019.8	102.0	

At test concentration of 87.7 mg a.s./L there was no fish mortality within the 96 hour duration of the study. Increasing the mean measured test concentration by a factor of about 1.5 to 135 mg a.s./L the mortality resulted in 100 % within the first 48 h of exposure. Based on these findings, the 96-h LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate technical was estimated to be between 87.7 and 135 mg a.s./L. The mortality in the control was 0%. The effects of glyphosate technical on mortality in rainbow trout are shown below.

Table 8.2.1-15: Effects of glyphosate technical on mortality of rainbow trout

Nominal concentration of glyphosate technical [mg a.s./L]	**Control**	**95**	**171**	**309**	**556**	**1000**
Geometric mean measured concentrations of glyphosate technical [mg a.s./L]	**Control**	**87.7**	**135**	**188**	**497**	**1019**
Mortality (24 h) [%]	0	0	50	100	100	100
Mortality (48 h) [%]	0	0	100	100	100	100
Mortality (72 h) [%]	0	0	100	100	100	100
Mortality (96 h) [%]	0	0	100	100	100	100

B. OBSERVATIONS

At glyphosate technical concentrations of 309 and 1000 mg a.s./L a sediment of the test material on the bottom of the tanks was observed.
Clinical signs were recorded at glyphosate technical concentrations of 171 and 309 mg a.s./L, whereas in the control and in the 95 mg a.s./L tanks no sub-lethal effects were recorded.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The 96-h LC_{50} for rainbow trout (*Oncorhynchus mykiss)* exposed to glyphosate technical was estimated to be between 87.7 and 135 mg a.s./L based on geometric mean measured concentrations. The NOEC after 96 h was 87.7 mg a.s./L.

Some precipitate observed at test concentrations 188 mg a.s./L and 1019 mg a.s./L. The validity criteria are fulfilled and so this study is considered valid and the acute LC_{50} value for rainbow trout exposed to glyphosate technical was estimated to be 87.7 - 135 mg a.e./L (geometric mean measured concentrations) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/006
Report author	[redacted]
Report year	1981
Report title	Acute Toxicity of MON 0139 (lot LURT 12011) (AB-81-072) to Rainbow Trout (*Salmo gairdneri*)
Report No	27202
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms
Deviations from current test guideline	Deviations from the current OECD 203 guideline (2019): Major: - No analytical verification of test concentrations none Minor: - Fish were acclimatised 48 hours prior to the test (7 days are required) - Fish lengths 25 - 31 mm (30 to 60 mm is required) - pH of the highest concentration (5.0) was not with the specified range of 6.0 - 8.5.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary of the study according to OECD format

Executive Summary

The effects of glyphosate isopropylamine salt (MON 0139) on the rainbow trout (*Salmo gairdneri,* currently known as *Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. Based on the results of a range finding test, a definitive toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L, corresponding to 62.5, 112, 200, 350 and 625 mg glyphosate isopropylamine salt/L (mg a.s./L) or 46.3, 83.3, 148, 259 and 463 mg glyphosate/L (mg a.e./L). In addition, a control group was exposed to dilution water (soft reconstituted water) and a reference product (Antimycin A). The mortality of fish was recorded in all test concentrations and the control at 24, 48 and 96 hours. No mortality was observed at any of the test concentrations up to and including 1000 mg test item/L, corresponding to 625 mg a.s./L or 463 a.e./L (nominal).

In a static acute fish toxicity test, the LC_{50} (96 h) for rainbow trout (*Salmo gairdneri*) exposed to glyphosate isopropylamine salt (MON 0139) was determined to be >1000 mg test item/L (nominal), corresponding to >625 mg a.s./L or 463 mg a.e./L (nominal).
The validity of the present study according to OECD guideline 203 is questionable, since the analytical part of the study was not performed and/or reported. The study is considered supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt (MON 0139)
Description:	Light yellow liquid
Lot/Batch #:	LURT 12011
Purity:	62.49 %

2. Vehicle and/or positive control: Vehicle: Deionised water
Positive control: Antimycin A

3. Test organism:

Species:	Rainbow Trout (*Salmo gairdneri*)
Age:	At least 14 days old
Size:	Length: 27 mm (mean)
Body weight:	0.22 g (mean)
Loading:	10 test individuals for 15 L test solution (=0.146 g/L)
Source:	[redacted]
Diet/Food:	Daily with Standard commercial fish food (Rangen's) except 48 prior to the test
Acclimation period:	48 hours prior to the test initiation

4. Environmental conditions:

Temperature:	12 ± 1°C
Photoperiod:	16 h light
pH:	7.0
Dissolved oxygen:	9.8 mg/L
Conductivity:	Not stated
Hardness:	45 mg $CaCO_3$/L.

5. Experimental dates of work: March 10th to March 14th 1981

B. STUDY DESIGN

Experimental treatments: Based on the results of a 48-h range finding test, a definitive toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L. In addition, a control group was exposed to dilution water (soft reconstituted water) and a reference product (Antimycin A). The mortality of fish was recorded in all test concentrations and the control at 24, 48 and 96 hours. There was one vessel per treatment, containing ten fish in 5 gallon (appr. 19 L) glass vessels containing 15 L test medium.

Observations: The fish mortality was recorded in all test concentrations and the control 24, 48 and 96 hours after the test initiation. Temperature, pH-value and oxygen saturation of the test solutions were measured on each observation date. Hardness of the test water was measured at the start of the test. The weight and length of the test fish were measured.

Statistical calculations: LC_{50} values were calculated using computer program by Stephan et al. (1978).
(Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W,. Andrew. 1978. A computer program for calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication

manuscript, August, 1978)

II. RESULTS AND DISCUSSION

A. FINDINGS

No analytical verification of the tested concentrations was conducted or reported.

The LC_{50} values are given below based on nominal concentrations.

Table 8.2.1-16: Endpoints

Endpoints (96 h)	Test item [mg/L]	Glyphosate isopropylamine salt [mg a.s./L]	Glyphosate [mg a.e./L]
LC_{50}	>1000	>625	>463

B. OBSERVATIONS

There was no mortality observed at any of the test concentrations up to and including 1000 mg test item/L. For the reference product Antimycin A, the LC_{50} was determined to be 0.000030 mg/L. The dissolved oxygen concentration which stayed between 40 and 100 % saturation was considered adequate for testing. The pH values dropped with increasing test concentrations.

Table 8.2.1-17: Lethal effects of glyphosate isopropylamine salt (MON 0139) to *Salmo gairdneri*

	Control					
Test item [mg/L]	**-**	**100**	**180**	**320**	**560**	**1000**
Glyphosate isopropylamine salt [mg a.s./L]	**-**	**62.5**	**112**	**200**	**350**	**625**
Glyphosate [mg a.e./L]	**-**	**46.3**	**83.3**	**148**	**259**	**463**
Mortality (24 h) [%]	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	0
Mortality (72 h) [%]	0	0	0	0	0	0
Mortality (96 h) [%]	0	0	0	0	0	0

The following validity criteria according to the OECD 203 (2019) were fulfilled:

- The dissolved oxygen concentration was maintained ≥60 % of the air saturation value (ranging from 9.9 to 9.4 mg/L through the study).
- The control mortality was lower than 10 % at the end of the study.

The following validity criterion according to the OECD 203 (2019) was not fulfilled:

- No analytical measurement of the test concentrations was reported.

The following points deviated from current guideline too:

- Fish were acclimatised 48 hours prior to the test instead of the 7 day requirement.
- Fish length varied between 25-31 mm instead of 30 to 60 mm required.
- Observations occurred after 24 h, 48 h and 96 h. The requirements are the following: a minimum of 2 observations within the first 24 hours of the study and on days 2-4 of the test, all vessels with living fish inspected twice per day (preferably early morning and late afternoon to best cover the 24-hour periods).
- The pH in the highest concentration was outside of the accepted range of 6.0-8.5 so the stock solution should have been adjusted to lie within this specified range.

These deviations may affect the outcome of the study, so the validity of the study is questionable.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a static acute fish toxicity test, the LC_{50} (96 h) for rainbow trout (*Salmo gairdneri*) exposed to glyphosate isopropylamine salt (MON 0139) was determined to be >1000 mg test item/L (nominal), corresponding to >625 mg a.s./L or >463 mg a.e./L (nominal).
Not all validity criteria according to the OECD 203 (2019) were fulfilled since the analytical part of the study was not performed and/or reported. Taking also into account the minor deviations, that may affect the outcome of the study, the study is therefore considered as supportive.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/007
Report author	[redacted]
Report year	1978
Report title	Acute Toxicity of Technical Glyphosate (AB-78-165) to Rainbow Trout (*Salmo gairdneri*)
Report No	AB 78-165
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms
Deviations from current test guideline	Deviations from the current OECD 203 guideline (2019): Major: - No analytical verification of test concentrations Minor: -
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary of the study according to OECD format

Executive Summary
The acute effects of glyphosate technical on rainbow trout (*Salmo gairdneri* – currently known as *Oncorhynchus mykiss)* were evaluated in a 96-hour static toxicity test. A definitive toxicity test was performed using nominal concentrations of 42, 87, 120, 180, 240 and 420 mg test item/L, corresponding to 34.9 72.2, 99.6, 149, 199 and 349 mg glyphosate technical/L (mg a.s./L), following a range-finding test. A control group was exposed to deionised water and a reference treatment group exposed to Antimycin A were also tested.

The mortality of fish was recorded at 24, 48 and 96 hours after test initiation. At 24 hours, there was 100 % mortality in the 240 and 420 mg test item/L treatment groups. At 48 hours, there was 100 % mortality in the 180 mg test item/L treatment group. At 96 hours, in the 120 mg test item/L group there was 100 % mortality recorded, 40 % mortality at 87 mg test item/L and no mortality in the control group or the lowest concentration (42 mg test item/L). The LC_{50} (96 h) was determined to be 86 mg test item/L, corresponding to 71.4 mg a.s./L (nominal). The NOEC was determined to be 42 mg test item/L, corresponding to 34.9 mg a.s./L (nominal).

According to the current OECD 203 test guideline, despite the control validity criteria of ≤10 % mortality being achieved, there was no chemical analysis performed to confirm glyphosate concentration in the test media. The test would therefore not be considered valid against the current criteria. Within the context of the Annex I renewal of glyphosate, this study may only be considered supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XHI-162
Purity:	83.0 %

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: Antimycin A

3. Test organism:

Species:	Rainbow trout (*Salmo gairdenri*)
Age:	Not stated
Size:	Length: 39 mm (mean, reference toxicant group: 34 mm)
Loading:	10 individual fish per vessel (19 L glass vessel) in 15 L test solution
Source:	[redacted]
Acclimation period:	48 hours prior to the test initiation
Body weight of the animals:	0.58 g (mean, reference toxicant group = 0.55g)

4. Environmental conditions:

Temperature:	12 ± 1°C
Photoperiod:	Not stated
pH:	7.0 – 7.2 (control); 4.4 – 5.8 (120 mg test item/L)
Dissolved oxygen:	7.6 – 8.7 mg/L
Conductivity:	Not stated
Hardness:	45 mg $CaCO_3$/L

5. Experimental dates of work: July 29th to August 2nd 1978

B. DESIGN AND METHODS

Experimental treatments: Following a range-finding test, a definitive test was conducted at nominal test concentrations of 42, 87, 120, 180, 240 and 420 mg test item/L, corresponding to 34.9, 72.2, 99.6, 149, 199

and 398 mg glyphosate a.s./L, in a static test setup. The test item was dissolved directly into dilution water.
A control group was also prepared using fish exposed to dilution water only (soft reconstituted water using deionised water).
A reference toxicant test was conducted in parallel with fish exposed to Antimycin A at rates between 0.000024 – 0.00032 mg /L. Acetone was used to prepare the reference toxicant media.
A single replicate vessel was prepared per treatment, control and reference toxicant group.

Observations: Mortality was recorded in all test concentrations and the control 24, 48 and 96 hours after test initiation in the glyphosate exposure test and additionally at 72 hours in the reference toxicant test. Temperature, pH-value and oxygen saturation of the test solutions were measured on each observation date. Hardness of the test water was measured at the start of the test. Weight and length of the test fish were equally measured.

Statistical calculations: LC_{50} values were calculated along with the 95% confidence limits using Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

At and above nominal concentrations of 120 mg test item/L, no fish survived. At a nominal concentration of 87 mg test item/L, 40% mortality was recorded whereas no mortality was observed at the lowest test concentration of 42 mg test item/L. For the highest concentration of the reference product Antimycin A (0.00032 mg/L), 100% mortality was observed 24 hours after the test initiation.

Table 8.2.1-18: Lethal effects of glyphosate to rainbow trout

Test item [mg/L]	**C**	**42**	**87**	**120**	**180**	**240**	**480**
Glyphosate technical [mg a.s./L]	**-**	**34.9**	**72.2**	**99.6**	**149**	**199**	**398**
Mortality (24 h) [%]	0	0	0	0	70	100	100
Mortality (48 h) [%]	0	0	0	60	100	100	100
Mortality (96 h) [%]	0	0	40	100	100	100	100

C = Control

The LC_{50} and NOEC values are given below based on nominal concentrations.

Table 8.2.1-19: Endpoints

Endpoints (96 h)	**Test item [mg/L]**	**Corresponding glyphosate technical concentration [mg a.s./L]**	**Reference [mg/L]**
LC_{50} (95% C.I.)	86 (70 - 106)	71.4 (58.1 - 88.0)	4.2×10^{-5} (3.6×10^{-5} - 4.9×10^{-5})
NOEC	42	34.9	-

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a static acute fish toxicity study of glyphosate, the LC_{50} (96 h) for rainbow trout (*Oncorhynchus mykiss*) exposed to the glyphosate technical was determined to be 86 mg test item/L, corresponding to 71.4 mg a.s./L (nominal). The NOEC was determined to be 42 mg test item/L, corresponding to 34.9 mg a.s./L (nominal).
No chemical analysis was performed to confirm glyphosate concentration in the test media. The test would therefore be considered supportive for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/008
Report author	[redacted]
Report year	1972
Report title	Four-day static fish toxicity studies with CP 67573 in rainbow trout and bluegills.
Report No	BTL-72-104
Document No	-
Guidelines followed in study	Not mentioned.
Deviations from current test guideline	Deviations from the current OECD 203 guideline (2019): Major: - No analytical verification of test concentrations - 60 % of the air saturation was not maintained throughout the test. Minor: - Oxygen, pH and temperatures were not daily measured. - The weight of the fish were not provided, so the loading cannot be calculated. - The length of bluegill ranged between 3.5 and 7.5 cm. - Temperature of bluegill test was 18°C.
Previous evaluation	Rainbow trout: Yes, accepted in RAR (2015) Bluegill: Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary of the study according to OECD format

Executive Summary
The acute effects of glyphosate acid (CP 67573) to rainbow trout (*Oncorhynchus mykiss*) and bluegills (*Lepomis macrochirus*) were evaluated in a 96-hour static toxicity tests. These tests were conducted at nominal test concentrations of 10, 18, 32, 56 and 78 mg a.s./L for rainbow trout and 32, 56, 56, 70, 85 and 100 mg a.s./L for bluegill. A control and a toxic reference item (Toxaphene) were also included in the test. Ten fish were exposed in the control and in each treatment. All fish were observed at daily intervals over

the 96-hour study duration, with mortality and sub-lethal signs of toxicity recorded. Dissolved oxygen and pH values were measured for all solutions in which mortalities occurred. The temperature was maintained at 13° C for rainbow trout and 18 °C for bluegills. Glyphosate acid (CP 67573) was found to have a very low solubility in water. No analytical measurements were performed. Only one of the three validity criteria according to the guideline OECD 203 was fulfilled (control mortality < 10 %).
The 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid (CP 67573) was determined to be 38 mg a.s./L with a 95 % confidence interval of 25 to 56 mg a.s./L.
The 96-hour LC_{50} value for the bluegills exposed to glyphosate acid (CP 67573) was determined to be approximately 78 mg a.s./L (95% confidence interval was not recorded).

The study was previously considered valid (RAR 2015) and was part of the list of endpoints, being the lowest available fish acute toxicity endpoint. However, as no analytical verification of test item was performed and oxygen levels decreased below 60 %, this are major deviations to the guideline. Taking also into account that some minor deviations were pointed out, the study is not considered valid according to OECD 203.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid (CP 67573)
Description:	Low solubility in water
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control:

Vehicle: reconstituted water
Positive control: Toxaphene

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
	Bluegill (*Lepomis macrochirus*)
Age:	Juvenile
Size:	Length: 35-75 mm
Body weight of the animals:	Not reported
Loading:	Not reported
Source:	Not reported
Diet/Food:	Brine shrimp or purina trout chow no feeding for 3 days prior to test
Observation period:	14 days prior to experimental use
Acclimation period:	24 hours

4. Environmental conditions:

Temperature:	13 °C for rainbow trout and 18 °C for bluegill
Photoperiod:	Not reported
pH range from start to 96h	

for rainbow trout:	Control: 7.0 - 7.3 18 mg/L: 6.2 32 mg/L: 6.0 – 6.4 56 mg/L: 5.5 – 6.2 78 mg/L: 4.0
for bluegill:	Control: 6.9 – 7.1 85 mg/L: 4.0 100 mg/L: 3.9 – 4.1
Dissolved oxygen range from start to 96h	
for rainbow trout:	Control: 5 - 7.6 mg O_2/L 18 mg/L: 0.8 mg O_2/L 32 mg/L: 3.0 – 3.4 mg O_2/L 56 mg/L: 2.6 – 6.0 mg O_2/L 78 mg/L: 6.7 mg O_2/L
for bluegill:	Control: 4.1 – 6.8 mg O_2/L 85 mg/L: 6.8 mg O_2/L 100 mg/L: 7.1 – 8.2 mg O_2/L
Conductivity:	Not recorded
Hardness:	Not recorded
5. Dates of experimental work:	Not reported

B. STUDY DESIGN

Experimental treatments

The toxicity test was performed with glyphosate acid (CP 67573) at nominal concentrations of 10, 18, 32, 56 and 78 mg a.s./L for rainbow trout and 32, 56, 70, 85 and 100 mg a.s./L for bluegill, prepared using reconstituted water. The bioassay vessels prepared for the control and at each treatment level, were lined with disposable polyethylene bags and then filled with 12.5 L of reconstituted water, with ten fish then added to each vessel. After an acclimation period of 24 hours, the test material was added directly to the vessels containing the fish. The tests were conducted under static test conditions. A negative control (water only) was also prepared. Toxaphene was used as toxic reference item and dispensed in the form of a 0.01 % w/v solution in acetone.

Observations

Fish in all vessels were observed for 96 hours after the introduction of the test material directly to the vessels, with sublethal effects (e.g. quiescence, mucosa shedding) and mortality recorded daily. The pH-value and oxygen saturation of test solutions were measured in all solutions in which mortalities occurred. Hardness and conductivity of the test water were not measured. Analytical measurements were not performed.

Statistical calculations

The four-day median tolerance level TL_{50} (equivalent to an LC_{50} value) and corresponding 95 % confidence intervals, were calculated using the technique of Litchfield, J. T., Jr. and Wilcoxon, F., "A Simplified Method of Evaluating Dose-Effect Experiments," J. Pharm. & Exp. Ther. 96, 99 (1949).

II. RESULTS AND DISCUSSION

A.FINDINGS

Analytical data: No analytical verification of test concentrations was performed.

The 96 hour LC_{50} values are presented below.

Table 8.2.1-20: Endpoints

Endpoints (96h)	Glyphosate acid (CP 67573) [mg a.s./L]
Rainbow trout LC_{50} (95% CI)	38 (25 – 56)
Bluegill LC_{50} (95% CI)	≈ 78 (n.d.)

CI= Confidence interval
n.d.= not determined

The 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid (CP 67573) was determined to be 38 mg a.s./L with a 95 % confidence interval of 25 to 56 mg a.s./L.
The 96-hour LC_{50} value for bluegill exposed to glyphosate acid (CP 67573) was determined to be approximately 78 mg a.s./L (95 % confidence interval was not recorded).

B. OBSERVATIONS

For the rainbow trout:
At test concentrations of 18 and 32 mg a.s./L three fish died within 96 hours of exposure. At test concentration of 56 mg a.s./L four fish died within 96 hours of exposure. Increasing the test concentration by a factor of about 1.4 (78 mg a.s./L) the mortality resulted to be 100 % within the first 24 hours of exposure.
Sublethal effects of quiescence, swimming against tank side on bottom, patchy shedding of external mucosa were observed within the 6 hours after exposure at 78 mg test item/L and within 24 hours at concentrations up to 18 mg test item/L. There were no recovery until the end of the test when sublethal effects were detected.
The fish were in the recommended range length of 3 to 6 cm (actual values ranged between: 3.5 and 7.5 cm). The water quality parameters were not recorded except for control pH which was within the OECD 203 specifications of 6 to 8.5 (actual value: 7.6). The levels of pH declined with increasing concentration of the test item, with a pH of 4.0 being recorded at the highest rate. The biological observations recorded during the test are presented below.

Table 8.2.1-21: Effects of CP 67573 to rainbow trout

Nominal concentration of glyphosate acid [mg a.s./L]	Number of survivor/observed symptoms [1]					96 h Survival %
	1-6 h	24 h	48 h	72 h	96 h	
Control	10/no	10/no	10/no	10/no	10/no	100
10	10/no	10/no	10/no	10/no	10/no	100
18	10/no	10/Q	10/Q	10/Q	7/Q	70
32	10/no	10/Q	10/Q	10/Q	7/Q	70
56	10/no	9/Q	8/Q, S	7/Q, S	6/Q, S	60
78	10/Q, S, E, P	0	0	0	0	0

[1] Q = quiescence, S = swimming against tank side on bottom, E = external mucosa shedding and P = patchy

<u>For the bluegill</u>:
No mortality occurred up the concentration of 70 mg a.s./L within the 96 hours of exposure. At test concentration of 85 mg a.s./L four fish died within 96 hours of exposure. At the highest test concentration of 100 mg a.s./L, the mortality resulted in 100% within 72 hours of exposure.
Sublethal effects of quiescence, light discoloration, external mucosa shedding or patchy behaviour were observed at the concentration of 56 mg a.s./L and higher. There were no recovery until the end of the test when sublethal effects were detected.
The fish were not in the recommended range length of 1 to 3 cm (actual values ranged between: 3.5 and 7.5 cm). The water quality parameters were not recorded except for control pH which was within the OECD 203 specifications of 6 to 8.5 (actual value: 6.8). The levels of pH declined with increasing concentration of the test item. The temperature was not in the required range of 21 to 25°C (actual value: 18°C). The biological observations recorded during the test are presented below.

Table 8.2.1-22: Effects of CP 67573 to bluegill

Nominal concentration of glyphosate acid [mg a.s./L]	Number of survivor/observed symptoms [1]					96 h Survival %
	1-6 h	24 h	48 h	72 h	96 h	
Control	10/no	10/no	10/no	10/no	10/no	100
32	10/no	10/no	10/no	10/no	10/no	100
56	10/no	10/Q	10/Q	10/Q	10/Q	100
70	10/no	10/Q, L	10/Q, L	10/Q, L	10/Q, L	100
85	10/Q, L, E, P	10/ Q, L, E, P	6/ Q, L, E, P	6/ Q, L, E, P	6/ Q, L, E, P	60
100	10/Q, L, E, P	7/ Q, L, E, P	4/ Q, L, E, P	0	0	0

[1] Q = quiescence, L = light discoloration, E = external mucosa shedding and P = patchy

General observations:
The test material, CP 67573, was found to have a very low solubility in water. At higher dose levels (56 mg a.s./L and upward) the test material displayed a very high acidity. Primarily those fish which came into direct contact with the test material (as it dropped to the bottom) were more affected.

The following points deviated from the current guideline:
- Oxygen, pH and temperatures were not daily measured.
- The weight of the fish were not provided, so the loading cannot be calculated.
- The length of bluegill ranged between 3.5 and 7.5 cm.
- Temperature of bluegill test was 18°C.

Validity criteria

In order to consider the test to be valid according to OECD 203, the following conditions should be fulfilled:
- Control mortality should not exceed 10% at the end of the exposure. No mortality was recorded in the control for both tests.
- The dissolved oxygen concentration should be ≥60 % of the air saturation value in all test vessels throughout the exposure. Air saturation was not reported. The dissolved oxygen values varied from 7.6 to 0.8 mg O_2/L, for rainbow trout. The dissolved oxygen values varied from 8.2 to 4.0 mg O_2/L, for bluegill. Hence, the dissolved oxygen concentration was not steady throughout the test.
- Analytical measurement of test concentrations is compulsory, however no analytical measurement was performed.

According to the current validity criteria of OECD 203 guideline, this study is not valid. The dissolved oxygen concentration above 60 % of air saturation and evidence that the concentration of the chemical

being tested has been satisfactorily maintained (at least 80 % of the nominal concentration) throughout the test, cannot be concluded.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid (CP 67573) was determined to be 38 mg a.s./L with a 95% confidence interval of 25 to 56 mg a.s./L.
The 96-hour LC_{50} value for bluegill exposed to glyphosate acid (CP 67573) was determined to be approximately 78 mg a.s./L (95% confidence interval was not recorded).

The study was previously considered valid (RAR 2015) and was part of the list of endpoints, being the lowest available fish acute toxicity endpoint. However, as no analytical verification of test item was performed and oxygen levels decreased below 60%, this are major deviations to the guideline. Taking also into account that some minor deviations were pointed out, the study is not considered valid according to OECD 203. Other valid studies with comparable results are available. This study is not considered acceptable for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CACA 8.2.1/009
Report author	[redacted]
Report year	1995
Report title	Glyphosate acid: Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*)
Report No	BL5553/B
Document No	-
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1.
Deviations from current test guideline	Deviations from the current OECD 203 guideline (2019): None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The acute effects of glyphosate acid to bluegill sunfish (*Lepomis macrochirus*) was evaluated in a 96-hour static toxicity test performed at nominal test concentrations of 10, 18, 32, 56, 100 and 180 mg a.s./L. A dilution water only control was also included in the test. Ten fish were exposed in the control and in each treatment. All fish were observed at daily intervals over the 96 hour study duration, with mortality and sub-lethal signs of toxicity recorded.

Dissolved oxygen, pH and temperature were measured daily in each test vessel. Samples of control and test media were analysed for glyphosate acid at 0 hours (before fish addition) and after 48 and 96 hours. Glyphosate acid was not detected in the control group. The overall mean measured concentrations of glyphosate acid in the treatment groups ranged from 94.4 to 97% of nominal concentrations.

There was no fish mortality or sublethal effects observed in the control group, and in the 10, 18 and 32 mg a.s./L treatments. By 96 hours, there was 90% mortality in the 56 mg a.s./L treatment and 100 % mortality in the 100 and 180 mg a.s./L treatments. All validity criteria according to the OECD guideline 203 were fulfilled.
The 96 hour LC_{50} value for bluegill sunfish (*Lepomis macrochirus)* exposed to glyphosate acid was 47 mg a.s./L (nominal concentration) with 95% confidence interval of 35 to 66 mg a.s./L. The NOEC after 96 hours was 32 mg glyphosate acid/L (nominal concentration). The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 % a.s.

2. Vehicle and/or positive control:

Vehicle: dechlorinated, filtered tap water
Positive control: none

3. Test organism:

Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	Juvenile
Size:	Length: 26 to 35 g (mean = 30 mm)
Body weight:	0.29 to 0.96 g (mean = 0.54 g)
Loading:	10 test individuals for 20 L test solution
Source:	[redacted]
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimation period:	19 days at 22 °C prior to the test initiation

4. Environmental conditions:

Temperature:	22 ± 1 °C
Photoperiod:	16 hours with 20 min transition period
pH:	Control (start – 96 h): 7.3–6.8 10 mg/L (start – 96 h): 5.9 – 6.4 18 mg/L (start – 96 h): 5.2 – 5.8 32 mg/L (start – 96 h): 4.6 – 4.8 56 mg/L(start – 96 h): 3.8 – 3.9 100 mg/L (start – 24 h): 3.4 180 mg/L (start – 24 h): 3.1
Dissolved oxygen:	6.2 – 9.0 mg/L
Conductivity:	100 µS/cm
Hardness:	16.0 mg $CaCO_3$/L.

5. Dates of experimental work: November 20th to November 24th 1995

B. STUDY DESIGN

Experimental treatments: The acute toxicity test was performed at nominal concentrations of 10, 18, 32, 56, 100 and 180 mg a.s./L prepared using filtered and dechlorinated tap water treated with ultra violet steriliser. The test was conducted under static test conditions (no media renewal). A negative control group (dilution water only) was also prepared. A single vessel was prepared for the control and each test media group, each containing ten fish (27.5 L borosilicate glass vessels containing 20 L test medium).

hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. Samples of test media were analysed for glyphosate acid content using HPLC analysis at test initiation and after 48 and 96 hours.

Analytical procedures: Samples were taken from the centre of the test solutions. Glyphosate acid concentrations in the test solutions were determined at 0, 48 and 96 hours by high performance liquid chromatography method using a fluorescence detector. The samples were quantified against standards of glyphosate acid. Prior to analysis, samples and standards were derivatised using flourenylmethyl chlorformate, to prepare a fluorescing derivate.

Statistical calculations: The 96 hour LC_{50} values and 95% confidence intervals were calculated using non-linear interpolation. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The measured concentrations of glyphosate acid in fresh media at test initiation ranged between 96.9 and 110 % of nominal. In aged test media at 96 hours, mean measured glyphosate acid concentrations ranged between 94.4 and 97.0 % of nominal. At 100 and 180 mg a.s./L, no chemical analysis was performed at 48 and 96 hours, as all there was 100 % fish mortality within the first 24 hours following addition.

Table 8.2.1-23: Analytical results

Nominal concentration of glyphosate acid [mg a.s./L]	Measured concentration of glyphosate acid [mg a.s./L] at 48 hours	Measured concentration of glyphosate acid [mg a.s./L] at 96 hours	% of nominal
Dilution water control	< 0.023	< 0.023	-
10	10	9.7	100
18	19[2]	17[2]	100
32	33	31	100
56	57	54	98
100	100	[1]	100
180	180	[1]	100

[1] Not sampled, 100% mortality on previous sampling occasion
[2] mean of triplicate analysis

As measured concentrations of glyphosate acid were between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item. The limit of detection was 0.023 mg/L.

The 96 h LC_{50} value and corresponding NOEC value based on nominal concentrations are given below.

Table 8.2.1-24: Endpoints

Endpoints (96h)	Glyphosate acid [mg a.s./L]
LC_{50} (95% CI)	47 (35- 66)
NOEC	32

CI= Confidence interval

B. OBSERVATIONS

There were no mortalities in the control or the 10, 18 and 32 mg a.s./L treatments. At 56 mg a.s./L, there was 90 % mortality. There was 100 % mortality at 100 mg a.s./L and higher test concentrations that occurred after 24 hours.

There was a strong negative correlation between pH value and test item concentrations observed. At 56 mg a.s./L, the pH was reduced to 3.8 and lower.

The biological observations recorded during the test are presented in the table below.

Table 8.2.1-25: Effects of glyphosate acid to Lepomis macrochirus

Nominal concentration of glyphosate acid [mg a.s./L]	Number of dead fish / number of fish with intoxication symptoms [1] and observed symptoms			
	24 h	48 h	72 h	96 h
Control	0 / 0	0 / 0	0 / 0	0 / 0
10	0 / 0	0 / 0	0 / 0	0 / 0
18	0 / 0	0 / 0	0 / 0	0 / 0
32	0 / 0	0 / 0	0 / 0	0 / 0
56	4 / 4	8 / 8	9 / 9	9 / 9
100	[2]	[2]	[2]	[2]
180	[2]	[2]	[2]	[2]

[1]Dead fish are added to the sum of fish with symptoms
[2]All fish dead

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The 96 hour LC_{50} value for bluegill sunfish (*Lepomis macrochirus)* exposed to glyphosate acid was 47 mg a.s./L (nominal) with a 95% confidence interval of 35 to 66 mg a.s./L. The 96 hour NOEC was 32 mg a.s./L (nominal).

This study is considered valid and the acute LC_{50} value for bluegill sunfish exposed to glyphosate acid was 47 mg a.s./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CACA 8.2.1/010
Report author	[redacted]
Report year	1991
Report title	Glyphosate technical: 96-Hour Acute Toxicity Study (LC_{50}) in the Bluegill Sunfish
Report No	271642
Document No	-
Guidelines followed in study	EEC directive 92/69, Part C.1 OECD guidelines No. 203 (1992) EPA 540/9-82-024
Deviations from current test guideline	Deviation according to the current guideline OECD 203: -none.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate technical on bluegill sunfish (*Lepomis macrochirus*) were evaluated in a 96-hour static toxicity test conducted with nominal test concentrations of 59.3, 88.9, 133.3, 200 and 300 mg a.s./L, corresponding to 43.3, 91.0, 119, 173 and 243 mg a.s./L based on geometric mean measured concentrations. Furthermore, a blank control and a stability control with 300 mg a.s./L (nominal) was tested. Ten fish were exposed to each treatment.

Mortality and sublethal effects were recorded 2, 24, 48, 72 and 96 hours after the start of the test. Prior to the start of the test, all animals were weighed and measured. Dissolved oxygen, pH and temperature were also measured and recorded prior to addition of the test article and 2, 24, 48, 72 and 96 hours after the start of the test in each test chamber. Concentration of the test item was determined by HPLC in the untreated control and for all test concentrations shortly after addition of the test item and 2, 48 and 96 hours after the start of the test except from test concentrations with 100% mortality. During the test period of 96 hours the fish were exposed to mean concentrations ranging between 59.6 and 144.2 % (average for test concentrations of 59.3 to 300 mg test item/L) of nominal concentration.

No mortality or sublethal effects occurred at geometric mean measured concentrations of up to 119 mg/L. The mortality was 100% at the 173 mg a.s./L test concentration, based on geometric mean measured concentration. At these high test concentrations the pH was very low (3.2 – 3.6). All validity criteria according to the guideline OECD 203 were fulfilled.

The LC_{50} (96 h) for rainbow trout exposed to glyphosate technical ranged between 133.3 mg a.s./L and 200 mg a.s./L (nominal), corresponding to 119 mg a.s./L and 173 mg a.s./L (geometric mean measured). The 96 hour NOEC was 133.3 mg a.s./L (nominal), corresponding to 119 mg a.s./L (geometric mean measured).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	Glyphosate technical
Description:	solid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9 %

2. Vehicle and/or positive control: Vehicle: reconstituted water
Positive control: none

3. Test organism:

Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	juvenile; detailed age not stated
Size:	3.9 cm (mean), range: 3.5 – 4.4 cm
Body weight of the animals:	0.8 g (mean)
Loading:	0.4 – 0.7 g fish/L (10 fish per 15 litres of test medium)
Source:	[redacted]
Diet/Food:	none
Acclimation period:	7 days

4. Environmental conditions:

Temperature:	18.5 – 20.5 °C
Photoperiod:	16 hours light / 8 hours dark (500 – 1500 lux)
pH:	3.1 – 8.5
Dissolved oxygen:	8.9 – 11.2 mg O_2/L
Conductivity:	Not stated
Hardness:	250 mg $CaCO_3$/L (reconstituted water)

5. Experimental dates of work: September 17th to September 21th 1990

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, the definitive toxicity test was performed using nominal concentrations of 59.3, 88.9, 133.3, 200 and 300 mg a.s./L dissolved in reconstituted water. Also a stability test with 300 mg a.s./L without fish was conducted. The test was conducted in a static test setup. In addition, a control group was exposed to the test medium without test substance or other additives. There was one vessel for each test concentration and one for the control group, each containing 10 fish (15 L glass containers).

Observations: Assessment of sublethal effects of after 2, 24, 48, 72 and 96 hours was conducted, while mortality was recorded daily. Temperature, pH-value and oxygen saturation of the test solutions were measured at the same time points as sublethal effects and on test initiation. Prior to the start of the test, all animals were weighed and measured. Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis using samples taken at test start and after 2, 48 and 96 h (except where the mortality was already 100%)

Statistical calculations: Descriptive statistics; the Logit-Model could not be used, since the mortality rates of 0 and 100 % were within two concentrations.

II. RESULTS AND DISCUSSION

A. FINDINGS

The NOEC, LOEC and LC_{50} value are given below based on geometric mean measured concentrations.

Table 8.2.1-26: Endpoints

Endpoints (96 h)	Glyphosate technical [mg a.s./L]
LC_{50}	between 119 and 173
LOEC	between 119 and 173
NOEC	119

Analytical data: At nominal concentrations of 88.9, 133.3 and 200 mg a.s./L, the concentration of glyphosate technical was recorded to be within the range of 80 - 120 % of nominal. At 300 mg a.s./L the concentration at test start was 79.7 % and after 2 h at 82.3 % of nominal. At the lowest test concentration (59.3 mg/L) the concentration ranged between 59.6 and 84.1 % of nominal. Therefore, the toxicity values are based on (geometric) mean measured concentrations. Analytical results are shown below.

Table 8.2.1-27: Analytical results

Nominal concentration of glyphosate technical [mg a.s./L]	Time (hours)	Mean concentration of Samples A and B	% of nominal	Geometric mean measured concentrations [mg a.s./L]
59.3	0	44.75	75.5	43.3
59.3	2	44.65	75.3	
59.3	48	49.81	84.1	
59.3	96	35.37	59.6	
88.9	0	96.35	108.4	91
88.9	2	128.15	144.2	
88.9	48	75.10	84.5	
88.9	96	74.03	83.3	
133.3	0	120.3	90.2	119
133.3	2	123.1	92.3	
133.3	48	113.3	85.0	
133.3	96	120.0	90.0	
200	0	176.4	88.2	172
200	2	169.1	84.5	
300	0	239.1	79.7	243
300	2	146.9	82.3	

B. OBSERVATIONS

No mortality occurred at concentrations of up to 119 mg a.s./L. At the nominal concentrations of 173 and 243 mg a.s./L there was 100% mortality detected. At these high test concentrations the pH was below the critical point of 4 for *Lepomis macrochirus*. At 173 mg a.s./L sublethal effects like loss of righting reflex and an enhanced respiratory rate were observed. Supine positions at the tank bottom, affection of the

motoric function, remaining at the tank bottom and an enhanced respiratory rate were notices at 243 mg a.s./L.

Table 8.2.1-28: Effects of glyphosate technical on survival of *Lepomis macrochirus*

Nominal concentration of glyphosate technical [mg a.s./L]	Control	59.3	88.9	133.3	200	300
Geometric mean measured concentrations of glyphosate technical [mg a.s./L]	Control	43.3	91.0	119	173	243
Mortality (0h) [%]	0	0	0	0	0	10
Mortality (2 h) [%]	0	0	0	0	0	100
Mortality (24 h) [%]	0	0	0	0	90	100
Mortality (48 h) [%]	0	0	0	0	100	100
Mortality (72 h) [%]	0	0	0	0	100	100
Mortality (96 h) [%]	0	0	0	0	100	100

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
The LC_{50} (96 h) for bluegill sunfish exposed to glyphosate technical ranged between 133.3 mg a.s./L and 200 mg a.s./L (nominal), corresponding to 119 mg a.s./L and 173 mg a.s./L (geometric mean measured). The 96 hour NOEC was 133.3 mg a.s./L (nominal), corresponding to 119 mg a.s./L (geometric mean measured).

This study is considered valid and the acute LC_{50} value for bluegill sunfish exposed to glyphosate technical ranged between 119 mg a.e./L and 173 mg a.e./L (geometric mean measured) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/011
Report author	[redacted]
Report year	1981
Report title	Acute Toxicity of MON 0139 (lot LURT 12011) (AB-81-073) to Bluegill Sunfish (*Lepomis macrochirus*)
Report No	27201
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms
Deviations from current test guideline	Deviations from the current OECD 203 guideline (2019): Major: - No analytical verification of test concentrations - Dissolved oxygen concentration decreased below 60 % of saturation (from 9.5 mg/L to 5.5 mg/L in all tested groups: control, 100 and 1000 mg test item/L) Minor: - Fish were acclimatized for 48 hours prior to the test (7 days are required) - pH of the highest concentration (1000 mg test item/L) was not with the specified range of 6.0-8.5 (pH measured: 4.5 – 5.1)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary of the study according to OECD format

Executive Summary

The effects of glyphosate isopropylamine salt (MON 0139) on the bluegill sunfish (*Lepomis macrochirus*) were evaluated in a 96-hour static toxicity test. Based on the results of a range finding test, a definitive toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L, corresponding to 62.5, 112, 200, 350 and 625 mg glyphosate isopropylamine salt/L (mg a.s./L) or 46.3, 83.3, 148, 259 and 463 mg glyphosate/L (mg a.e./L). In addition, a control group was exposed to dilution water (soft reconstituted water) and a reference product (Antimycin A). The mortality of fish was recorded in all test concentrations and the control at 24, 48 and 96 hours. There was no mortality observed at any of the test concentrations up to and including 1000 mg test item/L, corresponding to 625 mg a.s./L or 463 a.e./L (nominal).

Not all validity criteria according to the OECD 203 (2019) were fulfilled since the analytical part of the study was not performed and/or reported. Taking also into account that the oxygen levels decreased below 60 %. And further minor deviations, that may affect the outcome of the study, the study is therefore considered as invalid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item:	Glyphosate isopropylamine salt (MON 0139)
Description:	Light yellow liquid
Lot/Batch #:	LURT 12011
Purity:	62.49%
2. Vehicle and/or positive control:	Vehicle: Soft reconstituted water Positive control: Antimycin A
3. Test organism:	
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	At least 14 days old
Size:	Length: 19 mm (mean)
Body weight:	0.14 g (mean)
Loading:	10 test individuals for 15 L test solution (= 0.09 g fish/L)
Source:	[redacted]
Diet/Food:	Daily with Standard commercial fish food (Rangen's) except 48 prior to the test
Acclimation period:	48 hours prior to the test initiation
4. Environmental conditions:	
Temperature:	22 ± 1°C
Photoperiod:	16 h light
pH:	7.1
Dissolved oxygen:	9.5 mg/L
Conductivity:	Not stated
Hardness:	45 mg $CaCO_3$/L
5. Experimental dates of work:	March 19th to March 23rd 1981

B. STUDY DESIGN

Experimental treatments: Based on the results of a 48-h range finding test, a definitive toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L. In addition, a control group was exposed to dilution water (soft reconstituted water) and a reference product (Antimycin A). The mortality of fish was recorded in all test concentrations and the control at 24, 48 and 96 hours. There was one vessel per treatment, containing ten fish in 5-gallon (appr. 19 L) glass vessels containing 15 L test medium.

Observations: The fish mortality was recorded in all test concentrations and the control 24, 48 and 96 hours after the test initiation. Temperature, pH-value and oxygen saturation of the test solutions were measured on each observation date. Hardness of the test water was measured at the start of the test. The weight and length of the test fish were measured.

Statistical calculations: LC_{50} values were calculated using computer program by Stephan et al. (1978) (A computer program for calculating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-

publication manuscript, August, 1978.)

II. RESULTS AND DISCUSSION

A. FINDINGS

No analytical verification of the tested concentrations was conducted or reported.
The LC_{50} value is given below based on nominal concentrations.

Table 8.2.1-29: Endpoints

Endpoints (96 h)	Test item [mg/L]	Glyphosate isopropylamine salt [mg a.s./L]	Glyphosate [mg a.e./L]
LC_{50}	>1000	>625	>463

B. OBSERVATIONS

There was no mortality observed at any of the test concentrations up to and including 1000 mg test item/L. For the reference product Antimycin A, the LC_{50} was determined to be 0.00010 mg/L. The dissolved oxygen concentration slightly dropped under 60% saturation. The pH values dropped with increasing test concentrations.

Table 8.2.1-30: Lethal effects of glyphosate isopropylamine salt (MON 0139) to *Lepomis macrochirus*

	Control					
Test item [mg/L]	-	**100**	**180**	**320**	**560**	**1000**
Glyphosate isopropylamine salt [mg a.s./L]	-	**62.5**	**112**	**200**	**350**	**625**
Glyphosate [mg a.e./L]	-	**46.3**	**83.3**	**148**	**259**	**463**
Mortality (24 h) [%]	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	0
Mortality (72 h) [%]	0	0	0	0	0	0
Mortality (96 h) [%]	0	0	0	0	0	0

The following validity criterion according to the OECD 203 (2019) was fulfilled:

- The control mortality was lower than 10 % at the end of the study.

The following validity criteria according to the OECD 203 (2019) were not fulfilled:

- No analytical measurement of the test concentrations was reported.
- The dissolved oxygen concentration was slightly below the trigger value of ≥60 % of the air saturation value (ranging from 9.5 to 5.5 mg/L in all tested groups: control, 100 and 1000 mg test item/L through the study).

The following points deviated from current guideline:

- Fish were acclimatised 48 hours prior to the test instead of the 7 required
- Observations occurred after 24h, 48h and 96h. The requirements are the following: a minimum of 2 observations within the first 24 hours of the study and on days 2 - 4 of the test, all vessels with living fish inspected twice per day (preferably early morning and late afternoon to best cover the 24-hour periods).
- The pH was outside of accepted range of 6.0-8.5 (pH measured: 4.9 – 5.1) in the highest concentration (1000 mg test item/L) and therefore the stock solution should have been adjusted.

These deviations may affect the outcome of the study, so the validity of the study is questionable.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a static acute fish toxicity test, the LC_{50} (96 h) for bluegill sunfish (*Lepomis macrochirus*) exposed to glyphosate isopropylamine salt (MON 0139) was determined to be >1000 mg test item/L, corresponding to >625 mg a.s./L or >463 mg a.e./L (nominal).
Not all validity criteria according to the OECD 203 (2019) were fulfilled since the analytical part of the study was not performed and/or reported. Taking also into account that the oxygen levels decreased below 60 % and further minor deviations that may affect the outcome of the study, the study is therefore considered as invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/012
Report author	[redacted]
Report year	1978
Report title	Acute Toxicity of Technical Glyphosate to Bluegill Sunfish (*Lepomis macrochirus*)
Report No	AB 78-123
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms
Deviations from current test guideline	Deviations from the current OECD 203 guideline (2019): Major: - No analytical verification of test concentrations.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary of the study according to OECD format

Executive Summary
The acute effects of glyphosate technical on bluegill sunfish (*Lepomis macrochirus*) in a 96-hour static toxicity test. A definitive toxicity test was performed with glyphosate technical at nominal concentrations of 28, 42, 56, 75, 100, 120, 140 and 180 mg glyphosate technical/L. A control group was exposed to deionised water and a reference treatment group exposed to Antimycin A were also tested.

In the definitive test, the mortality of fish was recorded at 24, 48 and 96 hours after test initiation. After 96 hours of exposure to glyphosate technical, there was 100% mortality recorded in the 140 and 180 mg a.s./L treatment groups. In the 120 mg a.s./L treatment group, there was 50% mortality recorded, with no mortality recorded at or below nominal concentrations of 100 mg a.s./L. The LC_{50} (96 h) was determined to be 120 mg a.s./L (nominal concentration of glyphosate technical).

According to the current OECD 203 test guideline, despite the control validity criteria of <10 % mortality being achieved, the validity of the present study according to OECD guideline 203 is questionable, since the analytical part of the study was not performed and/or reported. The study is considered supportive.

I. MATERIALS AND METHODS

A. MATERIALS	
1. Test material:	
Test item:	Glyphosate technical
Description:	Not stated
Lot/Batch #:	Not stated
Purity:	Technical grade (stated)
2. Vehicle and/or positive control:	Vehicle: Deionised water Positive control: Antimycin A
3. Test organism:	
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	Not stated
Size:	Length: 3.42 cm (mean)
Body weight:	0.96 g (mean)
Loading:	10 individuals test per vessel (19 L glass vessels) in 15 L test solution (0.64 g fish/L)
Source:	[redacted]
Diet/Food:	Daily with Standard commercial fish food (Rangen's No. 1 Fry) except 48 prior to the test
Acclimation period:	48 hours prior to the test initiation
4. Environmental conditions:	
Temperature:	21 ± 1°C
Photoperiod:	Not stated
pH:	6.8 – 7.0
Dissolved oxygen:	6.2 – 8.2 mg/L
Conductivity:	Not stated
Hardness:	46 mg $CaCO_3$/L
5. Experimental dates of work:	Test start: February 10th 1978

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, definitive toxicity test was performed with glyphosate technical at nominal concentrations of 28, 42, 56, 75, 100, 120, 140 and 180 mg a.s./L in a static test setup. The test item was dissolved directly into deionised water. A control group was also prepared using fish exposed to deionised water only (soft reconstituted water).
A reference toxicant test was conducted in parallel using Antimycin A at rates between 0.024 and 0.21 mg/L, with acetone used to prepare the reference toxicant group treatment media.
A single replicate vessel was prepared per treatment, control and reference toxicant group.

Observations: Mortality was recorded in all test concentrations and the control 24, 48 and 96 hours after test initiation in the glyphosate exposure test and additionally at 72 hours in the reference toxicant test.

Temperature, pH-value and oxygen saturation of the test solutions were measured on each observation date. Hardness of the test water was measured at the start of the test. Weight and length of the test fish were equally measured.

Statistical calculations: LC_{50} values were calculated along with the 95% confidence limits using Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} values are given below based on nominal concentrations.

Table 8.2.1-31: Endpoints

Endpoints (96 h)	Glyphosate [mg a.s./L]
LC_{50} (95% C.I.)	120 (111 - 130)

B. OBSERVATIONS

At and above the nominal concentration of 140 mg test item/L, 100 % mortality was observed 96 hours after test initiation. At the nominal concentration of 120 mg test item/L, 50 % mortality was recorded whereas no mortality was observed at and below the nominal concentration of 100 mg test item/L. For the highest concentration of reference product Antimycin A (0.021 mg/L), 70 % mortality was observed 24 hours after the test initiation and no fish survived 48 hours after test initiation.

Table 8.2.1-32: Lethal effects of glyphosate technical to *Lepomis macrochirus*

Glyphosate [mg a.s./L]	C	28	42	56	75	100	120	140	180
Mortality (24 h) [%]	0	0	0	0	0	0	30	100	100
Mortality (48 h) [%]	0	0	0	0	0	0	40	100	100
Mortality (72 h) [%]	0	0	0	0	0	0	50	100	100
Mortality (96 h) [%]	0	0	0	0	0	0	50	100	100

C = Control

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a static acute fish toxicity test, the LC_{50} (96 h) for bluegill sunfish (*Lepomis macrochirus*) exposed to the test item glyphosate was determined to be 120 mg a.s./L (nominal).

According to the current OECD 203 test guideline, despite the control validity criteria of <10% mortality being achieved, there was no chemical analysis performed to confirm glyphosate concentration in the test media. The study is therefore not be considered valid against the current criteria.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CACA 8.2.1/013
Report author	[redacted]
Report year	2006
Report title	Glyphosate Technical: Acute Toxicity to Common Carp (*Cyprinus carpio*)
Report No	2060/015
Document No	-
Guidelines followed in study	OECD Guideline 203 (1992); JMAFF Testing Guideline for Toxicology Studies, 12 NohSan No. 8147, Guideline 2-7-1(2000)
Deviations from current test guideline	Deviation compared with OECD 203 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate acid to common carp (*Cyprinus carpio*) were evaluated in a 96-hour semi-static toxicity test (48 hour renewal of test media) conducted as limit test at a nominal test concentration of 100 mg a.s./L. A negative control (dechlorinated tap water) was prepared in parallel. Duplicate control and test vessels were prepared, each containing seven fish.

All fish were observed for sub-lethal effects and mortality at 3, 6, 24, 48, 72 and 96 hours after the start of the test (fish addition). Dissolved oxygen, pH and temperature were measured and recorded daily in each test vessel. Glyphosate acid concentrations were measured at 0, 24 and 96 hours. Glyphosate acid was not detected in the control group. Mean measured concentrations ranged from 90 to 98 % of nominal concentrations.

No mortality or sub-lethal effects to common carp (*Cyprinus carpio*) were observed, when exposed to glyphosate acid at the nominal concentration of 100 mg a.s./L. All validity criteria according to the guideline OECD 203 were fulfilled.

Glyphosate acid resulted in no mortality or sub-lethal effects in common carp at 100 mg a.s./L. The 96 h LC_{50} value for common carp exposed to glyphosate acid was determined to be > 100 mg a.s./L, the highest concentration tested. The NOEC was 100 mg glyphosate acid/L. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item:	Glyphosate acid
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 %

2. Vehicle and/or positive control:	Vehicle: Dechlorinated tap water Positive control: Pentachlorophenol sodium salt (tested in a different study)
3. Test organism:	
Species:	Common carp (*Cyprinus carpio*)
Age:	Juvenile
Size:	4.2 ± 0.1 cm
Body weight:	2.05 ± 0.13 g
Loading:	0.72 g body weight/L test solution
Source:	[redacted]
Diet/Food:	no feeding during the total test period
Acclimation period:	12 days at test conditions
4. Environmental conditions:	
Temperature:	20.6 – 21.2 °C
Photoperiod:	16 hours light / 8 hours dark, with 20 minutes dawn and dusk transition
pH:	7.4 – 8.3 (control), 6.3 – 8.0 (treatment)
Dissolved oxygen:	8.1 - 8.8 mg/L (91 – 99 % saturation at 20.6 – 21.2 °C)
Conductivity:	359 – 610 µS/cm
Hardness:	Approx. 100 mg $CaCO_3$/L.
5. Dates of experimental work:	2005-05-31 to 2005-06-04

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, a final toxicity test was performed under semi-static test design as limit test using a single nominal concentration of glyphosate acid of 100 mg a.s./L. The control and test media at 100 mg a.s./L were renewed at 48 hours. A negative control group (derchlorinated water) was also prepared in parallel. There were duplicate glass vessels for the test concentration and control, each containing seven test fish in 20 L test medium.

Observations: All fish were observed for sub-lethal effects and mortality after 3, 6, 24, 48, 72 and 96 hours after test initiation (fish addition). Test solutions were renewed after 48 hours. Water temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Water hardness was measured in fresh media only. Samples of fresh media were taken at o hours and samples of old test media were taken at 24 and 96 hours to be analysed for glyphosate using a HPLC method of analysis.

Statistical calculations: Since the mortality was <50 %, no statistical calculation of LC_{50} values was possible. Therefore, NOEC and LC_{50} were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Mean measured test item concentrations ranged from 90 % to 98 % of nominal test concentration. Therefore, endpoints were evaluated using nominal test item concentrations.

Table 8.2.1-33: Analytical results

Sample	Nominal concentration of glyphosate acid [mg a.s./L]	Measured concentration glyphosate acid [mg a.s./L]	% of nominal
0 h (fresh media)	control	<LOQ	-
	100	95.2	95
	100	97.8	98
24 h (old media)	control	<LOQ	-
	100	90.3	90
	100	92.9	93
96 h (old media)	control	<LOQ	-
	100	98.1	98
	100	98.4	98

LOQ= Limit of quantification (5.3 mg/L)

The 96 h LC_{50} and corresponding NOEC values based on nominal concentrations are given below.

Table 8.2.1-34: Endpoints

Endpoints (96 h)	Glyphosate acid [mg a.s./L]
LC_{50}	>100
NOEC	100

Reference test: The 96 h LC50 for the reference item pentachlorophenol was 0.26 mg/L, which is within the normal range of the reference material. The reference item was tested in a separate study.

B. OBSERVATIONS

During the acclimation the fish were fed with ZM Large Granule Feed as opposed to Commercial Car Pellets as this feed type was considered to be more suitable for the size of the fish. This deviation did not have any negative impact on the study validity.

At the 100 mg a.s./L concentration, there was no mortality during the 96 hours of exposure to glyphosate acid. In addition, no sub-lethal effects were observed.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The 96 h LC_{50} for common carp (*Cyprinus carpio*) exposed to glyphosate acid in a limit test was determined to be >100 mg a.s./L, with a 96 hour NOEC of 100 mg glyphosate a.s./L.

This study is considered valid and the acute LC_{50} value for common carp exposed to glyphosate acid was determined to be >100 mg a.s./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.1/014
Report author	[redacted]
Report year	1973
Report title	Information not available
Report No	95-00015
Document No	-
Guidelines followed in study	Information mentioned in the Monograph: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, accepted in RAR (2015).
Short description of study design and observations:	Toxicity of technical glyphosate (purity> 94%) to aquatic organisms (*Cyprinus carpio*) in a 96 hours static test
Short description of results:	LC_{50} = 115 mg a.e./L
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The full study report is not available to the applicant. However these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR (2015).
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 8.2.1/015
Report author	[redacted]
Report year	2000
Report title	Acute Toxicity of Glifosate Técnico Nurfarm to Zebrafish (*Brachydanio rerio*)
Report No	RF-D61.47/99
Document No	-
Guidelines followed in study	OECD Guideline 203 (1993)
Deviations from current test guideline	Deviation compared with OECD 203 – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary of the study according to OECD format

Executive Summary

The acute effects of glyphosate technical on zebra fish (*Brachdanio rerio* – also known as *Danio rerio*) were evaluated in a 96-hour semi-static toxicity test (48 hour media renewal) conducted at nominal test concentrations of 10, 32, 56, 100, 180, 320 mg a.s./L. A control (reconstituted water) was also prepared. Vessels were prepared in duplicate with ten fish per vessel.

Observations for fish mortality and sub-lethal effects were performed at 3, 24, 48, 72 and 96 hours after the start of the test (fish addition). Dissolved oxygen, pH and temperature were measured and recorded daily in each test vessel. Glyphosate technical concentrations were measured in new and old control and test media on each day of the test. Glyphosate technical was not detected in the control group. Overall mean measured concentrations of glyphosate techncial ranged between 95.9 and 108.8 % of nominal concentrations.

During the 96-hour exposure period to glyphosate technical, at nominal concentrations up to 56 mg a.s./L, there were no sub-lethal effects or mortality recorded. At the concentration of 100 mg a.s./L, there was 15 % mortality with hyperactivity observed in test fish at 48 hours onwards. At a concentration of 180 mg a.s./L and above, there was 100% mortality observed after 24 hours.

The 96 hour LC_{50} for zebra fish exposed to glyphosate technical was determined to be 122.91 mg a.s./L (nominal) with a 95% confidence interval of 111.97 to 134.92 mg a.s./L. The 96-hour NOEC was 56 mg a.s./L (nominal concentrations of glyphosate technical).

I. MATERIALS AND METHODS

A. MATERIALS **1. Test material:**	
Test item:	Glyphosate Tecnico Nufarm
Description:	White powder
Lot/Batch #:	037-919-113
Purity:	95.0 % a.s. (nominal), 95.49 % a.s.(analysed)
2. Vehicle and/or positive control:	Vehicle: Reconstituted water Positive control: Potassium dichromate ($K_2Cr_2O_7$)
3. Test organism:	
Species:	Zebra fish (*Brachydanio rerio*)
Age:	Not stated
Size:	Not stated
Body weight of the animals:	0.191 -0.239 g
Loading:	(0.38 to 1.44 g fish/L) 10 specimens exposed in 3 L test solution
Source:	In-house culture, previously obtained from the commercial supplier [redacted]
Diet/Food:	no feeding during the total test period
Acclimation period:	72 h (to dilution water) prior to the test initiation (no feeding 24 h prior to test start and during the test)
4. Environmental conditions:	
Temperature:	24.1 – 24.5 °C
Photoperiod:	16 hours
pH:	Control (start – 96 h): 7.4 – 7.5 10 mg/L (start – 96 h): 7.3 – 7.1 32 mg/L (start – 96 h): 7.0 – 6.6 56 mg/L(start – 96 h): 6.5 – 5.3 100 mg/L (start – 96 h): 5.1– 4.8 180 mg/L (start – 24 h): 4.1 – 4.0 320 mg/L (start – 24 h): 3.5 – 3.6
Dissolved oxygen:	4.9 – 5.8 mg O_2/L (61.72 % - 73.06 % of saturation value at 24.5 °C)
Conductivity:	691 - 711 µS/cm
Hardness:	229.7 – 249.9 mg $CaCO_3$/L.
5. Dates of experimental work:	18th October to 22nd October 1999

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, a definitive toxicity test was performed with glyphosate technical at nominal concentrations of 10, 32, 56, 100, 180, 320 mg a.s./L in a semi-static test setup, with test media renewal after 48 hours. A negative control (reconstituted water only) was also prepared. There were two vessels per treatment, containing ten fish each (4000 mL glass vessels containing 3000 mL test medium).

Observations: All fish were observed for sublethal effects and mortality after 3, 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Weight measurements were conducted of each individual fish at test initiation. Samples of test media were analysed using HPLC analysis at test initiation and after 48 and 96 hours.

Analytical procedures: Aliquots of exposure concentrations were collected at each test solution renewal. The active ingredient was analysed by Liquid Chromatography HP 1050 (according to SOP-M.365 – Determination of Active Ingredient Metsulfuron metil in Formulation).

Statistical calculations: LC_{50} values, along with respective 95% confidence limits were calculated using the Trimmed Spearman-Karber Method. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Mean measured concentrations of glyphosate acid ranged between 95.5 % and 108.8 % of the nominal test concentrations. As values were between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal test item concentration.

Table 8.2.1-35: Analytical results

Nominal concentration of glyphosate technical [mg a.s./L]	Mean measured concentration of glyphosate technical [mg a.s./L]	% of nominal
Dilution water control	0	-
10	10.83	108.3
32	33.28	104.0
56	58.37	104.2
100	108.80	108.8
180	171.96	95.5
320	346.34	108.2

The 96 h LC50 and corresponding NOEC values based on nominal concentrations are given below.

Table 8.2.1-36: Endpoints

Endpoints (96 h)	Glyphosate technical [mg a.s./L]
LC_{50} (95% CI)	122.91 (111.97 – 134.92)
NOEC	56

CI= Confidence interval

B. OBSERVATIONS

At the 180 mg a.s./L concentrations and higher, 100% mortality was observed after 24 hours exposure to glyphosate technical. At 100 mg a.s./L, there was 20% mortality after 72 hours and 30 % mortality after 96 hours, with hyperactivity observed in test fish at 48 hours onwards. At 56 mg a.s./L and lower, no fish mortalities or sub-lethal effects were observed throughout the test period.
The biological observations recorded during the test are presented in the table below.

Table 8.2.1-37: Lethal effects of glyphosate acid to zebra fish

Nominal concentration of glyphosate technical [mg a.s./L]	Number of dead fish and observed symptoms				
	3 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
10	0	0	0	0	0
32	0	0	0	0	0
56	0	0	0	0	0
100	0	0	0 HA	2 HA	3 HA
180	0 LE	10	10	10	10
320	9 LE	10	10	10	10

[1] Dead fish are added to the sum of fish with symptoms
LE loss of equilibrium
HA hyperactivity

The 96 h LC_{50} (95% CL) for the reference product was calculated to be 79.54 (68.87 – 91.88) mg/L based on nominal concentrations.
All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
The 96 hour LC_{50} for zebra fish (*Brachydanio rerio*) exposed to glyphosate technical was 123 mg a.s./L (nominal) with a 95% confidence interval of 111.97 to 134.92 mg a.s./L. All validity criteria according to OECD 203 were fulfilled. The 96-hour NOEC was 56 mg a.s./L (nominal concentration of glyphosate technical).
Since the analytical methods and substance verification were not documented in detail the study is therefore considered as supportive for the risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/016
Report author	[redacted]
Report year	1993
Report title	Acute Toxicity Testing in Fish, Test Article: 'Glyphosate isopropylamine salt'
Report No	80-91-2328-02-93
Document No	-
Guidelines followed in study	OECD Guideline 203; EEC Directive 92/69
Deviations from current test guideline	Deviations to OECD 203 (2019): Major: - None. Minor: - Test species: *Leuciscus idus* - Loading rate: slightly above 1 g fish/L (1.065 g fish/L)
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2.Full summary of the study according to OECD format

Executive Summary

The effects of glyphosate isopropylamine salt on golden orfe (*Leuciscus idus*) were evaluated in a 96-hour static toxicity test. The toxicity test was performed using nominal concentrations of 498, 887, 1578, 2809 and 5000 mg test item/L, corresponding to 307, 546, 972, 1730 and 3080 mg glyphosate isopropylamine salt/L (mg a.s./L) or 227, 405, 720, 1282 and 2282 mg glyphosate/L (mg a.e./L). Further a dechlorinated and deionised tap water control was used. Ten fish were exposed to each treatment level.

Mortality was recorded after 2, 4, 24, 48, 72 and 96 hours after the start of the test. Records on visible abnormalities were equally made. At termination of the test, all animals were weighed and measured.

Analytical control measurements of the actual concentrations of the test item were performed by mean of HPLC analysis. Glyphosate isopropylamine salt levels were determined based on the concentrations of glyphosate. Three representative concentrations (498, 1578 and 5000 mg test item/L, corresponding to 307, 972 and 3080 mg a.s./L or 227, 720 and 2282 mg a.e./L) were analysed at 24 h intervals.

At and below the nominal concentration of 5000 mg test item/L, no mortality was observed during the exposure period. In comparison to the control group, no abnormal effects were seen at or below the highest concentration tested. All validity criteria according to the guideline OECD 203 were fulfilled.

In a static acute toxicity study of glyphosate isopropylamine salt, the LC_{50} (96 h) for golden orfe (*Leuciscus idus*) was determined to be > 5000 mg test item/L, corresponding to 3080 mg glyphosate isopropylamine salt/L (mg a.s./L) or 2282 mg glyphosate/L (mg a.e./L) (nominal). The NOEC was determined to be ≥5000 mg test item/L, corresponding to ≥3080 mg glyphosate isopropylamine salt/L or ≥ 2282 mg glyphosate (mg a.e./L) (nominal). The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt
Description:	viscous liquid
Lot/Batch #:	01/06/93
Purity:	61.6% Glyphosate isopropylamine salt
Density:	1.23 g/cm^3 at 20°C
2. Vehicle and/or positive control:	Vehicle: dechlorinated deionised tap water Positive control: none
3. Test organism:	
Species:	Golden orfe (*Leuciscus idus*)
Age:	not stated
Size and Weight:	5.90 cm (mean length of 10 representative individuals), 2.13 g mean body weight
Loading:	10 L for 5 fish (1.065 g fish/L)
Source:	[redacted]
Diet/Food:	no feeding during test
Acclimation period:	≥ 48 h in a 250 L glass aquarium under general test conditions
Body weight of the animals:	2.13 g (mean body weight of all individuals)
4. Environmental conditions:	
Temperature:	18.8-21.6°C
Photoperiod:	16 hours light / 8 hours dark, 600 – 800 lux
pH:	7.5 – 8.5
Dissolved oxygen:	> 60% of air saturation (approx. 6.0 mg O_2/L)
Conductivity:	not stated
Hardness:	14° dH (1dH= 10 mg CaO/L)
5. Experimental dates of work:	03rd September to 19th September 1993

B. STUDY DESIGN

Experimental treatments

Based on the results of a range finding test, the definitive toxicity test was performed using nominal concentrations of 498, 887, 1578, 2809 and 5000 mg test item/L in a static test setup. In addition, a control group was exposed to dechlorinated and deionised tap water only. There were two vessels per treatment, each containing five fish (12 L glass containers containing 10 L test medium).

Observations

Assessment of effects and mortality of test fish after 2-4, 24, 48, 72 and 96 hours was conducted. Temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Hardness of the test water was measured at the start of the test. Mortality was recorded on each observation date. Records on visible abnormalities were equally made. At start and termination of the test, all animals were weighed and measured. Analytical control measurements of the actual concentrations of the test item were performed by mean of HPLC analysis. Glyphosate isopropylamine salt levels were determined based on

the concentrations of glyphosate. Three representative concentrations (498, 1578 and 5000 mg test item/L, corresponding to 307, 972 and 3080 mg a.s./L or 227, 720 and 2282 mg a.e./L) were analysed at 24 h intervals.

Statistical calculations: Descriptive statistics

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} values are given below based on nominal concentrations.

Table 8.2.1-38: Endpoints

Endpoints (96 h)	Test item [mg/L]	Glyphosate isopropylamine salt [mg a.s./L]	Glyphosate [mg a.e./L]
LC_{50}	>5000	>3080	>2282
NOEC	5000	3080	2282
LOEC	5000	3080	2282

Analytical data: Analytical control measurements were performed on three representative concentration levels of glyphosate isopropylamine salt, at 498, 1578 mg test item/L and 5000 mg test item/L. Before introduction of the fish 81.8%, 94.6% and 96.2% of glyphosate were recovered at 498, 1578 and 5000 mg test item/L, respectively. In the aged test media 85.3%, 103.9% and 90.8% of the nominal concentration were recovered. Consequently, during the test period of 96 hours the fish were exposed to a mean concentration of 93.3% (average for test concentrations of 498, 1578 and 5000 mg test item/L, respectively) of nominal concentration.
As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 8.2.1-39: Analytical results

Nominal concentration of the test item [mg/L]	Nominal concentration of glyphosate [mg a.e./L]	Time (hours)	Measured concentration of glyphosate [mg a.e./L]	% of nominal
498	227	0	186	81.8
		48	194	85.5
		96	194	85.3
1578	720	0	681	94.6
		48	665	92.4
		96	748	103.9
5000	2282	0	2196	96.2
		48	2215	97.1
		96	2072	90.8

B. OBSERVATIONS

Clinical observations:

At or below the nominal concentration of 5000 mg test item/L, no mortality was observed during the exposure period.
In comparison to the control group, no abnormal effects were seen at or below the concentration of 5000 mg test item/L.

Table 8.2.1-40: Lethal effects of glyphosate isopropylamine salt to golden orfe

	Control					
Test item [mg/L]	**-**	**498**	**887**	**1578**	**2809**	**5000**
Glyphosate isopropylamine salt [mg a.s./L]	**-**	**307**	**546**	**972**	**1730**	**3080**
Glyphosate [mg a.e./L]	**-**	**227**	**405**	**720**	**1282**	**2282**
Mortality (2-4 h) [%]	0	0	0	0	0	0
Mortality (24 h) [%]	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	0
Mortality (72 h) [%]	0	0	0	0	0	0
Mortality (96 h) [%]	0	0	0	0	0	0

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a static acute toxicity study of glyphosate isopropylamine salt, the LC_{50} (96 h) for golden orfe (*Leuciscus idus*) was determined to be > 5000 mg test item/L, corresponding to 3080 mg glyphosate isopropylamine salt/L (mg a.s./L) or 2282 mg glyphosate/L (mg a.e./L) (nominal). The NOEC was determined to be ≥5000 mg test item/L, corresponding to ≥3080 mg glyphosate isopropylamine salt/L or ≥ 2282 mg glyphosate (mg a.e./L) (nominal).

This study is considered valid and the acute LC_{50} value for golden orfe exposed to glyphosate isopropylamine salt was determined to be >2282 mg a.e./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/017
Report author	[redacted]
Report year	1998
Report title	96-Hour Acute Toxicity Study in Rainbow trout with (Aminomethyl)Phosphonic Acid (Static)
Report No	232469
Document No	-
Guidelines followed in study	EEC directive 92/69, Part C.1 OECD guidelines No. 203 (1992).
Deviations from current test guideline	Deviation compared with OECD 203 – none
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2.Full summary of the study according to OECD format

Executive Summary

The toxicity of AMPA (Aminomethyl- phosphonic acid) on rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour static toxicity test conducted as a limit test at a nominal test concentration of 100 mg/L. A negative control (dilution water only) was prepared in parallel. Seven fish were added to the control and each AMPA treated vessel.

Observations for sub-lethal effects and mortality were performed at 2, 24, 48, 72 and 96 hours after the start of the test (fish addition). Dissolved oxygen, pH and temperature were measured and recorded daily in each test chamber. AMPA concentrations were measured at 0 (freshly prepared test media before fish addition) and 96 hours (test end). AMPA was not detected in the control group. Mean measured concentrations ranged between 97 to 105% of nominal concentrations. Toxicity was evaluated based on the nominal concentrations.

There were no sub-lethal effects or fish mortality observed at the nominal 100 mg/L concentration during the 96 h exposure to AMPA. All validity criteria according to the guideline OECD 203 were fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item::	Aminomethyl - phosphonic acid (AMPA)
Description:	White powder
Lot/Batch #:	A010047101
Purity:	99%
2.Vehicle and/or positive control:	Vehicle: Tap water Positive control: Pentachlorophenol
3. Test organism:	
Species:	Rainbow trout (*Oncorhynchus mykiss*)

Age:	Juveniles
Size:	4.14 ± 0.34 cm
Body weight of the animals:	0.54 ± 0.20 g (mean weight of 10 representative individuals)
Loading:	0.38 g fish/litre (7 fish per 10 litres of test medium)
Source:	[redacted]
Diet/Food:	Last feeding at about 30 hours prior to the test and no feeding during the total test period
Acclimation period:	At least 12 days after delivery
4. Environmental conditions:	
Temperature:	14.2 – 14.8°C
Photoperiod:	16 hours light / 8 hours dark
pH:	7.3 – .8.4
Dissolved oxygen:	9.3 – 9.7 mg O_2/L
Conductivity:	Not stated
Hardness:	2.4 mmol/L
5. Experimental dates of work:	24th May to 29th 1998

B. STUDY DESIGN

Experimental treatments: The test was conducted as a static (without renewal) 96 hour limit test at a nominal test concentration of 100 mg/L of AMPA, based on the results of a range finding test. The test media was prepared by direct addition of AMPA to tap water. A negative control (dilution water) was prepared in parallel. Single vessels (18-L glass aquariums) containing 10 litres of control, or test media were prepared. Seven fish were added to each vessel at the start of the test.
Observations: All fish were observed for sub-lethal effects and mortalities after 2, 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Hardness of the test water was measured at test initiation only.
Prior to the start of the test, ten representative fish from the fish stock used in the test were weighed (wet weight (g)) and measured (total length (cm)).
Samples of control or test media were taken at test start (0 hours) before fish addition and at 96 hours (test end). Concentrations of AMPA in each sample were determined using an HPLC method of analysis.
Statistical calculations: Since the mortality was < 50%, no statistical calculation of LC_{50} values was possible. Therefore, NOEC and LC_{50} were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

Analytical data: Measured concentrations of AMPA in media samples taken at the start of the test before fish introduction were 105% of nominal. At the end of the test, concentrations in the aged test media were 97 % of nominal.

Table 8.2.1-41: Analytical results

Nominal concentration of AMPA [mg/L]	Time [hours]	Measured concentration of AMPA [mg/L]	% of nominal
water control	0	n.d.	-
100	0	105	105
water control	96	n.d.	-
100	96	96.7	97

n.d. = not determined

The mean measured concentration of AMPA ranged between 80 and 120% of nominal, therefore the ecotoxicological endpoints were evaluated based on the nominal AMPA concentrations.

The 96 hour LC_{50} and NOEC values for rainbow trout exposed to AMPA are given below.

Table 8.2.1-42: Endpoints

Endpoints (96 h)	Aminomethyl-phosphonic acid (AMPA) [mg/L]
LC_{50}	>100
NOEC	≥100

Reference test: The determined 96 h-LC_{50} for the reference item pentachlorophenol was 0.30 mg/L, which correspond well with the historical range of 0.10 - 0.46 mg/L. Thus, the sensitivity of trout from the present batch corresponded with the historical data.

B.OBSERVATIONS

There were no sub-lethal effects or mortality observed in fish exposed to AMPA during the 96 hours limit test at 100 mg/L.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Under the conditions of the present test AMPA induced no visible effects in rainbow trout at 100 mg/L (nominal). The 96 h LC_{50} for rainbow trout exposed to AMPA was determined to be >100 mg/L (nominal). The 96 hour NOEC for rainbow trout exposed to AMPA was considered to be ≥100 mg/L (nominal), the maximum concentration tested.

This study is considered valid and the acute LC_{50} value for rainbow trout exposed AMPA was determined to be ≥100 mg/L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.1/018
Report author	Anonymous
Report year	1994
Report title	No information available
Report No	94-00499
Document No	-
Guidelines followed in study	Information mentioned in the Monograph 2001: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Information mentioned in the Monograph: The data presented below were generated in accordance with […] the appropriate GLP-requirements.
Previous evaluation	Yes, accepted in RAR (2015).
Short description of study design and observations	Acute toxicity of the metabolite aminomethyl phophenic acid (AMPA) to Rainbow trout (*Oncorhynchus mykiss*) static test, 96 hours.
Short description of results	Test item: AMPA LC_{50} 96 h >180 mg/L NOEC 96 h > 8 mg/L
Reasons for why the study is not considered relevant/reliable or not considered as key study	The full study report is not available to the applicant. However, these data were provided in the Monograph 2001. Study was considered as valid in the Monograph 2001 but it was not mentioned in the RAR 2015. The study is therefore not considered valid.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a “request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 8.2.1/019
Report author	[redacted]
Report year	1991
Report title	Acute Toxicity of AMPA to Rainbow Trout (*Oncorhynchus mykiss*)
Report No	AB-90-402
Document No	-
Guidelines followed in study	OECD Guideline 203; Guideline 72-1; U.S. EPA-FIFRA, 40 CFR, Section 158.145
Deviations from current test guideline	Deviation compared with OECD 203 (2019) – none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The effects of AMPA on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. The toxicity test was performed with AMPA at nominal concentrations of 32, 56, 100, 180, 320, 560 and 1000 mg/L. In addition, a control group was exposed to dilution water (soft blended water). There was one vessel per treatment containing ten fish (19 L glass vessels containing 15 L test medium).

The fish mortality, loss of equilibrium, light discoloration, dark discoloration, fish on the bottom of test chamber, surfacing, quiescence, erratic swimming, excitability and/or laboured respiration were observed in all test concentrations and the control every 24 hours until finalisation of the test (24, 48, 72 and 96 hours). Dead individuals were removed from the test vessels after each observation.

80% and 90% mortality after 96 hours was observed in the 560 and 1000 mg/L test item treatments, respectively. Laboured respiration was noted in the 56 and 100 mg/L test item treatments only at 3 hours of exposure to AMPA. No abnormal effects were noted in these two chambers after this time. All validity criteria according to the OECD guideline 203 were fulfilled.

In a static acute fish toxicity test, the LC_{50} (96 h) for rainbow trout (*Oncorhynchus mykiss*) exposed to AMPA was determined to be 520 mg/L. The NOEC of 32 mg/L is based on the assessment after 3 hours, therefore the relevant NOEC at 96 h was determined to be 100 mg /L. The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item:	AMPA ((Aminomethyl)phosphonic acid)
Description:	White powder
Lot/Batch #:	HET-9001-M63T
Purity:	94.38%
2. Vehicle and/or positive control:	Vehicle: Soft blended water Positive control: none
3. Test organism:	

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	not stated
Size:	3.9 ± 0.3 cm
Body weight:	0.79 ± 0.19 g
Loading:	0.53 g/L test solution
Source:	[redacted]
Diet/Food:	none
Acclimation period:	72 h (To the test temperature) prior to the test initiation (No feeding during the acclimation period)
4. Environmental conditions:	
Temperature:	12°C
Photoperiod:	16 hours light daily, with 30 minutes transition period (110 footcandles)
pH:	4.2 - 7.6.
Dissolved oxygen:	7.1 - 9.4 mg/L (69 – 91 % saturation at 12°C)
Conductivity:	130 µMhos/cm
Hardness:	40-48 mg $CaCO_3$/L.
5. Dates of experimental work:	26th to 30th October, 1990

B. STUDY DESIGN

Experimental treatments

Based on the results of a range finding test, definitive toxicity test was performed with AMPA at nominal concentrations of 32, 56, 100, 180, 320, 560 and 1000 mg/L with one vessel per treatment, each containing ten fish (19 L glass vessels containing 15 L test medium).

Observations

Mortality, loss of equilibrium, light discoloration, dark discoloration, fish on the bottom of test chamber, surfacing, quiescence, erratic swimming, excitability and/or laboured respiration were monitored in all test concentrations and the control every 24 hours for 96 hours test duration (24, 48, 72 and 96 hours). Any dead individuals were removed from the test vessels after each observation. Temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis in all test concentrations with live fish. Hardness of the test water was measured at the start of the test. Mortality was recorded on each observation date. Records on visible abnormalities were equally made. Weight and length measurements were made on the control group of fish at the termination of the test. Analytical control measurements of the actual concentrations of the test item were performed and the results are reported in a separate study (study number: ML-90-403).

Statistical calculations

The LC_{50} values, along with their respective confidence limits were calculated using a computerized program developed by Stephan *et al*. (1978) (A computer program for calculating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.)

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: According to the results presented in the analytical study (study number ML-90-403), mean recovery of the test item was 102 ± 1.6% of the nominal test concentrations. Therefore, the ecotoxicological endpoints were based on nominal concentrations of the test item.

According to the current requirements the 3 hours observation time point is not relevant, and therefore based on 24h and 72h observations, the NOEC can be set to 100 mg/L (data detailed in the effect table in observation part of the summary). The LC_{50} and NOEC values are given below based on nominal concentrations.

Table 8.2.1-43: Endpoints

Endpoints (96 h)	**AMPA [mg/L]**
LC_{50} (95% CI)	520 (410 - 660)
NOEC	100

CI= Confidence interval

B. OBSERVATIONS

Environmental observations:

The pH decreased as the concentration of AMPA increased.

Clinical observations:

80% and 90% mortality was observed in the 560 and 1000 mg/L test concentrations after 96 hours exposure to AMPA, respectively. Laboured respiration was noted in the 56 and 100 mg/L concentrations only after 3 hours of exposure to AMPA. No abnormal effects were noted in these two chambers after this time. At or above the concentration of 320 mg/L, different abnormalities were observed and reported in the table below.

Table 8.2.1-44: Lethal effects of AMPA to rainbow trout

	Control	**AMPA [mg/L]**						
	-	**32**	**56**	**100**	**180**	**320**	**560**	**1000**
Mortality (3h) [%]	0	0	0	0	0	0	0	0
Symptoms (3h) [%]	100[1]	100[1]	60[1] 40[2]	30[1] 70[2]	10[1] 90[2]	100[2]	100[2]	100[2]
Mortality (24h) [%]	0	0	0	0	0	0	0	10
Symptoms (24h) [%]	100[1]	100[1]	100[1]	100[1]	90[1] 10[2]	60[1] 40[2]	20[1] 80[2]	100[2]
Mortality (48h) [%]	0	0	0	0	0	0	10	10
Symptoms (48h) [%]	100[1]	100[1]	100[1]	100[1]	100[1]	100[2]	100[2]	100[2]
Mortality (72h) [%]	0	0	0	0	0	0	20	70
Symptoms (72h) [%]	100[1]	100[1]	100[1]	100[1]	90[1] 10[2]	100[2]	100[2]	100[2]
Mortality (96h) [%]	0	0	0	0	0	0	80	90
Symptoms (96h) [%]	100[1]	100[1]	100[1]	100[1]	100[1]	100[2]	100[2]	100[2]

[1] normal;

[2]affected (this could be surfacing; on bottom of test vessel, quiescent, laboured respiration and loss of equilibrium; dark discoloration)

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a static acute fish toxicity test of AMPA, the LC_{50} (96 h) for rainbow trout (*Oncorhynchus mykiss*) exposed to AMPA was determined to be 520 mg/L. The NOEC (96 h) was determined to be 100 mg/L.

This study is considered valid and the acute LC_{50} value for rainbow trout exposed to AMPA was determined to be 520 mg/L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/020
Report author	[redacted]
Report year	1993
Report title	AMPA: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)
Report No	X582/A
Document No	
Guidelines followed in study	OECD 203
Deviations from current test guideline	Deviations to OECD 203 (2019): none
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of AMPA technical (Aminomethylphosphonic acid) to rainbow trout (*Oncorhynchus mykiss*) was evaluated in a 96-hour static toxicity test conducted with nominal test concentrations of 18, 32, 56, 100 and 180 mg/L. Furthermore, a dilution water control was tested. Ten fish were exposed to each treatment (1 replicate per concentration).

Mortality was recorded, 24, 48, 72 and 96 hours after the start of the test. Records on visible abnormalities were equally made. Dissolved oxygen, pH and temperature were measured and recorded daily in each test chamber. Test item concentrations were verified at 0, 48 and 96 hours by HPLC. Mean measured concentrations ranged from 100 to 111% of nominal concentrations.

No mortality occurred during the 96 h exposure time. Sub-lethal effects like dark discolouration, sounding and loss of balance were recorded starting at a concentration of 32 mg test item/L. All validity criteria according to the guideline OECD 203 were fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item::	AMPA technical (Aminomethylphosphonic acid)
Description:	Not stated
Lot/Batch #:	Not stated
Purity:	85%
2. Vehicle and/or positive control:	Vehicle: Dechlorinated, filtered tap water Positive control: none
3. Test organism:	
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	juvenile
Size:	45 - 60 mm (mean: 50 mm)
Body weight of the animals:	1.14 – 2.82 g/ fish (mean: 1.70 g)
Loading:	0.85 g fish/L (in the dilution water control)
Source:	[redacted]
Diet/Food:	none
Acclimation period:	18 days
4. Environmental conditions:	
Temperature:	14.2 – 15.2°C
Photoperiod:	16 hours light / 8 hours dark with a 20 minute transition period
pH:	7.22 – 7.66
Dissolved oxygen:	9.4 - 10 mg O_2/L
Conductivity:	227 µS/cm³ in the dilution water
Hardness:	41.3 mg $CaCO_3$/L
5. Dates of experimental work:	6th December to 10th December 1993

B. STUDY DESIGN

Experimental treatments

The toxicity test was performed using nominal concentrations of 18, 32, 56, 100 and 180 mg AMPA technical/L prepared using dechlorinated and filtered tap water treated with ultraviolet steriliser.
The test was conducted 96 h in a static test setup. In addition a control group was exposed to the test medium without test substance or other additives. There was one vessel per test concentration and one for the control group, each containing ten fish (27 L borosilicate glass vessel containing 20 L test medium).

Observations

Assessment of sublethal effects and mortality of test fish was conducted after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water were controlled at test initiation.
Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis at test start and after 48 and 96 hours.

Statistical calculations: Descriptive statistic

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} values and the NOEC are given below based on nominal concentrations.

Table 8.2.1-45: Endpoints

Endpoints	AMPA technical [mg/L]
LC_{50} (24 h)	> 180
LC_{50} (48 h)	> 180
LC_{50} (72 h)	> 180
LC_{50} (96 h)	> 180
NOEC (96 h)	18

Analytical data:
The mean measured concentrations of AMPA technical ranged from 100 to 111 %.
As the mean measured content of the test item always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 8.2.1-46: Analytical results

Nominal concentration of AMPA technical [mg/L]	Measured concentration of AMPA technical [mg/L]			Mean measured concentration of AMPA technical [mg/L]	% of nominal
	0 h	48 h	96 h		
Control	<7.9	<7.9	<7.9	<7.9	-
18	22	21	18	20	111
32	35[1]	34[1]	32	34	106
56	58	58	56	57	102
100	110	91	98	100	100
180	190	160	180	180	100

[1]triplicate analyses

B. OBSERVATIONS

No mortality occurred up to the highest test AMPA technical concentration of 180 mg/L. Sub-lethal effects like dark discolouration, sounding and loss of balance were observed at 32, 100 and 180 mg/L respectively. The results of the test are depicted in the following tables.

Table 8.2.1-47: Effects of AMPA technical to rainbow trout

Nominal concentration of AMPA technical [mg/L]	Number of dead fish / observed symptoms (% affected)			
	24 h	48 h	72 h	96 h
Control	0 / -	0 / -	0 / -	0 / -
18	0 / -	0 / -	0 / -	0 / -
32	0 / -	0 / -	0 / S, LB (11 – 30%)	0 / S, LB, DC (11 – 30%)
56	0 / -	0 / -	0 / -	0 / -
100	0 / -	0 / -	0 / S, LB (< 10%)	0 / S, LB (< 10%)
180	0 / -	0 / -	0 / S (11 – 30%)	0 / S, LB (> 30%)

S: Sounding
DC: Dark colouration
LB: Loss of balance

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

3. Assessment and conclusions

Assessment and conclusion by applicant:
The LC_{50} (96 h) for rainbow trout exposed to AMPA technical was >180 mg/L (nominal). The NOEC after 96 h exposure to AMPA was 18 mg/L (nominal).

This study is considered valid and the acute LC_{50} value for rainbow trout exposed to AMPA technical was >180 mg/L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.1/021
Report author	Antunes, A. M. *et al.*
Report year	2017
Report title	Gender-specific histopathological response in guppies *Poecilia reticulata* exposed to glyphosate or its metabolite aminomethylphosphonic acid
Document No	DOI 10.1002/jat.3461 E-ISSN: 1099-1263
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Ecotoxicity of glyphosate (GLY) and its metabolite aminomethylphosphonic acid (AMPA) was investigated in guppies, *Poecilia reticulata*. The median lethal concentration after 96 hours of exposure (LC_{50}, 96 h) of both test item was determined in male and female guppies

Both genders showed similar median lethal concentration (LC_{50}) at 96 hours for glyphosate and AMPA. The acute 96 hour-LC_{50} of glyphosate obtained for male and female guppies *P. reticulata* were 68.78 mg/L (95 % C.I.: 64.59–73.24 mg/L and 70.87 mg/L (95 % C.I.: 65.91–76.26 mg/L), respectively. The 96 hour-LC_{50} values for AMPA for male and female guppies were 180 mg/L (95 % C.I.: 175.12–184.54 mg/L) and 164.3 mg/L (95 % C.I.: 160.6–168.54 mg/L), respectively.

Materials and methods

Tested products; GLY and AMPA 96% and 99%, respectively, were purchased from Sigma-Aldrich (São Paulo, SP, Brazil). The stock solutions of GLY and AMPA were prepared in ultrapure water with a nominal concentration of 250 mg l^{-1}.

Animal collection and maintenance; *P. reticulata* used in the experiments was part of the animals group kept in the Aquatic Animal Biotery of the Cell Behavior Laboratory (Institute of Biological Sciences IV, Universidade Federal de Goiás, Goiânia, Brazil). All of them were 3-month-old F1 generation animals born in the biotery from a wild parental generation. 318 mature male and 318 mature female guppies (vitellogenic oocyte occurred) of an average weight of 252 ± 20 mg and 178.6 ± 14.4 mg, and total average length of 2.98 ± 0.3 cm and 2.47 ± 0.2 cm, respectively.

Toxicity test: LC_{50}; For each experimental condition, eight males or eight females guppies were maintained in 2 liter tanks (4 fish L) and exposed to different nominal concentrations of GLY (50, 55, 60.5, 66.5 and 73.2 mg/L) or AMPA (86.8, 104.2, 125, 150 and 180 mg/L) during 96 h in the static test under 12: 12 h light/dark cycles. These concentrations were determined in the preliminary tests. The control group that consisted of eight fish kept in dechlorinated water. All treatments were performed in a triplicate design and the fish were not fed during the experimental period (USEPA, 1993). The mortality was reported at different exposure times (2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h). Physical and chemical parameters of water were analyzed every morning and did not show any changes over the experimental period, such as temperature 24 ± 1 °C, dissolved oxygen 8 mg/L, ammonia 0.002 mg/L, pH 7.0 ± 1, nitrite 0.025 mg/L and nitrate 0.5 mg/L. Cumulative mortality data obtained at the end of the experiments (96 h) were analyzed by the trimmed Spearman–Karber method to estimate the LC_{50} of a 96 h exposure to GLY and AMPA.

Statistical analysis; All statistical analyses were performed using the Statistica 7.0 software (Statsoft Inc., 2005, Tulsa, OK, USA). The differences between the treatments of the analyzed variables were identified using parametric tests (two-way ANOVA, followed by the Tukey's test) and/or non-parametric tests (Kruskal–Wallis), depending on the distribution of the data and homogeneity of variances (Shapiro–Wilk and Levene's tests). Linear and non-linear regression analyses were also applied to verify the relationship between variables.

Results

Median lethal concentration (LC_{50}); No mortality was observed for both genders in the control group during the experimental period of 96 h. The LC_{50} results showed that the GLY is more toxic to the guppies than to its metabolite AMPA, whereas no significant difference was observed between the genders ($P > 0.05$). The GLY LC_{50} values obtained for male and female *P. reticulata* were, respectively, 68.78 mg/L (95 % confidence interval = 64.59–73.24 mg/L) and 70.87 mg/L (95 % confidence interval = 65.91–76.26 mg/L). The AMPA LC_{50} in turn, were 180 mg/L (95 % confidence interval = 175.12–184.54 mg/L) and 164.3 mg/L (95 % confidence interval = 160.6–168.54 mg/L), respectively. The GLY and AMPA toxicity increased linearly with the increasing concentration for females (GLY: $y = 0.6281x - 30.141$, $r = 0.96$, $P < 0.05$; AMPA: $y = 0.150x - 11.193$, $r = 0.93$, $P < 0.05$) and males (GLY: $y = 0.666x - 30.653$, $r = 0.93$, $P < 0.05$; AMPA: $y = 0.168x - 11.898$, $r = 0.88$, $P < 0.05$).

Discussion

The results of the LC_{50} values of GLY (male 68.78 mg/L and female 70.87 mg/L) and AMPA (male 180 mg/L and female 164.3 mg/L) based on the mortality test indicated a low sensitivity of *P. reticulata* in

comparison to the other teleost species, as reported by the USEPA. In addition, it was observed that the AMPA is less toxic to *P. reticulata* than GLY (male 2.6-fold, female 2.3-fold).

Conclusion
The present study determined the acute 96 hour-LC_{50} of glyphosate and AMPA. The glyphosate LC_{50} values obtained for male and female guppies *P. reticulata* were 68.78 mg/L (95 % C.I.: 64.59–73.24 mg/L and 70.87 mg/L (95 % C.I.: 65.91–76.26 mg/L), respectively.
The 96 hour-LC_{50} values for AMPA for male and female guppies were 180 mg/L (95 % C.I.: 175.12–184.54 mg/L) and 164.3 mg/L (95 % C.I.: 160.6–168.54 mg/L), respectively.

Assessment and conclusion by applicant:
The acute 96 hour-LC_{50} values for male and female guppies *P. reticulata* after exposure to glyphosate were 68.78 mg/L and 70.87 mg/L, respectively. The acute 96 hour-LC_{50} values for AMPA for male and female guppies were 180 mg/L and 164.3 mg/L, respectively.
In the material and methods part some important is missing. No information on preparation of test solution and application is given. Source and composition of media are unclear. Furthermore, there was no analytical verification of test concentrations reported. The study is considered as reliable with restrictions.

1. Information on the study

Data point:	CA 8.2.1/022 CA 8.2.1/023
Report author	Gholami, S.J. *et al.*
Report year	2013
Report title	Toxicity evaluation of Malathion, Carbaryle and Glyphosate in common carp fingerlings (Cyprinus carpio, Linnaeus, 1758)
Document No	ISSN: 2008-2525
Guidelines followed in study	OECD 203
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Fingerlings of the common carp (*Cyprinus carpio*, Linnaeus, 1758) are often exposed to a wide range of pesticides when they are released introduced into the sea at the estuaries of the rivers flowing into the Caspian Sea. The present study investigated effects of lethal concentrations (expressed as 96-hour LC_{50s}) and sublethal concentrations (determined by acetylcholinesterase assay) of glyphosate on these fingerlings. The results indicated that the 96-hour LC_{50} of glyphosate for the fingerlings was 6.75 mg/L. In addition, the lowest observed effective concentrations (LOECs) (96-hour LC_{10}) was 5.548 mg/l for glyphosate.
Materials and methods
Chemicals: Sodium carbonate, sodium hydroxide, copper sulfate, potassium sodium tartrate, bovine serum albumin, phosphoric acid, tris and hydrochloric acid were purchased from the official representative of the German Company Merck in Iran. Glyphosate was purchased from Bazargan Kala (Iran). Absorbance was read using an ELISA Microplate Reader (ELx 808, BioTek).

Reactants: 0.1 mol phosphate buffer solution (PBS) (pH 7 with no Tritone), the Folin–Ciocalteu reagent (FCR) (diluted with an equal volume of distilled water), DTNB (dissolved in TRIS/HCl buffer) and acetylthiocholine iodide were used in the experiments.

Two thousand fingerlings with the mean weight of 2±0.4 g were obtained from the Shahid Rajaee Fish Breeding and Rearing Center, Sari, Mazandaran Province, and were transferred to the Fish Breeding and Rearing Research Center in the Department of Fisheries at the College of Agriculture & Natural Resources (UTCAN) in University of Tehran (Karaj). In order to adapt to the new environmental conditions, the fish were kept in two 1000-liter fiberglass tanks for 15-20 days. The physicochemical parameters of water were controlled as follows: pH=7, total water hardness ($CaCO_3$) =175 mg/l, dissolved oxygen=more than 7 ppm and temperature=20±2°C. The stock solution of glyphosate was prepared with the concentration of 10,000 ppm.

Lethal concentration experiments (bioassays): To perform bioassay, the range of concentrations of glyphosate and the logarithmic distances were determined in a pilot test and then the main experiment was carried out. Based on the results of this pilot test, the fingerlings were exposed to the following concentrations of glyphosate for 96 hours: 5.5, 6.5, 7.5, 8.5, and 9.5 mg/l. Effects and LC_{50s} were determined in accordance with the OECD Guidelines for the Testing of Chemicals (No. 203) in static water. Bioassay for each pesticide was performed on 150 fingerlings (a total of 450) that were randomly and equally put in fifteen 100-N fiberglass tanks (three replicates for each concentration with 10 fish in each tank). The experimental conditions were close to those during the adaptation period. The fingerlings were not fed during the experiment. All experimental groups were monitored twice a day and the behavior of the fingerlings was studied. Moreover, the number of deaths was recorded at 24, 48, 72, and 96 hours after the toxin was added.

Sublethal toxicity experiment: The fingerlings were randomly placed in nine 100-N fiberglass tanks. Each tank contained 40 fingerlings, and the experimental conditions were the same as in the previous experiments. As in the rearing and adaptation periods, the subjects were fed 2% of their body weight and the feeding was stopped 24 hours before they were killed. In the sublethal toxicity experiment, the fingerlings were exposed to three different concentrations of glyphosate, each with three replicates for 15 days. The treatments were as follows: 0 (control), 0.6, and 1.2 mg/l of glyphosate. These concentrations were determined based on the LC_{50} values. About 10 % of the water in each tank was siphoned off every day in order to remove waste materials and reduce ammonia levels in the water. To maintain the stability of experimental conditions, the removed water was replaced by an equal volume of water with the initial concentrations of the pesticide.

Sampling and extract preparation (upper layer): A number of fingerlings from each treatment were sampled on the fifth, tenth, and fifteenth days after their first exposure to pesticides. Because of the very small size of the fingerlings, it was not possible to take blood or tissue samples. Therefore, they were beheaded and both parts (head and trunk) were frozen at -70° C to be later used for extract preparation. The obtained tissues were manually homogenized in 0.1 mmol PBS (pH 7 and containing 1 % of Tritone X-100). The samples were centrifuged and the resulting extract (upper layer) was removed to be used as the enzyme source.

Total protein assay and AChE activity measurement: Total protein concentration in the tissues was measured by using the Lowry method at 540 nm utilizing an ELISA microplate reader. In this method, FCR was used as the color reagent. Protein concentration in tissue samples was then determined using the resulting curve and its linear equation. The specific activity of cholinesterase (in µU/min/mg protein) was measured based on Ellman's method at 420 nm using a microplate reader. To this end, a mixture of the extract (upper layer), 0.1 mol PBS, DTNB (Ellman's reagent) and acetylthiocholine iodide were added to each tube. Finally, 100 ml of the final solution was poured into each well of the microplate and absorbance per minute (O.D. /min) was read.

Calculations and statistical analysis: The data obtained from the bioassay and mortality rate of the fingerlings determined by using the probit model were analyzed. The values obtained from bioassays were then estimated using the POLO-PC 2002 software (under license of the University of Tehran). The specific activity of the enzyme (in µU/min/mg protein) was calculated as the dependent variable. The data were statistically analyzed using two-way ANOVA. The concentrations of the pesticides and the durations of exposure to them were the independent variables. The difference between means was also evaluated using Duncan's test with type-I error level of 0.05.

Results
Bioassay results: No mortality was observed during the adaptation period of the fingerlings. The results showed that their mortality rate increased with at the higher concentrations. Based on the mortality rates in the bioassays, the mean LC_{10}, LC_{50}, and LC_{90} values of glyphosate for the fingerlings at 24, 48, 72, and 96 hours were calculated (α=0.95) (see table below).
The results indicated that the 96-hour LC_{50} of glyphosate for the fingerlings was 6.75 mg/L. In addition, the lowest observed effective concentrations (LOECs) (96-hour LC_{10}) was 5.548 mg/l for glyphosate.

Table 8.2.1-1: The mean values obtained from bioassays in Caspian Sea common carp fingerlings

Chemical's name	Lethal concentration (mg/l)	24-hour	48-hour	72-hour	96-hour
Glyphosate	LC_{10}	5.995	5.976	5.865	5.548
	LC_{50}	7.202	7.172	6.985	6.753
	LC_{90}	8.651	8.606	8.319	8.168

Conclusion
The results indicated that the 96-hour LC_{50} of glyphosate for the fingerlings was 6.75 mg/L. In addition, the lowest observed effective concentrations (LOECs) (96-hour LC_{10}) was 5.548 mg/l for glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The acute 96 hours- LC_{50} for common carp fingerlings was determined to be 6.75 mg/L by static exposure to glyphosate at 5 test concentrations between 5.5 and 9.5 mg/L.

The test was conducted according to OECD 203, but validity criteria are missing. No information on the test item such as purity is given. The results for the control are not stated. Furthermore, there was no analytical verification of test concentrations reported. The study is considered as reliable with restrictions.

CA 8.2.2 Long-term and chronic toxicity to fish

Studies considering the effects of glyphosate on longterm and chronic toxicity to fish were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. An expert opinion is available as indicated in the table below, which provides a detailed evaluation on the *Brachydanio* study. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

CA 8.2.2.1 Fish early life stage toxicity test
Early life stage studies are available and provided below.

Table 8.2.2.1-1: Studies on long-term and chronic toxicity of glyphosate and metabolites to fish

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.2.1/001	[redacted] 2010	Chronic, flow-through	*Oncorhynchus mykiss*	Glyphosate acid	Valid	
CA 8.2.2.1/002	[redacted], 2000	Chronic, semi-static	*Brachydanio rerio*	Glyphosate acid	Invalid	Refer to CA 8.2.2.1/003 for expert opinion
CA 8.2.2.1/003	[redacted] 2020	Expert opinion				Expert opinion regarding the study CA 8.2.2.1/002
CA 8.2.2.1/004	[redacted] 2011	Chronic, flow-through	*Pimephales promelas*	AMPA	Valid	

Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the long-term impact of glyphosate on fish are summarised in the table below. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to fish, please refer to document M-CP Section 10.2.

Table 8.2.2.1-2:Literature on long-term and chronic toxicity of glyphosate and metabolites to fish

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.2.1/005	Rodrigues *et al.*, 2019*).* Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms	acute toxicity to zebrafish embryos	Glyphosate and AMPA	reliable with restrictions	Glyphosate and AMPA caused no acute toxic effect (LC_{50}-96h > 100 mg/L) in zebrafish.
CA 8.2.2.1/006	Schweizer *et al.*, 2019. How glyphosate and its associated acidity affect early development in zebrafish (Danio rerio).	Acute toxicity to zebrafish embyros. Based on OECD 236.	glyphosate	reliable with restrictions	For Zebrafish (*Danio rerio*) embryos acutely exposed to glyphosate at concentrations between 1.69 and 1690.7 mg glyphosate/L for 96 hours post fertilization.

Endpoints of studies considered valid for glyphosate are shown in the table below. Studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate technical are automatically expressed as acid equivalent.

Table 8.2.2.1-3: Endpoints: Early life-stage toxicity of glyphosate to fish

Reference	Test item	Species	Test design/ GLP	LC_{50} (mg a.e./L)	NOEC (mg a.e./L)
[redacted] 2010 CA 8.2.2.1/001	Glyphosate acid	*Oncorhynchus mykiss*	Chronic, 85 d (60 days post-hatch) ELS, flow-through	-	**≥ 9.63 (gm)**

a.e.: acid equivalents
gm: geometric mean measured
Endpoints in **bold** are used for risk assessment

Endpoints of studies considered valid for AMPA are shown in the table below.

Table 8.2.2.1-4: Endpoints: Early stage toxicity of AMPA to fish

Reference	Test item	Species	Test design/ GLP	LC_{50} (mg/L)	NOEC (mg/L)
[redacted] 2011 CA 8.2.2.1/003	AMPA	*Pimephales promelas*	Chronic, 33 d (7 days post-hatch) ELS, flow-through	-	**≥ 12 (nom)**

nom: nominal
Endpoint in **bold** is used for risk assessment

Study summaries are provided below.

1. Information on the study

Data point:	CA 8.2.2.1/001
Report author	[redacted]
Report year	2010
Report title	Glyphosate acid: Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions
Report No	1005.029.321
Document No	
Guidelines followed in study	OECD Guideline 210 (1992)
Deviations from current test guideline	Deviations from the current OECD 210 guideline (2013): none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The effects of glyphosate acid on the early life-stages of rainbow trout was determined under flow-through (continuous renewal) exposure conditions. Fertilized eggs of *Oncorhynchus mykiss* were exposed for 85 days to nominal glyphosate acid concentrations of 0.095, 0.305, 0.977, 3.125 and 10.0 mg a.s./L. Initially, 50 fertilized eggs were exposed in duplicate exposure vessels at each of the five concentrations, with duplicate negative control groups (dilution water only) run in parallel.

Eggs were fertilized in the laboratory directly before addition to egg cups and remained undisturbed in the test system in the dark until hatching success was determined on days 22 to 26, based on the number of viable eggs. On day 26 (complete hatch), twenty fish fry per replicate i.e. 40 organisms per treatment level and control were transferred from egg cups to surrounding test media, where the development and survival was evaluated until test termination. Dissolved oxygen (DO) concentrations, pH and temperature were measured and recorded in each test vessel at experimental start and weekly thereafter until test termination (day 85). Glyphosate acid concentrations were measured on test days 0, 6, 13, 20, 27, 33, 41, 48, 55, 62, 70, 76 and 85. Glyphosate acid was not detected in the control group. Mean measured concentrations were substantially achieved and ranged between 85.7 and 96.3% of nominal concentrations. Ecotoxicological endpoint evaluation was based on overall mean measured glyphosate acid concentrations.
No statistical significant differences were detected for normal fry at hatch, hatching success, survival at test termination and growth (total length, wet and dry weight), when compared to the control group. All validity criteria according to OECD 210 were satisfied.
In a fish early life stage study performed with rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate acid, the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) were determined to be ≥ 9.63 and > 9.63 mg a.s./L, respectively, based on geometric mean measured concentrations. The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

Materials and Methods **1. Test material:**	
Test item:	Glyphosate acid
Lot/Batch #:	GLP-0807-19475-T
Purity:	96.03%
2. Vehicle and/or positive control:	Vehicle: reconstituted well water Positive control: none
3. Test organism:	
Species:	Rainbow trout (*Oncorhynchus mykiss*) eggs and milt
Age of eggs:	Eggs and milt were less than 36 hours old at fertilization. The time between fertilization and egg addition to test system was less than 3.5 hours
Number of animals/dose level:	40 organisms per replicate i.e. 40 organisms per treatment level and control
Supplier:	[redacted]
Mean loading rate (biomass per volume of test solution)	0.31 g/L per 24 hours
4. Environmental conditions:	
Temperature:	Continuously measured temperature: 9.4 to 13.1°C Single-point measured temperature: 11.3 to 13.9ºC
pH:	7.14 to 8.44
Dissolved oxygen:	> 60 % ASV for study duration
Conductivity of test medium:	340 to 450 µS/cm
Hardness of test medium:	153 to 184 mg/L $CaCO_3$
Photoperiod:	16 hours with a 30 minute transition from Day 32 until test completion. Light intensity was 137 to 377 lux. Eggs and larvae were shielded from all light during the incubation and hatching phases until one week after hatching

5. Dates of experimental work:	May 14th to August 10th 2009

B. STUDY DESIGN

Experimental treatments

The fish early life-stage toxicity test was performed under flow-through exposure conditions, using a constant-flow test item delivery system, supplying the appropriate test medium to duplicate exposure vessels at each of the five concentrations and the duplicate negative dilution water control vessels. The fertilized eggs were exposed to glyphosate acid at test concentrations of 0.095, 0.305, 0.977, 3.125 and 10.0 mg a.s./L for 85 days.

Twelve impartially located exposure vessels were maintained in a temperature-controlled water bath designed to maintain the test solution temperatures at 12 ± 2 °C. During the egg exposure phase and until one week after hatching the test area was maintained in continuous darkness. From test day 32 until test completion, the vessels were illuminated to a light intensity of 137 to 377 lux using fluorescent tubes. A photoperiod of 16 hours was employed with a 30 minute (dawn/dusk) transition period.

Preparation of test solution: A 1 g glyphosate acid/L stock solution was prepared directly prior to test initiation and as required during the exposure period, by dissolving approximately 11.737 g of glyphosate acid in 10 L of dilution water. The stock solution was further diluted (dilution water) by the test item delivery system to achieve the required concentrations in each of the exposure vessels. For the control group, dilution water only without test item was used.

Test units: The test vessels measured 39.0 cm × 19.2 cm, with an approximate water depth of 14.6 cm maintained at a constant volume of 10 L. Two replicates (A and B) were maintained for all treatments and the control.

Test initiation: Prior to fertilization, freshly collected rainbow trout milt and eggs were acclimatized in their respective delivery containers to the approximate test temperature of 12 ± 2 °C, using a water bath and then mixed carefully together. The 'apparently' fertilized eggs were impartially distributed to egg incubation cups in groups of five, until each cup contained 50 eggs. The incubation cups were suspended in the respective exposure vessel with two cups per replicate vessel, resulting in 100 eggs per replicate. The test was initiated once all vessels contained eggs within 3.5 hours of receipt of the gametes and within two hours of fertilisation.

Hatching success was determined on days 22 to 26 based on the number of viable eggs. Any eggs exhibiting embryonic development, whether dead or alive, at the time of assessment, were considered fertile for purposes of determining percent viability. All non-viable eggs were counted and discarded at day 26. The percent viability was calculated based on the actual number of fertilized embryos on day 26. Hatching success was calculated based on the actual number of viable embryos.

Egg exposure: Dead and alive eggs were counted daily. All eggs observed to be clear were considered to be alive, all eggs observed to be opaque and milky were considered to be dead. All eggs observed to be dead were removed and preserved in Stockard's solution for clearing and determination of embryonic development. Fry which hatched prior to the determination of viability were collected in an auxiliary egg cup.

Post hatch exposure: At completion of hatch on day 26, twenty organisms per replicate i.e. 40 organisms per treatment level and control were transferred directly from the first egg cup (i.e., A1 and B1) to the surrounding test media in the test vessels and the egg cups were removed.

For replicate A of the control and the 0.095 mg glyphosate acid/L treatment, 20 fry in the auxiliary egg cup containing the early hatched fry were randomly selected. For replicate A of the 10 mg a.s./L treatment, only eight viable eggs hatched of the 20 randomly selected eggs and therefore only eight hatched fry were released into the test vessel.

All remaining alive and dead eggs were preserved in Stockard's solution. The remaining fry were recorded and then discarded. After evaluation of the developmental status of the cleared eggs, the viability of all eggs was calculated.

During the post-hatch exposure period, developing fry in all vessels were observed daily; recording behaviour and appearance. Dead fry were removed during these observations. Survival was estimated daily

throughout the post-hatch period. At 60-days post-hatch exposure (experimental completion), the percentage fish survival was calculated.
Fry feeding: At the beginning of fry swim-up, the fry were fed live brine shrimp nauplii (*Artemia salina*), harvested from hydrated cysts (24 to 36 hours post-hydration) three times per day. Fish were not fed during the 24 hours prior to study termination.
Length and weight: At day 60 post-hatch all of the surviving fish in each replicate vessel were euthanized with MS-222 (tricain methane-sulfonate), measured and weighed individually to determine fish total lengths and wet weights, respectively for each treatment.

Observations
The dissolved oxygen (DO) concentrations, pH and temperature were measured and recorded in each test vessel at experimental start and weekly thereafter until test termination (day 85). On test day 75, the DO levels decreased to between 6.31 to 7.50 mg O_2/L, so aeration was provided to each test vessel until test completion.
Temperature was continuously monitored in one replicate (replicate A of the control) throughout the study. Total hardness, alkalinity and specific conductivity were monitored at experimental start and on test days 5, 11, 19, 25, 32, 39, 46, 53, 61, 67, 74 and 81 in one replicate of the highest treatment level and the control during the exposure.

Analytical procedures
Prior to the start of the exposure phase, i.e., day -2, samples from one replicate of the treatment level solutions and control solutions were collected and analysed for the active ingredient. Results of the pre-test analyses were used to assess correct dosage of the system before test initiation.
During the in-life phase, water samples of approximately 10 mL were removed from both replicates of each treatment level and control on test days 0, 6, 13, 20, 27, 33, 41, 48, 55, 62, 70, 76 and 85 and the content of glyphosate acid was determined. Samples of the stock solutions were also analysed at each sampling interval.

Statistical calculations
The data for percent normal fry at hatch, hatching success, survival at test termination and growth (total length, wet and dry weight) were first checked for normality using Shapiro-Wilks' Test (Weber *et al.*, 1989) and for homogeneity of variance using Bartlett's Test (Bartlett, 1937).
The data set for hatching success and survival at test termination were arc-sine (square root) transformed prior to determination of the NOEC and the LOEC by using one-way ANOVA and the parametric post-hoc Dunnett's Test (Dunnett, 1955, 1964). The data sets for growth passed the tests for homogeneity and normality, and Dunnett's Test was used to determine the NOEC and the LOEC.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS
Analytical data: The mean measured concentrations (calculated as geometric means) of 0.305, 0.977, 3.125 and 10.0 mg a.s./L ranged between 85.7 and 96.3% of the nominal test concentrations, with the exception of the lowest test concentration (0.095 mg a.s./L), where a mean recovery of 66.9% of the nominal concentration was calculated. Based on these results, the mean measured concentrations (calculated as geometric means) of 0.064, 0.261, 0.846, 2.804 and 9.63 mg a.s./L were used for the evaluation of the biological data.

Table 8.2.2.1-5: Analytical results

Nominal concentration [mg a.s./L]	Mean measured concentration [mg a.s./L]	% of nominal
Control	-	-
0.095	0.064	66.9
0.305	0.261	85.7
0.97	0.846	86.6
3.125	2.804	89.7
10.0	9.63	96.3

The water quality parameters measured were not affected by test item concentrations. The results of the water quality measurements carried out during this study established that conditions maintained throughout the 85-day exposure were satisfactory for the promotion of normal rainbow trout embryo hatchability, fry survival and growth.
The effects of glyphosate acid on embryo viability, hatching success, number of normal fry at hatch, survival at test termination and growth (total length, wet and dry weight) are provided in the table below.

Table 8.2.2.1-6: Egg viability, hatching success and normal fry at completion of hatch (test day 26) and survival, total length, wet weight and dry weight of rainbow trout (*Oncorhynchus mykiss*) at test termination of the 85-day exposure to glyphosate acid

Mean measured concentration (mg a.s./L)	Egg viability [%][1]	Hatching success [%][1]	Normal fry at hatch [%]	60 days post-hatch			
				Survival [%]	Total length [mm]	Wet weight [mg]	Dry weight [mg]
Control	35±3.3	92±6.9	97±0.56	85±7.1	46.38±0.41	942.6±34.9	195.1±14.3
0.064	43±4.9	84±20.2	96±5.2	95[2]±7.1	45.33±0.83	899.6±10.7	188.7±5.9
0.261	40±4.0	99±1.7	100±0.0	95±0.0	46.75±0.65	932.2±60.5	190.7±7.5
0.846	38±9.9	95±1.5	100±0.0	93±10.6	46.37±1.7	908.6±84.3	189.1±23.0
2.804	41±2.1	91±5.5	99±2.0	95±7.1	46.19±0.33	889.7±23.7	188.4±10.7
9.63	27±9.2	80±28.3	98±2.1	100±0.0	46.38±1.7	947.3±135	203.0±36.5

[1] Based on total number of viable eggs
[2] On test day 59, one fish of replicate A was inadvertently injured during the cleaning process of the test vessel. One day later this fish had died. Since this mortality was not test item related, the fish was therefore excluded from further statistical evaluation.

The NOEC and LOEC values for survival and growth of rainbow trout (*Oncorhynchus mykiss*) after 85-day exposure to glyphosate acid are based on geometric mean measured concentrations.

Table 8.2.2.1-7: Endpoints

Endpoint	Glyphosate acid [mg a.s./L]	
	NOEC	LOEC
Percent normal fry at hatch	≥ 9.63	>9.63
Hatching success	≥ 9.63	>9.63
Survival at test termination	≥ 9.63	>9.63
Total length	≥ 9.63	>9.63
Wet weight	≥ 9.63	>9.63
Dry weight	≥ 9.63	>9.63

All validity criteria according to OECD 210 were fulfilled, as dissolved oxygen concentration was between 60% and 100% of air saturation, water temperature was within the range specified for the test species and constant exposure conditions have been maintained (i.e. within ±20% of nominal concentration were recovered, except for the lowest concentration which does not affect the results of the study), and overall survival of fertilised eggs in the controls was greater than or equal to the limits defined in Annexes 3 and 6 of OECD 210.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
In a 85-day (60 days post-hatch) chronic study with rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate acid, the NOEC and LOEC values for percent normal fry at hatch, hatching success, fry survival, length and weight were ≥ 9.63 and > 9.63 mg a.s./L, respectively, based on geometric mean measured concentrations.

The study is considered valid and the NOEC for rainbow trout exposed to glyphosate acid was ≥ 9.63 mg a.s./L (nominal) and is considered to be appropriate for use in ecotoxicological risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.2.1/002
Report author	[redacted]
Report year	2000
Report title	Chronic Toxicity of Glifosate Técnico Nufarm to Zebrafish larvae (*Brachydanio rerio*)
Report No	RF-D62.16/99
Document No	
Guidelines followed in study	IBAMA 1990: Manual de testes para avaliacao da ecotoxicidade de agentes quimicos
Deviations from current test guideline	Deviations compared from the current OECD 212 guideline (1998): Major: • The study was not conducted according to the OECD 212 test guideline. • Free swimming fish larvae were exposed for 168 h without feeding, therefore the influence of the lack of feeding on the achieved results during the study cannot be excluded. • Larvae were added to the test vessels and not fresh eggs 'as soon as possible after fertilisation (early gastrula stage) to 5 days post-hatch (8-10 days) within 30 mins to 8 hours of fertilisation as stated in the test guideline. • Active ingredient concentrations were determined in the stock solutions only. • Survival of fertilised eggs and differences of water temperature between test chambers or successive days is not reported. • Holding stock tank was maintained at 28 ºC. Temperature of test media at fish addition was 24.1ºC. The temperature difference between the holding tank and the test tank, exceeds the variability in temp range permitted for this study type ± 1ºC (25±1ºC stated in Annex 3 of OECD 212). • For the batch of eggs received from which the larvae used in the test, were sourced, it is not possible to validate the quality of the eggs used in the test as there is no information on the hatching success reported. • Validity criteria based on hatching success and post hatching survival are not reported.
Previous evaluation	Not accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 5

2. Full summary of the study according to OECD format

Executive Summary

A fish short term toxicity test with glyphosate acid with larvae of *Danio rerio* (formerly named *Brachydanio rerio*) was performed under semi-static conditions with test medium each 48 hours. Three replicates with 30 fish per concentration were exposed for 168 hours to seven concentrations of glyphosate acid, ranging from 0.32 to 32 mg a.s./L. A control treatment containing reconstituted water and a toxic reference using potassium dichromate was maintained concurrently.

Observations for mortality and sub lethal responses were made every 24 hours. Dissolved oxygen, pH and temperature were measured and recorded daily. Glyphosate acid concentrations were measured by liquid chromatography in the stock solutions. Mean measured concentrations were at least 80% of nominal concentrations. Glyphosate acid was not detected in the control group.
A significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L, behavioural responses such as lethargy was observed at 3.2, 5.6, 10 and 32 mg a.s./L. Several validity criteria according to the current OECD guideline 212 were not fulfilled.
The No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) for zebra fish larvae (*Danio rerio*) exposed to glyphosate acid were determined to be 3.2 mg a.s./L and 5.6 mg a.s./L, respectively, based on nominal concentrations. The LC_{50} after 168 hours was determined to be 24.71 mg a.s./L. Overall this study is not reliable, invalid and does not address any current data requirements.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	954.9 g/kg acid equivalent
2. Vehicle and/or positive control:	Vehicle: Tap water Positive control: Potassium dichromate ($K_2Cr_2O_7$)
3. Test organism:	
Species:	Zebra fish (*Danio rerio*) larvae
Age:	Larvae; approx. 48 hours old
Size:	Not stated
Loading:	1 L for 10 larvae (bodyweight not specified)
Source:	Eggs: in-house. Matrix fish: Peixe [redacted]
Diet/Food:	Fish were not fed during acclimation or during the 168 h exposure period.
Acclimation period:	48 hours prior to testing during embryo incubation and hatching
4. Environmental conditions:	
Temperature:	23.8 - 24.3 °C
Photoperiod:	16 hours light / 8 hours dark
Dissolved oxygen:	60-100 %
Conductivity of test medium:	168 µS/cm
Hardness of test medium:	44.1 mg/L $CaCO_3$
5. Dates of experimental work:	03rd November to 19th November 1999

B. STUDY DESIGN

Experimental treatments

The fish early life-stage toxicity test was performed under semi-static exposure conditions renewing the test solution every 48 hours. Following a range finding test, the freshly hatched fry (48 h post hatch) of *Danio rerio* were exposed to glyphosate acid at test concentrations of 0.32, 0.56, 1.0, 3.2, 5.6, 10 and 32 mg a.s./L for 168 hours. A control consisting of reconstituted water and five toxic reference concentrations (32, 56, 100, 140 and 180 mg $K_2Cr_2O_7$/L) were maintained concurrently.

Observations
Observations for mortality and sublethal responses were made every 24 hours. Dead individuals were removed at each observation. Temperature, dissolved oxygen, pH and conductivity were measured daily. The active ingredient analysis of stock solutions was performed by liquid chromatography.

Statistical calculations
LC_{50} and its confidence limits were determined using trimmed Spearman-Karber method. Fisher's Exact test was used for determination of significant differences in survival between control and exposure. The NOEC and LOEC were determined by Fisher's Exact test.

II. RESULTS AND DISCUSSION

A. FINDINGS
Analytical results: The active ingredient concentration in each stock solution was at least 80% of the nominal concentration. Ecotoxicological relevant endpoints were therefore evaluated using nominal concentrations of the test item.
The 168h LC_{50}, NOEC and LOEC values are given below based on nominal concentrations.

Table 8.2.2.1-8: Endpoints

Endpoints (168 h)	Glyphosate acid [mg a.s./L]
LC_{50}	24.7 (95% C.I. 13.75 – 44.40 mg a.s./L)
LOEC	5.6
NOEC	3.2

B. OBSERVATIONS
A significant increase of mortality after exposure to glyphosate acid was observed at concentrations of 5.6, 10 and 32 mg a.s./L. Behavioural responses such as lethargy was observed at 3.2, 5.6, 10 and 32 mg a.s./L. The results of the test are depicted in the following table.

Table 8.2.2.1-9: Lethal effects of glyphosate acid to zebra fish

Glyphosate acid [mg a.s./L]	C	0.32	0.56	1.0	3.2	5.6	10	32
Mortality (168 h) [%]	0	0	0	0	10	16.7	26.7	56.7

C = Control

For the reference compound potassium dichromate ($K_2Cr_2O_7$) a 168 hour LC_{50} value of 124.66 mg/L (95 % C.I. 112.08 – 138.67 mg/L) was determined.
With regard to the validity criteria of the OECD guideline 212 (1998), survival of fertilised eggs and differences of water temperature between test chambers or successive days is not reported. Additionally no information on timing of fertilization is provided. Mortality in control group did not exceed 10 %, dissolved

oxygen concentration was between 60 and 100 % of air saturation. Analysis of test item treatments was performed only for the stock solutions.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
The NOEC and the LOEC for zebra fish (*Danio rerio*) exposed to glyphosate acid were determined to be 3.2 mg a.s./L and 5.6 mg a.s./L, respectively, based on nominal concentrations. The LC_{50} after 168 hours was determined to be 24.7 mg a.s./L (nominal).

This study type is based on OECD 212 which is not part of current data requirements and therefore receives a category 5 for studies in AIR 5 dossiers and typically a summary would not be presented. However, for completeness purposes and since the chronic aquatic endpoint in the RAR 2015 was based on this study, it is presented here.
Despite the study having been conducted according to GLP, there are several validity criteria according to the current OECD test guideline 212, that were not fulfilled, with multiple major and minor deviations to the test guideline identified in the summary above, that would make the study unreliable for use in risk assessment.
To further support this evaluation, a further reliability assessment has been conducted using the criteria applied to public domain literature according to EFSA [EFSA Journal 2011;9(2):2092] and is presented in the table below.
Additionally - to ensure an appropriate evaluation of the studies validity and relevance for use in EU level risk assessment, the opinion of an independent Expert is provided in CA 8.2.2.1/003.
Conclusions of the Expert are that this study would not hold up to scientific scrutiny and would not be accepted for a scientific publication.
Based on the reliability assessment and on the opinion of the independent Expert, the study is not therefore considered relevant for use in EU level ecotoxicological risk assessment. Therefore, the study will not be used in ecotoxicological risk assessment for the EU renewal of glyphosate.

ECOTOXICOLOGY: Reliability criteria for the detailed assessment of full-text documents

Data requirements (indicated by the corresponding EU data point)	Criteria for "Reliable" articles	Criteria met? Yes / No / Uncertain
General criteria for reliability considered for all data requirements indicated by the corresponding EU data points as specified in	1. For guideline-compliant studies (GLP studies): OECD, OPPTS, ISO, and others. The validity/quality criteria listed in the corresponding guidelines met.	**Yes** – the study was GLP, but validity criteria of the OECD 212 test guideline were **not** stated / met.
	2. Not previous exposure to other chemicals is documented (where relevant).	**No** – no information in the report to confirm the source / quality of the fish.
	3. For aquatic studies, the test substance is dissolved in water or where a carrier is required, it is appropriate (non-toxic) and a carrier control / positive control is considered in the test design.	**Yes.**

EC Regulation (EU) No 283/2013	4. Glyphosate or Its metabolites (AMPA and HMPA), is sufficiently documented , and reported (i.e. purity, source, content, storage conditions)	**Yes**
	5. For tests including vertebrates, compliance of the batches used in toxicity studies compared to the technical specification	**Uncertain** – no information stated in report.
	6. Species used in the experimental clearly reported, including source, experimental conditions (where relevant): strain, adequate age/life stage, body weight, acclimatization, temperature, pH, oxygen (dissolved oxygen for aquatic tests) content, housing, light conditions, humidity (terrestrial species) incubation conditions, feeding.	**No** - Source of fish not stated. Fertilisation and hatching success of egg batches used in test **not** reported. **No** fish body weights reported therefore fish loading rates could not be determined (g fish/L).
	7. The validity criteria from relevant test guidelines can be extrapolated across different species but not necessarily across different test designs. If different, then the nature of the difference and impact should ideally be discussed.	**No** - Validity criteria were not stated. See summary above.
	8. Only glyphosate or Its metabolites is the test substance (excluding mixture), and information on application of the test substance is described.	**Yes**
	9. The endpoint measured can be considered a consequence of glyphosate (or a glyphosate metabolite)	**Uncertain** – Starvation and temperature issues may have also contributed to the observed effects.
	10. Study design / test system is well described, including when relevant: concentration in exposure media (dose rates, volume applied, etc), dilution/mixture of test item (solvent, vehicle) where relevant.	**No** – Definitive test media preparation cannot be confirmed from report - **no** prep details reported. Renewal frequency in the definitive test **cannot** be confirmed. Exposure **cannot** be confirmed in the test system, as there was **no** chemical analysis of test media during the test
	11. Analytical verifications performed in test media (concentration)/ collected samples, stability of the test substance in test medium should be documented	**No** – Test media was not analysed during the test. Report indicates that stock solutions were stable during the test – but this cannot be confirmed from the report
	12. An endpoint can be derived. Findings do deliver a regulatory endpoint, and/or is useful as supporting information	**Uncertain** – as the validity of the test against a relevant

		guideline set of criteria cannot be confirmed. The test guideline requires freshly fertilized embryos to be exposed and not fish larvae – as was the case.
	13. The test has been tested in several dose levels (at least 3) including a positive/negative control where relevant	**Yes**
	14. Suitable exposure throughout the whole exposure period was demonstrated and reported	**No** – there was no analysis of test media during the test
	15. A clearly concentration response relationship is reported, in studies where the dose response test design is employed.	Uncertain – cannot be confirmed as exposure concentrations were not reported
	16. There is included a sufficient number of animals per group to facilitate statistical analysis: mortality in control groups reported, observations/findings in positive/negative control clearly reported (where relevant).	**Yes**
	17. Assessment of the statistical power of the assay is possible with reported data.	**No**
	18. If statistical methodology was applied for findings reported, then the data analysis applied is clearly reported (e.g., checking the plots and confidence intervals)	**Yes**
	19. Description of the observations (including time-points), examinations, and analyses performed, with (where relevant) dissections being well documented.	**No** – detailed timepoint observations of fish and appearance of the test media were not reported.
	20. For terrestrial ecotox studies in the lab or the field, the substrates used should be adequately described e.g. nature of substrate i.e. species of leaf or soil type.	-
	20.1. Field locations relevant/comparable to European conditions. Soils not completely matching the OECD criteria but from Europe or to some extent representative for the European Agriculture.	-
	20.2. Characterization of soil: texture (sandy loam, silty loam, loam, loamy sand), pH (5.5-8.0), cation exchange capacity, organic carbon (0.5-2-5%), bulk density, water retention, microbial biomass (~1% of organic carbon)	
	20.3. Other soils where information on characterization by the parameters: pH, texture, CEC, organic carbon, bulk density, water holding capacity, microbial biomass	-
	20.4. For tests including agricultural soils, they should not have been treated with test substance or similar substances for a minimum of 1 year	-
	20.5. For soil samples, sampling from A-horizon, top 20 cm layers; soils freshly from field preferred (storage max 3 months at 4 +/- 2°C).	-

	20.6. Data on precipitation is recorded	-
	21. For lab terrestrial studies, the temperature was appropriate to the species being tested and generally should fall within the range between 20-25°C and soil moisture / relative humidity was reported.	-
	22. For bee studies, temperature of the study should be appropriate to species.	-
	23. For lab aquatic studies	
	23.1. The source and / or composition of the media used should be described	**Uncertain**
	23.2. The temperature of the water should be appropriate to the species being tested and generally fall within the 15-25ºC	**No** – see deviations section in summary above
	24. The residue data can be linked to a clearly described GAP Table appropriate in the context of the renewal of approval of Glyphosate (crop, application method, doses, intervals, PHI).	**No**
	25. Analytical results present residues measurements which can be correlated with the existing residues definition of glyphosate, and where relevant Its metabolites	**No**
	26. Analytical methods clearly described and adequate Statement of specificity and sensitivity of the analytical methods is included.	**No** – There is no analytical method information presented in the report
	27. Assessment of the ECX for the width of the confidence interval around the median value; and the certainty on the level of protection offered by the median ECX.	**Yes** – The presented LC_{50} value is presented with confidence intervals, that exceed the range of concentrations tested in the study. A NOEC is also presented.

Assessment and conclusion by RMS:

1. Information on the expert opinion

Data point:	CA 8.2.2.1/003
Report author	[redacted]
Report year	2020
Report title	External expert opinion to the study No. RF-D62.16/99
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No

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

2. General evaluation

The study by [redacted] (2000) (CA 8.2.2.1/002) was evaluated by an independent fish expert not associated with industry. The expert was not provided with the name of test substance, study director, performing laboratory nor the data owner or sponsor of the study.

The following observations and statements were made:

Overall, the study has been based on OECD TG 212. However, there are several shortcomings in the study or in the report:

(1) A major deviation is that the renewal intervals for the test solution has been extended from 24 h to 48 h. Basically, this can be done; however, in such a case, precise chemical-analytical data documenting the stability of the test solutions must be provided. A general reference such as "Roberts, 1998 (P. 6 of report) is certainly not sufficient and is inadequate. Given that the report lacks any chemical-analytical data, the extension of the renewal time of the test solutions is a serious deviation from OECD TG 212. Since the reviewer was not provided with the name of the test substance, he could not check whether such an extension can be accepted as an exception. In any case, the extension of the renewal time of the test solutions should have explicitly been reported as a deviation from the guideline.

(2) Another critical deviation from OECD TG 212 is the fact that the information about the age of the embryos upon initiation of chemical exposure is confusing, if not lacking. Both OECD TG 212 and the more recent OECD TG 236 clearly require an exposure start as early as possible, if not within the first 1 h after fertilization. The report does not provide any information about the exact timing of the fertilization process and the time of egg collection.

(3) Further deviations from OECD TG 212 are a pH of ~ 7.4 (recommendation OECD TG 212: 7.8) and a temperature of ~ 24 °C (recommendation OECD TG 212: 28 °C). The consequences of these deviations cannot be assessed, as long as the name of test substance is not disclosed. Given the rather wide limits of tolerance of the zebrafish embryo, both pH and temperature deviations may have had an impact on the outcome of the test (chemical speciation, metabolism), however not necessarily.

(4) The terminology for the general description of the assay is scientifically not correct: OECD TG 212 does not measure chronic toxicity, nor does it use larvae.

(5) The origin of the fish is very poorly defined: no information about the strain of zebrafish used, no information about the age of the parental fish.

(6) Likewise, the report lacks data on fertilization rate, which is an important parameter to assess the quality of the egg batch used for the experiment (cf. information required for, e.g., OECD TG 236 [fish embryo test]). Maybe, in 1999, this was acceptable; today it would be not

(7) The report completely fails to provide details on behavioural observation; the term "lethargy" is definitely not satisfying and could have been specified much more precisely.

Additional specific comments:

(1) Although OECD TG 212 also mentions zebrafish as *Brachydanio rerio*, the title of this species has been changed to *Danio rerio*.
(2) The term "larvae" should be avoided for the early developmental stages used in this study. The official title of OECD TG 212 also reads "Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages". Seven days old individuals of zebrafish are scientifically correctly termed "eleutheroembryos", since they still live on the remnants of the yolk, but have not yet completely initiated external food uptake
(3) The term "chronic toxicity" should be avoided, since OECD TG 212 does not use this term for the test itself. OECD TG 212 explicitly states that "Guideline does not replace Guideline 210 but it would provide useful information in that it could (a) form a bridge between lethal and sublethal tests, (b) be used as a screening test for either a Full Early Life Stage test (Guideline 210) or for chronic toxicity
(4) Composition of reconstituted water is missing.
(5) Lack of information on the strain, age of the fish used for egg production.
(6) Lack of information on the parental fish: the water used for the maintenance, maintenance conditions, composition of breeding groups (Loading).
(7) The quality of the chemical analysis cannot be assessed, since reference to an internal SOP is not sufficient as long as the SOP is not provided
(8) Oxygen saturation occasionally drops below 60 % (e.g. 4.4 mg/L in some replicates of 0.56 mg/L test solution, which is equivalent to 53 % [saturation: 8.3 mg/L at 23.5 °C]). The minimal acceptable oxygen saturation for OECD TG 212 is 60 %. Since such low oxygen saturation were measured repeatedly (Table p. 30 of report), this parameter is somewhat borderline

Summarized Deviations from the test guideline:
As per deviations compared from the current OECD 212 guideline (1998):
Major:

- The renewal intervals for the test solution has been extended from 24 h to 48 h.
- The information about the age of the embryos upon initiation of chemical exposure was confusing, if not lacking.
- pH of ~ 7.4 (recommendation OECD TG 212: 7.8) and a temperature of ~ 24 °C (recommendation OECD TG 212: 28 °C).

Minor:

- Lack of data on fertilization rate.
- No details on behavioural observation provided

Given these major problems and the relatively long list of specific comments listed below, **this report would not be acceptable as a *scientific publication*.**

Assessment and conclusion by applicant:
According to expert opinion, study RF-D62.16/99 would not be accepted for scientific publication due to various deficiencies and should also not be considered a chronic study in the assessment of effects of glyphosate on fish.
As a publication, this study would not be considered reliable and would not be considered for risk assessment.

Assessment and conclusion by RMS:

I. Information on the study

Data point:	CA 8.2.4/004
Report author	[redacted]

Report year	2011
Report title	AMPA (Aminomethylphosphonic acid): An early life-stage toxicity test with the fathead minnow (*Pimephales promelas*)
Report No	139A-39A
Document No	-
Guidelines followed in study	OECD Guideline 210 (1992) OPPTS 850.1400 ASTM E 1241-05
Deviations from current test guideline	Deviations from the current OECD 210 guideline (1992): none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of AMPA (Aminomethyl-phosphonic acid) on the time of hatch, hatching success, survival and growth of fathead minnow (*Pimephales promelas*), was evaluated in a fish early life-stage toxicity test performed under flow-through exposure conditions, using a continuous flow test item delivery system. The appropriate test medium was supplied to four replicates at each of five concentrations and a negative control (dilution water only) group. The fertilized eggs were exposed to AMPA at nominal test concentrations of 0.75, 1.5, 3.0, 6.0 and 12 mg/L for a 5 day hatching period followed by a 28 day post hatch growth period. AMPA concentrations in test media were measured on day 0, 7, 14, 21, 28 and 33. Mean measured concentrations ranged from 82.5 to 117% of nominal concentrations. AMPA was not detected in the control group.

No significant differences in the time to hatch, hatching success, survival at test termination and growth (total length, wet and dry weight) were observed, when compared to the control. All validity criteria according to the current guideline OECD 210 were fulfilled.

In an fish early life stage test (OECD 210), performed using fathead minnows (*Pimephales promelas*) the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) for fathead minnow (*Pimephales promelas*) exposed to AMPA were determined to be ≥ 12.0 and > 12.0 mg/L, respectively, based on mean measured concentrations. The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item:	AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7%
2. Vehicle and/or positive control:	Vehicle: moderately hard well water Positive control: none
3. Test organism:	
Species:	Fathead minnow (*Pimephales promelas*) embryos <24 hours old
Age of eggs:	<24 hours old

Number of animals/dose level:	20 organisms per replicate i.e. 80 organisms per treatment level and control
Supplier:	[redacted]
Mean loading rate (biomass per volume of test solution)	0.05 g fish/L per 24 hours; instantaneous loading at the end of test: 0.32 g fish/L
Diet/Food:	live brine shrimp nauplii (*Artemia* sp.), Brine Shrimp Direct, Ogden, Utah, USA
4. Environmental conditions:	
Temperature:	25±1°C
pH:	7.8 to 8.2
Dissolved oxygen:	≥ 89% of saturation (7.3 mg/L)
Conductivity of test medium:	361 - 395 µS/cm
Hardness of test medium:	132 - 140 mg/L $CaCO_3$
Photoperiod:	16 hours with a 30 minute transition period; Light intensity = 296 lux
5. Dates of experimental work:	13th January to 03rd February 2011

B. STUDY DESIGN

Experimental treatments

The fish early life-stage toxicity test was performed under flow-through exposure conditions, using a constant-flow test item delivery system, supplying the appropriate test medium to the exposure vessels at each of the five concentrations and a negative control (dilution water only) group. The embryos of fathead minnow (*Pimephales promelas*) were exposed to AMPA at test concentrations of 0.73, 1.5, 2.9, 6.0 and 12.0 mg/L for 33 days. The test was conducted in a temperature controlled environmental chamber. The test vessels were 9 L glass aquaria with a constant volume of 7 L of test solution. Embryos were held in incubation cups constructed from glass cylinders 50 mm in diameter with 425 µm nylon screen mesh. Four replicates vessels were maintained for all treatments and the control.

At test initiation, embryos <24 hours old were impartially distributed to incubation cups. After a hatching period of 5 days, larvae were released into test chambers. Newly hatched larvae were fed live brine shrimp nauplii (*Artemia sp*.) harvested from hydrated cysts 2 - 3 times per day.

Observations

During the first day of exposure, embryos were observed twice for mortality and fungal infection. Thereafter, until hatching was complete, observations of embryo mortality and the removal of dead embryos was performed once per day. Once hatching had reached >90% in the control groups on day 5 of the test, the larvae were released into their respective test vessels and the post-hatch period began. During the 28-day post-hatch exposure period, the number of fry mortalities and numbers of individuals exhibiting clinical signs of toxicity or abnormal behaviour was recorded. From these observations, the time to hatch, hatching success, and post-hatch growth and survival were evaluated. On day 28 of the post-hatch exposure period – test termination, the total length for all surviving fish was measured to the nearest 1 mm using a metric ruler and wet and dry weights of all fish was measured to the nearest 0.1 mg using an analytical balance. Fish were euthanized (MS-222) and dried to constant weight in an oven at approximately 60 °C for approximately 47 hours to establish fish dry weight data.

Dissolved oxygen, temperature and pH were measured in alternating replicates of each treatment and control group at the beginning of the test, weekly during the test, and at the end of the test. Hardness, alkalinity and specific conductance were measured in alternating replicates of the negative control (dilution water) and the highest concentration treatment group at the beginning of the test, weekly during the test and at the end of the test.

Analytical procedures
Analytical measurements were performed by HPLC analysis using UV detection. Water samples were collected from one test chamber of each treatment and control group four days prior to test initiation to confirm the operation of the diluter. Water samples were collected from alternating replicate test chambers of each treatment and control group on day 0, 7, 14, 21, 28 and 33 (test termination) to determine concentrations of the test substance in the test chambers. All samples were collected at mid-depth in the test chambers, placed in glass vials and analysed immediately.

Statistical calculations
Data were statistically tested using Chi-square and Fisher's Exact test (discrete-variable data; $\alpha = 0.05$) and Dunnett's t-test (one-tailed, normal distributed data; $\alpha = 0.05$).The NOEC and LOEC were determined by visual interpretation of the observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS
Analytical data: Analytical measurements were performed on samples of representative test concentrations. Recoveries ranged from 82.5 % to 117% relative to nominal concentrations for all test concentrations and ranged from 97 to 100% of nominal for overall mean measured concentrations.

Table 8.2.2.1-10: Analytical results

Nominal concentration of AMPA [mg/L]	Mean measured concentration of AMPA [mg/L]	% of nominal
Control	Control	-
0.75	0.73	97
1.5	1.5	100
3.0	2.9	97
6.0	6.0	100
12	12	100

The water quality parameters measured were not affected by test item concentrations. The results of the water quality measurements carried out during this study established that conditions maintained throughout the 33-day exposure were satisfactory for the promotion of normal fathead minnow embryo hatchability, fry survival and growth.

B. OBSERVATIONS
The effects of AMPA on embryo viability, hatching success and growth (total length, wet and dry weight) are provided in the table below.

Table 8.2.2.1-11:Hatching success, larval survival and total length, wet weight and dry weight of fathead minnow (*Pimephales promelas*) at test termination of the 33-day exposure to AMPA.

Mean measured concentration of AMPA [mg/L]	Hatching success [%]	Survival to day 28 post hatch [%]	Growth 28 days post-hatch		
			Mean total length [mm]	Mean wet weight [mg]	Mean dry weight [mg]
Control	99	91	25.2 ±0.57	112.0 ±11.5	24.1 ±1.4
0.73	100	91	25.2 ±0.27	120.7 ±7.4	24.6 ±1.0
1.5	100	93	25.5 ±0.39	119.3 ±14.2	24.9 ±2.1
2.9	100	90	25.7 ±0.62	117.4 ±3.8	23.5 ±0.42
6.0	100	91	25.4 ±0.22	117.4 ±4.2	23.6 ±0.70
12	99	92	26.2 ±0.62	135.2 ±11.0	26.5 ±2.9

The majority of the fish in the control group and in the AMPA treatment groups appeared normal throughout the test. Through Day 7 post-hatch, in the control group and in the AMPA treatment groups, a low frequency of larvae were noted as either weak, lying on the bottom of the test chambers, curled, or having a curled or curved spine/crooked spine. The frequency of curved/curled or curled spine/crooked spine observed in the treatment groups were comparable to historical frequencies observed in control treatments in early life-stage studies with fathead minnows performed at the test facility and consequently concluded to be not treatment related. Additionally, the frequencies of the occurrence of smaller fish visually observed in the control and treatment groups were comparable and consistent with the individual dry weight measurements.
The 33-day NOEC values are given below based on mean measured concentrations.

Table 8.2.2.1-12: Endpoints table

Endpoints (33 days)	AMPA [mg/L]
LOEC (hatching success, survival or growth)	>12
NOEC (hatching success, survival or growth)	≥ 12

All validity criteria according to OECD 210 were fulfilled, as dissolved oxygen concentration was between 60 % and 100 % of air saturation, water temperature was within the range specified for the test species and constant exposure conditions have been maintained (i.e. within ± 20% of nominal concentration were recovered), and overall survival of fertilised eggs/embryos in the controls was greater than or equal to the limits defined in Annexes 3 and 6 of OECD 210.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a fish early life stage test (OECD 210) performed using fathead minnow (*Pimephales promelas*) exposed to AMPA, the NOEC and LOEC values for hatching success, fry survival, length and weight were ≥ 12 and >12 mg/L, respectively, based on mean measured concentrations.

The study is considered valid and the NOEC for fathead minnow exposed to AMPA was ≥ 12 mg/L (mean measured concentrations) and is considered to be appropriate for use in ecotoxicological risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.2.1/005
Report author	Rodrigues, L.B. *et al.*
Report year	2019
Report title	Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms
Document No	doi.org/10.1016/j.mrgentox.2019.05.002 E-ISSN: 1873-135X
Guidelines followed in study	OECD 236
Deviations from current test guideline	Not reported
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The present study assessed the acute toxicity of glyphosate, as well as the main metabolite aminomethylphosphonic acid (AMPA) on non-target aquatic organisms. The toxic effects of these chemicals were evaluated in a zebrafish (*Danio rerio*) embryo-larval toxicity test according to OECD Test Guideline 236 at 6 concentrations between 1.7 and 100 mg/L. Three replicates with 20 fertilized eggs per concentration were used.

Glyphosate and AMPA caused no acute toxic effect (LC_{50}-96 h > 100 mg/L).

Materials and methods

Test chemicals; Technical-grade glyphosate (GLY; Glyphosate PESTANAL®; purity 99 %, CAS No. 1071-83-6) and aminomethylphosphonic acid (AMPA, purity 99 %, CAS No. 106651-9) were purchased from Sigma-Aldrich.

Zebrafish maintenance and egg production; Adult male and female zebrafish (*D. rerio*) were provided by the zebrafish facility (ZebTec Tecniplast) at the Institute of Biology, University of Brasília and kept in separate tanks (ethical approval UFG Nº 102/2014). Fish were maintained in a Rack Hydrus (Alesco) recirculating system using water filtered by reverse osmosis, where water passes through several levels of filtration (activated carbon filters and biological filters), is then disinfected by ultraviolet (UV) light and automatically adjusted for pH and conductivity. The temperature was maintained at 26 ± 1 °C, conductivity at 750 ± 50 µS, pH at 7.5 ± 0.5 and dissolved oxygen of 8 ppm. Nitrate, nitrite and ammonia were regularly monitored. This water was used in preparing the test solutions of all assays performed. Adult organisms were fed with commercial dry flake food (TetraColor Flakes®) and live brine shrimp. On the day of the test, zebrafish eggs were collected about 30 min after natural mating, rinsed in water and examined under a stereomicroscope (Bel Photonics STM PRO). Unfertilized or damaged eggs were discarded. The fertilization success was checked, and only batches of eggs with a minimum fertilization rate of 90% were used.

Fish embryo acute toxicity (FET) test; The zebrafish embryo-larval toxicity test was carried out according to OECD Test Guideline 236. Twenty fertilized eggs per concentration were randomly selected and carefully distributed in a 24-well plate, filled with 2 mL of GLY, AMPA at 1.7, 5, 10, 23, 50 and 100 mg/L and controls (negative control – NC: maintenance water and positive control – PC: 3,4-dichloroaniline at 4.5 mg/L). Tests were performed in triplicates (three independent experiments) in a climate chamber at 26 ± 1 °C and 12 h light under static conditions. Neither food nor aeration was provided during the bioassays. Embryo development was assessed at 24, 48, 72 and 96 h post-fertilization (hpf), using a stereomicroscope (Bel Photonics STM PRO) with 3x magnification. The distinction between the normal and abnormal development of embryos was established according to the zebrafish development descriptions reported previously. Lethal (egg coagulation, no somite formation, nondetachment of the tail from yolk sac and no heart beating) and sublethal (effects on the eye and body pigmentation, absorption of the yolk sac, hatching rate, swimming bladder inflation, otolith, presence of edemas and blood accumulation, tail deformities) parameters were observed and reported.
Statistical analysis; The FET and Comet data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Each experimental value was compared to its corresponding negative control and the statistical difference was considered significant when $p < 0.05$. With respect to the FET, the toxicity was expressed as the lethal concentration (LC50), which was calculated using GraphPad Prism software (version 5.0, GraphPad Software, San Diego, CA, USA) with 95% confidence interval.

Results

Acute effects for zebrafish early-life stages; The present study investigated the effects of active ingredient GLY and its metabolite AMPA on the zebrafish embryonic development (survival and malformations) at 24, 48, 72 and 96 h of exposure. According to Fig. 1, no significant mortality was observed in zebrafish early-life stage after exposure to different concentrations (1.7–100 mg/L) of GLY and AMPA (Fig. 1), which presented survival rate ≥90 % in all exposure periods.

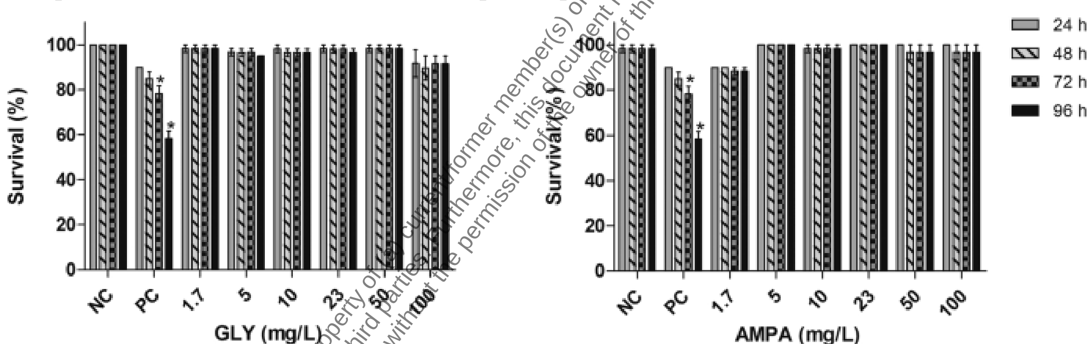


Fig. 1. Survival rate of zebrafish at different developmental stages exposed to GLY and AMPA for 24, 48, 72 and 96 h. Twenty fertilized eggs per experimental group were evaluated. Bars represent the mean ± standard error of the mean of three independent experiments. *$p < 0.05$ statistically different from the respective negative control (NC) based on one-way ANOVA and Dunnett's post hoc test. PC = positive control (3,4-dichloroaniline at 4.5 mg/L after 24, 48, 72 and 96 h of exposure).

In relation to sublethal effects, Fig. 2 shows that GLY induced some morphological abnormalities, however, these malformations were not statistically significant when compared to their respective negative control.

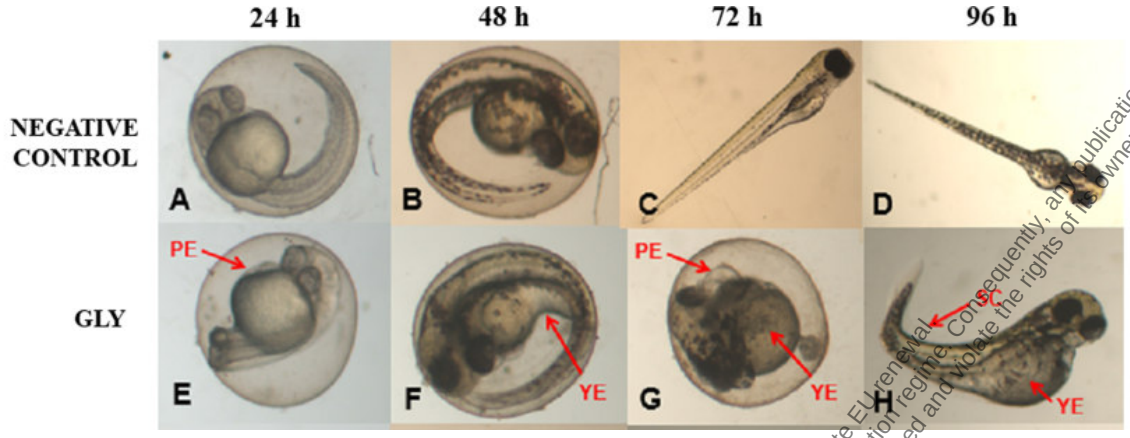


Fig. 2. Zebrafish embryos and larvae abnormalities after GLY exposure: pericardial edema (PE), yolk sac edema (YE), spinal curvature (SC). Embryos control after 24 h and 48 h of exposure, respectively (A–B); larvae control after 72 h and 96 h of exposure, respectively (C–D); embryos exposed to GLY at 23 mg/L and 100 mg/L for 24 h and 48 h, respectively (E–F); non-hatching embryo exposed to GLY at 10 mg/L for 72 h (G); larvae exposed to GLY at 100 mg/L for 96 h (H).

Discussion

The current results showed that glyphosate and AMPA did not induce acute toxicity in zebrafish early-life stage with LC_{50}-96 h > 100 mg/L. Similar effect was observed by researchers in assessing the acute effects of glyphosate (0.005; 0.05; 5; 10 and 50 mg/L) on early-life stages of zebrafish and common carp (Cyprinus carpio) for 120 h. The authors demonstrated that all tested concentrations, except the highest concentration (50 mg/L), induced cumulative mortality ≤ 10% after 96 h of exposure. Glyphosate at 50 mg/L caused the highest cumulative mortality, reaching 17.5% after 120 h of exposure while in this study, there were no significant differences between this group (glyphosate at 50 mg/L) and control with 1.7 % of larvae mortality after 96 of exposure. It is worth noting that according to OECD 236, the survival of embryos in the NC must be ≥ 90 % (validation criterion of the test), and therefore mortality ≤ 10 % in the experimental groups is acceptable.

Conclusion

Glyphosate and AMPA caused no acute toxic effect (LC_{50}-96 h > 100 mg/L) in zebrafish.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The acute toxicity of technical glyphosate and its metabolite aminomethylphosphonic acid (AMPA) to zebrafish embryos was investigated.

Glyphosate and AMPA caused no acute toxic effect (LC_{50}-96 h > 100 mg/L) in zebrafish.

The study was stated to have been conducted according to OECD guideline 236, but there is no information on hatching rates in the treatment and control groups, so exposure of the embryo without a potential barrier function of the chorion cannot be confirmed.

Concerning the validity of the study, four of the six validity criteria from the test guideline are mentioned in the paper (fertilization rate of embryo batches used was >90%, survival in the negative control group was > 90%, temperature was maintained at 26 ±1ºC and dissolved oxygen was at an acceptable level 8ppm). There is no information presented on the performance of the positive control group (3, 4-dichloroaniline) and no information provided on the hatching rates in the negative control group at 96 hours, which for the control group should exceed 80%. As these information are not presented and the

fact that there was no analytical verification of test concentrations reported, this study considered as reliable with restrictions.

1. Information on the study

Data point:	CA 8.2.2.1/006
Report author	Schweizer, M. *et al.*
Report year	2019
Report title	How glyphosate and its associated acidity affect early development in zebrafish (*Danio rerio)*
Document No	DOI 10.7717/peerj.7094 ISSN: 2167-8359
Guidelines followed in study	OECD Guideline 236
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Zebrafish (*Danio rerio*) embryos exposed to concentrations between 10 µM and 10 mM glyphosate (corresponding concentrations between 1.69 and 1690.7 mg glyphosate/L) in an unbuffered aqueous medium, as well as at pH 7, for 96 hours post fertilization (hpf). Furthermore, for investigations of the influence of pH, the test concentration 1 mM glyphosate (169.07 mg glyphosate/L) was tested at different pH values ranging between pH 3 and 8 vs. the respective pH controls. A total of 32 embryos were used per treatment with 8 replicates of 4 embryos each. The observed endpoints included mortality, the hatching rate, developmental delays at 24 hpf, the heart rate at 48 hpf, hatching success from 60 to 96 hpf and malformations at 96 hpf. $LC_{10/50}$, EC_{10} and, if reasonable, EC_{50} values were determined for unbuffered glyphosate.
In unbuffered glyphosate medium the lethal concentrations were calculated to be 385 mM (LC_{10}) and 582 mM (LC_{50}) at 96 hpf. Regarding heart rates the EC_{10} was 43 mM. Concerning the hatching rate, EC_{10} and EC_{50} levels at 96 hpf were 155 and 224 mM, respectively. For developmental delays at 24 hpf the EC_{10} was 126 mM.

Materials and methods

Glyphosate; Glyphosate (N-(phosphonomethyl)glycine, 96% pure substance, molecular weight: 169.07 g/mol, CAS: 1071-83-6; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was used to prepare the test solutions. A stock solution with a concentration of 25 mM was prepared as follows: glyphosate was diluted in reconstituted water (0.23 g KCl, 2.59 g $NaHCO_3$, 4.93 g MgS_4O x 7 H_2O and 11.76 g $CaCl_2$ x 2 H_2O were dissolved separately in one L double-distilled water, then 25 mL of each stock solution was added to 900 mL double-distilled water). The stock solution was then diluted to the following test concentrations: 10, 50, 100, 250, 500, 750 mM, one and 10 mM glyphosate. All those concentrations were tested unbuffered and at pH 7. For pH adjustments, 1M HCl and NaOH solutions were used as recommended in the Organisation for Economic Co-operation and Development (OECD) 236 (2013) guideline. For investigations of the influence of pH, 1 mM glyphosate was tested at different pH values ranging between pH 3 and 8 vs. the respective pH controls. Due to preliminary results from the broad-scale pH testing, particular attention was paid to the range between pH 3 and 4. Measurements of pH were conducted with a pH meter (SevenCompactDuo; Mettler Toledo, Gießen, Germany) directly prior to the exposure.

Maintenance of zebrafish and test procedure; The embryos used in this study stem from our own breeding stock of the D. rerio West aquarium strain established in the Animal Physiological Ecology group,

Tübingen University. Adult zebrafish were kept in 90 L aquaria filled with a 1:1 mixture of purified water and filtered tap water (AE-2L water filter with an ABL-0240-29 activated carbon filter, 0.3 mm; Reiser, Seligenstadt, Germany) at 26 ± 1 °C and an oxygen saturation of 100% ± 5%. Conductivity ranged from 260 to 350 mS/cm, nitrite and nitrate concentrations from 0.025 to 0.1 mg/L, one and five mg/L, respectively, and total water hardness from eight to 12 dH. Fish were subjected to an artificial 12:12 h day/night cycle and fed three times daily with flake food (TetraMin; Tetra GmbH, Melle, Germany) supplemented with frozen black mosquito larvae and glass worms (Poseidon Aquakultur Freeze, Ruppichteroth, Germany) prior to spawning to ensure sufficient dietary protein. The day before the test, pre-exposure and test Petri dishes (90 and 30 mm in diameter) were filled with the respective solutions and stored at 26 ± 1 °C overnight to saturate the glass (the same was done with the Schott flask used for the stock solution, beforehand). On the morning of the test, Petri dishes were emptied and refilled with 70 mL (pre-exposure) and three mL (test Petri dishes) solution. For spawning, Plexiglas boxes 20 x 20 x 6 cm in size and covered with a mesh grid to keep zebrafish from feeding on their own eggs were used as breeding boxes. They were topped with artificial sea grass acting as an optical spawning stimulus and were placed into the fish tanks the evening before the start of the test. Zebrafish spawn at sunrise; therefore, spawning in the laboratory starts with the onset of light the next morning. Eggs were collected with a sieve, rinsed with tepid tap water, transferred into pre-exposure Petri dishes and incubated for 2 h at 26 ± 1 °C. Following the pre-exposure, eggs for the test were chosen with regard to their age and developmental stage (0 hours post fertilization (hpf) ≙ 8 a m.), placed into the small 30 mm Petri dishes and stored in a heated cabinet at 26 ± 1 °C. A total of 32 individuals were used per treatment, that is, four per Petri dish and eight replicates each. Embryos were checked every 12 to 24 h. Endpoints investigated under a stereo microscope (Stemi 2000-C; Zeiss, Oberkochen, Germany) included mortality, developmental delays at 24 hpf, heart rate at 48 hpf, hatching success from 60 to 96 hpf and malformations at 96 hpf. Except for mortality, analysis of all endpoints, including hatching success, was based on living embryos/larvae at the respective time point of evaluation.

Table 8.2.2.1-13: Overview of observed lethal and sublethal endpoints at respective time points.

Endpoint	12 hpf	24 hpf	48 hpf	60 hpf	72 hpf	96 hpf
Mortality	✓	✓	✓	✓	✓	✓
Developmental delays		✓				
No somites		✓				
Non-detachment of the tail		✓				
No development of the eyes		✓				
Heart rate			✓			
Hatching success				✓	✓	✓
Malformations						✓
Oedema						✓
Eye/brain defects						✓
Deformation of the spine						✓
Light pigmentation						✓

Heart rates were determined from two out of four individuals per Petri dish for 20 s, and values were extrapolated to 1 min. Coagulated eggs, dead larvae and empty egg shells were removed from the Petri dishes to avoid depletion of oxygen due to biological degradation processes. The embryo test was run three times and conducted according to Organisation for Economic Co-operation and Development (OECD) 236 (2013). The compound 3,4-dichloraniline (98%, CAS: 95-76-1; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) at a concentration of four mg/L served as a positive control and reconstituted water, as a negative control. According to the Directive 2010/63/EU of the European Parliament and the Council on the protection of animals for scientific purposes, D. rerio embryos and larvae that do not feed independently are not regarded as animals, thus regulations and permissions for animal testing do not apply. Nevertheless, all embryos in our tests were handled in the least stressful way possible and with the utmost care. After test termination embryos/larvae were euthanized with MS222.

Statistics; All statistical analyses were conducted in JMP 11.2.0 (SAS Institute Inc., Cary, NC, USA). Mortality, hatching success and the malformation rate at 96 hpf, as well as developmental delays at 24 hpf, were analysed with a likelihood-ratio w2 test, followed by Fisher's exact test. Finally, the sequential Bonferroni-Holm method was applied accounting for multiple testing. A Cox regression was used to assess mortality and hatching success over time. For the analysis of heart rate, the data were averaged per Petri dish and checked for a normal distribution and homogeneity of variances. Subsequently an ANOVA with Tukey's HSD or Dunnett's test was conducted. If data did not meet the criteria for an ANOVA and transformation of the data did not lead to the desired result, a non-parametric Steel-Dwass test was conducted instead. Additionally, for assessing the pH range in which pH control and glyphosate treatments differed in heart rate across the whole span of tested pH, non-linear regression analysis, including calculation of 95% confidence intervals (TableCurve 2D v5.01; SYSTAT Software Inc., San Jose, CA, USA), was applied. Non-linear regression analysis by TableCurve was also used for determining LC10/EC10 and LC50/EC50 values of endpoints in unbuffered glyphosate treatments.

Results

After 96 hpf, mortality and hatching success were 0% and above 80%, respectively, in control embryos. The 3,4-dichloraniline positive control induced high mortalities, with rates consistently above 80 % after 96 hpf. Thus, the validity criteria according to Organisation for Economic Co-operation and Development (OECD) 236 (2013), including sensitivity of zebrafish, were met.

Unbuffered glyphosate: At the two highest concentrations tested (1 and 10 mM), it was already difficult to select well-developed eggs after the 2 h pre-exposure period. The yolk sac, which usually has a regular spherical shape, was found to be asymmetric and partly oval, and the chorion fluid, which is naturally clear, was murky in some cases and contained indefinable streaks.

As early as 12 hpf, all individuals, without exception, in the 10 mM treatment died. Mortality in the 1 mM exposure experiment was beyond 85% at 12 hpf and reached 100% within the first 24 h. Within the 750 mM glyphosate treatment, only six out of a total of 96 individuals survived until the end of the test at 96 hpf, whereas concentrations of 250 mM and below resulted in negligible or no mortality (3.125 %). Regarding mortality at 96 hpf, all treatments 500 mM were highly significantly different from the control (likelihood ratio w2, $p < 0.001$). Lethal concentrations were calculated to be 385 mM (LC10) and 582 mM (LC50) at 96 hpf. Heart rates showed a concentration-dependent relationship, decreasing with increasing glyphosate concentration. The mean heart rate was 149 beats per minute (bpm) for the control and between 130 and 140 bpm for low (10, 50 mM), 120 and 130 bpm for medium (100, 250 mM) and 110 and 120 bpm for the higher (500, 750 mM) concentrations. Thus, differences between the control and the 750 mM concentration ranged between 30 and 40 bpm. The treatments with the highest concentrations of glyphosate (one mM, 10 mM) could not be evaluated due to 100% mortality at that time point. Only two individuals out of those exposed to one mM glyphosate survived until 60 hpf and seemed to continue the observed relationship between glyphosate and heart rate by showing even lower rates (93 and 96 bpm). As single individuals, they were not included in the statistical analysis. All remaining treatments were significantly different from the control (ANOVA with Tukey's HSD, $p < 0.001$) and the relationship between glyphosate concentration and heart rate could be described by linear regression analysis ($R2 = 0.546074$, $p < 0.001$). The EC10 was 43 mM. Concerning the hatching rate, we observed a clear division between a cluster of treatments that comprised the control treatment and lower concentrations of glyphosate (10, 50, 100 mM) and another treatment cluster comprising higher concentrations (250, 500, 750 mM).

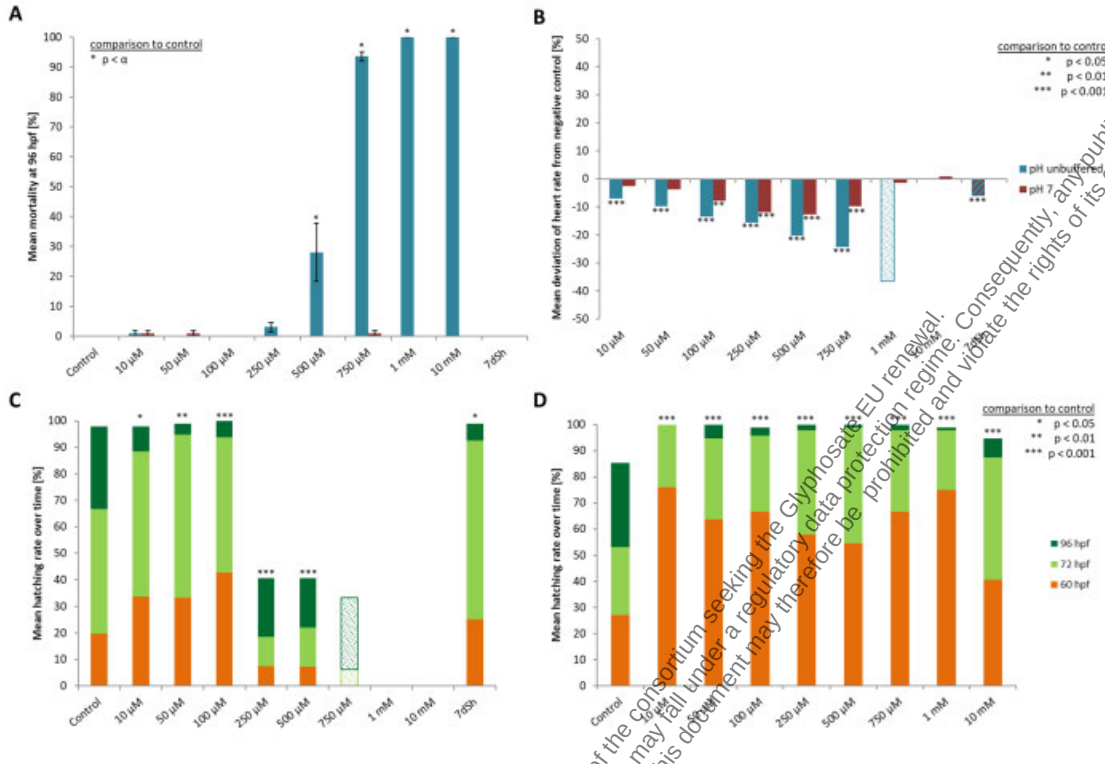


Figure 8.2.2.1-1: Mortality, heart rate and hatching success in percentage of unbuffered and pH 7 treatment. (A) Mortality after 96 hpf (likelihood ratio χ^2, Fisher's exact test, Bonferroni-Holm, $p < \alpha$), (B) heart rate at 48 hpf relative to the negative control (Steel-Dwass, $p < 0.01$), (C) hatching rate over time in unbuffered treatments (Cox regression, $p < 0.05$), (D) hatching rate in pH 7 treatments over time (Cox regression, $p < 0.001$); shaded bars mark treatments with $n < 5$ that show tendencies but are not included in the statistical analyses.

Embryos exposed to lower concentrations hatched in 98–100% of cases, whereas hatching success in the experiments with 250 and 500 mM glyphosate was approximately 40%. All glyphosate treatments showed significant differences compared with the control (Cox regression, $p < 0.05$). EC10 and EC50 levels at 96 hpf were 155 and 224 mM, respectively. There were no developmental delays at 24 hpf for glyphosate concentrations between 10 and 100 mM, whereas in treatments with 250 to 750 mM, rates varied from 15 % to 25 %. The EC10 for this endpoint was 126 mM. Results for all concentrations 250 mM were highly significant (likelihood ratio w2, $p < 0.001$) compared with the control. A direct concentration dependency could not be observed. Rather, it seemed that a distinct concentration threshold had to be exceeded to induce those developmental delays and failures, which later approached the same level. Prevalent defects were a lack of tail detachment, sometimes combined with apically curved tails; a lack of somite formation and an impairment of eye development was not detected. Occasionally, embryos were fully developed but either the complete tail or just the posterior end of their tails remained attached to the yolk sac. Under normal conditions, movement begins after tail detachment. Yet, even the embryos in glyphosate treatments that lacked tail detachment, overall development had progressed to a point at which muscular contractions were already visible. But due to the undetached tails, embryos were unable to turn around and their movement was very limited. Additionally, some embryos had the posterior end of their tails detached but displayed severe spine deformations.
Those embryos could not move their tails in the same fluid manner as normally developed embryos could. Malformations could be found in embryos of all glyphosate treatments but with rates below 20 %. All glyphosate treatments were significantly different from the control. Among the malformations recorded, lightly pigmented embryos and larvae were particularly frequen). Furthermore, reduced eye size occurred regularly, and some individuals suffered from cardiac or yolk sac oedemas. Two individuals showed a

notable shortening of the tail. Deformations of the spine at 96 hpf were observed surprisingly rarely, despite the high rates of tail and spine malformations at 24 hpf.

Table 8.2.2.1-14: Results for concentration-dependent glyphosate treatments, as well as for pH-dependent control and glyphosate treatments, as percentages.

	Mortality		Hatching		HR	D	M
	96 hpf (%)	Over time	96 hpf (%)	Over time	48 hpf (bpm)	24 hpf (%)	96 hpf (%)
Unbuffered							
Neg. control	0	–	97.92	–	148.75	0	0.26
10 µM	1.04	n.s.	97.92	*	**138.38*** **	0	**2.36***
50 µM	0	n.s.	98.96	*	**134.19*** **	0	**5.47***
100 µM	0	n.s.	100	***	**128.69*** **	0	**4.69***
250 µM	3.13	n.s.	**40.65***	***	**125.56*** **	**22.23***	**13.57***
500 µM	**28.13***	*	**40.58***	***	**118.63*** **	**30.36***	**16.06***
750 µM	**93.64***	***	**33.33***	n.a.	**94.50*** **	**19.04***	**18.06***
1 mM	**100***	***	n.a.	n.a.	n.a.	n.a.	n.a.
10 mM	**100***	***	n.a.	n.a.	n.a.	n.a.	n.a.
LC_{10}/EC_{10}	385 µM		155 µM		43 µM	126 µM	179 µM
LC_{50}/EC_{50}	582 µM		224 µM		–	–	–
7dSh	0	n.s.	98.93	*	**139.25*** **†**	0.35	**0.27†**
Neutral (pH 7)							
Neg. control	0	–	85.42	–	148.04	0	0.52
10 µM	1.04	n.s.	**100***	***	144.25	0	1.04
50 µM	1.04	n.s.	**100***	***	142.56	1.04	1.87
100 µM	0	n.s.	**98.96***	***	**136.57****	0	2.60
250 µM	0	n.s.	**100***	***	**130.31*** **	0	0.26
500 µM	0	n.s.	**100***	***	**129.19*** **	0	1.56
750 µM	1.04	n.s.	**100***	***	**133.50***	0	2.35
1 mM	0	n.s.	**98.96***	***	145.94	0.69	**6.56***
10 mM	0	n.s.	**94.79***	***	149.38	0	**7.29***
pH range—control							
Neg. control	**0**	–	94.69	–	160.38	0	0.26
pH 3	**100***	***	n.a.	n.a.	n.a.	n.a.	n.a.
pH 3.1	**100***	***	n.a.	n.a.	n.a.	n.a.	n.a.
pH 3.2	**100***	***	n.a.	n.a.	n.a.	n.a.	n.a.
pH 3.3	**84.03***	***	**61.11***	***	**140.19*** **	**9.36***	**4.17***
pH 3.4	**51.04***	***	**77.78*†**	***	**141.38*** **	**6.93***	**3.51***
pH 3.5	**8.33***	n.s.	**15.77*†**	***†	**144.25*** **	**8.32***	**2.0***
pH range—glyphosate							
pH 3	**100***	***	n.a.	n.a.	n.a.	n.a.	n.a.
pH 3.1	**100***	***	n.a.	n.a.	n.a.	n.a.	n.a.
pH 3.2	**100***	***	n.a.	n.a.	n.a.	n.a.	n.a.
pH 3.3	**72.57***	***	83.33	***	**136.46*** **	**13.33***	0
pH 3.4	**28.47***	***	**60.61*†**	***	**141.49*** **	**4.54***	**3.80***
pH 3.5	**17.36***	n.s.	**66.57*†**	***†	**143.50*** **	**10.31***	**2.04***

Notes:
Asterisks (*) and bold indicate statistically significant differences from the negative control (Cox regression, ANOVA. $^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$. Likelihood ratio χ^2, Fisher's exact test, Bonferroni-Holm: $^{*}p < \alpha$).
Crosses (†) denote additional statistical significances between pH control and glyphosate within the same pH range or, in the case of 7dSh, differences from 1 mM glyphosate at pH 7. For unbuffered glyphosate concentrations, endpoint-related LC_{10}/EC_{10} and LC_{50}/EC_{50} values are given.
HR, heart rate; D, developmental delays; M, malformations; n.s, not significant; n.a., not available (no sufficient sample sizes for statistical analysis).

Glyphosate at pH 7; When the glyphosate solutions were adjusted to pH 7, almost no mortality or developmental delays occurred, and malformation rates were below 10% but were still significantly elevated in 1 and 10 mM treatments (likelihood ratio w2, $p < 0.001$). In the concentration range of 10 to 500 mM, heart rates showed a similar trend to those in unbuffered treatments but at a lower level: bpm decreased with increasing concentration. Still, treatments between 100 and 500 mM differed significantly from the negative control (Tukey's HSD, $p < 0.01$). At 750 mM, heart rates increased again, with a higher frequency than at 250 and 500 mM. At the two highest concentrations (1, 10 mM), heart rates were, on the one hand, marginally decelerated (1 mM) and on the other hand, marginally accelerated (10 mM) compared with the negative control. Thus, it seems that there is a turning point between 500 and 750 mM, at which

the relationship between increasing concentration and heart rate shifts from deceleration to acceleration in comparison with the negative control. As already seen for lower concentrations in unbuffered treatments, glyphosate tends to induce early hatching, even at the lowest concentration and independently of concentration. This effect unfolded to its true extent in the pH-neutral treatments. At least twice as much larvae had hatched across all glyphosate treatments at 60 hpf compared with the negative control. After 72 hpf, all larvae were hatched in glyphosate treatments, except for single individuals that hatched at 96 hpf or did not hatch at all, whereas in the negative control, only 53 % of the embryos were hatched at 72 hpf and even about 15% remained unhatched at 96 hpf.

pH range , In a first step, one mM glyphosate was tested at pH 3, 4, 5, 6, 7 and 8 in comparison with negative controls at the respective pH but without the pesticide. Mortality was 100% for both treatments at pH 3, independent of the presence of glyphosate. Only a single individual survived the first 12 hpf. In contrast, only one individual died throughout all other exposures within 96 hpf. Morphological aberrations described for high glyphosate concentrations under unbuffered conditions also applied to low pH treatments, independent of glyphosate addition. Concerning sublethal endpoints, results between different acidities in the range of pH 4 to 8, as well as between control and glyphosate within the same pH range, were inconspicuous for the most part. Thus, the pH 3 to 8 series was tested just once, and subsequent testing concentrated on the range from pH 3 to 4. Thus, in the next step, pH 3, 3.25, 3.5, 3.75 and 4 were investigated in detail. As embryos exposed to pH 3.75 and 4 did not show any prominent effects, only a single run was conducted, and the final testing scheme was determined from pH 3 to 3.5 in 0.1 increments. Additionally, a test with unbuffered glyphosate at a one mM concentration (which resulted in a pH of 3.2 in the test solution) was included for direct comparison. Mortality decreased with increasing pH. Treatments with a pH of 3.2 and lower induced 100% mortality after 96 hpf. Whereas embryos exposed to pH 3 and 3.1 died within 48 hpf at the latest, embryos in pH 3.2 treatments survived considerably longer.

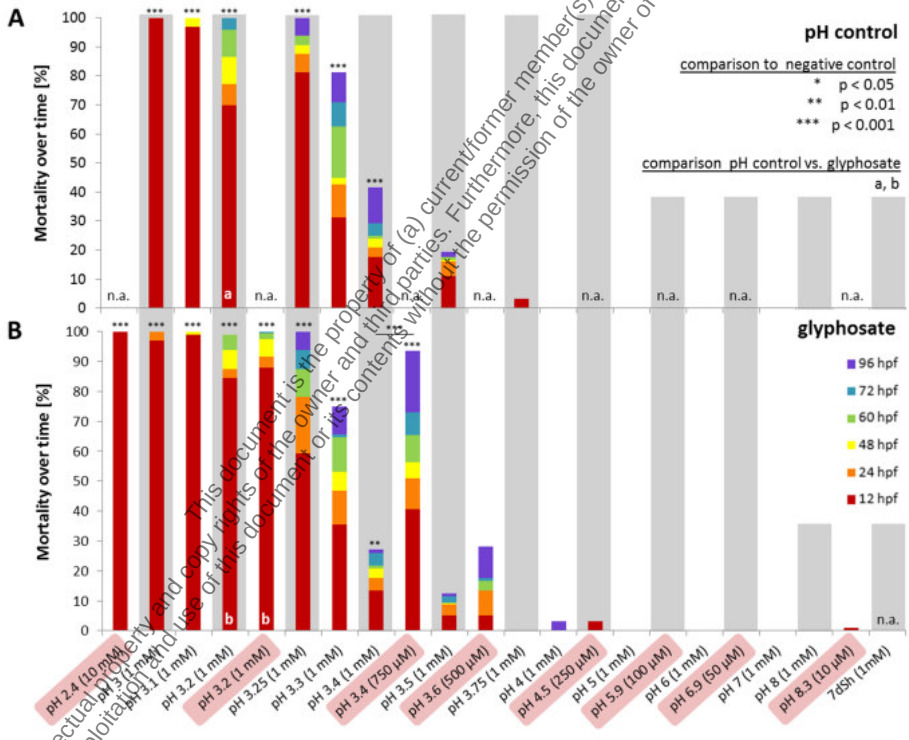


Figure 8.2.2.1-2: Mortality over time as percentages of embryos exposed to the pH control (A) and glyphosate (B). Respective concentrations of glyphosate are given in brackets. Results from unbuffered treatments (50 µM–10 µM glyphosate; highlighted in red) are combined with pH range results and positioned according to their measured pH. Treatments not conducted in the pH control or glyphosate scheme are labelled n.a. (not available). Significant differences from the negative control are marked with asterisks (*), except glyphosate pH 3.4 with an additional significant comparison between unbuffered and

pH 7 treatment. Significances between pH control and glyphosate treatments within respective pH ranges are denoted with letters (a and b) (Cox regression, $p < 0.01$).
Apart from pH 3.5 without glyphosate, all treatments showed elevated mortality rates compared with the negative control (Cox regression, $p < 0.05$). There were no differences between control and glyphosate treatments with corresponding pH values, except for the elevated mortality in unbuffered glyphosate compared with the respective pH 3.2 control. Compared with the negative control, hatching was significantly delayed and also reduced in both glyphosate and pH control treatments (Cox regression, $p < 0.001$). Whereas 30 % of the control embryos hatched at 60 hpf, in the pH control and glyphosate exposures, the hatching rate at 60 hpf was consistently below 5% (see Supplementary File, hatching rate). The tendency toward glyphosate-induced premature hatching at 60 hpf that was observed in pH-neutral treatments was not evident at low pH. Although not statistically significant (except for pH 3.5: Cox regression, $p < 0.001$), embryos exposed to glyphosate tended to hatch earlier and more frequently than embryos in the respective pH controls. Heart rates were significantly lowered by glyphosate at pH 3.3 to 3.5, as well as by the corresponding control pH treatments (Steel-Dwass, $p < 0.001$). Differences between glyphosate and the respective controls at the same pH value could only be detected when the full pH range dataset (including results for pH 3 to 8) was analyzed. At a pH between 5.55 and 6.02, glyphosate elevated the embryonic heart rate significantly compared with pH controls (TableCurve 2D v5.01). Developmental delays and malformations occurred in the low pH treatments, but they did not vary in a pH-dependent manner, and there was no detectable difference between glyphosate and the respective pH controls.
Comparison; When datasets for the unbuffered glyphosate treatment and the pH range were merged regarding mortality in relation to pH, interestingly, embryos exposed to unbuffered glyphosate showed higher mortalities at 500 and 750 mM compared with their 1 mM counterparts at pH 3.5 and 3.4, respectively. The unbuffered 750 mM treatment with a pH of 3.4, in particular, resulted in a mortality rate more than twice as high as that in the glyphosate pH 3.4 treatment (1 mM), mirroring mortality effects seen in treatments ranging rather between pH 3.25 and 3.3.

Conclusion

In unbuffered glyphosate medium the lethal concentrations were calculated to be 385 mM (LC_{10}) and 582 mM (LC_{50}) at 96 hpf. Regarding heart rates the EC_{10} was 43 mM. Concerning the hatching rate, EC_{10} and EC_{50} levels at 96 hpf were 155 and 224 mM, respectively. For developmental delays at 24 hpf the EC_{10} was 126 mM.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant:</u>

For Zebrafish (*Danio rerio*) embryos acutely exposed to glyphosate at concentrations between 1.69 and 1690.7 mg glyphosate/L (10 µM to 10 mM) for 96 hours post fertilization (hpf) the LC_{10} and LC_{50} values (96 hpf) were calculated to be 65.1 mg a.s./L (385 µM) and 98.4 mg a.s./L (582 µM), respectively (in unbuffered glyphosate medium). Regarding heart rates the EC_{10} was 7.27 mg a.s./L (43 µM). Concerning hatching rate, 96 hpf -EC_{10} and EC_{50} values were 26.2 mg a.s./L (155 µM) and 37.9 (224 µM), respectively. For developmental delays at 24 hpf the EC10 was 21.3 mg a.s./L (126 µM). The test was conducted according to OECD 236 test guideline.
Concerning the validity criteria in the OECD 236, despite the stated > 80% mortality in the positive control (>30% required) there are no details presented to confirm the level of mortality. The fertilisation rate of the batch of eggs used was not reported. Finally, acute endpoints based on developmental delay and heart rate are not relevant to an EU level risk assessment for Annex I renewal purposes.
The test design is adequately described, however, there was no analytical verification of test concentrations reported. The study is considered as reliable with restrictions.

CA 8.2.2.2 Fish full life cycle test

A full life cycle study is available and presented below.

Table 8.2.2.1-15: Studies on fish full life cycle test

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.2.2/001	Anonym., 1975	Chronic, 255 d FFLC, flow-through	Glyphosate acid	Valid	

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on fish full life cycles. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. For discussions of literature regarding toxicity to fish, please refer to document M-CP Section 10.2.

Endpoints of studies considered valid are shown in the table below.

Table 8.2.2.1-16: Endpoints: Full life cycle toxicity of glyphosate to fish

Reference	Test item	Species	Test design/ GLP	NOEC (mg a.e./L)
Anon., 1975, CA 8.2.2.2/001	Glyphosate acid	*Pimephales promelas*	Chronic, 255 d FFLC, flow-through /non-GLP	≥ 25.7 (mm)

a.e.: acid equivalents
mm: mean measured; cannot be determined from study report if arithmetic or geometric mean measured

Study summaries are provided below.

1. Information on the study

Data point:	CA 8.2.2.2/001
Report author	Anonymous
Report year	1975
Report title	Chronic Toxicity of Glyphosate to the Fathead Minnow (*Pimephales promelas*, Rafinesque)
Report No	BN-75-129
Document No	-
Guidelines followed in study	EPA: Recommended bioassay procedures for fathead minnow (Pimephales promelas, Rafinesque) chronic tests. By the Bioassay Committee, National Water Quality Laboratory, Duluth, USA (1971)
Deviations from current test guideline	Deviations from the current EPA guideline OPPTS 850.1500 (1996): - none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed

Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The effects of glyphosate on fathead Minnow (*Pimephales promelas*) were evaluated in a full life cycle test in flow-through test conditions. The test was performed using mean measured concentrations of 0.7, 2.8, 7.0, 13.0 and 25.7 mg glyphosate/L (mg a.s./L). In addition, a control group was exposed to the dilution water. At test initiation, thirty fathead minnow eggs were incubated in each test aquarium and observed for effects at all developmental steps of the full life cycle. Forty fish were divided into two groups of twenty each, were randomly selected, and distributed to growth chambers in each aquarium. Two growth chambers were used to facilitate handling of fry for 30 and 60 day measurements by a photographic method. Percent survival based on cumulative mortality was also determined at these intervals. After 60 day measurements, the number of fish released to each spawning chamber was impartially reduced to fifteen after combining fish from the growth chambers. When secondary sexual characteristics were well developed (circa day 134), the number of fish in each tank was reduced initially to four males and four females and subsequently (day 179) to two males and four females which were allowed to spawn.

During the full life cycle test, adult fecundity (approx. day 112) and survival (day 30, 60 and day 134) were recorded. The egg hatchability was determined on the first generation eggs 4 days after the test initiation. Total length (day 30, day 60 and day 255), total wet weight (day 254), sex ratio (day 134 and day 254) and gonadal conditions (day 254) were equally determined for each adult fish.

Temperature and dissolved oxygen were measured on a daily basis. The alkalinity, acidity and hardness of the test water were measured on a weekly basis. All validity criteria according to EPA guideline OPPTS 850.1500 were fulfilled. None of the parameters studied (adult fecundity, parental and juvenile mortality, total length, wet weight, sex ratio and gonadal conditions), were significantly affected by the chronic exposure to the test item.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Description:	None
Lot/Batch #:	Not stated
Purity:	87.3%

2. Vehicle and/or positive control:

Vehicle: dilution water

Positive control: none

3. Test organism:

Species:	Fathead minnow (*Pimephales promelas*, Rafinesque)
Age:	Not stated
Size:	Not stated
Loading:	40 fish per aquarium of 41 L test solution (at test initiation)
Source:	In-house stock culture
Diet/Food:	3 - 4 times per day *ad libitum* with brine shrimp nauplii (first 45 days);

	Twice a day *ad libitum* with frozen brine shrimp (after 45 days)
Acclimation period:	Not stated
Body weight of the animals:	1.5 g

4. Environmental conditions:

Temperature:	25 ± 1 °C (chronic test)
Photoperiod:	16 hours light / 8 hours dark
pH:	6.5 – 7.6
Dissolved oxygen:	6.3 – 9.0 mg O_2/L
Conductivity:	not stated
Hardness:	32 - 42 mg $CaCO_3$/L

5. Experimental dates of work: Test start: January 27th 1975

B. STUDY DESIGN

Experimental treatments: A fish chronic toxicity tests (full life cycle) was performed with glyphosate using concentrations 0.7, 2.8, 7.0, 13.0 and 25.7 mg a.s./L (mean measured) in a flow-through test. In addition, a control group was exposed to the dilution water. The test medium in aquaria was exchanged continuously through a flow-through system. A glass flow-splitting chamber was calibrated to deliver an equal flow rate to the growth chambers. There were six duplicate test vessels, containing 41L test solution each. At test initiation, thirty eggs were incubated in each test vessel. Dead eggs were removed and counted each day until hatching was completed (4 days at 25°C). 40 fish (selected from the hatched fish) were randomly distributed to growth chambers in each vessel. Percent survival based on cumulative mortality was determined at these intervals. After 60 day, the number of fish released to each spawning chamber was impartially reduced to fifteen after combining fish from the growth chambers. On day 64, five spawning sites were made. When secondary sexual characteristics were well developed (circa day 134) the number of fish in each tank was reduced initially to four males and four females and subsequently (day 179) to two males and four females. When spawning began (circa day 112), eggs were daily removed from the underside of spawning tiles and counted. Fifty eggs from each of the first ten spawning were then oscillated in their respective test waters and dead eggs were removed and counted daily, until hatching was completed. Twenty fry from the first two spawns in each tank, in which at least 80 % live hatch was observed, were placed in their respective growth chambers and observed for 30 days, after which fry groups were terminated and total lengths determined by the photographic method. Total length, wet weight, sex and gonadal conditions were determined for each adult fish at the termination of the experiment.

Observations: During the full life cycle test, adult fecundity was determined approximately on day 112 and survival was observed on day 30, day 60 and day 134. The egg hatchability was determined on the first generation (F_1) eggs 4 days after the test initiation. Total length, wet weight, sex and gonadal conditions were equally determined for each adult fish at termination of the experiment after 254 days. Temperature and dissolve oxygen were measured on a daily basis. The alkalinity, acidity and hardness of the test water were measured on a weekly basis. Chemical analyses were performed on samples of the test solutions (taken weekly) to quantify glyphosate in test solution with colorimetric measurements. Indirect quantification of glyphosate was used by quantifying ortho-phosphate and total phosphorus, and then to correct the quantification of the difference between the two analyses for background (i.e. controls) and results were expressed as mg/L phosphorus calculated as glyphosate.

Statistical calculations: ANOVA, Duncan's Multiple Range Test at $\alpha = 0.05$ as post hoc test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.2.2.1-17: Endpoints

Endpoints	Glyphosate [mg a.s./L]
NOEC (255 days)	≥ 25.7

Analytical results: Chemical analyses were performed on samples of the test solutions (taken weekly) to quantify glyphosate in test solution. The mean measured concentrations of the test item in test solutions were 43.75%, 87.50%, 110.11%, 104.0% and 102.80% for the nominal test concentrations of 1.6, 3.2, 6.3, 12.5 and 25 mg a.s./L respectively.

Table 8.2.2.1-18: Analytical results

Nominal concentration of glyphosate [mg a.s./L]	Mean measured of glyphosate [mg a.s./L]	% of nominal
Control		-
1.6	0.7	43.8
3.2	2.8	87.5
6.3	7.0	110.1
12.5	13.0	104.0
25.0	25.7	102.8

B. OBSERVATIONS

Clinical observations: Analyses of variance indicated that continuous exposure of fathead minnows to concentrations of glyphosate as high as 25.7 mg a.s./L had no significant effects on any of the parameters studied during 254 days of continuous exposure. Hatchability of eggs was >94 % in all test item treatments. Mortality and total length of fathead minnows after 30 through 134 days of exposure to concentrations of glyphosate in the treatment groups did not differ significantly from control fish. At termination, total length and wet weight of the female fathead minnows were similar to controls among fish exposed to all concentrations of glyphosate. The number of spawning, eggs per female and eggs per spawn did not differ significantly between controls and fish exposed to the test item treatments.
Percentage of live fry hatching in test item treatments was similar to that which was observed in the controls. Survival and total length and wet weight of second generation fathead minnows was similar to controls for fish exposed 30 days to concentrations of glyphosate. The number of spawnings, eggs per female and eggs per spawn did not differ significantly between controls and fish exposed to concentrations of glyphosate as high as 25.7 mg/l. One spawn of 33 eggs was recovered from the B replicate of 25.7 mg/l before the accidental death of fish due to a diluter malfunction early in the spawning period. Prior to that time, all fish appeared healthy and had reached sexual maturity.

Table 8.2.2.1-19: Survival and growth of fathead minnows during chronic exposure to glyphosate (mean values)

Glyphosate (mg a.s./L)		Control	0.7	2.8	7.0	13.0	25.7
Egg hatchability		99.5	97	96	97	99	97
Day 30	Survival [1]	98.5	81.5	78	89	73	89
	Total length	16	16	16	14.5	16.5	16
Day 60	Survival	93	81.5	76.5	82.5	73	89
	Total length	25.5	25	27.5	26.5	27.5	26
Day 140	Survival [2]	100	93	96.5	96.5	76.5	96.5
Day 254	Total length ♂	59	62	62	63	65	61[3]
	Total length♀	47	46	48	45	48	42
	Total weight ♂	3.8	3.3	3.4	3.0	3.2	2.4
	Total weight ♀	1.08	1.03	1.18	0.91	1.05	0.94

[1] Survival based on 40 fish per duplicate.
[2] Survival based on 15 fish per duplicate.
[3] Fish accidentally killed on day l68 due to diluter malfunction.

Table 8.2.2.1-20: Spawning and egg hatchability of fathead minnows continuously exposed to glyphosate (mean values)

Glyphosate (mg a.s./L)	Control	0.7	2.8	7.0	13.0	25.7 [A]
Number females	4	4	4	4	4	4
Spawning/♀	9.5	4.5	10.0	5.5	5.0	4.5
Eggs spawned/♀	340	207	619	323	298	263
Eggs/spawning	66.5	51.0	62.5	60.0	65.0	51.0
Hatchability	93.5	90.0	87.5	91.5	89.5	86.5 [B]
N [C]	7.5	6.0	10.0	9.0	5.5	6.0

[A] All fish killed on day 168 due to diluter malfunction in only one compartment of the aquarium.
[B] Eggs from unexposed parents (in the aquarium compartment, in which all fish were killed)
[C] Number of egg groups exposed.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a flow through full life cycle study of fathead minnows exposed to glyphosate, none of the parameters studied (adult fecundity, parental and juvenile mortality, total length, wet weight, sex ratio and gonadal conditions), were significantly affected by the chronic exposure to glyphosate. The NOEC was determined to be ≥ 25.7 mg a.s./L (mean measured).

This flow through full life cycle study is considered valid and the NOEC value for fathead minnow exposed to glyphosate was determined to be >25.7 mg a.e./L (mean measured) and can be used in risk assessment.

Assessment and conclusion by RMS:

CA 8.2.2.3 Bioconcentration in fish

Bioconcentration of glyphosate in fish has been evaluated and presented below.

Table 8.2.2.1-21:Studies on bioconcentration in fish

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.2.3/001	[redacted] 1989	BCF (part 1): 56 d /flow-through	*Lepomis macrochirus*	Radiolabelled glyphosate acid	valid	-
CA 8.2.2.3/002	[redacted] 1989	BCF (part 2): 56 d /flow-through	*Lepomis macrochirus*	Radiolabelled glyphosate acid	valid	-

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on bioconcentration. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document.

Endpoints of studies considered valid are shown in the table below.

Table 8.2.2.1-22: Endpoints: Bioconcentration of glyphosate in fish

Reference	Test item	Species	Test design/ GLP	BCF
[redacted] 1989 CA 8.2.2.3/001	Glyphosate acid	*Lepomis macrochirus*	BCF (part 1) - 56 d flow-through/ GLP	1.1 ± 0.61
[redacted] 1989 CA 8.2.2.3/002	Glyphosate acid	*Lepomis macrochirus*	BCF (part 2) - 56 day flow-through/ GLP	1.1 ± 0.61

Study summaries are provided below.

1. Information on the study

Data point:	CA 8.2.2.3/001
Report author	[redacted]
Report year	1989
Report title	Uptake, Depuration and Bioconcentration of ^{14}C Glyphosate to Bluegill Sunfish (*Lepomis macrochirus)* Part I
Report No	MSL-9304
Document No	-
Guidelines followed in study	Guideline 72-6
Deviations from current test guideline	Deviations according to the current OECD 305 guideline (2012): - none.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid

Category study in AIR 5 dossier (L docs)	Category 2a

1. Information on the study

Data point:	CA 8.2.2.3/002
Report author	[redacted]
Report year	1989
Report title	Uptake, Depuration and Bioconcentration of ^{14}C Glyphosate to Bluegill Sunfish (*Lepomis macrochirus)* Part II: Characterization and Quantitation of Glyphosate and Its Metabolites
Report No	MSL-9303
Document No	-
Guidelines followed in study	Guideline 72-6
Deviations from current test guideline	Deviations according to the OECD guideline 305; Minor: fish loading range of 0.1 g – 1.0g/L, actual loading is slightly outside this range at 1.5 g/L.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

In a dynamic flow-through laboratory study, the bioconcentration potential was determined in bluegill sunfish (*Lepomis macrochirus*). A flow-through proportional diluter system was used to maintain a mean measured water concentration of 12 ± 0.7 mg ^{14}C glyphosate/L for a 35-day exposure period. Subsequently, the fish were exposed for 21-days to flowing uncontaminated well water. During the uptake phase, water was sampled on day 0 and then water and fish were sampled after 2 and 6 hours, and after 1, 3, 7, 14, 21, 28 and 35 days. During the depuration period, water and fish were sampled on day 1, 3, 7, 10, 14 and 21 (corresponding to day 36, 38, 42, 45, 49 and 56 after test initiation).

Five fish per sampling date were collected from each replicate and pooled into control and treated samples. Six of the control and treated fish were dissected into fillet/edible (body muscle, skin and skeleton) and viscera/non-edible (fins, head and internal organs). Four fish of the control and treated samples per sampling date were used for whole fish analysis. For metabolite characterisation, 12 fish from the control and treatment group from each aquarium were sampled and dissected on days 7, 14, 21 and 28 of the uptake phase.

The daily bioconcentration factor ranged from <0.11 to 0.38 for fillet, from <0.11 to 0.52 for whole fish, and from <0.11 to 0.63 for viscera, respectively. Uptake tissue concentrations of ^{14}C-glyphosate ranged from <1.4 to 4.6 mg a.s./kg for fillet, from <1.3 to 6.2 mg a.s./kg for whole fish, and from <1.3 to 7.6 mg a.s./kg for viscera, respectively. ^{14}C-residue levels were below minimum quantifiable limits until day 21 for fillet and day 7 for whole fish and viscera samples. Radio-analysis on day 21 of the depuration period indicated 35%, 52% and 51% depuration from fillet, whole fish and viscera, respectively.

The uptake rate constant (K_1) of ^{14}C glyphosate was estimated to be 0.022 ± 0.004 mg a.a./kg in fish/mg/L per day while the depuration rate constant (K_2) was of 0.020 ± 0.01/day. The 50% clearance was estimated to be to 35 ± 18 days.

All validity criteria according to the OECD guideline 305 were fulfilled.

In a flow-through dynamic uptake study of ^{14}C-glyphosate (12 mg a.s./L) by *Lepomis macrochirus,* the time to reach 90 % of steady state was estimated to be 120 ± 59 days. The bioconcentration factor (BCF) was estimated to be 1.1 ± 0.61. The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	^{14}C glyphosate (N-phosphonomethylglycine-methyl-^{14}C)
Description:	White powder
Lot/Batch #:	C-1106-4; C-1106-5 (FJGT-07-000)
Purity:	99.2 %

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: none

3. Test organism:

Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	juvenile
Size:	Length: 6.3 ± 01.8 mm
Body weight:	8.1 ± 0.9 g
Loading:	1 specimen/0.6 L (1.5g fish/L)
Source:	[redacted]
Diet/Food:	Daily with Zeigler Brothers #l Salmon Starter equivalent to approximately 3 % of fish body weight.
Acclimation period:	>14 days

4. Environmental conditions:

Temperature:	22 ± 1 °C
Photoperiod:	16/8 hours light/dark
pH:	7.8 – 8.2
Dissolved oxygen:	6.4 – 8.4 mg/L (76 – 100 % of oxygen saturation)
Conductivity:	480 – 540 µS/cm³
Hardness:	238 – 278 $CaCO_3$/L.

5. Dates of experimental work: January 26th to March 22nd 1988

B. STUDY DESIGN

Experimental treatments:
Based on the results of a range-finding test, a 56-days laboratory bioconcentration study of bluegill sunfish (*Lepomis macrochirus*) exposed to glyphosate was conducted using a nominal test concentration of 12 mg ^{14}C glyphosate/L under flow-through conditions. The test was conducted in glass aquaria containing 70 L test solution. A modified proportional diluter system (Hamilton Model 420 dual syringe dispenser), was used for intermittent introduction of test item and water solution at an average rate of 340 mL/min., replacing test volume approximately 7 times/day.
The uptake phase (day 0 – 35) was initiated by transferring groups of 110 specimens to each replicate. Water was sampled on day 0 and water and fish were sampled 0.17 (2 – 6 hours), 1, 3, 7, 14, 21, 28 and 35 of the uptake phase and on day 1, 3, 7, 10, 14 and 21 of the depuration period (corresponding to day 36, 38, 42, 45, 49 and 56 after test initiation) and radio-assayed.

All measurements of radioactivity were made using either a Searle Model Delta 300® Liquid Scintillation Counting (LSC) System or a TM Analytic Model Delta 300® LSC System optimized for carbon-14 sample analysis.

Observations: On sampling days, five fish from each chamber were collected and pooled into control and treated samples. Six of the pooled fish were dissected into fillet/edible (body muscle, skin and skeleton) and viscera/non-edible (fins, head and internal organs). The remaining four fish of the pooled control and treated samples were reserved for whole fish analysis. Additional fish (12 fish from the control and treatment group) were collected and dissected for metabolite characterization on days 7, 14, 21 and 28 of the uptake phase.

Analytical procedures: The levels of ^{14}C-activity calculated as concentrations of ^{14}C-glyphosate in whole fish, fillet and viscera samples were determined by triplicate analysis of homogenised samples using sample combustion followed by liquid scintillation counting.

Statistical calculations: A non-linear kinetic modelling computer program (Dow BIOFAC) was used to determine the uptake rate constant (K_1) and depuration rate constant (K_2). The Bioconcentration factors for the uptake period were determined by dividing the ^{14}C-glyphosate concentration in tissue by the mean ^{14}C-glyphosate concentration in water for corresponding exposure time.

II. RESULTS AND DISCUSSION

A. FINDINGS

Initial water concentrations are shown below, throughout the 35-day study water concentrations ranged from 11 to 13 mg ^{14}C/L, equivalent to 91.7% and 108.3% of the nominal test concentration respectively.

Table 8.2.2.3-1: Initial water concentrations – Radiochemical/HPLC analysis

Glyphosate in water [mg a.s./L]	% in final concentrate	% Glyphosate	% AMPA
12.3	74.3	95	1.2
12.5	97.8	95.9	1.9
13.2	82.9	95.8	1.8
12.3	85.6	96.6	1.1

Total ^{14}C-radioactivity calculated as ^{14}C-glyphosate in test water and fish tissue during 35 days exposure and 21 days depuration with bluegill sunfish is given below.

Table 8.2.2.3-2: Summary of results

Parameter	Endpoints
K_1, Uptake rate constant [ppm fish/ppm water/day]	0.022 ± 0.004
K_2, Depuration rate constant [/ day]	0.020 ± 0.010
50% Depuration [days]	35 ± 18
90% Steady-State [days]	120 ± 59
Bioconcentration factor	1.1 ± 0.61
Symptoms	none

Table 8.2.2.3-3: Total ^{14}C-radioactivity calculated as ^{14}C-glyphosate in test water and bluegill sunfish tissue

	Fillet		Whole fish		Viscera	
Days ↓	[mg a.s./kg]	BCF	[mg a.s./kg]	BCF	[mg a.s./kg]	BCF
3	< LOD	< 0.11	< LOD	< 0.11	< LOD	< 0.11
14	< LOD	< 0.11	4.3	0.36	5.1	0.42
21	1.8	0.15	3.9	0.32	7.6	0.63
28	3.6	0.30	6.2	0.52	6.8	0.57
35	4.6	0.38	4.6	0.38	7.2	0.60

LOD: Limit of detection

Table 8.2.2.3-4: Depuration of total ^{14}C calculated as ^{14}C-glyphosate from bluegill sunfish during a 21-day clearance period

	Fillet			Whole fish			Viscera		
Days ↓	Conc. [mg a.s./kg]	Depuration		Conc. [mg/kg]	Depuration		Conc. [mg/kg]	Depuration	
		[mg a.s./kg]	[%]		[mg a.s./kg]	[%]		[mg a.s./kg]	[%]
0	4.6	0	0	4.6	0	0	7.2	0	0
1	2.7	1.9	41	13	0	0	5.2	2.0	28
3	2.8	1.8	39	4.1	0.5	11	5.6	1.6	22
7	4.8	0	0	10	0	0	6.2	1.0	14
10	2.1	2.5	54	6.8	0	0	3.4	3.8	53
14	3.0	1.6	35	2.5	2.1	46	3.9	3.3	46
21	3.0	1.6	35	2.2	2.4	52	3.5	3.7	57

B. OBSERVATIONS

Due to the nature of the test compound, a steady-state plateau was never achieved during the 35 days of uptake. No mortality or abnormal behaviour was observed during the conduct of this study. All validity criteria according to the OECD guideline 305 were fulfilled as the temperature variation was < 2°C and the concentration of dissolved oxygen was ≥ 60% saturation. The concentration of the test substance in the chambers was maintained within ± 20% of the mean of measured values during uptake phase and no mortality or abnormal behaviour was observed during the conduct of this study.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a flow-through dynamic uptake study of ^{14}C-glyphosate by Bluegill sunfish (*Lepomis macrochirus),* the time to reach 90% of steady state was estimated to be 120 ± 59 days. The bioconcentration factor (BCF) was estimated to be 1.1 ± 0.61.

This flow through dynamic uptake study of ^{14}C-glyphosate by Bluegill sunfish (*Lepomis macrochirus)* is considered valid and the bioconcentration factor (BCF) was estimated to be 1.1 ± 0.61 and can be used in risk assessment.

Assessment and conclusion by RMS:

CA 8.2.3 Endocrine disrupting properties

According to the endocrine disrupting (ED) criteria laid down in Regulation (EU) 2018/605, endocrine mediated adversity as well as activity and the biological link between those two must be apparent to identify a substance as an endocrine disruptor. A detailed evaluation of endocrine disrupting properties has been made according to EFSA Journal 2018;16(6):5311 incorporating relevant regulatory studies and reliable literature articles. The results are summarised below, see report CA 5.8.3/010 for full details.

Concerning the ED assessment of non-target organisms, EATS-mediated adversity of glyphosate has not been observed in any of the ecotoxicological studies conducted with glyphosate in birds, fish, amphibians and invertebrates. Regarding the assessment of potential EAS-mediated adversity, only secondary effects as a consequence of systemic toxicity are observed. The effects are ranked as "sensitive to, but not diagnostic of EATS" modalities and "systemic toxicity". Potential EAS-mediated activity has been investigated within a Fish Short-Term Reproduction Assay and is therefore sufficiently investigated. No indication for EAS-related endocrine activity was observed. T-mediated activity was investigated within an amphibian metamorphosis assay and is therefore sufficiently investigated. No effects on relevant parameters rated as "T-mediated" were found. This result is sufficient to conclude that T-mediated adversity is unlikely, as no T-related endocrine activity has been observed. Hence, the ED criteria for glyphosate with regards to non-target organisms are therefore not met.

In conclusion, glyphosate does not induce EATS-mediated adversity and no EATS-related endocrine activity was observed *in silico, in vitro,* and *in vivo* for humans and mammals as well as for non-target organisms. This conclusion is in concordance with the current Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate (EFSA Journal 2017; 15(9): 4979) as well as with the conclusion of EPA on the Endocrine Screening Program (EDSP) Tier l (US EPA, 2015).

Since glyphosate has not been shown to induce EATS-mediated adversity or endocrine activity, it is concluded that the ED criteria with regard to EATS-modalities in humans and mammals as well as non-target organisms are not met for glyphosate.

Specific studies considering the effects of glyphosate on the endocrine system were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below. These studies are also incorporated into the ED assessment report (CA 5.8.3/010).

Table 8.2.3-1: Studies on endocrine properties

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.3/001	[redacted] 2012	Fish short-term reproduction assay	Glyphosate	Valid	-
CA 8.2.3/002	[redacted] 2012	Amphibian metamorphosis assay	Glyphosate	Valid	-

Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on endocrine disrupting properties are summarized in the report CA 5.8.3/010.

Endpoints of studies considered valid for glyphosate are shown in the table below. Studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate technical are automatically expressed as acid equivalent.

Table 8.2.3-2: Endpoints: endocrine disrupter properties

Reference	Test item	Species	Test design	Endpoints based on	EC_{50} (mg a.e./L)	NOEC (mg a.e./L)
[redacted] 2012 CA 8.2.3/001	Glyphosate acid	*Pimephales promelas*	Fish short-term reproduction assay (FSTRA)	am	-	≥33
[redacted] 2012 CA 8.2.3/002	Glyphosate acid	*Xenopus laevis*	Amphibian metamorphosis assay (AMA)	nom	-	≥100

am= arithmetic mean measured, nom: nominal

Study summaries are provided below.

1. Information on the study

Data point:	CA 8.2.3/001
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Fish Short-Term Reproduction Assay (FSTRA) with the Fathead Minnow (*Pimephales promelas*)
Report No	707A-102A
Document No	-
Guidelines followed in study	OECD Guideline 229 (2009) OPPTS/OCSPP Guideline 890.1350 (2009)
Deviations from current test guideline	Deviations from guideline OECD 229 (2012): Minor: - Temperature range was greater than 2°C for a short time period (< 24 hours).
Previous evaluation	Yes, EFSA ED Conclusion (2017)[12]
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

[12] EFSA (European Food Safety Authority), 2017. Conclusion on the peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate. EFSA Journal 2017;15(9):4979, 20 pp. https://doi.org/10.2903/j.efsa.2017.4979

2. Full summary

Executive Summary

The 21-day short-term reproduction assay of MON 77973 (glyphosate acid) with the fathead minnow (*Pimephales promelas*) was conducted under flow-through conditions to determine the impact of glyphosate acid on the hypothalamus-pituitary-gonadal (HPG) endocrine axis by evaluating effects on the reproductive system, such as fecundity, fertility, secondary sexual characteristics (tubercles and fatpad scores), gonadosomatic index (GSI) histopathology of gonads as well as plasma vitellogenin. Four groups of adult males and females (2 males and 4 females in each group), were exposed to glyphosate acid at nominal concentrations of 0 (negative control), 0.048, 0.24, 1.2, 6.0, and 30 mg a.s./L, (the highest test concentration was based on one-third of a 96-hr LC_{50} value of a previous acute toxicity test) with a total of 24 fish exposed per treatment and control group. Following a pre-exposure period of 19 days, groups of actively spawning fish, were exposed to glyphosate acid according to the aforementioned treatment groups, for a 21-day exposure period, with survival, fecundity, fertility and general observations recorded daily. The remaining reproductive endpoints were evaluated at test termination, along with fish lengths and fish weights.

The overall arithmetic mean measured glyphosate acid concentrations were (negative control; <LOQ), 0.046, 0.23, 1.2, 6.2, and 33 mg a.s./L, respectively. All performance criteria were met for this study, except for a slight deviation in temperature. Recorded temperatures exceeded the recommended range (25 ± 1 °C), for less than 24 hours on Day 7 when the maximum recorded temperature reached 29.1 °C (range of 28.6 - 29.1 °C); deviation occurred in three replicates each in the 1.2 mg a.s./L and 6.2 mg a.s./L groups). This deviation was due to a loose wiring between the temperature probe and the heat plates beneath these replicates, which was quickly rectified. Temperature measurements repeated on Day 7, across all affected replicates fell within a 24.4 to 4.7 ºC range. This minor deviation is not considered to have had any impact on study integrity.

There was 100 % fish survival in the negative control, 0.046, 0.23, 6.2, and 33 mg a.s./L treatment groups with 91.7 % survival in the 1.2 mg a.e./L treatment group.

Glyphosate acid did not result in any significant increases or decreases in weight or length for either sex at any treatment level. There were no observed effects on secondary sex characteristics or clinical signs (i.e., behavioral and other sub-lethal effects) in males or females in any treatment group. The mean number of eggs per female reproductive day in the negative control was 23.5 eggs/day (range: 23.2-23.9 eggs/female/day); fertilization success in the negative control was 97.3 %. Fecundity and fertilization success were not significantly different from the negative control for any treatment group.

There were no effects on survival, growth, reproduction, secondary sex characteristics, GSI, VTG or gonad histopathology in male or female fish exposed to glyphosate acid for 21 days. Based on the endpoints evaluated, glyphosate acid is concluded to not impact the function of the hypothalamus-pituitary-gonadal (HPG) endocrine axis in fathead minnows. The study is considered valid.

Table 8.2.3-3: Summary of FSTRA Findings

Treatment (mg a.e./L) [mean-measured]	Fecundity	Fertilization Success	Tubercle Score		GSI		Gonadal Histopathology		Plasma VTG	
			M	F	M	F	M	F	M	F
0.046	No	No	No	No	No	No	No	No	No	No
0.23	No	No	No	No	No	No	No	No	No	No
1.2	No	No	No	No	No	No	No	No	No	No
6.2	No	No	No	No	No	No	No	No	No	No
33	No	No	No	No	No	No	No	No	No	No

F = Female; GSI = Gonado-Somatic Index; M = Male; VTG = Vitellogenin

The fish short-term reproduction assay (FSTRA) with breeding groups of fathead minnow (*Pimephales promelas*) exposed to glyphosate acid is considered valid. The overall NOEC was ≥ 33 mg a.s./L (arithmetic mean measured).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: MON 77973 (glyphosate acid)
Description: White powder
Lot/Batch #: GLP-1103-21149-T
Purity: 85.14 % before drying (95.93 % glyphosate acid, dried)
CAS #: 1071-83-6
Stability of test compound: Stable. Mean-measured concentrations yielded recoveries of 96-110% of nominal.

2. Vehicle and/or positive control: Vehicle: dilution water (filtered well water)
Positive control: none

3. Test organism:

Species/sex: Fathead minnow (*Pimephales promelas*)
Strain: Not specified
Age at start of dosing: 5.5 months
Weight at start of dosing: 0.9 g (females) – 1.6 g (males)
Source: [redacted]
Acclimation period: 2 months, plus 19-day pre-exposure period
Diet: Commercial flake food (Sera Vipan, Sera North America) supplemented with shrimp brine nauplii (Brine Shrimp Direct, Ogden, UT, USA), 2 times/day

Housing:

Exposure System: Continuous flow-through diluter system
Flow-through Rate: 44 mL/min
Exposure Vessel: 12 L Glass Aquaria (10 L fill volume)
Spawning Substrate Material: Inverted semi-circular PVC pipe section (~10 cm)

Source of dilution water:	Fresh filtered and sterilized well water (0.45 µm)

4. Environmental conditions:

Temperature:	25.4 °C (24.3 °C – 29.1 °C) A minor temperature deviation occurred on Day 7 due to loose wiring between a temperature probe and heat plates beneath replicates B, C and D of the 1.2 mg a.s./L treatment group and replicates A, B and C of the 6.2 mg a.s./L treatment group, with a maximum temperature of 29.1 ºC recorded. The wiring was reattached, and measurements were re-taken later on Day 7. The second measurements in Replicates B, C and D of the 1.2 mg a.s./L and Replicates A, B and C of the 6.2 mg a.s./L treatment groups respectively were 24.7, 24.6, 24.5, 24.4, 24.4 and 24.5ºC, respectively.
pH:	8.1 (8.0 – 8.3)
Dissolved Oxygen	7.2 mg/L (6.0 – 7.9 mg/L)
Total Alkalinity:	173.5 mg/L as $CaCO_3$ (166 – 180 mg/L as $CaCO_3$)
Hardness:	144.5 mg/L as $CaCO_3$ (140 – 148 mg/L as $CaCO_3$)
Photoperiod:	16 h light/ 8 h dark (30-minute transition of low light between light and dark periods).
Light Intensity at Water's Surface:	Mean = 1170 ± 412 lux (range 450 – 1976 lux)
5. Dates of experimental work:	17th October 2011 to 11th January 2012

B. STUDY DESIGN

Experimental treatments

A 14-day range-finding test was conducted at 1.9, 3.8, 7.5, 15 and 30 mg a.s./L, for 14 days, the highest concentration tested, being based on the results of a 96 hour acute toxicity study[13], being approximately one-third of the achieved LC_{50}. In the range-finding test, one incidental mortality occurred at 15 mg a.s./L, with no other signs of toxicity observed in any control or treatment group throughout the test duration.

The definitive test concentration range was 0.048, 0.24, 1.2, 6.0 and 30 mg a.s./L, conducted under flow-through exposure conditions. A nominal stock solution of 225 mg a.s./L – corrected for purity, was pumped into mixing chambers according to treatment group at rates (mL/min) required to achieve the final required test concentrations. Test solutions were then pumped into the test chambers (12-L glass aquaria) filled with approximately 10 L of test water. The volume in the test chambers was maintained by an overflow port on one end of each chamber. Into each chamber, a spawning substrate or tile was placed into each chamber. A tile consisted of a semi-circular section of PVC pipe approximately 10 cm in length.

Four replicates were used in each treatment group (including the control group); each replicate consisted of two males and four females, except the fourth replicate at 33 mg a.s./L, where there were three males and three females due to a mis-sexed fish at pre-exposure allocation. Water samples were collected from two alternating replicate test chambers in each treatment and control group for concentration analysis on Days 0, 7, 14, and 21. The limit of quantification (LOQ) was 0.0300 mg a.s./L.

Nominal and arithmetic mean measured glyphosate acid concentrations can be found in the table below.

[13] [redacted]. 1975. Chronic toxicity of glyphosate to the fathead minnow (*Pimephales promelas* Rafinesque). Monsanto unpublished study BN-75-129. MRID 108171.

Table 8.2.3-4: Summary of Treatment Concentrations in the FSTRA with Glyphosate acid

Treatment ID	Nominal Concentration (mg a.e./L)	Measured Concentration (mg a.e./L)	Mean CV (%)
Negative Control	0.00	< LOQ	NA
Treatment 1	0.048	0.046	9.0
Treatment 2	0.24	0.23	9.9
Treatment 3	1.2	1.2	2.3
Treatment 4	6.0	6.2	3.4
Treatment 5	30	33	7.8

CV = Coefficient of variation; LOQ = Limit of Quantification (0.0300 mg a.e./L)

Observations:

Mortality, Clinical Signs: Survival and general observations were made daily during the 21-day exposure period. External abnormalities and abnormal behavior were noted if observed. Dead fish were removed as soon as possible but were not replaced in either the control or treatment test chambers.

Body Weight and Length: The wet weight and total length of each fish was recorded at test termination. Fish were blotted dry and weighed to the nearest 0.1 mg. Total length was measured to the nearest millimeter.

Secondary Sex Characteristics: Detailed observations of secondary sex characteristics including pigmentation patterns, tubercles, fatpads, and ovipositors were recorded and the external sex was determined at test termination.

Spawning and Mean Fecundity: Spawning tiles were removed from the test chambers daily and any eggs that were present were counted. Fecundity was calculated as the number of eggs per surviving female per reproductive day per replicate. After eggs were counted, they were evaluated for fertilization success. The number of infertile eggs was counted, and the number of fertile eggs was calculated as the difference between the total number of eggs and the number of infertile eggs on the tile. Fertilization success (%) was calculated as the number of embryos divided by the number of eggs, multiplied by 100.

Plasma Vitellogenin (VTG): At study termination, at least two blood samples were collected from the caudal vein/artery of each fish using heparinized microhematocrit tubes. Male fish were processed before female fish to avoid contamination of VTG samples. After collection, the plasma was separated by centrifugation and transferred to a microcentrifuge tube containing lyophilized protease inhibitor (aprotinin). Analysis for vitellogenin was conducted with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosense Laboratories, Bergen, Norway) using an antibody raised against fathead minnow VTG. The procedures used to collect, prepare and analyze the plasma samples were based upon methodology provided by the ELISA system manufacturer and those presented by the U.S. EPA.

Plasma Sex Steroid Levels: No plasma sex steroids were measured.

Gonadal Histology and Histopathology: Immediately following blood collection, gonads were fixed *in situ* with Davidson's solution, removed from the abdominal cavity, gently blotted and weighed to the nearest 0.1 mg to determine the gonadosomatic index (GSI = gonad wt/body wt × 100). After weighing, each pair of gonads (right and left) was enclosed in a plastic tissue cassette that was then placed in a container of fixative (Davidson's solution). After at least 24 hours of fixation, the gonads in the cassettes were rinsed with 70 % ethanol and placed in neutral-buffered formalin. Gonads were then subjected to routine histological processing, embedded in paraffin, and longitudinally sectioned. At the largest cross-sectional area of the gonads, three step sections (each 4-6 microns thick) were cut at approximately 50-micron

intervals and all three sections were mounted on a single glass slide. Slides were stained with hematoxylin and eosin, cover-slipped, and then evaluated by a histopathologist.

Gonadal staging for the male fathead minnow was as follows: 0 = undeveloped, 1 = early spermatogenic,2 = mid-spermatogenic, 3 = late spermatogenic, 4 = spent. Gonadal staging for the female fathead minnow was as follows: 0 = undeveloped, 1 = early development, 2 = mid-development, 3 = late development, 4 = late development/hydrated, 5 = post-ovulatory.

Histomorphologic parameters assessed included relative germ cell numbers, alterations in numbers and sizes of non-germ cells (e.g., testicular interstitial cells and ovarian perifollicular cells), and increased degenerative changes. When appropriate, the pathologist used a scoring system to indicate the severity of these changes and other abnormalities according to the following scale: Grade 0 = not remarkable, Grade 1 = minimal, Grade 2 = mild, Grade 3 = moderate, and Grade 4 = marked. Any changes not amenable to grading were designated as “Present”. In addition, the stage of developmental maturity of each gonad pair was indicated according to guideline recommendations.

Analytical procedures: Water samples were collected from two alternating replicate test chambers in each treatment and control group for concentration analysis on Days 0, 7, 14, and 21. Samples were collected from mid-depth at each interval, placed in glass vials, and processed immediately for analysis and analyzed by reverse-phase high performance liquid chromatography (HPLC) using variable wavelength detection set at 500 nm. Chromatographic separations were achieved using a YMC-PACK ODS-AM analytical column (150 mm x 4.6 mm, 3-µm particle size). Fresh calibration standards (range: 0.0300 - 0.300 mg a.s./L) were prepared and analyzed with each sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of glyphosate acid in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equation. The limit of quantification (LOQ) was 0.0300 mg a.s./L. Four matrix blank samples were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses.

Statistical calculations: Analyses were performed to evaluate differences between treatment and control groups for each of the following endpoints: survival, wet weight, total length, fecundity, fertility, gonado-somatic index (GSI), vitellogenin (VTG) concentration, tubercle score, gonad developmental stage, and incidence and severity of gonad abnormalities. Measurements of VTG are inherently variable, and boxplots of log transformed VTG values were used to identify potential outliers (Tukey’s method) that might need special handling in the analyses. No outliers were excluded from analyses in this study. Unless otherwise noted, replicate test chambers were used as the unit of statistical analysis. Males and females were analysed separately for each endpoint when appropriate. Endpoints were first evaluated for monotonicity. Since the responses for all endpoints except male tubercle scores appeared to be monotonic, a step-down Jonckheere-Terpstra trend test was used to evaluate possible trends in the ranks of replicate means to determine concentration responsive trends among the treatment groups. Dunnett’s test was used to evaluate male tubercle scores. Survival was analyzed using Fisher’s Exact test, and histopathology severity scores and stages of individuals were analyzed using step-down Jonckheere- Terpstra trend tests. Statistical tests used to evaluate treatment effects were performed at confidence level of $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of glyphosate acid in test solution samples ranged from 96 to 110 % of nominal concentrations.

B. OBSERVATIONS

Mortality, Clinical Signs: No treatment-related effects on survival were observed in any treatment group. There were incidental mortalities of one female and one male fish in the 1.2 mg a.s./L treatment group resulting in an overall survival of 91.7 %. Survival was 100 % in the remaining treatment groups.

No clinical signs were observed in any males or females in the negative control and treatment groups.

Body Weight and Length: No treatment-related effects were observed on mean body weight or mean length in males or females (see table below).

Table 8.2.3-5: Body Weight and Length at Test Termination in Fathead Minnow (*Pimephales promelas*)

Treatment [mg a.s./L] (mean measured)	Body Weight						Length					
	Males			Females			Males			Females		
	n	Mean [g]	± SD	n	Mean [g]	± SD	n	Mean [mm]	± SD	n	Mean [mm]	± SD
Negative Control	4	2.20	0.462	4	1.14	0.047	4	55	2.8	4	46	0.7
0.046	4	2.17	0.348	4	1.11	0.056	4	53	1.8	4	46	0.9
0.23	4	2.28	0.397	4	1.04	0.069	4	55	3.5	4	45	1.1
1.2	4	2.20	0.185	4	1.12	0.051	4	54	1.6	4	46	0.2
6.2	4	2.15	0.272	4	1.07	0.108	4	53	2.3	4	45	1.3
33	4	2.05	0.209	4	1.13	0.094	4	52	1.0	4	46	0.8

Secondary Sex Characteristics: Overall, there were no treatment-related effects on secondary sex characteristics in males or females in all treatment groups. No treatment-related effects were observed on median tubercle scores. Male nuptial median tubercle scores ranged from 15 at 33 mg a.s./L to 19 at 0.046 and 1.2 mg a.s./L; no nuptial tubercles were observed for females. There were 3 males instead of the recommended 2 due to a mis-sexing error in the fourth replicate of the 33 mg a.s./L treatment group. The median scores are unaffected when this fish is removed from the results.

Spawning and Mean Fecundity: No treatment-related effects were observed on mean fecundity and mean fertilization success (see table below).

Table 8.2.3-6: Fecundity and Fertilization Success in Fathead Minnow (*Pimephales promelas*)

Treatment [mg a.s./L] (mean measured)	Fecundity (Eggs per Female per Reproductive Day)		Fertilization Success (%)	
	Mean	± SD	Mean	± SD
Negative Control	23.5	0.33	97.3	0.4
0.046	29.3	5.3	97.6	1.0
0.23	22.7	5.4	98.4	1.4
1.2	24.9	0.89	96.0	2.7
6.2	28.1	6.4	98.1	1.1
33	23.6	2.2	96.7	2.0

Plasma Vitellogenin (VTG): The mean VTG concentration in males in the negative control, 0.046, 0.23, 1.2, 6.2 and 33 mg a.s./L treatment groups was 1.01, 0.77, 1.34, 0.75, 0.39 and 0.33 µg/mL, respectively. There were no statistically significant effects on VTG among males in any treatment group in comparison to the negative control ($p > 0.05$). The mean VTG concentration in females in the negative control, 0.046, 0.23, 1.2, 6.2 and 33 mg a.s./L treatment groups was 3191, 2124, 2226, 2195, 1442 and 2142 µg/mL, respectively. There were no statistically significant effects on VTG among females in any treatment group in comparison to the negative control ($p > 0.05$).

Table 8.2.3-7: Plasma Vitellogenin (VTG) in Fathead Minnow (*Pimephales promelas*)

Treatment [mg a.s./L] (mean measured)	Males			Females		
	n	Mean [µg/mL plasma]	± SD	n	Mean [µg/mL plasma]	± SD
Negative Control	4	1.01	1.143	4	3191	1170
0.046	4	0.77	0.312	4	2124	807
0.23	4	1.34	2.068	4	2226	624
1.2	4	0.75	1.240	4	2195	403
6.2	4	0.39	0.368	4	1442	550
33	4	0.33[1]	0.210	4	2142	356

[1] In the fourth replicate of the 33 mg a.s./L treatment group, there were 3 males instead of the recommended 2 due to a mis-sexing error. If this fish is removed from analysis of VTG, the treatment means are very similar as when retained (327 when retained vs. 299 when mis-sexed removed). The values in this table reflect data excluding the mis-sexed male.

Gonadal Histology and Histopathology:

There were no treatment-related effects on GSI (see table below) or on median gonadal staging in males or females.

Table 8.2.3-8: Gonado-Somatic Index (GSI) in Fathead Minnow (*Pimephales promelas*)

Treatment [mg a.s./L] (mean measured)	Males			Females		
	n	Mean GSI (%)	±SD	n	Mean GSI (%)	±SD
Negative Control	4	1.48	0.218	4	14.7	3.28
0.046	4	1.11	0.202	4	14.4	2.04
0.23	4	1.43	0.393	4	13.1	1.66
1.2	4	1.33	0.087	4	14.0	2.58
6.2	4	1.35	0.324	4	15.5	2.06
33	4	1.51[1]	0.325	4	15.8	3.12

[1] In the fourth replicate of the 33 mg a.s./L treatment group, there were 3 males instead of the recommended 2 due to a mis-sexing error. If this fish is removed from analysis of GSI, the treatment means are very similar as when retained (1.51 when retained vs. 1.52 when removed).

No treatment-related effects in gonadal staging were observed. Testes and ovaries from the five treatment groups showed no changes in gonadal staging or increased abnormalities when compared with the negative control.

There were no treatment-related effects or statistically significant differences observed in the histological evaluations of the testes and ovaries. Testes and ovaries from the five treatment groups showed no changes in gonadal staging or increased abnormalities when compared with the negative control.

Minimal and mild granulomatous inflammation was found in male gonads from the mean-measured 0.046 mg a.s./L treatment group, but these observations were not considered to be treatment-related (see table below). No other male gonadal histopathological observations were made.

Table 8.2.3-9: Gonadal histopathology in male fathead minnow (*Pimephales promelas*)—Selected parameters as discussed above

Treatment (mg a.e./L) [mean-measured]	Severity	Granulomatous Inflammation	
		n	Incidence
Negative Control	0	8	8
	1	8	0
	2	8	0
	3	8	0
	4	8	0
0.046	0	8	4
	1	8	1
	2	8	3
	3	8	0
	4	8	0
0.23	0	8	6
	1	8	0
	2	8	0
	3	8	0
	4	8	0
1.2	0	7	7
	1	7	0
	2	7	0
	3	7	0
	4	7	0
6.2	0	8	8
	1	8	0
	2	8	0
	3	8	0
	4	8	0
33[1]	0	9	9
	1	9	0
	2	9	0
	3	9	0
	4	9	0

[1] In the fourth replicate of the 33 mg a.e./L treatment group, there were 3 males instead of the recommended 2 due to a mis-sexing error. Because the mis-sexed male in the fourth replicate of the mean-measured 33 mg a.e./L treatment group was not explicitly identified, it was included in the histopathologic evaluations.

Mild increased oocyte atresia in females was observed in the negative control, low, and mid concentration treatments, and a single incident of moderate increased oocyte atresia was noted in the high concentration treatment group (see table below). Moderate to marked increases in mature oocytes were observed in two, five, and one females in the negative control and mean-measured 1.2 and 33 mg a.s./L treatment groups, respectively, and were therefore not considered to be treatment-related. Mild granulomatous inflammation was noted in a single female in the negative control and mean-measured 6.2 mg a.s./L treatment group and therefore was not considered to be treatment-related (see table below).

Table 8.2.3-10: Gonadal histopathology in female fathead minnow (*Pimephales promelas*)—Selected parameters as discussed above

Treatment (mg a.e./L) [mean-measured]	Severity	Increased Ooctye Atresia		Granulomatous Inflammation		Increased Mature Oocytes	
		n	Incidence	n	Incidence	n	Incidence
Negative Control	0	16	15	16	15	16	16
	1	16	0	16	0	16	0
	2	16	1	16	1	16	0
	3	16	0	16	0	16	0
	4	16	0	16	0	16	2
0.046	0	16	15	16	16	16	16
	1	16	0	16	0	16	0
	2	16	1	16	0	16	0
	3	16	0	16	0	16	0
	4	16	0	16	0	16	0
0.23	0	16	16	16	16	16	16
	1	16	0	16	0	16	0
	2	16	0	16	0	16	0
	3	16	0	16	0	16	0
	4	16	0	16	0	16	0
1.2	0	15	13	15	15	15	10
	1	15	0	15	0	15	0
	2	15	2	15	0	15	0
	3	15	0	15	0	15	1
	4	15	0	15	0	15	4
6.2	0	16	16	16	15	16	16
	1	16	0	16	0	16	0
	2	16	0	16	1	16	0
	3	16	0	16	0	16	0
	4	16	0	16	0	16	0
33	0	15	14	15	15	15	14
	1	15	0	15	0	15	0
	2	15	0	15	0	15	0
	3	15	1	15	0	15	0
	4	15	0	15	0	15	1

Following point is a minor deviation from guideline OECD 229 (2012):
- Temperature range was greater than 2 °C for a short time period (< 24 hours).
This deviation did not have any adverse impact on the study.

The test is regarded as valid, since criteria for test acceptability according to OECD 229 guideline (2012) were met:

- The dissolved oxygen concentration was at least 60 % of the air-saturation value throughout the exposure period.
- Water temperature did not differ by more than 1 ºC between test vessels at any one time during the exposure period and was maintained within ±1 ºC of the 25 ºC temperature specified, except on Day 7 of the test when the maximum temperature was 29.1 ºC for a short duration (< 24 hours). This deviation did not have any adverse impact on the study
- There was more than 90% survival of control animals over the duration of the chemical exposure.
- Mean measured concentrations of the test substance remained within an acceptable range throughout the test (CV < 20 %)

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Breeding groups of fathead minnows (*Pimephales promelas*) were exposed to glyphosate acid at arithmetic mean measured concentrations of 0.046, 0.23, 1.2, 6.2 and 33 mg a.s./L for 21 days. The endpoints evaluated were adult survival, body length and wet weight, fecundity (cumulative egg production and eggs per female reproductive day), fertilization success, secondary sex characteristics (including fatpad and tubercle scores), GSI, VTG and gonad histopathology. There were no effects on survival, growth, reproduction, secondary sex characteristics, GSI, VTG or gonad histopathology in male or female fish exposed to glyphosate acid for 21 days. Based on the endpoints evaluated, glyphosate acid is concluded to not affect the function of the hypothalamus-pituitary-gonadal (HPG) endocrine axis in fathead minnows.

The fish short-term reproduction assay (FSTRA) with breeding groups of fathead minnow (*Pimephales promelas*) exposed to glyphosate acid is considered valid and the overall NOEC ≥33 mg a.s./L (arithmetic mean measured) can be used for ecotoxicological risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.3/002
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances
Report No	707A-103
Document No	-
Guidelines followed in study	OECD Guideline 231 (2009) OPPTS/OCSPP Guideline 890.1100 (2009)
Deviations from current test guideline	Deviations from guideline OECD 231 (2009): Minor: - Measured test concentrations CV% >20 % due to low recoveries in the low treatment group on Day 14 and in the high treatment group on Day 21.
Previous evaluation	Yes, EFSA ED Conclusion (2017)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The 21-day assay of MON 77973 (glyphosate acid) on amphibian metamorphosis of the African clawed frog (*Xenopus laevis*) was conducted under flow-through conditions, to determine the potential for glyphosate to interfere with the normal function of the hypothalamic-pituitary thyroid (HPT) axis and the African clawed frog (*Xenopus laevis*). Amphibian larvae at Nieuwkoop-Faber (NF) stage 51 (80 per control and treatment group) were exposed to glyphosate acid at nominal concentrations of 0 (negative control),

0.16, 0.80, 4.0, 20, and 100 mg a.s./L. Arithmetic mean-measured concentrations were < 0.100 (<LOQ; control), 0.13, 0.79, 4.3, 20, and 90 mg a.s./L.
All performance criteria were met in this study, except for the test solution coefficient of variance (CV) for the 0.16 and 100 mg a.s./L treatment groups, in which the CVs were 41 and 31%, respectively, both greater than the recommended maximum of 20%. However, this deviation did not impact the interpretation of the results.
Tadpole survival to Day 21 in the negative control group and in the 0.13, 0.79, 4.3, 20 and 90 mg a.s./L treatment groups was 98.8, 100, 100, 100, 96.3 and 98.8%, respectively. The numbers of tadpoles in the treatment groups with tail curvature were comparable to the number in the control group and the tail curvature was not considered to be a thyroid-related effect, but rather a dietary effect. In feeding trials done at the testing lab it was shown that feeding rates during acclimation contribute to the amount of curvature observed.
Glyphosate acid caused no significant acceleration or delay of median NF developmental stage throughout the test. Further, no asynchronous development was observed. No tadpoles in the control and treatment groups developed beyond NF stage 57. Glyphosate acid exposure did not cause significant effects on Day 7 or Day 21 normalized hind-limb lengths (HLL) at any concentration tested. Snout-vent length (SVL) was not significantly affected at any treatment concentration at Day 7, but was significantly increased ($p < 0.05$) in the 4.3, 20, and 90 mg a.s./L treatment concentrations at Day 21 (5.2 %, 2.5 %, and 6.7 % increase, respectively) compared to the control. Additionally, there was a significant increase in Day 21 body weight at 90 mg a.s./L (17% increase). However, growth should never be solely relied upon to determine thyroid toxicity. Rather, growth, in conjunction with developmental stage and thyroid histopathology, should be used to determine thyroid activity.

There were no treatment-related effects on thyroid gland histopathology at any treatment level, with comparable incidence and severity of thyroid gland atrophy and hypertrophy, and follicular cell hypertrophy and hyperplasia in the control and treatment concentrations. While there appeared to be an increased incidence of mild thyroid gland hypertrophy at the highest treatment concentration, the same incidence was observed at the lowest treatment concentration and the effect was not concentration responsive. Similar findings were observed for follicular cell height increase: an apparent increase in mild severity at the top concentration with a similar incidence at the lowest treatment concentration and no concentration-responsive pattern. Finally, the pathologist report indicated that there were no treatment-related changes in the thyroid glands of tadpoles exposed to glyphosate acid when compared to those in the negative control.

Table 8.2.3-11: Summary of AMA Findings

Treatment (mg a.e./L) [mean-measured]	NF Developmental Stage		Normalized Hind-Limb Length[1]		Asynchronous Development		Thyroid Gross and Histopathology
	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21	Day 21
0.13	No	No	No	No	No	No	No
0.79	No	No	No	No	No	No	No
4.3	No	No	No	No	No	No	No
20	No	No	No	No	No	No	No
90	No	No	No	No	No	No	No

[1] Hind-limb length is normalized to snout-vent length (SVL).

The Amphibian metamorphosis assay (AMA) with the African clawed frog (*Xenopus laevis*) exposed to glyphosate acid is considered valid and the overall NOEC was ≥100 mg a.s./L (arithmetic mean measured).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 77973 (glyphosate acid)
Description:	White powder
Lot/Batch #:	GLP-1103-21149-T
Purity:	85.14% before drying (95.93% glyphosate acid, dried)
CAS #:	1071-83-6
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Vehicle: dilution water (filtered well water)
Positive control: none

3. Test animals

Species/sex:	African clawed frog (*Xenopus laevis*)
Strain:	Not specified
Age at start of dosing:	NF Stage 51, 16 days post-fertilization; all tadpoles were derived from eggs spawned on the same day
Source:	Tadpoles were from eggs collected from adult male frogs and female frogs injected with Hcg induced to spawn in the laboratory; healthy adults obtained from Xenopus I (Dexter, MI; USA)
Diet:	Sera Micron (Sera North America, PA, USA), 3 times/day
Housing	
Exposure System:	Continuous flow-through diluter system
Flow-through Rate:	69 mL/min
Exposure Vessel:	12 L Glass Aquaria (10 L fill volume)
Source of dilution water:	Filtered fresh well water

4. Environmental conditions:

Temperature:	21.9°C (21.4 - 22.3 °C)
Hardness:	142.5 mg/L as $CaCO_3$ (140 - 144 mg/L as $CaCO_3$)
pH:	8.0 (7.0 – 8.3)
Dissolved Oxygen	8.2 mg/L (7.6.0 – 8.7 mg/L)
Iodide:	3 - 6 µg/L
Aeration:	No
Photoperiod:	12 h light/ 12 h dark
Light Intensity at Water's Surface:	911 – 1387 lux

5. Experimental dates: October 19th to November 14th 2011

B. STUDY DESIGN

Experimental treatments:

Test concentrations were 0 (dilution water only), 0.16, 0.80, 4, 20, and 100 mg a.s./L. The highest test concentration was selected based on results of a 14-day range-finder and is the guideline-recommended highest test concentration. All test solutions were adjusted for test substance purity. Water samples were collected from each replicate test chamber on Days 0, 7, 14, and 21 to measure concentrations of the test substance. The limit of quantification (LOQ) was 0.100 mg a.s./L. Nominal and mean measured glyphosate acid concentrations can be found in the table below. Additional water samples were collected as needed during the test when previous results were questionable, or when there were interruptions in test substance

delivery. The number of replicates per treatment was four (4); the number of larvae per replicate per treatment at test initiation was 20 (total: 80 larvae/treatment).

Table 8.2.3-12: Summary of Treatment Concentrations in the Amphibian Metamorphosis Assay with Glyphosate

Treatment ID	Nominal Concentration (mg a.e./L)	Measured Concentration (mg a.e./L)	Mean CV (%)
Control (dilution water only)	0.00	<LOQ	NA
Treatment 1[a]	0.16	0.13	41
Treatment 2	0.80	0.79	3.2
Treatment 3	4.0	4.3	4.4
Treatment 4	20	20	6.8
Treatment 5	100	90	31

[a] In Treatment 1, Day 0, 7 and 21 concentrations were >80% of nominal. Day 14 samples were <LOQ due to a diluter malfunction. Day 16 samples confirmed that concentrations were returning to nominal once the diluter was repaired. Values of ½ the LOQ (0.050 mg a.e./L) were used for Day 14 samples to calculate the mean measured concentration.

Arithmetic mean measured concentrations are 81, 99, 108, 100 and 90% for the 0.16, 0.80, 4, 20, and 100 mg a.s./L treatment groups.

Observations:

Mortality, Clinical Signs: Survival and clinical signs of toxicity, including any abnormal behavior, were assessed daily. Dead tadpoles were not replaced in either the control or treatment test chambers.

Developmental Stage: Developmental stage was determined under a dissection microscope based on the developmental stages described by Nieuwkoop and Faber (NF). Developmental stage was determined on Day 7 for five tadpoles randomly selected from each test chamber and on Day 21 for all remaining tadpoles.

Tadpole Growth: Tadpoles were measured for total length to the nearest 1 mm using a metric ruler and were weighed to the nearest 0.1 mg. Digital images were used to determine snout-to-vent length and hind-limb length for each tadpole, using a computer image-processing program. For consistency, the left hind limb of each tadpole was measured. Hind-limb length was normalized by dividing by snout-to-vent length. Any tadpoles beyond Stage 60 by Day 21 were excluded from analyses of growth.

Histopathology: On Day 21, the tadpoles were fixed in Davidson's solution for at least 48 hours, rinsed with 70% ethanol, and placed in neutral buffered formalin. When possible, stage-matched tadpoles (5 from each replicate test chamber) were selected for histopathological processing and evaluation based on the median developmental stage of the negative controls. When there were fewer than five tadpoles at that stage, where available in a replicate, additional tadpoles were randomly selected from the developmental stages just above or below the median control developmental stage.

Histomorphologic parameters assessed included relative increases or decreases in the overall size of the thyroid glands, changes in follicular epithelial cell numbers or height, and alterations in colloid consistency. When appropriate, a scoring system to indicate the severity of these changes was used (Grade 0 = unremarkable, Grade 1 = mild, Grade 2 = moderate, and Grade 3 = severe).

Analytical procedures: Water samples were collected from each replicate test chamber on Days 0, 7, 14 and 21 to measure concentrations of the test substance. Samples were collected from mid-depth at each interval, placed in glass vials, and processed immediately for analysis. The analytical method consisted of diluting the samples in freshwater, derivatizing and filtering. The samples were then analyzed by high performance liquid chromatography (HPLC) using variable wavelength detection set at 500 nm. Concentrations of glyphosate acid in the samples were determined using an Agilent Series 1100/1200 High Performance Liquid Chromatograph with an Agilent Series 1100 Variable Wavelength Detector. Chromatographic separations were achieved using a YMC-PACK ODS-AM column (150 mm × 4.6 mm, 3-µm particle size). Calibration standards (range: 0.100 – 1.00 mg a.s. mg/L) were analyzed with each sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of glyphosate acid in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equation. The limit of quantitation (LOQ) for the analysis of glyphosate acid in freshwater was 0.100 mg a.s./L.

Statistical calculations: Analyses were performed on survival, developmental stage, body weight, snout-vent length (SVL), normalized hind-limb lengths (HLL), and incidence and severity of thyroid abnormalities. Unless otherwise noted, the unit of statistical analysis was the replicate test chamber. If necessary, endpoints were analyzed using two complementary statistical approaches. For growth parameters, endpoints were first evaluated for monotonicity. Since responses for these endpoints appeared to be monotonic, a step-down Jonckheere-Terpstra trend test was used to determine possible concentration responsive trends among the treatment groups. Body weight and SVL data also were analysed by performing pair-wise comparisons using Dunnett's multiple comparison test to further evaluate if those treatment groups differed statistically from the control group. Data for endpoints analyzed by Dunnett's test were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Levene's test ($\alpha = 0.01$).

Survival was analyzed using Fisher's Exact test and histopathology severity scores of individuals were analyzed using step-down Jonckheere- Terpstra trend tests only. Statistical tests used to evaluate treatment effects were performed at confidence level of $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Measured concentrations of the pretest samples ranged from approximately 57 to 107% of nominal concentrations. Arithmetic mean measured concentrations are 81, 99, 108, 100 and 90% for the 0.16, 0.80, 4, 20, and 100 mg a.s./L treatment groups.

B. OBSERVATIONS

Mortality, Clinical Signs: There were no treatment-related effects on survival during the 21-day test. Mean percent survival to Day 7 was 100% in all treatment groups including control, except at 20 and 90 mg a.s./L, where mean survival was 97.5% and 98.8%, respectively. Mean percent survival to Day 21 was 98.8, 100, 100, 100, 96.3 and 98.8% in the 0, 0.13, 0.79, 4.3, 20, and 90 mg a.s./L treatment groups, respectively.
Control and treatment tadpoles generally appeared normal and healthy throughout the test. Beginning on Day 2 and continuing until test termination, tail curvature was observed in control and treatment tadpoles. By test termination, tail curvature was observed in 64, 63, 65, 53, 53, and 78% of the tadpoles in the negative control, 0.13, 0.79, 4.3, 20, and 90 mg a.s./L treatment groups, respectively. The tail curvature was not considered to be a treatment-related effect. Tail curvature was not considered to be a thyroid-related effect, but rather, a dietary effect. In feeding trials done at the testing lab, it was shown that feeding rates during acclimation contribute to the amount of curvature observed.

Developmental stage: No treatment-related effects on the median developmental stage were observed on Day 7 or Day 21. The median developmental stage of the tadpoles on Days 7 and 21 were 53 and 57,

respectively, in all treatment groups including control. No observations of asynchronous development were noted.

Tadpole Growth:
Hind-limb Length
No treatment-related effects on absolute or normalized hind-limb lengths were apparent on Days 7 or 21 (see table below).

Table 8.2.3-13: Larval Development in African Clawed Frog (*Xenopus laevis*) – Hind- Limb Length

Treatment (mg a.e./L) [mean-measured]	Day 7				Day 21			
	n	Mean (mm)	±SD	HLL:SVL	n	Mean (mm)	±SD	HLL:SVL
Negative Control	4	2.08	0.10	0.13	4	7.65	0.68	0.33
0.13	4	2.10	0.08	0.13	4	8.48	0.22	0.36
0.79	4	2.15	0.17	0.13	4	7.78	0.43	0.33
4.3	4	1.75	0.21	0.11	4	8.20	0.50	0.34
20	4	2.08	0.10	0.13	4	8.00	0.69	0.34
90	4	2.10	0.14	0.13	4	8.25	0.79	0.33

Snout-to-Vent Length (SVL) and Body Weight
Mean SVL was not significantly affected by glyphosate acid treatment on Day 7 (see table below). On Day 21, SVL was significantly increased ($p < 0.05$) compared to control in the 4.3, 20 and 90 mg a.s./L treatment groups by 5.2 %, 2.5 % and 6.7 %, respectively, but this difference was not significant when normalized for hind-limb length. Additionally, there was a significant increase (17 %) in Day 21 body weight at 90 mg a.s./L. However, growth should never be solely relied upon to determine thyroid toxicity. Rather, growth, in conjunction with developmental stage and thyroid histopathology, should be used to determine thyroid activity.

Table 8.2.3-14: Larval Growth in African Clawed Frog (*Xenopus laevis*)

Treatment (mg a.e./L) [mean-measured]	Snout-Vent Length (SVL)						Body Weight[a]					
	Day 7			Day 21			Day 7			Day 21		
	n	Mean (mm)	±SD	n	Mean (mm)	±SD	n	Mean (g)	±SD	n	Mean (g)	±SD
Negative Control	4	15.8	0.98	4	23.2	0.43	4	0.267	0.040	4	0.864	0.038
0.13	4	15.9	0.38	4	23.6	0.66	4	0.273	0.021	4	0.925	0.100
0.79	4	16.1	0.75	4	23.5	0.83	4	0.288	0.031	4	0.907	0.078
4.3	4	16.1	0.80	4	**24.4***	0.15	4	0.290	0.042	4	0.973	0.037
20	4	16.1	0.34	4	**23.8***	0.45	4	0.282	0.022	4	0.920	0.056
90	4	16.1	0.99	4	**24.8***	0.38	4	0.300	0.048	4	**1.01***	0.060

SD= standard deviation
[a] Also referred to as "wet weight" in the test guideline.
* Statistically significant p<0.05 but this difference was not apparent when normalized for hind-limb length

Histopathology: There were no apparent treatment-related trends in thyroid histopathology. Observations and severity of thyroid atrophy and hypertrophy, and follicular cell hypertrophy and hyperplasia were comparable between the stage matched control and treatment groups (see table below). While there appears

to be an increased incidence of mild thyroid gland hypertrophy in the highest treatment concentration, the same incidence was observed at the lowest treatment concentration and the effect was not concentration responsive. Similar findings were observed for follicular cell height: an apparent increase in mild severity at the top concentration, but again, this incidence was similar to the lowest treatment concentration and no concentration-responsive pattern was seen. In addition, the pathology analysis indicated that there were no treatment related changes in the thyroid glands of tadpoles exposed to glyphosate acid when compared to organisms in the negative control.

Table 8.2.3-15: Gross Histopathology of the Thyroid Gland in African Clawed Frog (*Xenopus laevis*)

Treatment (mg a.e./L) [mean-measured]	Diagnostic Observations								
	Severity*	Thyroid Gland Hypertrophy		Thyroid Gland Atrophy		Follicular Cell Hypertrophy		Follicular Cell Atrophy	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence
Negative Control	0	20	17	20	19	20	17	20	17
	1	20	3	20	1	20	1	20	2
	2	20	0	20	0	20	2	20	1
	3	20	0	20	0	20	0	20	0
0.13	0	20	14	20	17	20	14	20	16
	1	20	4	20	3	20	4	20	2
	2	20	2	20	0	20	1	20	2
	3	20	0	20	0	20	1	20	0
0.79	0	20	17	20	17	20	13	20	17
	1	20	1	20	2	20	3	20	3
	2	20	2	20	1	20	3	20	0
	3	20	0	20	0	20	1	20	0
4.3	0	20	18	20	18	20	16	20	17
	1	20	1	20	2	20	2	20	3
	2	20	1	20	0	20	2	20	0
	3	20	0	20	0	20	0	20	0
20	0	20	18	20	19	20	18	20	15
	1	20	0	20	1	20	1	20	4
	2	20	2	20	0	20	1	20	1
	3	20	0	20	0	20	0	20	0
90	0	20	14	20	18	20	14	20	17
	1	20	6	20	2	20	2	20	3
	2	20	0	20	0	20	4	20	0
	3	20	0	20	0	20	0	20	0

* Thyroid histopathology graded 0 – 3 based on severity: 0 = not remarkable, 1 = Mild, 2 = Moderate, 3 = Severe.

The test is regarded as valid, since performance criteria for test acceptability according to OECD 231 guideline (2009) were met with one exception which did not affect the outcome of the study:

- The dissolved oxygen concentration was at least 40% of the air-saturation value throughout the exposure period.
- Water temperature did not differ by more than 1ºC between test vessels at any one time during the exposure period, and were maintained within ±1ºC of the 22ºC temperature specified.
- There was at least 90 % survival of control animals over the duration of the exposure period, and mortality in any one control replicate did not exceed two tadpoles.
- Test concentrations were consistent over the course of the study (i.e., contained at ≤20 % CV over the 21-day test), except for low recoveries in the low treatment group on Day 14 and in the high treatment group on Day 21.
- The minimum median stage of the control tadpoles at the end of the test was at least 57.
- The 10th and the 90th percentiles of the developmental stage distribution did not differ by more than 4 stages.

- There were less than two non-control test concentrations with overt toxicity.
- There were less than two replicates across the test that were compromised.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Arithmetic mean measured concentrations are 81, 99, 108, 100 and 90% for the 0.16, 0.80, 4, 20, and 100 mg a.s./L treatment groups. Therefore, results can be expressed as nominal concentrations.

There were no treatment related effects on survival, stage, or normalized hind-limb length during the 21-day test. Histopathologic analysis showed no treatment related changes in the thyroid glands of *Xenopus laevis* tadpoles when compared to negative control animals. There was a slight increase in wet weight in the 100 mg a.s./L treatment group and in snout-to-vent length in the 4.0 and 100 mg a.s./L treatment groups at the end of the 21-day test, however, this difference in snout-vent length was not significant when normalized with hind-limb length. Since there were no effects observed on normalized hind-limb length, stage, or thyroid histology, these increases are not indicative of a thyroid effect. Glyphosate acid was not found to interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis of African clawed frog tadpoles in this study.

The Amphibian metamorphosis assay (AMA) with the African clawed frog (*Xenopus laevis*) exposed to glyphosate acid is considered valid and the overall NOEC ≥100 mg a.s./L (arithmetic mean measured) can be used for ecotoxicological risk assessment.

Assessment and conclusion by RMS:

CA 8.2.4 Acute toxicity to aquatic invertebrates

Studies on acute effects of the active substance glyphosate and its relevant metabolites on aquatic invertebrates to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

Studies considering the acute toxicity of glyphosate to aquatic invertebrates were assessed for their validity to current and relevant guidelines for glyphosate, glyphosate salts and the metabolites AMPA and HMPA, and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 8.2.4- 1: Studies on acute toxicity of glyphosate and metabolites to aquatic invertebrate

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.4.1/001	[redacted] 2003	48 hour acute	Glyphosate K - salt	Valid	-
CA 8.2.4.1/002	[redacted] 2000	48 hour acute	IPA salt	Valid	-
CA 8.2.4.1/003	[redacted] 2000	48 hour acute	Glyphosate technical	Valid	-
CA 8.2.4.1/004	[redacted] 1996	48 hour acute	Glyphosate acid	Valid	
CA 8.2.4.1/005	[redacted] 1995	48 hour acute	Glyphosate acid	Supportive	Report not available
CA 8.2.4.1/006	[redacted], 1995	48 hour acute	Glyphosate	Valid	-
CA 8.2.4.1/007	[redacted] 1994	48 hour acute	IPA salt	Valid	-
CA 8.2.4.1/008	[redacted] 1993	48 hour acute	IPA salt	Supportive	Report not available
CA 8.2.4.1/009	[redacted] 1990	48 hour acute	Glyphosate technical	Valid	-
CA 8.2.4.1/010	[redacted] 1981	48 hour acute	IPA salt	Supportive	No analytical verification of test concentrations
CA 8.2.4.1/011	[redacted] 1978	48 hour acute	Gyphosate	Supportive	No analytical verification of test concentrations
CA 8.2.4.1/012	[redacted] 1998	48 hour acute	AMPA	Valid	-
CA 8.2.4.1/013	[redacted] 1994	48 hour acute	AMPA	Valid	-
CA 8.2.4.1/14	[redacted] 1991	48 hour acute	AMPA	Valid	-
CA 8.2.4.1/15	[redacted] 2011	48 hour acute	HMPA	Valid	-
CA 8.2.4.2/001	[redacted] 1996	96 hour acute	Glyphosate acid	Valid	-
CA 8.2.4.2/002	[redacted] 1978	96 hour acute	Glyphosate	Supportive	No analytical verification of test concentrations
CA 8.2.4.2/003	[redacted] 1996	48 hour acute	Glyphosate acid	Valid	-
CA 8.2.4.2/004	[redacted] 1985	48 hour acute	Glyphosate technical	Supportive	No analytical verification of test concentrations

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the acute impact of glyphosate or its relevant metabolites on aquatic invertebrates. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. For discussions of literature regarding toxicity to aquatic invertebrates, please refer to document M-CP Section 10.2.

Endpoints of studies considered valid for glyphosate are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.2.4- 2: Endpoints: Acute toxicity of glyphosate to Aquatic Invertebrates

Reference	Test item	Species	Test design	Endpoints based on	EC_{50} (mg a.e./L)	NOEC (mg a.e./L)
[redacted] 2003 CA 8.2.4.1/001	Glyphosate K - salt	*Daphnia magna*	48h static	am	278	149
[redacted] 2000CA 8.2.4.1/002	IPA salt	*Daphnia magna*	48h static	im	> 471	≥ 471
[redacted] 2000CA 8.2.4.1/003	Glyphosate technical	*Daphnia magna*	48h static	im	420.59	179.56
[redacted] 1996 CA 8.2.4.1/004	Glyphosate acid	*Daphnia magna*	48h static	nom	136.5	100
[redacted] 1995 CA 8.2.4.1/006	Glyphosate	*Daphnia magna*	48h static	nom	> 100	≥ 100
[redacted] 1994 CA 8.2.4.1/007	IPA salt	*Daphnia magna*	48h static	nom	> 45.64	≥ 45.64
[redacted] 1990 CA 8.2.4.1/009	Glyphosate technical	*Daphnia magna*	48h static	mm	74.0	53
[redacted] 1996 CA 8.2.4.2/001	Glyphosate acid	*Mysidopsis bahia*	96h static	nom	80	32
[redacted] 1996 CA 8.2.4.2/003	Glyphosate acid	*Crassostrea gigas*	48h static	nom	**40**	32

a.e.: acid equivalents
nom: nominal, mm mean measured, im initial measured; am: arithmetic mean measured
Endpoint in **bold** is used for risk assessment

Endpoints of studies for AMPA and HMPA considered valid are shown in the table below.

Table 8.2.4- 3: Endpoints: Acute toxicity of AMPA and HMPA to *Daphnia magna*

Reference	Test item	Species	Test design	Endpoints based on	EC_{50} (mg/L)	NOEC (mg/L)
[redacted] 1998 CA 8.2.4.1/012	AMPA	*Daphnia magna*	48h static	nom	> 100	≥ 100
[redacted] 1994 CA 8.2.4.1/013	AMPA	*Daphnia magna*	48h static	nom	>180	≥ 180
[redacted] 1991 CA 8.2.4.1/014	AMPA	*Daphnia magna*	48h static	nom	**690**	320

Table 8.2.4- 3: Endpoints: Acute toxicity of AMPA and HMPA to *Daphnia magna*

Reference	Test item	Species	Test design	Endpoints based on	EC_{50} (mg/L)	NOEC (mg/L)
[redacted] 2011 CA 8.2.4.1/015	HMPA	*Daphnia magna*	48h static	nom	**>100**	≥ 100

Endpoints in **bold** are used for risk assessment

Full study summaries are provided below.

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

1. Information on the study

Data point:	CA 8.2.4.1/001
Report author	[redacted]
Report year	2003
Report title	MON 78623: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)
Report No	139A-309
Document No	-
Guidelines followed in study	OECD Guideline 202 (1984) OPPTS 850.1010 (1996) EU Directive 67/548/EEC Method C2 (1992)
Deviations from current test guideline	Deviation from the guideline OECD 202 (2004): Minor: - Immobilisation was recorded after 19 h of exposure (this is in addition to the guideline requirement).
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of Mon 78623 (Glyphosate K-Salt) on *Daphnia magna* were evaluated in a 48-hour static toxicity test performed using nominal concentrations of 156, 313, 625, 1250 and 2500 mg test item/L, equivalent to 74, 149, 298, 596 and 1193 mg glyphosate acid equivalents/L. These nominal concentrations are equivalent to mean measured concentrations of 165, 312, 624, 1285 and 2582 mg test item/L. In addition, a negative control group (well water only) was run in parallel. There were two vessels prepared for the control and for each treatment, each containing ten daphnids.

The total number of immobile *Daphnia magna* was recorded at 19 h, 24 h and 48 h after test initiation. Mean measured concentrations were recorded at the beginning and at the end of the tests.

Mean overall measured concentrations of glyphosate (acid equivalents) ranged between 100 and 106 % of the nominal values. Glyphosate K-salt was not detected in the control group. At 624 mg test item/L 65 % of the daphnids were observed to be lethargic at the bottom of the test chamber at test termination. Immobility at 48 h at concentrations of 1285 and 2582 mg test item/L were 5 and 25 %, respectively and

all remaining daphnids at these two test concentrations were lethargic at the bottom of the test chamber. All validity criteria according to the guideline OECD 202 were fulfilled.
In conclusion, the 48 h EC_{50} for *Daphnia magna* exposed to Glyphosate K-salt was calculated to be > 2582 mg/L, equivalent to >1231.6 mg glyphosate acid/L based on mean measured concentrations. The 48- hour no-effect level (NOEC) for Glyphosate K-salt was determined to be 312 mg/L, equivalent to 148.8 mg glyphosate acid/L based on mean measured concentrations. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 78623 (Glyphosate K-salt)
Active substance	Glyphosate acid
Description:	Yellow liquid
Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7 % acid equivalents

2. Vehicle and/or positive control: Vehicle: Well water
Positive control: None

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	2 × 10 specimens for 250 mL test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None

4. Environmental conditions:

Temperature:	19.5 – 20.0 °C
Photoperiod:	16 hours light / 8 hours dark with 30 min transition period
pH:	5.7 – 8.1 (test item) 8.1 – 8.2 (control)
Dissolved oxygen:	≥ 8.6 mg/L (≥ 96 % saturation)
Conductivity:	310 µmhos/cm
Hardness:	140 mg $CaCO_3$/L.
Alkalinity:	184 mg $CaCO_3$/L

5. Experimental dates: December 3, 2002 to December5, 2002

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, a definitive toxicity test was performed using nominal concentrations of 156, 313, 625, 1250 and 2500 mg test item/L (mean 165, 312, 624, 1285 and 2582 mg test item/L in a static test setup. The test solutions were prepared using test facility well water (Dissolved oxygen ≥ 96 %, pH = 5.7 – 8.1, hardness 140 mg $CaCO_3$/L.). In addition, a control group was exposed to well water (negative control). There were two replicates per treatment, each containing ten daphnids. Test chambers were 250 mL glass beakers containing approx. 250 mL of test medium.
2. Observations: Total number of immobile *Daphnia magna* was recorded at 19h, 24 h and 48 h after the test initiation. Temperature of the test solutions was measured at the test initiation and termination.

Hardness, alkalinity and specific conductance of the dilution water were measured at test initiation. The pH value and oxygen saturation were measured at test initiation and at 24 h and 48 h. For analysis of test substance concentration with HPLC, test medium was collected from the replicate test chambers at 0 and 48 h.
The validity criteria according to the current OECD 202 guideline are the following:

- In the control, not more than 10 per cent of the daphnids should have been immobilised or show or other signs of disease or stress.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

3. Statistical calculations: Since the immobility was < 50%, no statistical calculation of EC_{50} values was possible. Therefore, EC_{50} and NOEC values were determined by visual inspection.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analytics confirm the stability of the test substance, since the recovery was 99 – 105 % at test start and 97 – 107% at test end. Results are based on arithmetic mean measured concentrations.

Table 8.2.4- 4: Analytical results

Nominal concentration MON 78623 [mg/L]	Control	156	313	625	1250	2500
0 h mean measured concentration [mg/L]		163	311	636	1279	2548
48 h mean measured concentration [mg/L]		167	314	612	1291	2616
Mean measured over 48 h Glyphosate K-salt (MON 78623) [mg/L]		165	312	624	1285	2582
% of nominal		106	100	100	103	103
Mean Measured over 48 h Glyphosate acid [mg/L]	<	78.7	148.8	297.6	612.9	1231.6

The EC_{50} and NOEC are based on mean measured concentrations of 165, 312, 624, 1285 and 2582 mg test item/L and are given below.

Table 8.2.4- 5: Endpoints

Endpoints	Glyphosate K-salt [mg/L]	Glyphosate Acid [mg a.e./L]
48 h EC_{50}	> 2582	> 1231.6
NOEC	312	148.8

B. OBSERVATIONS

In the negative control and at mean measured concentrations of 165 and 312 mg test item/L no effects were observed. At 624 mg test item/L 65% of the daphnids were observed to be lethargic at the bottom of the test chamber at test termination. Immobility at 48 h at 1285 and 2582 mg test item/L was 5 and 25 %, respectively. All remaining daphnids were lethargic at the bottom of the test chamber.

Table 8.2.4- 6: Lethal effects of glyphosate K-salt to *Daphnia magna*

Mean measured Glyphosate K-salt (MON 78623) [mg/L]	**Control**	**165**	**312**	**624**	**1285**	**2582**
Mean Measured Glyphosate acid [mg a.e./L]	**-**	**78.7**	**148.8**	**297.6**	**612.9**	**1231.6**
Immobility (19 h) [%]	0	0	0	0	0	0
Immobility (24 h) [%]	0	0	0	0	0 (8C)	0 (17C)
Immobility (48 h) [%]	0	0	0	0 (13C+G)	1 (19C+G)	5 (15C+G)

C = lethargic; G = on bottom of test chamber; AN = appear normal

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 h EC_{50} for *Daphnia magna* exposed to Glyphosate K-salt was calculated to be > 2582 mg/L, equivalent to >1231.6 mg a.e./L based on mean measured concentrations. The 48- hour no-effect level (NOEC) for Glyphosate K-salt was determined to be 312 mg/L, equivalent to 148.8 mg a.e./L based on arithmetic mean measured concentrations.
Based on lethargy, RAR 2015 recalculated EC_{50} to be 278 mg a.e./L and NOEC to be 149 mg a.e./L, arithmetic mean measured.
The study is considered valid and reliable for the risk assessment of glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/002
Report author	[redacted]
Report year	2000
Report title	Acute toxicity of glifosato IPA tecnico Nufarm to *Daphnia magna*
Report No	RF-D51.017/00
Document No	-
Guidelines followed in study	OECD 202 (1984)
Deviations from current test guideline	Deviations from the guideline OECD 202 (2004): Minor: - The concentration of the test substance in the test media was measured only at the beginning of the study.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to 100, 180, 320, 560, and 1000 mg a.s./L nominal concentrations. In addition, 4 x 5 *Daphnia* were exposed to test water without test substance (blank control). Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test. The analysed test concentrations ranged between 75.90 and 139.70% of the nominal values. Therefore, the results reported are related to initial measured concentrations of the test item. The NOEC after 48 h based on immobilisation was ≥ 1397 mg test item/L (equivalent to ≥ 471 mg a.e./L). All validity criteria according to the guideline OECD 202 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate Isopropylamine Salt
Lot/Batch #:	MJRT 025-201-104
Purity:	612.7 g/kg salt equivalent (analysed on May 02, 2000)

2. Vehicle and/or positive control: Vehicle: Water
Positive control: Toxic standard (potassium dichromate)

3. Test organism:

Species:	*Daphnia magna*
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (30 mL glass beakers containing 20 mL

	test solution)
Source:	Carolina Biological Supply Company, Burlington, North Carolina (USA) and maintained as a stock culture at BIOAGRI

4. Environmental conditions:

Temperature:	21.1 to 21.2 °C
pH:	Start of the test: 5.56-7.39 End of the test: 5.54-7.81
Dissolved oxygen:	Start of the test: 6.10-6.27 mg O_2/L End of the test: 5.57-5.67 mg O_2/L
Conductivity:	603.0 mg/L µS/cm
Hardness:	248 mg $CaCO_3$
Photoperiod:	Light/dark 0/24 h

5. experimental dates: June 6th, 2000 to June 15th, 2000

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to 100, 180, 320, 560, and 1000 mg a.s./L nominal concentrations. In addition, 4 x 5 *Daphnia* were exposed to test water without test substance (blank control). A reference test using potassium dichromate was carried out in order to verify the sensitivity of the test system. The primary stock solution of nominal concentration of 1000 mg a.s./L was prepared by dissolving 500 mg test item in 500 mL water. Appropriate amounts of this stock solution were diluted to prepare the lower test concentrations of 100, 180, 320, and 560 mg a.s./L. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.
2. Observations: Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test.
3. Statistical calculations: The EC_{50} for glyphosate could not be quantified due to the absence of toxicity of the test item, therefore, no statistical analysis was performed. The EC_{50} value for the reference substance potassium dichromate was calculated by applying Trimmed Spearman-Karber method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analysed test concentrations ranged between 75.90 and 139.70 % of the nominal values. Therefore, the results reported are related to measured concentrations of the test item.

Table 8.2.4- 7: Analytical results

Nominal concentration [mg test item/L]	Measured concentration [mg test item/L]	% of nominal
Control	-	-
100	75.9	75.90
180	150.0	83.33
320	282.8	88.37
560	693.6	123.85
1000	1397	139.70

The EC_{50} value is given below based on nominal concentrations.

Table 8.2.4- 8: Endpoints

Endpoints	Test item mg/L	Glyphosate acid [mg/L]
EC_{50} (48 h)	> 1397	> 471

The reference substance potassium dichromate resulted in a 48-h EC_{50} of 1.22 mg/L (95% CL = 1.12-1.35 mg/L).

B. OBSERVATIONS

After 24 hours and 48 hours of exposure neither in the control nor in the test item concentration vessels immobilisation of *Daphnia* was observed.

The effects of glyphosate on *Daphnia magna* are shown below.

Table 8.2.4- 9: Effects of glyphosate on *Daphnia magna*

Nominal concentration [mg test item/L]	Measured concentration [mg test item/L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours	Number of immobile *Daphnia* after 48 hours
Control	-	20	0	0
100	75.9	20	0	0
180	150.0	20	0	0
320	282.8	20	0	0
560	693.6	20	0	0
1000	1397	20	0	0

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48-h EC_{50} for *Daphnia magna* exposed to glyphosate isopropylamine Salt was > 1397 mg test item/L (corresponding to 471 mg a.e./L) based on initial measured concentration. The NOEC after 48 h based on immobilisation was ≥ 1397 mg test item/L (corresponding to ≥ 471 mg a.e./L).

All validity criteria according to the OECD 202 were fulfilled, the study is therefore considered valid and the EC_{50} of 471 mg a.e./L and the NOEC of ≥ 471 mg a.e./L can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/003
Report author	[redacted]
Report year	2000
Report title	Acute toxicity of glifosate tecnico Nufarm to Daphnia magna
Report No	RF-D51.39/99
Document No	-
Guidelines followed in study	OECD 202 (1984)
Deviations from current test guideline	Deviations from the guideline OECD 202 (2004): Minor: • The concentration of the test substance in the test media was measured only at the beginning of the study.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 100, 180, 320, 560, and 1000 mg a.s./L (corresponding to 103.40, 179.56, 334.11, 597.06, and 1051.12 mg a.s./L measured concentrations). In addition, 4 x 5 *Daphnia* were exposed to test water without test substance (blank control).

Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test. The analysed test concentrations ranged between 99.75 and 106.61% of the nominal values. The results reported are related to initial measured concentrations of the test item. The NOEC after 48 h based on immobilisation was 179.56 mg a.e./L. All validity criteria according to the guideline OECD 202 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	037-919-113
Purity:	95 %

2. Vehicle and/or positive control: Vehicle: Water
Positive control: Toxic standard (potassium dichromate)

3. Test organism:

Species:	*Daphnia magna*
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (30 mL glass beakers containing 20 mL test solution)
Supplier:	Carolina Biological Supply Company, Burlington, North Carolina (USA) and maintained as a stock culture at BIOAGRI

4. Environmental conditions:

Temperature:	20.2 to 21.5 °C
pH:	Start of the test: 3.06-7.40 End of the test: 3.10-7.96
Dissolved oxygen:	Start of the test: 5.7-6.2 mg O_2/L End of the test: 4.4-4.6 mg O_2/L
Conductivity:	410 mg/L µS/cm
Hardness:	245 mg $CaCO_3$
Photoperiod:	Light/dark 0/24 h
5. Experimental dates:	October 13th, 1999 to October 28th, 1999

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 100, 180, 320, 560, and 1000 mg a.s./L (corresponding to 103.40, 179.56, 334.11, 597.06, and 1051.12 mg a.s./L measured concentrations). In addition, 4 x 5 *Daphnia* were exposed to test water without test substance (blank control). A reference test using potassium dichromate was carried out in order to verify the sensitivity of the test system. The primary stock solution of nominal concentration of 1000 mg a.s./L was prepared by dissolving 1000 mg test item in 1000 mL water. Appropriate amounts of this stock solution were diluted to prepare the lower test concentrations of 100, 180, 320, and 560 mg a.s./L. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations: Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test.

3. Statistical calculations: The EC_{50} value for glyphosate and reference substance potassium dichromate was calculated by applying Trimmed Spearman-Karber method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analysed test concentrations ranged between 99.75 and 106.61 % of the nominal values. The results reported are related to initial measured concentrations of the test item.

Table 8.2.4- 10: Analytical results

Nominal concentration [mg test item/L]	Measured concentration [mg test item/L]	% of nominal
Control	-	-
100	103.40	103.40
180	179.56	99.75
320	334.11	104.4
560	597.06	106.61
1000	1051.12	105.11

The effects of glyphosate on *Daphnia magna* are shown below.
The 24 and 48 hour EC_{50} values (based on measured concentrations) are given below:

Table 8.2.4- 11: Endpoints EC_{50} values for *Daphnia magna*

Time	EC_{50} (mg a.s./L)	95 % confidence interval (mg a.s./L)
24 h	530.42	471.64 - 596.52
48 h	420.59	388.02 – 455.90

The reference substance potassium dichromate resulted in a 48-h EC_{50} of 0.68 mg/L (95% CL = 0.63-0.75 mg/L).

B. OBSERVATIONS

Table 8.2.4- 12: Effects of glyphosate on *Daphnia magna*

Nominal concentration [mg test item/L]	Measured concentration [mg test item/L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours	Immobility after 24 hours [%]	Number of immobile *Daphnia* after 48 hours	Immobility after 48 hours [%]
Control		20	0	0	0	0
100	103.40	20	0	0	0	0
180	179.56	20	0	0	0	0
320	334.11	20	0	0	2	10
560	597.06	20	14	70	20	100
1000	1051.12	20	20	100	20	100

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The 48-h EC_{50} for *Daphnia magna* exposed to glyphosate technical was 420.59 mg a.e./L based on initial measured concentration. The NOEC after 48 h based on immobilisation was 179.56 mg a.e./L. All validity criteria according to the OECD 202 were fulfilled. The study is therefore considered valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute toxicity to *Daphnia magna*
Report No	AB0503/C
Document No	-
Guidelines followed in study	OECD 202 (1984), EPA FIFRA, Subdivision E, Guideline 72-2
Deviations from current test guideline	Deviations from guideline OECD 202 (2004): none
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate acid on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 10, 18, 32, 56, 100 and 180 mg/L of glyphosate acid and a pH adjusted 1000 mg/L test concentration of glyphosate acid. In addition, 4 x 5 *Daphnia* were exposed to test medium without test substance (blank control).

Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. The concentration of glyphosate acid in the test solutions were measured at 0 and 48 hours.

The analysed test concentrations ranged between 85 and 100 % of the nominal values, therefore, the results reported are related to nominal concentrations of the test item. All validity criteria according to the guideline OECD 202 were fulfilled. The 48 hour EC_{50} for Daphnia exposed to glyphosate acid falls between 100 and 180 mg/L, where there was zero and 100 % immobility, respectively. Using linear interpolation between these two concentrations, the EC_{50} is 136.5 mg/L. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	Not mentioned in the report
Purity:	95.6 %

2. Vehicle and/or positive control: Vehicle: Dilution water
Positive control: none

3. Test organism:

Species:	*Daphnia magna* Straus
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (250 mL glass beakers containing 200 mL test solution) which corresponds to 25 *Daphnia*/L.
Source:	Continuous laboratory cultures

4. Environmental conditions:

Temperature:	20.5-20.8 °C
pH:	4.21-8.98
Dissolved oxygen:	8.7-9.0 mg O_2/L
Conductivity:	693 mg/L µS/cm
Hardness:	263 mg $CaCO_3$
Photoperiod:	16 hours light / 8 hours dark with 20 minute transition periods

5. Experimental dates: July 24th, 1995 to July 26th, 1995

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate acid on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 10, 18, 32, 56, 100 and 180 mg/L of glyphosate acid and a pH adjusted 1000 mg/L test concentration of glyphosate acid. In addition, 4 x 5 *Daphnia* were exposed to test medium without test substance (blank control).

A stock solution of nominal concentration of 1000 mg a.s./L was prepared by dissolving 1000 mg test item in 1000 mL dilution water. The 10 to 180 mg a.s./L test solutions were prepared by dispersing aliquots of the stock solution to dilution water.

A further 1000 mg a.s./L stock solution was prepared by dissolving 1 g of glyphosate acid in 1 litre of dilution water. The pH of this stock solution was adjusted from 2.59 to 8.98 using 12 mL of 1 M sodium hydroxide. All stock and test solutions were observed to be clear and colourless. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations: Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. The concentrations of glyphosate acid in the test solutions were measured at 0 and 48 hours.

3. Statistical calculations: The EC_{50} values for the 10 and 180 mg a.s./L test concentrations were calculated with the binomial method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analysed test concentrations ranged between 85 and 100 % of the nominal values, therefore, the results reported are related to nominal concentrations of the test item.
Due to an oversight at 0 hours the pH adjusted 1000 mg a.s./L test solution was not sampled for analysis and therefore a sample was taken at 24 hours. The lack of 0 hour analysis for this concentration was considered not to have affected the validity of the study since analysis at 24 and 48 hours gave results which were close to the nominal value (100 and 83 %, respectively).

Table 8.2.4- 13: Analytical results

Nominal concentration [mg/L]	Measured concentration of Glyphosate acid [mg/L]		Mean measured concentration of Glyphosate acid [mg/L]	% of nominal
	0 hours	48 hours		
Control	< 0.0039	< 0.0039	< 0.0039	-
10	8.6	8.4	8.5	85
18	16[1]	16[1]	16	89
32	29	29	29	91
56	49	49	49	88
100	92	93	93	93
180	180	180	180	100
1000 (pH adjusted)	1000[2]	830	920	92

[1]Triplicate analysis
[2] measured at 24 hours.

The 24 and 48 hour EC_{50} values (based on nominal concentrations of glyphosate acid) are given below.

Table 8.2.4- 14: EC50 values for Daphnia magna

Time	EC_{50} [mg a.s./L]	95 % confidence interval [mg a.s./L]
24 h	130	100-180
48 h	130	100-180

The pH adjusted 24 and 48 hour EC_{50} values (based on nominal concentrations of glyphosate acid) are given below:

Table 8.2.4- 15: EC50 values for Daphnia magna (pH adjusted)

Time	EC_{50} [mg a.s./L]	95 % confidence interval [mg a.s./L]
24 h	>1000	-
48 h	>1000	-

B. OBSERVATIONS

The results obtained from this study indicate that the toxicity of glyphosate acid below 1000 mg/L was caused by pH values less than 5.

The effects of glyphosate acid on *Daphnia magna* are shown below.

Table 8.2.4- 16: Effects of glyphosate acid on Daphnia magna

Nominal concentration [mg a.s./L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours	Immobility after 24 hours [%]	Number of immobile *Daphnia* after 48 hours	Immobility after 48 hours [%]
Control	20	0	0	0	0
10	20	0	0	0	0
18	20	0	0	0	0
32	20	0	0	0	0
56	20	0	0	0	0
100	20	0	0	0	0
180	20	20	100	20	100
1000 (pH adjusted)	20	0	0	0	0

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Due to 0% mortality at 100 mg/L and 100% mortality at 180 mg a.s./L, the 48 hour EC_{50} for Daphnia exposed to glyphosate acid falls between 100 and 180 mg/L. Using linear interpolation between these two concentrations, the EC_{50} is 136.5 mg/L. The NOEC was 100 mg a.s./L (nominal).
All validity criteria according to the OECD 202 were fulfilled, so the study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.4.2.1/005
Report author	[redacted]
Report year	1995
Report title	The acute toxicity of glyphosate to *Daphnia magna*
Report No	710/22
Document No	-
Guidelines followed in study	Information mentioned in the Monograph: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015).
Short description of study design and observations	Toxicity of technical glyphosate (purity >94 %) to aquatic organisms (*Daphnia magna*), 48 hours test.
Short description of results	NOEC 24 h = 100 mg a.s./L LC_{50} 24 h >100 mg a.s./L NOEC 48 h = 18 mg a.s./L LC_{50} 48 h = 40 mg a.s./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	These data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015. The study is considered as supportive because the report is not available and therefore it cannot be concluded on the study validity according the current guideline requirements.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 8.2.4.1/006
Report author	[redacted]
Report year	1995
Report title	Acute Toxicity Study in *Daphnia magna* with Glyfosaat
Report No	141863
Document No	-
Guidelines followed in study	OECD Guideline 202 (1984)
Deviations from current test guideline	Deviation from the guideline OECD 202 (2004): Minor: - Only two replicates - Only one test concentration of 100 mg/L.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. The toxicity test was performed using three nominal concentrations, 1, 10 and 100 mg test item/L. Furthermore, a blank control was tested. Ten daphnids were exposed to the concentrations of 1 and 10 mg test item/L (in one test vessel for each test concentration). 2 replicates with 10 daphnids each were prepared for the highest test concentration of 100 mg test item/L and the control.

At or below the highest test nominal concentration, no immobilisation was observed in tested daphnids during the 48 h exposure period. Hence, the 48 h EC_{50} for *Daphnia magna* exposed to glyphosate was determined to be > 100 mg a.e./L. All validity criteria according to OECD 202 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	22021
Purity:	96%

2. Vehicle and/or positive control: Vehicle: ISO-medium (in milli-RO water)
Positive control: $K_2Cr_2O_7$

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 80 mL test medium
Source:	In-house culture

4. Environmental conditions:

Temperature:	19.5°C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.0 – 8.1 (control), 5.2 - 5.5 (100mg test item/L)
Dissolved oxygen:	8.9 – 9.5 mg O_2/L
Hardness:	250 mg $CaC0_3$/L

5. Experimental dates: April 12, 1995 to April 14, 1995

B: STUDY DESIGN AND METHODS

1. Experimental treatments: A range finding test, which was considered as the final test (since no immobility of daphnids was observed at or below the highest test concentration), was performed using three nominal concentrations, 1, 10 and 100 mg test item/L, prepared using ISO-medium (in milli-RO water). The test was conducted in a static test setup for 48 hours in 100 mL vessels containing 80 mL test solution each. In addition, a control group was exposed to test medium without test substance or other additives.

The test consisted of one vessel per treatment (containing 10 daphnids each) for the test concentrations of 1 and 10 mg test item/L and two vessels (containing 10 daphnids each) for the highest test concentration of 100 mg test item/L and for the control.
2. Observations: Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation.
The pH-values were measured at test initiation and termination, for all concentrations and the control. The oxygen saturation was measured at test initiation for the control and the highest test concentration and at test termination for all concentrations and control. The temperature was controlled daily in one control vessel, starting from the beginning of the test.
Analytical control measurements of the actual concentration of the test item were performed by mean of HPLC analysis using samples taken at test start (0 h) and test termination (48 h).
3. Statistical calculations: Descriptive statistics

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements were performed on samples of the highest test concentration. Before introduction of the daphnids 112 % of the nominal glyphosate concentration was recovered in the test media. In the aged test media 109 % of the nominal concentration was recovered.
As the mean measured content of the test item ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 8.2.4- 17: Analytical results

Nominal Concentration [mg/L]	Time [hours]	Measured	% of nominal
0	0	0	-
0	48	0	-
100	0	112	112
100	48	109	109

The EC_{50} value is given below based on nominal concentrations.

Table 8.2.4- 18: Endpoints

Endpoints	Glyphosate [mg a.e./L]
EC_{50} (48 h)	> 100

Reference item: The 48h-EC_{50} for the reference item was 0.52 mg/L (95% CL = 0.50 – 0.55 mg/L), which was within the range of expected responses. Hence, the sensitivity of this batch of D*aphnia magna* was in agreement with the historical data collected at test facility.

B. OBSERVATIONS

At or below the highest test nominal concentration, no immobilisation was observed in tested daphnids during the 48 h exposure time. Also, all validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
Under the conditions of the present test, glyphosate induced no visible effects in *Daphnia magna* at 100 mg a.e./L, the only concentration tested. Hence, the 48 h EC_{50} for *Daphnia magna* exposed to glyphosate was determined to be > 100 mg a.e./L and the NOEC ≥ 100 mg a.e./L. Although this limit test was conducted with only two replicates, all validity criteria according to the OECD 202 were fulfilled. Therefore, the study is considered valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/007
Report author	[redacted]
Report year	1994
Report title	Acute Toxicity in *Daphnia magna*; Test Article: 'Glyphosate isopropylamine salt'
Report No	83-91-0737-00-93
Document No	-
Guidelines followed in study	OECD Guideline 202
Deviations from current test guideline	Deviation from the guideline OECD 202 (2004): Minor: - Limit test with one concentration (100 mg test item/L)
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute effects of glyphosate isopropylamine salt on *Daphnia magna* were evaluated in a 48-hour static toxicity test. The test was conducted to supplement the results of the acute toxicity test already performed as a range finding study for the 21 d reproduction test in *Daphnia magna* (IBR Project No. 89-91-2328-05-93).

The acute toxicity test was performed under static conditions as limit test using only one test concentration of nominal 100 mg test item/L, equivalent to 61.6 mg glyphosate isopropylamine salt/L or 45.64 mg glyphosate/L. In addition, a control group was exposed to reconstituted water (Elendt-medium). As a toxic reference, daphnids were exposed to 0.4 and 1.4 mg/L of the reference substance $K_2Cr_2O_7$.

There were four test vessels per treatment, each containing five *Daphnia magna* (25 mL volumetric cylinder containing 10 mL test medium).

Temperature, pH-value and oxygen saturation of the test solutions were measured at test initiation and termination. Total number of mobile daphnids and the rate of immobilisation were recorded 24 and 48 h after test initiation. At 100 mg test item/L, none of the *Daphnia magna* was found to be immobilised. The EC_{50} was determined to be >100 mg test item/L, equivalent to 61.6 mg glyphosate isopropylamine salt/L

or 45.64 mg a.e./L (nominal). All validity criteria according to OECD 202 were fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt
Description:	viscous liquid
Lot/Batch #:	01/06/93
Purity:	61.6 % Glyphosate isopropylamine salt
Density:	1.23 g/cm^3 at 20°C

2. Vehicle and/or positive control: 0.4 and 1.4 mg/L $K_2Cr_2O_7$

3. Test organism:

Species:	*Daphnia magna* Strauss
Age:	neonates (6 - 24 h old)
Loading:	10 mL for 5 specimens
Source:	Laboratory bred
Diet/Food:	none
Acclimation period:	Daphnids were held in groups of 25-30 organisms in 1000 mL glass vessels at test conditions. Specimens were fed on green algae and water was renewed 3 times a week.

4. Environmental conditions:

Temperature:	18 – 22 °C (± 1 °C during the test)
Photoperiod:	16 hours light / 8 hours dark, 600 – 700 lux
pH:	7.5 – 8.5
Dissolved oxygen:	> 60 % of air saturation (approx. 6.0 mg O_2/L)
Conductivity:	0.049 µS/cm
Hardness:	14.5° dH

5. Experimental dates: January 4th, 1994 to January 6th, 1994

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The acute toxicity test was performed under static conditions as limit test using a nominal test concentration of 100 mg test item/L, corresponding to 61.6 mg glyphosate isopropylamine salt/L or 45.64 mg glyphosate/L in glass vessels containing reconstituted water (Elendt-medium). In addition, a control group was exposed to Elendt-medium. Two reference groups were equally exposed to 0.4 and 1.4 mg/L of $K_2Cr_2O_7$. There were four vessels per treatment, each containing five *Daphnia magna* (25 mL volumetric cylinder containing 10 mL test medium).

2. Observations: The *Daphnia magna* were observed 24 and 48 hours after initiation of the test. Temperature, pH-value and oxygen saturation of the test solutions were measured at initiation and test termination.

Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation.

Analytical measurement of the test item concentration was performed by mean of HPLC analysis at the beginning (0 h) and end (48h) of the limit test. Glyphosate isopropylamine salt concentrations were determined based on the concentrations of glyphosate.

3. Statistical calculations: Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The average recovery of glyphosate in the test media at the beginning (0 h) and end (48h) of the limit test were 103.7%, and 103.2% respectively. As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 8.2.4- 19: Analytical results

	Nominal concentration [mg/L]	Measured concentration [mg/L]		% of nominal	
		24 hr	48 hr	24 hr	48 hr
test item	100	-	-	-	-
glyphosate isopropylamine salt	61.6	-	-	-	-
glyphosate	45.65	47.32	47.09	103.7%	103.2%

The EC_{50} values are given below based on nominal concentrations.

Table 8.2.4- 20: Endpoints

Endpoints	test item [mg/L]	Glyphosate isopropylamine salt [mg/L]	Glyphosate [mg a.e./L]
EC_{50} (48 h)	> 100	> 61.6	> 45.64

B. OBSERVATIONS

The immobility rate in the control group did not exceed 10% (0% in the test) at any stage of the test. At the concentration level of 100 mg test item/L, none of the daphnids tested were found to be immobilised, 24 h and 48 h after the start of the test.

Table 8.2.4- 21: Immobilisation of daphnids exposed to glyphosate isopropylamine salt

	Control	Test item [mg/L]	Reference [mg/L]	
test item	-	100		
glyphosate isopropylamine salt	-	61.6	0.4	1.4
glyphosate	-	45.64	0.4	1.4
24 h	0	0	0	85
48 h	0	0	5	100

The 48 h EC_{50} obtained for the reference substance was within the range of 0.4 to 1.4 mg/L, documenting the functional integrity of the test system. All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In a 48-hours static acute toxicity study with *Daphnia magna* exposed to glyphosate isopropylamine salt, the EC_{50} was determined to be >100 mg test item/L, corresponding to 61.6 mg a.s/L or 45.64 mg a.e./L (nominal). As this was conducted as a limit test, the NOEC corresponds to ≥ 45.64 mg a.e./L.
All validity criteria according to the OECD 202 were fulfilled, the study is therefore considered valid and reliable for the regulatory risk assessment for glyphosate..

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.4.2.1/008
Report author	[redacted]
Report year	1993
Report title	Information not available
Report No	94-00549 (typo error in the Monograph: 9**5**-00549)
Document No	-
Guidelines followed in study	Information mentioned in the Monograph 2001: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015).
Short description of study design and observations	Acute and chronic toxicity of glyphosate isopropylamine salt (purity 61 – 65 %) to aquatic organisms (*Daphnia magna*), 48 hours static test.
Short description of results	LC_{50} (48 h) >1000 mg a.s./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	The study is considered as supportive because the report is not available; so it cannot be concluded on the study validity according the current guideline requirements. However, these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 8.2.4.1/009
Report author	[redacted]
Report year	1990
Report title	48-Hour Acute Toxicity of Glyphosate Technical to *Daphnia magna* (OECD-Immobilization Test)
Report No	272968
Document No	-
Guidelines followed in study	OECD Guideline 202 (1984)
Deviations from current test guideline	Deviation from the guideline OECD 202 (2004): Minor: • The pH was not in a range of 6-9, but from 2.3 – 7.6.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate technical on *Daphnia magna* were evaluated in a 48-hour static toxicity test. The toxicity test was performed using five nominal concentrations, 62.5, 125, 250, 500 and 1000 mg test item/L. Furthermore, a blank control consisting of reconstituted water and a stability control with 1000 mg test item/L and no daphnids were tested. Two replicates with ten daphnids each were exposed to the test item concentrations and the control. Immobilisation was recorded 24 and 48 hours after the test initiation. Dissolved oxygen and pH were recorded at the beginning and at the end of the tests.

Test item concentrations were verified in the freshly prepared and in the aged test media. During the test period of 48 hours the daphnids were exposed to a mean concentration of 86.1% of nominal concentration. Therefore, all reported results are related to nominal concentrations of the test item.

The immobilisation of *Daphnia magna* increased with increasing test concentration, while at increasing test concentrations, the pH decreases beyond the pH range of 6 - 9 given in the guideline. The EC_{50} (48 h) was 84.0 mg a.e./L with a 95% confidence interval of 73.3 to 96.6 mg a.e./L based on nominal concentrations. All validity criteria according to the guideline OECD 202 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	solid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9%

2. Vehicle and/or positive control: Vehicle: Test medium
Positive control: Reference item: Potassium dichromate ($K_2Cr_2O_7$)

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 20 mL test medium
Source:	In-house culture
Diet/Food:	Not stated
Acclimation period:	~ 24 h (acclimatisation started on July 2nd, test started on July 3rd).

4. Environmental conditions:

Temperature:	21.0 ± 0.5 °C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.3 – 8.2 (control) 6.3 – 7.6 (62.5 mg test item/L) 4.8 – 5.2 (125 mg test item/L) 3.2 – 3.4 (250 mg test item/L) 2.7 – 2.9 (500 mg test item/L) 2.3 – 2.6 (1000 mg test item/L)
Dissolved oxygen:	8.3 – 8.1 mg O_2/L (mean)
Conductivity:	Not stated
Hardness:	250 mg $CaCO_3$/L (reconstituted water)

5. Experimental dates: July 3rd, 1990 to July 5th, 1990

B: STUDY DESIGN AND METHODS

1. Experimental treatments: Five test concentrations (nominal 62.5, 125, 250, 500 and 1000 mg test item/L), prepared with reconstituted water according to EEC directive, were tested in duplicate.
The test was conducted in a static test setup for 48 hours in 50 mL beakers containing 20 mL test solution each. In addition, a control group was exposed to test medium without test substance or other additives. The test vessels contained 10 daphnids each. Also a stability control with 1000 mg test item/L without daphnids was tested.
2. Observations: Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after test initiation. The pH-values and oxygen saturation were measured ion each test vessel at test initiation and termination. Analytical control measurements of the actual concentration of the test item were performed by mean of HPLC analysis using samples taken at test start (0 h) and test termination (48 h).
3. Statistical calculations: The EC_{50} was estimated by using the Logit-model, EC_0, EC_{50} and EC_{100} values were determined by linear regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements were performed on all test concentrations and the stability control at test initiation and test termination. At 62.5, 125, 250 and 500 the test concentrations were in the range of 78.5 – 94.9 % of nominal at test initiation and 77.6 – 95.2 % at test termination. At the highest test concentration of 1000 mg test item/L, the concentration at test initiation was 69.7 % of nominal and at test termination 85.3 %, respectively. During the test period of 48 hours the daphnids were exposed to a mean concentration of 86.1 % of nominal concentration. Therefore, all reported results are related to nominal concentrations of the test item.

Table 8.2.4- 22: Analytical results

Nominal concentration [mg test item/L]	% of nominal	
	0 hrs	48 hrs
62.5	80.9	89.1
125	78.5	77.6
250	92.4	93.4
500	94.9	95.2
1000	69.7	85.3

The EC_{50} value is given below based on nominal concentrations.

Table 8.2.4- 23: Endpoints

Endpoints	Glyphosate technical [mg a.e./L]
48 h EC_{50} (95% CL), Logit-model	84.0 (73.3 – 96.6)

Reference item: The 48h-EC_{50} for the reference item was 1.32 mg/L (95% CL = 1.203 – 1.426 mg/L), which was within the range of expected responses. Hence, the sensitivity of this batch of D*aphnia magna* was in agreement with the historical data collected at test facility.

B. OBSERVATIONS

The immobilisation increases with increasing test concentration. Beginning with 125 mg test item/L, all daphnids are immobilised after 48 h. At increasing test concentrations, the pH decreases beyond the pH range of 6 - 9 given in the guideline.

Table 8.2.4- 24: Observations of pH and immobilisation

	Control	Glyphosate [mg a.e./L]									
Test parameters	-	**62.5**		**125**		**250**		**500**		**1000**	
Replicate No.		1	2	1	2	1	2	1	2	1	2
% immobile daphnids after 24 h	0	10	0	30	60	100	100	100	100	100	100
% immobile daphnids after 48 h	0	10	0	100	100	100	100	100	100	100	100
pH after 24 h	8.4	6.3		4.8		3.2		2.7		2.3	
pH after 48 h	7.9	7.6		5.2		3.4		2.9		2.6	

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The EC_{50} (48 h) for *Daphnia magna* exposed to glyphosate technical was 84.0 mg a.e./L with a 95% confidence interval of 73.3 to 96.6 mg a.e./L based on nominal concentrations.

In the RAR 2015, results were recalculated based on mean measured concentrations using probit analysis which provided an EC50 value of 74 mg a.e./L (95% CL: 16.966 -130.338). The NOEC was determined to be 53 mg a.e./L.

The validity criteria according to the OECD 202 were fulfilled, the study is therefore considered valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/010
Report author	[redacted]
Report year	1981
Report title	Acute Toxicity of MON 0139 (Lot LURT 12011) (AB-81-074) to *Daphnia magna*
Report No	27203
Document No	-
Guidelines followed in study	Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, US EPA, Ecol Res. Ser. 660/3-75009
Deviations from current test guideline	Deviation from the current guideline OECD 202 (2004): Major: - No analytical measurements of the lowest and highest treatment solutions were performed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of MON 0139 on *Daphnia magna* were evaluated in a 48-hour static toxicity test. The test was performed using six nominal concentrations of 56, 100, 180, 320, 560 and 1000 mg test item/L in duplicates and included a blank control group (daphnia medium only). Twenty daphnids (2 replicates, 10 individuals per replicate) were exposed to each treatment level and in the control group. Total number of immobile *Daphnia magna* in each vessel were recorded at 24 and 48 hours after the test initiation. The pH-values and oxygen saturation of the test solutions were measured at test initiation and termination. In addition, total hardness and specific conductivity of the dilution water was analysed.

The 48 h LC_{50} for *Daphnia magna* exposed to MON 0139 was determined to be 930 mg test item/L. The no effect level (NOEC) observed for MON 0139 was 320 mg test item/L after 48 hours. According to the points deviated from the current guideline OECD 202 recommendations, the study is considered as supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 0139
Description:	Light yellow liquid
Lot/Batch #:	LURT 12011
Purity:	62.49 %

2. Vehicle and/or positive control: water

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (1st instar, < 24 h old)
Loading:	10 specimens in 200 mL test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None

4. Environmental conditions:

Temperature:	20 ± 1 °C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.6 (control, test start), 7.9 – 8.6 (at test end)
Dissolved oxygen:	8.8 mg/L (control, test start), 3.5 – 7.8 mg/L (at test end)
Conductivity:	50 µmhoS/cm
Hardness:	255 ppm ($CaCO_3$).

5. Experimental dates: April 21 to April 24 1981

B. STUDY DESIGN

Experimental treatments

The toxicity of MON 0139 on *Daphnia magna* was evaluated in a 48-hour static toxicity test, using nominal concentrations of 56, 100, 180, 320, 560 and 1000 mg test item/L. In addition, a control group was exposed to dilution water. The test solutions were prepared using water prepared to a total hardness of 255 mg $CaCO_3$/L. There were two glass jars per treatment, each containing ten daphnids (250 mL glass jars containing 200 mL test medium). The vessels were kept at 20 ± 1 °C. The photoperiod was controlled to give 16 hours daylight and 8 hours darkness.

Observations

Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation. The pH-values and oxygen saturation of the test solutions were measured at test initiation (only in control) and at test termination (control and three test concentrations). In addition, total hardness and specific conductivity of the dilution water was analysed.

The validity criteria according to the current OECD 202 guideline are the following:

- In the control, not more than 10 per cent of the daphnids should have been immobilised or show or other signs of disease or stress.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

Statistical calculations

The LC_{50} values were obtained by employing a computerised LC_{50} program developed by Stephan et. al. (1978) performing binomial, moving average and probit tests.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 48 hours LC_{50} and NOEC values are given below based on nominal concentrations.

Table 8.2.4- 25: Endpoints

Endpoints	MON 0139 [mg/L]	Glyphosate [mg a.s./L]
48 hours LC_{50} (95% C.I.)	930 (800 - 1200)	581 (500 - 750)
48 hours NOEC	320	200

C.I. = Confidence interval

B. OBSERVATIONS

No mortality to *Daphnia magna* from exposure to MON 0139 was observed at test concentrations ≤ 560 mg test item/L. At 1000 mg test item/L, some behavioural effects were notified after 48 hours and 10% and 60% mortality was observed after 24 and 48 hours, respectively (see table below).

Table 8.2.4- 26: Mortality of *Daphnia magna* exposed to MON 0139

Test concentration (mg MON 0139/L)	Mortality (%)	
	24 hours	48 hours
Control	0	0
56	0	0
100	0	0
180	0	0
320	0	0
560	0	5
1000	10	60

The following points deviated from the current guideline OECD 202 recommendations:

- No analytical measurements of the lowest and highest treatment solutions were performed.
- The hardness is slightly higher than 250 mg/L CaCO3 (actual value: 255 mg/L)

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The 48 h LC_{50} for *Daphnia magna* exposed to MON 0139 was determined to be 930 mg test item/L equivalent to 581 mg a.e./L. The no effect level (NOEC) observed for MON 0139 was 320 mg test item/L after 48 hours, equivalent to 200 mg a.e./L. No chemical analysis was performed to confirm glyphosate concentration in the test media. The test would therefore be considered as supportive for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/011
Report author	[redacted]
Report year	1978
Report title	Acute Toxicity of Technical Glyphosate (AB-78-201) to *Daphnia magna*
Report No	AB 78-201
Document No	-
Guidelines followed in study	Committee on methods for toxicity tests with aquatic organisms.
Deviations from current test guideline	Deviations from guideline OECD 202 (2004): Major: • no analytical verification of test concentrations
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Based on the results of a range finding test, a definite toxicity test was performed using nominal concentrations of 560, 650, 750, 870 and 1000 mg test item/L, equivalent to 464.8, 539.5, 622.5, 722.1, and 830.0 mg glyphosate/L. In addition, a control group was exposed to dilution water. There were three vessels per treatment, each containing ten daphnids.

The total number of immobile *Daphnia magna* was recorded at 24 h and 48 h after test initiation.

At and above nominal concentrations of 870 mg test item/L, 100 % immobilisation was observed, while no immobilisation was observed at a nominal concentration of 560 mg test item/L, 48 hours after the test initiation. The 48 h EC_{50} for *Daphnia magna* exposed to glyphosate was calculated to be 780 mg test item/L. The 48- hour no-effect level (NOEC) was determined to be 560 mg/L. All validity criteria according to the guideline OECD 202 were fulfilled, however no analytical verification of test concentrations was made and the study was not conducted to GLP. This study is therefore considered supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Technical Glyphosate
Description:	White powder
Lot/Batch #:	XHI-162
Purity:	83.0 %

2. Vehicle and/or positive control: Vehicle: Well water
Positive control: None

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 18 h old)
Loading:	10 specimens for 250 mL test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None

4. Environmental conditions:

Temperature:	19 ± 1 °C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.0 (at test termination)
Dissolved oxygen:	7.5 mg/L
Conductivity:	Not stated
Hardness:	> 250 mg $CaCO_3$/L.

5. Experimental dates: August 29th, 1978 to August 31st, 1978

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, definite toxicity test was performed using nominal concentrations of 560, 650, 750, 870, 1000 mg test item/L, equivalent to 464.8, 539.5, 622.5, 722.1, and 830.0 mg glyphosate/L in a static test setup. The test solutions were prepared using well water of the test facility (Dissolved oxygen = 8.6 mg/L, pH = 7.8, hardness > 250 mg $CaCO_3$/L.). In addition, a control group was exposed to dilution water. There were three replicates per treatment, each containing ten daphnids (500 mL glass beakers containing each 250 mL test medium).
2. Observations: Total number of immobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation. Temperature, pH-value and oxygen saturation of the test solutions were measured at the test termination. Hardness of the test water was measured at test initiation.
3. Statistical calculations: EC_{50} values were calculated along with the 95 % confidence limits using Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

No analytical verification reported.

The EC_{50} and NOEC values are given below based on nominal concentrations as no analytical verification

of test concentrations was made.

Table 8.2.4- 27: Endpoints

Endpoints (48 h)	Test item[mg/L]	Glyphosate [mg a.e./L]
EC_{50} (95% C.I.)	780 (696 - 874)	647.4 (577.7 - 725.4)
NOEC	560	464.8

B. OBSERVATIONS

At and above nominal concentrations of 870 mg test item/L, 100 % immobilisation was observed while no immobilisation was observed at the nominal concentration of 560 mg test item/L, 48 hours after the test initiation. At concentrations of 650 and 750 mg test item/L, immobilisation of 3.3 % and 33.3 % of specimens was observed.

Table 8.2.4- 28: Lethal effects of glyphosate to *Daphnia magna*

Test item [mg/L]	Control	560	650	750	870	1000
Glyphosate [mg a.e./L]	**-**	**464.8**	**539.5**	**622.5**	**722.1**	**830.0**
Immobility (24 h) [%]	0	0	0	6.7	73.3	100
Immobility (48 h) [%]	0	0	3.3	33.3	100	100

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 h EC_{50} for *Daphnia magna* exposed to technical glyphosate was calculated to be 780 mg test item/L, equivalent to 647.4 mg a.e./L. The 48- hour no-effect level (NOEC) was determined to be 560 mg/L, equivalent to 464.8 mg a.e./L.

All validity criteria according to the guideline OECD 202 were fulfilled, however no analytical verification of test concentrations was made. This study is therefore considered supportive for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/012
Report author	[redacted]
Report year	1998
Report title	Acute Toxicity Study in *Daphnia magna* with (Aminomethyl)Phosphonic Acid (Static)
Report No	232471
Document No	-
Guidelines followed in study	OECD Guideline 202, Part I (1984) ECC Directive 92/69, Part C.2 (1992) ISO International Standard 6341 (1996)
Deviations from current test guideline	Deviation from the guideline OECD 202 (2004): none
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of (Aminomethyl) phosphonic acid (AMPA) on *Daphnia magna* were evaluated in a 48-hour static toxicity test conducted as a limit test with a nominal concentration of 100 mg test item/L. Furthermore, a blank control was tested. Twenty daphnids (2 replicates, 10 individuals per replicate) were exposed to each treatment level.
Immobilisation was recorded 24 and 48 hours after the start of the test.
At the tested nominal concentration of 100 mg test item/L, no immobilisation was observed in tested daphnids during the 48 h exposure time. The 48-h EC_{50} for *Daphnia magna* exposed to AMPA was determined to be > 100 mg test item/L. All validity criteria according to OECD 202 were fulfilled. The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	(Aminomethyl)phosphonic acid
Description:	White powder
Lot/Batch #:	A010047101
Purity:	99 %

2. Vehicle and/or positive control: Reference item: $K_2Cr_2O_7$

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 80 mL of test medium

Source: In-house culture

4. Environmental conditions:

Temperature: 20.4 – 20.6 °C
Photoperiod: 16 hours light / 8 hours dark
pH: 8.0 – 8.2 (control), 6.2 - 6.4 (test solution)
Dissolved oxygen: 8.8 – 9.0 mg O_2/L
Hardness: 250 mg $CaC0_3$/L

5. Experimental dates: May 18th, 1998 to May 27th, 1998

B: STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, the final toxicity test was performed using a unique nominal concentration of 100 mg test item/L prepared using ISO-medium (in milli-RO water). The test was conducted in a static test setup as limit test. In addition, a control group was exposed to the test medium without test substance or other additives. The test consisted of two replicates per treatment group (100 mL vessels containing 80 mL test solution each). Per replicate 10 daphnids were exposed.

2. Observations: Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation.

The pH-values and oxygen saturation of the test solutions were measured at test initiation and termination. The temperature was controlled daily in one control vessel, starting from the beginning of the test.

Analytical control measurements of the actual concentration of the test item were performed by mean of HPLC analysis using samples taken at test start (0 h) and test termination (48 h).

3. Statistical calculations: Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} value is given below based on nominal concentrations.

Table 8.2.4- 29: Endpoints

Endpoints	(Aminomethyl) phosphonic acid [mg/L]
EC_{50} (48 h)	> 100

Analytical data: Before introduction of the daphnids 98 % of (Aminomethyl)phosphonic acid was recovered. In the aged test media 95 % of the nominal concentration was recovered. The results are summarised below.

As the mean measured content of the test item always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 8.2.4- 30: Analytical results

Time [hours]	Nominal [mg/L]	Analysed [mg/L]	% of nominal [mg/L]
0	100	98.2	98
48	100	95.4	95

Reference test: The 48h-EC_{50} for the reference item was 0.5 mg/L (95 % CL = 0.4 – 0.6 mg/L), which was within the range of expected responses. Hence, the sensitivity of this batch of D*aphnia magna* was in agreement with the historical data collected at test facility.

B. OBSERVATIONS

At the tested nominal concentration, no immobilisation was observed in tested daphnids during the 48 h exposure time. Also, all validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Under the conditions of the present test (Aminomethyl) phosphonic acid induced no visible effects in *Daphnia magna* at nominal concentrations of 100 mg/L. Hence, the 48-h EC_{50} for *Daphnia magna* exposed to AMPA was determined to be > 100 mg/L, the maximum nominal concentration tested, and the NOEC ≥ 100 mg/L.

The study is considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/013
Report author	[redacted]
Report year	1994
Report title	AMPA: Acute toxicity to *Daphnia magna*
Report No	X582/C
Document No	-
Guidelines followed in study	OECD No 202
Deviations from current test guideline	Deviation from the guideline OECD 202 (2004): none
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of AMPA on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 18, 32, 56, 100 and

180 mg/L of AMPA. In addition, 4 x 5 *Daphnia* were exposed to test medium without test substance (blank control).
Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. The concentration of AMPA in the test solutions were measured at 0 and 48 hours.
The mean measured test concentrations of AMPA ranged from 93 to 128% of the nominal values, therefore, the results reported are related to nominal concentrations of the test item. The 48-h EC_{50} for *Daphnia magna* exposed to AMPA was >180 mg/L. The NOEC after 48 h based on immobilisation was 180 mg AMPA/L. All validity criteria according to OECD 202 were fulfilled. The study is therefore considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA technical (metabolite of glyphosate))
Description:	White solid
Lot/Batch #:	Not mentioned in the report
Purity:	85 %

2. Vehicle and/or positive control: Vehicle: Dilution water
Positive control: None

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Less than 24 hours
Loading:	5 organisms per vessel (250 mL glass beakers containing 200 mL test solution) which corresponds to 25 *Daphnia*/L.
Source:	Continuous laboratory cultures

4. Environmental conditions:

Temperature:	19.9-20.1 °C
pH:	8.23-847
Dissolved oxygen:	8.8-9.1 mg O2/L
Conductivity:	545 mg/L µS/cm
Hardness:	161.6 mg $CaCO_3$
Photoperiod:	16 hours light / 8 hours dark with 15 minute transition periods

5. Experimental dates: November 16th, 1993 to November 18th, 1993

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of AMPA on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 18, 32, 56, 100 and 180 mg/L of AMPA. In addition, 4 x 5 *Daphnia* were exposed to test medium without test substance (blank control). A stock solution of nominal concentration of 180 mg a.s./L was prepared by dissolving 0.36 mg test item in 2 L dilution water. This stock solution was observed to be clear and colourless. One litre of each test solution was prepared by the addition of aliquots of stock solution to dilution water. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations: Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24,

and 48 hours thereafter. The concentrations of AMPA in the test solutions were measured at 0 and 48 hours.
3. Statistical calculations: The EC_{50} could not be quantified due to the absence of toxicity of the test item, therefore, no statistical analysis was performed.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured test concentrations of AMPA ranged from 93 to 128 % of the nominal values. The limit of quantification of AMPA in this study was 6.9 mg/L. The results are summarised below. The variability of the chemical analysis was considered to be due to the analytical method used. A similar study with AMPA technical completed after this study with an improved analytical method, reported mean measured concentrations ranging from 100 to 111 % of the nominal values. Therefore, it was assumed that the nominal concentrations were maintained during this study and results have been provided using the nominal concentrations.

Table 8.2.4- 31: Analytical results

Nominal concentration [mg AMPA/L]	Measured concentration [mg AMPA/L]		Mean measured concentration	
	0 hrs	48 hrs	[mg AMPA/L]	% of nominal
Control	<6.9	<6.9	<6.9	-
18	34	11	23	128
32	45	30	38	119
56	73	47	60	107
100	99	86	93	93
180	170	200	190	106

The 24 and 48 hour EC_{50} values (based on nominal concentrations of AMPA) are given below.

Table 8.2.4- 32: EC_{50} values for *Daphnia magna*

Time	EC_{50} (mg a.s./L)	95 % confidence interval (mg a.s./L)
24 h	>180	-
48 h	>180	-

B. OBSERVATIONS

The effects of AMPA on *Daphnia magna* are shown below.

Table 8.2.4- 33: Effects of AMPA on *Daphnia magna* exposed for 48 hours

Nominal concentration [mg a.s./L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours	Immobility after 24 hours [%]	Number of immobile *Daphnia* after 48 hours	Immobility after 48 hours [%]
Control	20	0	0	0	0
18	20	0	0	0	0
32	20	0	0	0	0
56	20	0	0	0	0
100	20	0	0	1	5
180	20	0	100	0	0

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The 48-h EC_{50} for *Daphnia magna* exposed to AMPA was ≥ 180 mg/L based on nominal concentration. The NOEC after 48 h based on immobilisation was ≥ 180 mg/L.

All validity criteria according to OECD 202 were fulfilled, so the study is therefore considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/014
Report author	[redacted]
Report year	1991
Report title	Acute Toxicity of AMPA to *Daphnia magna*.
Report No	38988
Document No	-
Guidelines followed in study	Guideline No. 72-2, U.S. EPA-FIFRA 40 CFR. Part 158, 145
Deviations from current test guideline	Deviation from to the guideline OECD 202 (2004): none
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of AMPA on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Based on the results of a range finding test, the final toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L prepared using hard blended water (a combination of well water and reverse-osmosis water blended to a hardness of 160-180 mg/L as $CaCO_3$) Furthermore, a control group was exposed to the dilution water (hard blended water). The test consisted of two replicates per treatment group. Per replicate 10 daphnids were exposed.

Total number of immobile *Daphnia magna* was recorded at 3, 24 h and 48 h after the test initiation. In addition, other abnormal effects such as surfacing, clumping of the daphnids together and daphnids tending to the bottom of the test chambers were recorded.

At the highest test concentration (1000 mg test item/L), 85 % and 100 % immobility were observed at 24 and 48 hours after test initiation. At or below a concentration of 320 mg test item/L, no mortality was observed.

Immobility and abnormal effects, namely surfacing and daphnids trailing extraneous material were observed in the 560 and 1000 mg/L test concentrations. The abnormal effects such as fish on the bottom of the test vessel and immobility at 24- and 48- hours, respectively, in the control were considered aberrant since no toxic response was observed at 100, 180 and 320 mg/L test concentrations. The 48 h EC_{50} for *Daphnia magna* exposed to AMPA was determined to be 690 mg AMPA/L (nominal). The 48- hour no-effect level (NOEC) was determined to be 320 mg/L (nominal). All validity criteria according to the OECD guideline 202 were fulfilled. The study is therefore considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA
Description:	White powder
Lot/Batch #:	HET-9001-1463T
Purity:	94.38 %

2. Vehicle and/or positive control: None

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 200 mL of test medium
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None

4. Environmental conditions:

Temperature:	20 ± 1 °C
Photoperiod:	16 hours light / 8 hours dark (399-797 Lux), with 30 minute dawn dusk transition periods.
pH:	8.2 – 8.3 (control), 5.2 (highest test concentration)
Dissolved oxygen:	8.4 – 8.8 mg O_2/L (94 % - 101 % of O_2 saturation)
Conductivity:	370 µS/cm
Hardness:	160 mg $CaCO_3$/L

5. Experimental dates: November 24, 1990 to November 26, 1990

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, the final toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L dissolved in hard blended water (a combination of well water and reverse-osmosis water blended to a hardness of 160 mg/L as $CaCO_3$). The test was conducted in a static test setup. In addition, a control group was exposed to dilution water (hard blended water). The test consisted of two replicates per treatment group in 250 mL glass beakers containing 200 mL test solution. 10 daphnids were exposed per replicate.

2. Observations: Total number of immobile *Daphnia magna* was recorded 3, 24 h and 48 h after test initiation. In addition, other effects such as surfacing, clumping of the daphnids together and daphnids tending to the bottom of the test chambers were recorded.

The pH-values and oxygen saturation of the test solutions were measured at test initiation and termination (0 – 48 h). The temperature was recorded continuously in all test vessels, starting from the test initiation.

Analytical samples of the control water and each test level solutions were taken at the beginning and the end of exposure. These samples were frozen and sent to the study sponsor at test termination. The results of these analyses are reported separately Monsanto (Study No. ML-90-403/EHL-90187-Daphnia)

3. Statistical calculations: The EC_{50} values were determined by Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical results

The results of analytical part are reported in a separate study (Monsanto study No. ML-90-403/EHL-90187-Daphnia).

The EC_{50} and NOEC values are given below based on nominal concentrations.

Table 8.2.4- 34: Endpoints

Endpoints	AMPA [mg/L]
EC_{50} (48 h) (95% CI)	690 (560 – 1000)
NOEC (48 h)	320

B. OBSERVATIONS

At highest test concentration (1000 mg test item/L), 85 % and 100 % immobility were observed at 24 and 48 hours after test initiation. At or below a concentration of 320 mg test item/L, no mortality was observed. Immobility and abnormal effects such as surfacing and daphnids trailing extraneous material were observed in the 560 and 1000 mg/L test concentrations. The abnormal effects such as fish on the bottom of the test vessel and immobility at 24- and 48- hours, respectively, in the control were considered aberrant since no toxic response was observed at 100, 180 and 320 mg/L test concentrations.

Table 8.2.4- 35: Lethal and sublethal effects of AMPA to *Daphnia magna*

		Control	AMPA [mg/L]				
			100	180	320	560	1000
24 h	Cumulated Immobility [%]	5	0	0	0	0	85
	Symptoms	5% OB	-	-	-	5% OB	-
48 h	Cumulated Immobility [%]	0	0	0	0	15%	100
	Symptoms	-	-	-	-	5% SUR/TR	-

SUR = surfacing; OB = on bottom of test vessel; TR = trailing extraneous material

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The 48 h EC_{50} for *Daphnia magna* exposed to AMPA was determined to be 690 mg/L (nominal). The 48- hour no-effect level (NOEC) was determined to be 320 mg/L (nominal).

All validity criteria according to the OECD 202 were fulfilled. The study is therefore considered valid and reliable for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.1/015
Report author	[redacted]
Report year	2011
Report title	HMPA (Hydroxymethylphosphonic acid): A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*)
Report No	139A-395
Document No	-
Guidelines followed in study	OECD 202 (1984) EPA OPPTS 850.1010
Deviations from current test guideline	Deviation from the guideline OECD 202 (2004): none
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The toxicity of Hydroxymethylphosphonic acid (HMPA) on *Daphnia magna* was evaluated in a 48-hour static toxicity test. *Daphnia magna* neonates were exposed to a limit concentration of 100 mg HMPA/L and a negative control consisting of dilution water only. The test consisted of three replicates per treatment group and control with 10 daphnids exposed per replicate vessel. *Daphnia* were not fed during the test. All Daphnids were observed for immobilisation and other clinical signs of toxicity at 2.5, 24 and 48 hours after test initiation.

Temperature, pH-values and dissolved oxygen concentrations were measured at the beginning, at approximately 24 hours during the test and at the end of the test. Samples of the control and the test item treatment media were taken and analysed for HMPA concentration at the beginning of the test and at 48 hours from each replicate test chamber. HMPA was not detected in the control group. The measured test concentrations ranged between 86 and 103 % of the nominal values.

There was no immobility or overt signs of toxicity observed in the treatment group or in the control. The 48-hour EC_{50} for *Daphnia magna* exposed to HMPA was > 100 mg HMPA/L. The 48- hour NOEC was determined to be ≥ 100 mg HMPA/L. All validity criteria according to the OECD guideline 202 were fulfilled. The study is therefore considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	HMPA(Hydroxymethylphosphonic acid)
Description:	White powder
Lot/Batch #:	GLP-1003-20448-A
Purity:	97.0 %

2. Vehicle and/or positive control: Vehicle: Well water
Positive control: None

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 220 mL of test medium
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None

4. Environmental conditions:

Temperature:	19.7 – 20.7 °C
Photoperiod:	16 hours light (light intensity = 323 Lux), with 30 minute transition periods.
pH:	6.9 – 8.5
Dissolved oxygen:	8.3 – 9.4 mg O_2/L (≥92 % of O_2 saturation)
Conductivity:	386 µS/cm
Hardness:	140 mg $CaCO_3$/L

5. Experimental dates: January 25, 2011 to January 28, 2011

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity of Hydroxymethylphosphonic acid (HMPA) on neonates of *Daphnia magna* was evaluated in a 48-hour static toxicity test at a single nominal limit concentration of 100 mg HMPA/L dissolved in well water. A negative control group (well water only) was prepared in parallel. Thirty daphnids (3 replicates of 10 animals per test beaker) were exposed at the control and at the limit concentration.

2. Observations: The total number of immobile *Daphnia magna* was recorded at 2.5, 24 h and 48 h after test initiation. In addition, specimens were observed for clinical signs of toxicity.

Temperature, pH-values and oxygen saturation of the test solutions were measured at test initiation, after 24 hours and at test termination (48 h). The temperature of test media was monitored continuously in all test vessels. Hardness, alkalinity, specific conductance and total organic carbon (TOC) were measured at the beginning of the test.

Samples of test media were taken from each replicate test chamber at the start and end of the test for the determination of HMPA concentrations. Samples were analysed using an HPLC method of analysis with mass selective detection (LC/MS).

The validity criteria according to the current OECD 202 guideline are the following:

- In the control, not more than 10 per cent of the daphnids should have been immobilised or show or other signs of disease or stress.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

3. Statistical calculations: Descriptive only since no immobility of daphnids was observed in the test and control treatments.

II. RESULTS AND DISCUSSION

A. FINDINGS

The measured test concentrations ranged between 85.9 and 103 % of the nominal values.

Table 8.2.4- 36: Analytical results

Nominal HMPA [mg/L]	0 mg/L	100 mg/L
0 h	< LOQ[1]	85.9
48 h	< LOQ[1]	95.8
	< LOQ[1]	99.6
	< LOQ[1]	103.0
Mean measured HMPA [mg/L]	-	93
% of nominal	-	93

[1] LOQ = 1.00 mg/L

Therefore, the EC_{50} and NOEC values given below are based on nominal concentrations.

Table 8.2.4- 37: Endpoints

Endpoints	HMPA [mg/L]
48 h EC_{50}	>100 mg/L (nominal)
48 h NOEC	≥ 100 mg/L (nominal)

B. OBSERVATIONS

After 2.5, 24 and 48 hours of exposure, no immobilisation of *Daphnia* in the control nor in the test item concentration vessels was observed.

Table 8.2.4- 38: Acute toxicity of MON 52276 to *Daphnia magna* under flow-through conditions

Nominal concentration HMPA (mg a.s./L)	Time point (h)	Abnormalities/ Sublethal Effects	No. of *Daphnia* immobilised or dead[1]	Cumulative % mortality
0	2.5 24 48	None observed	0 0	0 0
100	2.5 24 48	None observed	0 0	0 0

[1] Of 30 total *Daphnia* in group.

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48-hour EC_{50} for *Daphnia magna* exposed to HMPA was >100 mg/L (nominal). The 48- hour NOEC was determined to be ≥ 100 mg/L (nominal).

All validity criteria according to the OECD 202 were fulfilled. The study is therefore considered valid and reliable for the regulatory risk assessment of glyphosate.

Assessment and conclusion by RMS:

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

As glyphosate is not an insecticide or insect growth regulator, studies on the acute toxicity to an additional aquatic invertebrate species are not required. Nevertheless, the following studies are available.

1. Information on the study

Data point:	CA 8.2.4.2/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute toxicity to mysid shrimp (*Mysidopsis bahia*)
Report No	AB0503/H
Document No	-
Guidelines followed in study	EPA FIFRA, Subdivision E, Guideline 72-3
Deviations from current test guideline	Deviation from the guideline OCSPP 850.1035 (2016): none
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The effects of glyphosate acid on mysid shrimp *Mysidopsis bahia* were evaluated in a 96-hour static toxicity test. Ten mysids were allocated to a single vessel (1000 mL glass beaker containing 800 mL test solution) for each test concentration and the dilution water control. The shrimps were exposed to nominal 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg a.s./L, together with pH adjusted 320, 560, and 1000 mg a.s./L.
The mysids were exposed to the test item for 96 hours at 25±1°C. The mysids were fed on days 0, 1, and 3 with *Artemia salina* nauplii.
Mortalities of the mysids and overt symptoms of toxicity were assessed after 24, 48, 72, and 96 hours. pH-values were determined in the test media at the beginning and at the end of the test. Dissolved oxygen concentrations were measured at 0, 48, and 96 hours. The water temperature in the test vessels was measured daily. The salinity of the dilution water control and 1000 mg/L solution was determined at the start and at the end of the test. The concentrations of glyphosate acid in the test solutions were measured at 0, 48, and 96 hours.
At the lowest test concentration of 3.2 mg/L, analytical results indicated that an error might have occurred during the solution preparation, leading to a value 150 % of nominal. Since this was a no effect concentration, and several higher concentrations gave no indication of toxicity, this data point was excluded from all calculations. Excluding this concentration, the mean measured concentrations ranged from 81 to 95 % of the nominal values. On the basis of the analytical data the nominal concentrations were used for the calculation and reporting of all results.
The 96-h LC_{50} for *Mysidopsis bahia* exposed to glyphosate acid was 80 mg/L based on nominal concentration. The NOEC after 96 h was 32 mg test item/L.
In test systems dosed with pH adjusted glyphosate acid, no mortalities at a nominal concentration of 560 mg a.s./L and 50 % mortality at 1000 mg a.s./L indicated this 96-h LC_{50} (80 mg/L) was caused by the low pH of the unneutralised glyphosate acid solutions.
The validity criteria of OCSPP 850.1035 were fulfilled so the study is therefore considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
2. Vehicle and/or positive control:	Vehicle: Dilution water (1:1 mix of dechlorinated tap water and full seawater Positive control: Not stated

3. Test organism:

Species:	Mysid shrimp *Mysidopsis bahia*
Source of organisms:	Continuous cultures at Brixham Environmental Laboratory
Age of animals:	Less than 24 hours
Loading:	0.8 mysids per litre of water

4. Environmental conditions:

Temperature:	23.7-25.9 °C
pH:	4.5-8.0 (unneutralised test solutions) 8.0-8.5 (neutralised test solutions)
Dissolved oxygen:	7.0-8.4 mg O_2/L
Salinity:	17%
Photoperiod:	16 hours light / 8 hours dark with 20 minute transition periods

5. Experimental dates: March 21, 1996 to March 25, 1996

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate acid on mysid shrimp *Mysidopsis bahia* were evaluated in a 96-hour static toxicity test. Ten mysids were allocated to a single vessel (1000 mL glass beaker containing 800 mL test solution) for each test concentration and the dilution water control. The shrimps were exposed to nominal 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg a.s./L, together with pH adjusted 320, 560, and 1000 mg a.s./L.

A stock solution of nominal concentration of 1000 mg a.s./L was prepared by dispersing 1.5 g test item in 1.5 L of dilution water. The unneutralised test solutions were prepared by dispersing aliquots of the stock solution to dilution water. Three further test solutions were prepared at 320, 560, and 1000 mg a.s./L from a stock solution of 1000 mg/L prepared by dispersing 2.0 g of glyphosate acid in approximately 2 L of dilution water and adjusted to pH 8.1 with 1M sodium hydroxide.

The mysids were randomly placed into the test beaker and exposed to the test item for 96 hours at 25±1 °C. The mysids were fed on days 0, 1, and 3 with *Artemia salina* nauplii.

2. Observations: Mortalities of the mysids and overt symptoms of toxicity were assessed after 24, 48, 72, and 96 hours. pH-values were determined in the test media at the beginning and at the end of the test. Dissolved oxygen concentrations were measured at 0, 48, and 96 hours. Treatments showing 100 % mortality were measured for pH and dissolved oxygen at that time. The water temperature in the test vessels was measured daily. The salinity of the dilution water control and 1000 mg/L solution was determined at the start and at the end of the test. The concentrations of glyphosate acid in the test solutions were measured at 0, 48, and 96 hours.

3. Statistical calculations: The LC_{50} values were calculated by the Brixham Environmental Laboratory computer program "LC_{50}" using Stephan's method.

II. RESULTS AND DISCUSSION

A. FINDINGS

At the lowest test concentration of 3.2 mg/L, analytical results indicated that an error might have occurred during the preparation of the test solution, leading to a value 150 % of nominal. Since this was a no effect concentration, and several higher concentrations gave no indication of toxicity, this data point was excluded from all calculations. Excluding this concentration, the mean measured concentrations ranged from 81 to 95 % of the nominal values. Based on the analytical data the nominal concentrations were used for the calculation and reporting of all results.

Table 8.2.4- 39: Analytical results

Nominal concentration of Glyphosate acid [mg/L]	Measured concentration of glyphosate acid [mg/L]			Mean measured concentration of glyphosate acid [mg/L]	% of nominal
	0 h	48 h	96 h		
Dilution water control	< 0.01	< 0.01	< 0.01	< 0.01	
3.2	4.8	4.1	5.5	4.8	150
5.6	4.7[1]	4.1[1]	5.5[1]	4.8	86
10	7.9	7.0	9.5	8.1	81
18	16	15	16	16	89
32	30	28	30	29	91
56	55	48	50	51	91
100	98	89	97	95	95
180	170	160	-	170	94
320 (pH adjusted)	300	270	290	290	91
560 (pH adjusted)	530	490	550	520	93
1000 (pH adjusted)	940	860	970	920	92

[1]mean of triplicate analysis. The LOQ was 0.01 mg glyphosate acid/L.

The LC_{50} values for *Mysidopsis bahia* (based on nominal concentrations of glyphosate acid) are given below.

Table 8.2.4- 40: Endpoints

Time	LC_{50} [mg a.s./L]	95 % confidence interval [mg a.s./L]
24 h	130	100-180
48 h	96	77-130
72 h	88	71-110
96 h	80	64-100

The 96-hour NOEC was 32 mg a.s./L.

B. OBSERVATIONS

The effects of glyphosate acid on *Mysidopsis bahia* are shown in the table below.

Table 8.2.4- 41: Effects of glyphosate acid on *Mysidopsis bahia*

Nominal concentration (mg a.s./L)	Cumulative percentage mortality observed			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
3.2	0	0	0	0
5.6	0	0	0	0
10	0	0	0	0
18	0	0	0	0
32	0	0	0	0
56	0	0	0	10
100	0	60	80	80
180	100	100	100	100
320 (pH adjusted)	10	10	10	10
560 (pH adjusted)	0	0	0	0
1000 (pH adjusted)	0	0	30	50

In test systems dosed with pH adjusted glyphosate acid, no mortalities at a nominal concentration of 560 mg a.s./L and 50% mortality at 1000 mg a.s./L indicated this 96-h LC_{50} (80 mg/L) was caused by the low pH of the unneutralised glyphosate acid solutions.

The validity criteria of OCSPP 850.1035 Mysid Acute Toxicity Test (October 2016) were fulfilled as:

- All test vessels were identical
- Individual test organisms were randomly assigned to test vessels.
- A dilution water control was included in the test
- Not more than 10% of the organisms in the dilution water control showed signs of disease, stress (*e.g.,* discoloration, unusual behaviour, immobilization), and/or death.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 96-h LC_{50} for *Mysidopsis bahia* exposed to glyphosate acid was 80 mg a.s./L based on nominal concentrations. The NOEC after 96 h was 32 mg a.s./L.

The validity criteria of OCSPP 850.1035 were fulfilled. The study is therefore considered valid and reliable for the regulatory risk assessment of glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.2/002
Report author	[redacted]
Report year	1978
Report title	Toxicity of seven test materials to mysid shrimp *Mysidopsis bahia*
Report No	BP-78-4-032
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)
Deviations from current test guideline	Deviation from the guideline OCSPP 850.1035 (2016): Major: • No analytical verification performed • No indication of the organisms randomisation
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed.
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The effects of seven test items, two solid test items (Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45) and five liquid test items (Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and Comp. 5A) on mysid shrimp, *Mysidopsis bahia,* were evaluated in a 96-hour static toxicity test. The test concentrations used for solid test items were using 3.2, 10, 32, 56, 100, 1000 mg test item/L. For the liquid test items, the concentrations used were 0.6, 1.0, 3.2, 10, 32 and 56 % effluent. The test solutions were prepared using seawater. In addition, a control group was exposed to seawater without test material. There was one replicate (3.5 L glass jar) per treatment (7 jars for each solid test material and 8 jars for each liquid test material), containing each ten mysids in 3 L test solution.

Mortality was recorded in all test concentrations and the control 24, 48, 72 and 96 hours after test initiation. For the two solid test materials (Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45) the highest mortality was 20 % in the 1000 mg test item/L treatment group for both test items after 96 hours of exposure. For the liquid materials, the highest mortality was observed with Comp. #3A, BN-78-48, while the lowest mortality was obtained with Comp. #l, BN-78-46 and "Comp. #4, BN-78-49. In Comp. #3A, BN-78-48, mortality was 40 % and 30 % in the non-aerated and aerated test solutions of the 10 % effluent treatment group, respectively; in Comp. 5A, mortality was 0 % and 10 % in the non-aerated and aerated treatments of the 10 % effluent treatment group, respectively. However, oxygen demand apparently contributed to the toxicity of these two samples in concentrations ≥ 32 % effluent. The study is considered to be supportive as no analytical verification was performed and organisms randomisation was not performed or reported.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item (Description):	Glyphosate, BN-78-44 (white, crystalline solid) Glyphosate intermediate, BN-78-45 (fine, white powder) Comp. #1, BN-78-46 (clear liquid) Comp. #2, BN-78-47 (clear liquid) Comp. #3A, BN-78-48 (murky liquid) Comp. #4, BN-78-49 (clear liquid) Comp. 5A. (clear liquid)
2. Vehicle and/or positive control:	Dodecyl sodium sulphate (DSS)

3. Test organism:

Species:	Mysid shrimp (*Mysidopsis bahia)*
Age:	6 – 8 days old
Size:	4 – 6 mm length
Loading:	10 test individuals for 3 L test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	48 hours prior to the test initiation
Body weight of the animals:	Not stated

4. Environmental conditions:

Temperature:	20 ± 1 °C
Photoperiod:	Not stated
pH or Salinity (‰):	Glyphosate, BN-78-44, (6.4 – 8.3) Glyphosate intermediate, BN-78-45. (6.8 – 8.3) Comp. #1, BN-78-46 (8 – 20 ‰) Comp. #2, BN-78-47 (8 – 20 ‰) Comp. #3A, BN-78-48 (20 – 32 ‰) Comp. #4, BN-78-49 (12 – 20 ‰)
Dissolved oxygen:	Glyphosate, BN-78-44, (6.4 – 7.7 mg O_2/L) Glyphosate intermediate, BN-78-45. (6.4 – 7.4 mg O_2/L) Comp. #1, BN-78-46 (6.1 – 7.6 mg O_2/L) Comp. #2, BN-78-47 (6.1 – 7.4 mg O_2/L) Comp. #3A, BN-78-48 (0.4 – 7.4 mg O_2/L) Comp. #4, BN-78-49 (4.9 – 7.6 mg O_2/L) Comp. 5A. (0.3 – 7.4 mg O_2/L)
Conductivity:	Not stated
Hardness:	Not stated
5. Experimental dates:	Not stated

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Toxicity tests for the seven test materials were performed using 3.2, 10, 32, 56, 100, 1000 mg test item/L for the two solid test materials (Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45) and the nominal concentrations of 0.6, 1.0, 3.2, 10, 32 and 56% effluent for liquid materials (Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.). For solid test materials, appropriate amounts were added to deionised water; the pH was

adjusted to 8.0, and the materials were finally diluted in seawater in the test containers to obtain appropriate concentrations. For liquid materials, the test solutions were prepared by adding appropriate volumes of test materials to seawater in the test containers: Two containers of 10 % test concentration were tested for each material, one aerated and one non-aerated. In addition, a control group was exposed to seawater without test material. Salinity controls were also maintained; mysids were exposed to salinities corresponding to the lowest and highest (8 and 32 ‰) salinity occurring in any of the test concentrations.
There was one replicate (3.5 L glass jar) per treatment (7 jars for each solid test material and 8 jars for each liquid test material), containing each ten mysids in 3 L test solution.
A separate test was conducted, in which mysids were exposed to the reference toxicant dodecyl sodium sulfate under the same test conditions as for the test materials.
2. Observations: Mortality was recorded in all test concentrations and the control 24, 48, 72 and 96 hours after test initiation. Temperature was constantly maintained at 20 ± 1 °C; pH-value and oxygen saturation of the test solutions were measured at test initiation and test termination.
3. Statistical calculations: The percentage of dead mysids was converted to a Probit (Finney, 1971) and the LC_{50} values were then calculated by linear regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} values are given below based on nominal concentrations.

Table 8.2.4- 42: Endpoints

Test materials	EC_{50} (96 h) [% effluent or mg test item/L)]
Glyphosate, BN-78-44	> 1000 mg test item/L
Glyphosate intermediate, BN-78-45	> 1000 mg test item/L
Comp. #1, BN-78-46	> 56 % effluent
Comp. #2, BN-78-47	5.6 % effluent
Comp. #3A, BN-78-48	2.8 % effluent
Comp. #4, BN-78-49	> 56 % effluent
Comp. 5A.	> 10, <32 % effluent

B. OBSERVATIONS

Clinical observations:
For the two solid test materials (Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45) the highest mortality was 20 % in the 1000 mg test item/L treatment group for both test items after 96 hours of exposure. For the liquid materials, the highest mortality was observed with Comp. #3, BN-78-48, while the lowest mortality was obtained with Comp. #l, BN-78-46 and “Comp. #4, BN-78-49.
Two of the liquid samples, Comp. #3 and Comp. 5A, had considerable oxygen demand. In test concentrations < 10 % effluent, the oxygen demand did not contribute appreciably to toxicity.
In Comp. #3A, BN-78-48, mortality was 40 % and 30 % in the non-aerated and aerated test solutions, respectively; in Comp. 5A, mortality was. 0 % and 10 % in the non-aerated and aerated treatments, respectively. However, oxygen demand apparently contributed to the toxicity of these two samples in concentrations ≥ 32 % effluent.

Table 8.2.4- 43: Lethal effects of Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45 on *Mysidopsis bahia*

Test items [mg/L] →	Control	3.2	10	32	56	100	1000
Glyphosate, BN-78-44							
Mortality (24 h) [%]	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	10	0
Mortality (96 h) [%]	0	0	10	0	0	10	20
Glyphosate intermediate, BN-78-45							
Mortality (24 h) [%]	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	10	10
Mortality (96 h) [%]	0	0	10	0	0	10	20

Table 8.2.4- 44: Lethal effects of Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49, and Comp. 5A. on *Mysidopsis bahia*

Test items [% effluent] →	Control	0.6	1.0	3.2	10	10 AE	32	56
Comp. #1, BN-78-46								
Mortality (24 h) [%]	0	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	10	0	0	0	0	0
Mortality (96 h) [%]	0	0	10	0	10	0	0	10
Comp. #2, BN-78-47								
Mortality (24 h) [%]	0	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	20	10	30	100
Mortality (96 h) [%]	0	0	10	10	70	70	90	100
Comp. #3A, BN-78-48								
Mortality (24 h) [%]	0	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	30	20	0	40	20	100	100
Mortality (96 h) [%]	0	30	20	20	40	30	100	100
Comp. #4, BN-78-49								
Mortality (24 h) [%]	0	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	20	10	20	0
Mortality (96 h) [%]	0	0	0	0	20	20	20	30
Comp. 5A								
Mortality (24 h) [%]	0	0	0	10	0	0	0	0
Mortality (48 h) [%]	0	0	0	10	0	0	100	100
Mortality (96 h) [%]	10	20	20	10	0	10	100	100

AE = aerated

Table 8.2.4- 45: Lethal effects of the toxic reference dodecyl sodium sulfate on *Mysidopsis bahia*

Test items [mg/L] →	Control	6	8	10
Mortality (24 h) [%]	0	0	20	20
Mortality (48 h) [%]	0	0	20	20
Mortality (96 h) [%]	0	30	60	70

The following points deviated from OCSPP 850.1035 Mysid Acute Toxicity Test (October 2016):

- Analytical confirmation of dissolved test concentrations were not performed.

The validity criteria of OCSPP 850.1035 guideline (2016) are the following:

- All test vessels were identical - achieved
- Individual test organisms were randomly assigned to test vessels - no information in the report.
- A dilution water control was included in the test - achieved
- Not more than 10% of the organisms in the dilution water control showed signs of disease, stress (*e.g.,* discoloration, unusual behaviour, immobilization), and/or death - achieved

III. CONCLUSIONS

Assessment and conclusion by applicant:

The effects of seven glyphosate-related test items on *Mysidopsis bahia* were studied in a static acute toxicity test. The EC_{50} (96 h) for *Mysidopsis bahia* exposed to Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and were > 56, 5.6, 2.8 and > 56% effluent respectively. The EC_{50} (96 h) for Comp. 5A was found to be between 10 and 32% effluent. For the test items Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45, no EC_{50} were calculated since the effects on mysid shrimps were low at the highest test concentration.

No analytical verification was performed and organism randomisation was not performed or reported. The study is therefore considered to be supportive for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.2/003
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute toxicity to larvae of the Pacific oyster (*Crassostrea gigas*)
Report No	AB0503/G
Document No	-
Guidelines followed in study	EPA FIFRA, Subdivision E, Guideline 72-3 ASTM (1989) E724/9-85-012 (OPPTS 850.1055)
Deviations from current test guideline	Deviation from OPPTS 850.1055 (1996): none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

The effects of glyphosate acid to pacific oyster (*Crassostrea gigas*) was evaluated in a 48-hour static toxicity test conducted with nominal test concentrations of 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg Glyphosate acid/L. Furthermore, a dilution water control was tested. To each test vessel 0.535 mL inoculum containing 22 embryos/ mL was added. For each test item concentration 2 replicates and for the control 4 replicates were tested. The number of normal and abnormal larvae was counted after 48 h. Dissolved oxygen and pH were measured at test start and test end, while the temperature was measured daily. The salinity was measured in the dilution water control and in the 180 mg/L test solution and the density of the embryo solution was determined by electronic particle counting before test start. Test item concentrations were verified by HPLC at 0 and 48 hours. Mean measured concentrations ranged from 91 to 100 % of nominal concentrations.

The reduction of oyster development was assessed with a parametric and a non-parametric test which both indicated no significant reduction of development up to nominal concentrations of 32 mg test item/L. The LC_{50} (48 h) for *Crassostrea gigas* was 40 mg a.s./L (nominal). The NOEC after 48 h was 32 mg a.s./L. All validity criteria according to OPPTS 850.1055 were fulfilled. The study is therefor considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Density:	Not stated

2. Vehicle and/or positive control:

Vehicle: Dilution water
Positive control: None

3. Test organism:

Species:	Pacific oyster (*Crassostrea gigas*), Brood stock batch OY17
Age:	Embryos, approx. 15 minutes after fertilisation
Source:	In-house culture originally obtained from Guernsey Sea Farms, Parc Lane, Vale, Guernsey, Channel Islands, UK
Density of embryo solution at test start:	22 embryos/mL

4. Environmental conditions:

Temperature:	19.4 – 20.5 °C
pH:	5.6 – 8.1
Dissolved oxygen:	7.0 – 7.8 mg O_2/L
Salinity:	31.0 – 31.5 ‰

5. Experimental dates: April 23, 1996 to April 25, 1996

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test was performed using nominal concentrations of 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg glyphosate acid/L prepared using natural sea water, filtered through 0.2 µm with adjusted salinity (32 ±2 ‰). In addition, a control was exposed to the test medium without test substance or other additives.

The test was conducted 48 h in a static test setup in 250 mL glass beakers with loose fitting lids. There were two vessels per test concentration and four for the control group, each containing 0.535 mL embryo solution with an embryo density of 22 embryos/mL (determined in three additional inoculated vessels). At test end, the test media were mixed, and 20 mL removed and fixed with 1 mL buffered formalin. The number of normal and abnormal larvae was counted. Larvae were defined as normal, if the bivalve shell was fully formed.

2. Observations: The number of normal and abnormal larvae was counted at test end in triplicate in 1 mL subsamples using an inverted microscope. The pH-value and the oxygen saturation were measured at test start and test end. The temperature was measured daily in one replicate of each test solution. The salinity was measured in the dilution water control and in the 180 mg/L test solution. The density of the embryo solution was determined by electronic particle counting before test start. Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis at test start and test end.

3. Statistical calculations: The EC_{50} value was calculated using Stephan's method. The significance of reduction in normal development was assessed using the Students t-test with Bonferroni adjustment (parametric) and Wilcoxon rank sum test (non-parametric).

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} value and the NOEC are given below based on nominal concentrations.

Table 8.2.4- 46: Endpoints

Endpoints	Glyphosate acid [mg/L]
EC_{50} (48 h) (95% CL)	40 (36 – 45)
NOEC (48 h)	32

Analytical data: The mean measured concentrations of glyphosate acid ranged from 91 to 100 % of nominal values. As the mean measured content of the test item always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 8.2.4- 47: Analytical results

Nominal concentration of glyphosate acid [mg/L]	Measured concentration of glyphosate acid [mg/L]		Mean measured concentration of glyphosate acid [mg/L]	% of nominal
	0 h	48 h		
Dilution water control	< 0.01	<0.01	<0.01	-
3.2	3.0	2.7	2.9	91
5.6	5.7	5.1	5.4	96
10	10	8.8	9.4	94
18	18	16	17	94
32	32	30	31	97
56	56	52	54	96
100	100	94	97	97
180	180	170	180	100

B. OBSERVATIONS

The reduction of oyster development was assessed with two statistical methods. The parametric test (Students t-test with Bonferroni adjustment) calculated a non significant reduction at nominal concentrations up to 32 mg/L. As these data were non-parametric, the Wilcoxon rank sum test for non-parametric data was conducted, which also indicated no significant development reduction up to nominal concentrations of 32 mg test item/L.

The results of the test are depicted in the following tables.

Table 8.2.4- 48: Effects of glyphosate acid to *Crassostrea gigas*

Nominal concentration of glyphosate acid [mg/L]	Number of normal / abnormal oysters after 48 h				Mean normal oysters [%]	Reduction [%]
	A	B	C	D		
Control	43/0	36/4	46/1	45/2	103	-
3.2	42/1		37/2		95	8
5.6	43/0		41/2		100	3
10	45/1		42/1		105	0
18	38/1		38/3		90	13
32	41/4		37/4		91	12
56	12/21		9/25		27	74*
100	0/26		0/29		0	100*
180	0/11		0/12		0	100*

*significant reduction

All validity criteria according to OPPTS 850.1055 were fulfilled, as mortality/ aberrant development in control group did not exceed 30 %, dissolved oxygen concentration was ≥ 60 % of air saturation and embryos were ≤ 4 h old at test start.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In conclusion, the LC_{50} (48 h) for *Crassostrea gigas* exposed to glyphosate acid was 40 mg a.s./L (nominal). The NOEC after 48 h was 32 mg a.s./L, based on nominal test concentrations.

The study is considered to be valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.4.2/004
Report author	[redacted]
Report year	1985
Report title	Acute Toxicity of Roundup (Technical) to Atlantic Oyster (*Crassostrea virginica*)
Report No	BN-73-79
Document No	-
Guidelines followed in study	Woelke, C. E. - "Measurement of Water Quality with the Pacific Oyster Bioassay." Water Quality Criteria, ASTM Spec. Tech. Publ. 416, Am. Soc. Testing Mats, 1967, p. 112-120.
Deviations from current test guideline	Deviation from OPPTS 850.1055 (1996): • No information about the dissolved oxygen concentration. • No analytical verification.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The effects of glyphosate technical on the normal embryonic development of the Atlantic oyster (*Crassostrea virginica*) were evaluated in a 48-hour static toxicity test. The test was performed using nominal concentrations of 0.75, 1.0, 2.4, 4.9, 7.5 and 10 mg glyphosate/L in triplicates. In addition, a control with test medium without test substance was tested.

The test was performed in 500 mL volumetric flasks, containing each 300 mL test solution, in which 15000 newly fertilised oyster eggs (at two-cell stage) were introduced for each test concentration and control. The

test flasks were incubated for 48 hours at 25° C. The salinity of the test solutions was measured at test initiation to range between 26 – 28 ‰ at test initiation.
At the end of this period cultures were sieved and larvae were preserved in 5 % formalin for microscopic examination to determine the percentage of fertilized eggs that had developed to a normal morphological stage.
Compared to the untreated control, no adverse effects of glyphosate on the normal embryonic development of oysters were observed up to the highest concentration tested (10 mg glyphosate/L). The EC_{50} and the NOEC were therefore determined to be > 10 mg/L and ≥ 10 mg/L, respectively. The study is considered to be supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	CP67573
Purity:	96.7 %

2. Vehicle and/or positive control: None

3. Test organism:

Species:	Atlantic oyster (*Crassostrea virginica*)
Age:	Fertilised eggs
Size:	Not stated
Loading:	50.000 fertilized eggs/L
Source:	U. S. Bureau of Commercial Fisheries Shellfish Research Laboratory in Milford
Diet/Food:	None
Acclimation period:	Sexually mature Atlantic oysters were collected from Milford harbour and held at the BCF' Shellfish Laboratory in filtered sea water for 7 days at a temperature of 22C.

4. Environmental conditions:

Temperature:	25 °C
Photoperiod:	Not stated
Salinity	26 – 28 ‰ (at test start)
Dissolved oxygen:	Not stated
Conductivity:	Not stated

5. Experimental dates: Not mentioned

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The static acute toxicity test was performed using nominal concentrations of 0.75, 1.0, 2.4, 4.9, 7.5 and 10 mg glyphosate/L in triplicates. In addition, a control with the test medium (without test substance) was tested under the same conditions as in the test groups. Ten hours prior to the test initiation, mature oysters were placed in a Pyrex tray filled with ultraviolet-light-treated water for eggs laying. About 30 minutes before spawning was desired, the water temperature was raised to 30°C and a sperm suspension from a sexually mature, sacrificed male oyster was added to the water. The combination of increased temperature and sperm induced one or more of the female oysters to spawn. Eggs from a single female were

selected for use in the bioassay and the number of eggs/unit volume was determined by sampling the sperm-egg suspension. The test was performed in 500 mL volumetric flasks, containing each 300 mL test solution with a salinity of 26 – 28‰, in which 15000 newly fertilised oyster eggs (at two-cell stage) were introduced for each test concentration and control. The test flasks were incubated for 48 hours at 25°C. At the end of' this period cultures were poured through a 37 µm sieve to obtain samples containing about 200 larvae and samples were preserved in 5% formalin for microscopic examination.
2. Observations: Quantitative samples were taken 48 hours after test initiation to determine the percentage of the fertilized eggs that had developed to a normal morphological stage (straight-hinged veliger larvae).
3. Statistical calculations: The concentrations tested and the corresponding observed percent normal development were transformed to log and Probit, respectively. The EC_{50} values were predicted using a linear regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} and NOEC values given below are based on nominal concentrations.

Table 8.2.4- 49: Endpoints

Endpoints	Test item [mg/L]
EC_{50} (48 h)	> 10
NOEC (48 h)	≥ 10

B. OBSERVATIONS

Compared to untreated control, no adverse effects of glyphosate on the normal embryonic development of oysters were observed up to the highest concentration tested (10 mg test item/L).

Table 8.2.4- 50: Percentage normal development of Atlantic oyster larva exposed to glyphosate for 48 hours

Glyphosate [mg/L]	Control	0.75	1.0	2.4	4.9	7.5	10.0
Normal embryonic development [%]	> 90	> 90	> 90	> 90	> 90	> 90	> 90

Results showed that glyphosate did not adversely affect the normal development of Atlantic Oyster larvae.

The validity criteria according to OPPTS 850.1055 are the following:

- The mortality/aberrant development in control group should not exceed 30 % - achieved
- The dissolved oxygen concentration should be ≥ 60 % of air saturation – no information in the report
- The embryos should be ≤ 4 h old at test start – two cell stage embryos were used.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In an acute toxicity test, Atlantic Oysters (*Crassostrea virginica*) were exposed to glyphosate technical for 48 hours. The EC_{50} and the NOEC were therefore determined to be > 10 mg a.e./L and ≥ 10 mg a.e./L, respectively.

Since no analytical verification was performed, the study is considered to be supportive and not considered reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

Studies on long-term and chronic effects of the active substance glyphosate and its relevant metabolites on aquatic invertebrates to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

Studies considering the reproductive toxicity of glyphosate to aquatic invertebrates were assessed for their validity to current and relevant guidelines for glyphosate, glyphosate salts and the metabolite AMPA and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 8.2.5- 1: Studies on long-term and chronic toxicity of glyphosate and metabolites to aquatic invertebrate

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.5.1/001	[redacted] 1999	21 d Reproduction	Glyphosate acid	Valid	-
CA 8.2.5.1/002	[redacted] 1995	21 d Reproduction	Glyphosate	Valid	-
CA 8.2.5.1/003	[redacted], 1993	21 d Reproduction	IPA salt	Valid	-
CA 8.2.5.1/004	[redacted], 1990	21 d Reproduction	Glyphosate	Valid	-
CA 8.2.5.1/005	[redacted], 1989	21 d Reproduction	Glyphosate	Valid	-
CA 8.2.5.1/006	[redacted] 1982	21 d Reproduction	Glyphosate	Valid	-
CA 8.2.5.1/007	[redacted] 2011	21 d Reproduction	AMPA	Valid	-
CA 8.2.5.3/001	[redacted] 2020	Water spiked	Glyphosate acid	Valid	-

Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate and the metabolites on aquatic invertebrates are summarised in the table below. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to aquatic invertebrates, please refer to document M-CP Section 10.2.

Table 8.2.5-2: Literature on long-term and chronic toxicity of glyphosate and metabolites to aquatic invertebrate

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.5.1/008	[redacted] 2015	21 d Reproduction	AMPA	Reliable	Chronic toxicity tests of the AMPA were performed with fathead minnow (*Pimephales promelas*) and *Daphnia magna*.

Endpoints of studies considered valid for glyphosate are shown in the table below.

Table 8.2.5-3: Endpoints: Long-term and chronic toxicity of glyphosate to Aquatic Invertebrates

Reference	Test item	Species	Test design	Endpoints based on	EC_{50} (mg a.e./L)	NOEC (mg a.e./L)
[redacted] 1999CA 8.2.5.1/001	Glyphosate acid	*Daphnia magna*	21 d Reproduction semi-static	nom	100	**12.5**
[redacted] 1995 CA 8.2.5.1/002	Glyphosate	*Daphnia magna*	21 d Reproduction semi-static	nom	> 100	56
[redacted] 1993 CA 8.2.5.1/003	IPA salt	*Daphnia magna*	21 d Reproduction semi-static	nom	267.93	42.90
[redacted] 1990 CA 8.2.5.1/004	Glyphosate	*Daphnia magna*	21 d Reproduction semi-static	nom	-	30
[redacted] 1989 CA 8.2.5.1/005	Glyphosate	*Daphnia magna*	21 d Reproduction semi-static	nom	> 100	≥ 100
[redacted] 1982 CA 8.2.5.1/006	Glyphosate	*Daphnia magna*	21-day flow-through	nom	-	50
[redacted] 2020 CA 8.2.5.3/001	Glyphosate acid	*Chironomus sp.*	Spiked water	nom	-	≥1000

a.e.: acid equivalents
nom: nominal
Endpoint in **bold** is used for risk assessment.

Endpoints of studies considered valid for AMPA are shown in the table below.

Table 8.2.5-4: Long-term toxicity of AMPA to Aquatic Invertebrates

Reference (Data owner)	Test item	Species	Test design	Endpoints based on	EC_{50} (mg/L)	NOEC (mg/L)
[redacted] 2011 8.2.5.1/007	AMPA	*Daphnia magna*	21 d Reproduction semi-static	nom	Immobility: > 120 Reprod: 90 Growth: 90	Immobility: ≥ 120 **Reprod: 15** Growth: 30

Endpoint in **bold** is used for risk assessment.

Study summaries are provided below.

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

1. Information on the study

Data point:	CA 8.2.5.1/001
Report author	[redacted]
Report year	1999
Report title	Glyphosate acid: Chronic toxicity to *Daphnia magna*
Report No	AF0497/B
Document No	-
Guidelines followed in study	OECD 202, Part II, Reproduction Test (1984)
Deviations from current test guideline	Deviation from guideline OECD 211 (2012): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The lethal and sub lethal effects of glyphosate acid on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. Ten replicates of one *Daphnia* per concentration were exposed to 12.5, 25, 50, 100, and 200 mg a.s./L nominal concentrations. In addition, 10 x 1 *Daphnia* were exposed to test medium without test substance (blank control). The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 21 days. The test *Daphnia* were fed daily with cultured algae (*Chlorella vulgaris*).

Mortality of P_0 generation of *Daphnia* and observation for the presence of alive and dead offspring (termed F_1 generation) were recorded daily in each test vessel. At the end of the test, the length of each surviving P_0 *Daphnia* was measured.

The pH was measured in each newly prepared test solution. The pH and dissolved oxygen concentration of two of the replicates of the old test solutions were measured after transfer of the P_0 generation of daphnids. Temperature measurements were recorded daily by means of a thermometer and hourly automatically. The concentration of glyphosate acid in the test solutions was determined on days 0, 2, 7, 9, 14, and 16. Old solutions were analysed on days 2, 7, 9, 14, and 21.

The mean measured concentrations of glyphosate acid in the new test solutions ranged from 100 to 104 % of the nominal values. The mean measured concentrations in the old test solutions ranged from 96 to 104 % of the nominal values. Therefore, the results are based on nominal glyphosate acid concentrations. The overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid was 50 mg/L based on nominal concentration. All validity criteria according to the pertinent OECD 211 guideline were fulfilled. The overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid was 50 mg/L based on nominal concentration. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Lot/Batch #:	P30
Purity:	97.6 %

2. Vehicle and/or positive control: Dilution water: Dilution water
Positive control: none

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	1 organism per vessel (glass beakers containing 80 mL test solution)
Source:	Continuous laboratory cultures

4. Environmental conditions:

Temperature:	19.4 to 20.2 °C
pH:	3.67-8.02 (new solutions) 3.46-8.00 (old solutions)
Dissolved oxygen:	9.2-9.2 mg O_2/L (dilution water, new) 8.8-9.2 mg O_2/L (test solutions, old)
Conductivity:	572-617 mg/L µS/cm (test solutions)
Hardness:	202.7-218.3 mg $CaCO_3$
Photoperiod:	16 hours light /8 hours dark, 20 minute dawn and dusk transition period; 480 lux

5. Experimental dates: November 16, 1998 to December 07, 1998

B. STUDY DESIGN AND METHODS

Experimental treatments: The lethal and sub lethal effects of glyphosate acid on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. Ten replicates of one *Daphnia* per concentration were exposed to 12.5, 25, 50, 100, and 200 mg a.s./L nominal concentrations. In addition, 10 x 1 *Daphnia* were exposed to test medium without test substance (blank control). The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 21 days. The test *Daphnia* were fed daily with cultured algae (*Chlorella vulgaris*).

A primary stock solution of 200 mg a.s./L was prepared on day 0 by dissolving 400 mg test item in 2000 mL of dilution water. On days 2, 4, 7, 9, 11, 14, 16, and 18 a primary stock solution of 100 mg a.s./L was prepared by dissolving 200 mg test item in 2000 mL dilution water. The test solutions were prepared by the addition of appropriate aliquots of the stock solutions to dilution water. At each renewal of the test solutions, the surviving P_0 generation of *Daphnia* were transferred to the new solutions. The F_1 generation of *Daphnia* were removed from each vessel and counted. The numbers of alive and dead F_1 *Daphnia* were recorded.

2. Observations: Mortality of P_0 generation of *Daphnia* and observation for the presence of alive and dead offspring (termed F_1 generation) were recorded daily in each test vessel. At the end of the test, the length of each surviving P_0 *Daphnia* was measured
The pH was measured in each newly prepared test solution. The pH and dissolved oxygen concentration of two of the replicates of the old test solutions were measured after transfer of the P_0 generation of daphnids. Temperature measurements were recorded daily by means of a thermometer and hourly automatically. The concentration of glyphosate acid in the test solutions was determined on days 0, 2, 7, 9, 14, and 16. Old solutions were analysed on days 2, 7, 9, 14, and 21.
The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: The reproduction and length data for each individual P_0 generation daphnid were entered into electronic data files and analysed using statistical procedures contained in the Brixham Environmental Laboratory computer programs 'STATS' (version 4.10) and 'EPA' (version1.04).

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of glyphosate acid in the new test solutions ranged from 100 to 104 % of the nominal values. The mean measured concentrations in the old test solutions ranged from 96 to 104 % of the nominal values. On the basis of the analytical data, the nominal concentrations were used for the calculation and reporting of all results.

Table 8.2.5-5: Analytical measurements

Nominal concentration (mg glyphosate acid/L)	Mean measured (new solutions) mg/L	Mean measured (old solutions) mg/L	% of nominal of overall mean measured concentrations
Control		-	-
12.5	13 (104%)	12 (96%)	100
25	25 (100%)	25 (100%)	100
50	50 (100%)	52 (104%)	102
100	100 (100%)	102 (102%)	101
200	200 (100%)	200 (100%)	100

The 21-day EC_{50} and NOEC values (based on nominal concentrations) are given below:

Table 8.2.5-6: Toxicity values for *Daphnia magna*

Mortality	
21-day EC_{50}	100 (95 % confidence interval 77-142)
21-day NOEC	50
21-day LOEC	100
Maximum allowable toxicant concentration (MATC)	71

Table 8.2.5-6: Toxicity values for *Daphnia magna*

Reproduction	
21-day NOEC	100 (considered 25 by RMS)
21-day LOEC	200
Maximum allowable toxicant concentration (MATC)	141
Length	
21-day NOEC	100
21-day LOEC	200
Maximum allowable toxicant concentration (MATC)	141
Overall result	
21-day NOEC	50 (considered 25 by RMS)
21-day LOEC	100
Maximum allowable toxicant concentration (MATC)	71

B. OBSERVATIONS

In the dilution water control and test concentrations up to and including 100 mg a.s/L all surviving P_0 *Daphnia* generation had released their first offspring by day 10. There was no reproduction at the concentration of 200 mg a.s./L due to mortality of the P_0 *Daphnia*.

The effects of glyphosate acid on *Daphnia magna* mortality and reproduction are shown below.

Table 8.2.5-7: Effects of glyphosate acid on *Daphnia magna* mortality and reproduction after 21 days of exposure

Nominal concentration (mg a.s./L)	Mean adult mortality [%]	Total number of off-spring per parent	Total offspring	Mean adult length [mm]
Control	10	108± 20	1028	4.28
12.5	0	100±21	1003	4.40
25	**0**	**84±12***	**840**	**4.31**
50	0	91±18	912	4.31
100	50	109±23	763	3.81
200	100	-	-	[A]

[A] mortality before day 21

* Statistically significant difference

All validity criteria according to OECD 211 were fulfilled, as immobility of adult daphnids was ≤20% in control groups and number of off-spring was >60 for the duration of the exposure.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid was 50 mg a.s./L based on nominal concentration. The EC50 was determined to be 100 mg a.s./L.
In the RAR 2015, the RMS considered the nominal NOEC to be 12.5 mg a.s./L based on statistical difference at the next higher test concentration.

The study is considered to be valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.5.1/002
Report author	[redacted]
Report year	1995
Report title	*Daphnia magna*, Reproduction Test with Glyfosaat
Report No	141874
Document No	-
Guidelines followed in study	OECD Guideline 202 ECC Draft Guideline XI/681/86 "Prolonged Toxicity Study with *Daphnia magna*: Effects on Reproduction"
Deviations from current test guideline	Deviation from guideline OECD 211 (2012): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate (glyfosaat) on *Daphnia magna* were evaluated in a 21-day reproduction test under semi-static conditions. The reproduction test was performed using six nominal test concentrations (5, 10, 18, 32, 56 and 100 mg test item/L) and a control. 10 replicates with one daphnid each were prepared per test concentration and 20 replicates with one daphnid each for the control.
The number of living, immobilised and dead parental *Daphnia magna* was observed on a daily basis. In addition, the presence of eggs in the brood pouch was observed on every workday. For the F1 generation, the appearance of the first brood was recorded. Every workday, the number of newborn daphnids were counted and the condition of the young recorded. The presence of eggs, which did not hatch was recorded, when observed. Incidental mortality was equally recorded, when occurred.
There was no test substance related mortality of parental daphnids at any test concentrations. The average numbers of offspring per parent at concentrations up to and including 56 mg/L were > 90 % when compared to the control group. The average number of offspring at 100 mg/L ranged from 54 to 74 % when compared to the controls. Statistical analysis demonstrated significant reduction of reproductive capacity of *Daphnia magna* at 100 mg/L. The EC_{50} for parental immobility and reproduction were both calculated to be > 100

mg a.e./L (nominal). The overall no observed effect concentration (NOEC) was 56 mg a.e./L based on nominal concentrations. All validity criteria according to the pertinent OECD 211 guideline were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	22021
Purity:	96 %

2. Vehicle and/or positive control: Vehicle: Dilution water (M4 medium)
Positive control: None

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	1 daphnid per 50 mL test medium
Source:	In-house culture
Diet/Food:	*Chlorella pyrenoidosa* at each solution renewal

4. Environmental conditions:

Temperature:	19.5- 21.0 °C
Photoperiod:	16 hours light / 8 hours dark, 600 lux
pH:	7.7 – 8.8 (control), 5.2 – 5.7 (100mg test item/L)
Dissolved oxygen:	≥ 8.9 mg O_2/L, (5.9 – 7.6 mg O_2/L on day 21 only)
Conductivity:	Not stated
Hardness:	250 mg $CaCO_3$/L

5. Experimental dates: May 5, 1995 to May 29, 1995

B. STUDY DESIGN AND METHODS

1. Experimental treatments: A 21-day reproductive toxicity test was conducted under semi-static conditions (renewal of test medium three times a week). *Daphnia magna* was exposed to nominal concentrations of 5, 10, 18, 32, 56 and 100 mg test item/L in ISO-medium (M4). In addition, a control group was exposed to test medium without test substance. Ten glass vessels (80 mL vessels containing 50 mL test medium each) were used per treatment group for the test item and 20 vessels for the control group. One daphnid was exposed per replicate (vessel).

2. Observations: The number of living, immobilised and dead parental *Daphnia magna* was observed on a daily basis. In addition, the presence of eggs in the brood pouch was observed on every workday.

For the F1 generation, the appearance of the first brood was recorded. Every workday the number of young newborn daphnids was counted and the condition of the young recorded. The presence of unhatched eggs was recorded, when observed. Incidental mortality was equally recorded, when occurred.

The pH-values and the oxygen saturation were measured at test initiation and just before the renewal of the test media in all treatments. The temperature was controlled at each renewal in one of the control vessels and on a daily basis in the climate room.

Analytical control measurements were performed by mean of HPLC analysis using samples taken from all test concentrations on day 0 for the freshly prepared solutions. For the aged test media, samples were taken

from 3 representative test concentrations.
The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20 % at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: Data were statistically tested using a mean comparison test (Williams' t-Test; $\alpha = 0.05$). EC_{50} (immobilisation) and the EC_{50} (reproduction) were estimated.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements were performed on samples of representative test concentrations. Recoveries ranged from 104 % to 118 % relative to nominal concentrations for test concentrations > 10 mg/L. Therefore, endpoints are based on nominal concentrations. At 5 and 10 mg/L recovery of glyphosate was significantly higher than nominal (> 120 %). The actual concentrations did not decrease significantly during the periods between renewal (48 or 72 hours).

Table 8.2.5-8: Analytical results

	mg glyphosate/L						
Nominal concentration	**Control**	**5**	**10**	**18**	**32**	**56**	**100**
Day 0	-	8.47	12.0	21.3	32.2	58.7	100
Day 3 (old)	-		14.3		35.7		110
Day 7 (fresh)	-		19.1		36.3		112
Day 14 (fresh)	-		16.7		36.7		111
Day 21 (old)	-				34.1	58.7	106
Mean measured over 21 d study		8.4	15.5	21.3	35.2	58.7	108.2
% of nominal		169	155	118	110	104	108

The 21-day EC_{50} and NOEC values are given below based on nominal concentrations.

Table 8.2.5-9: Endpoints

Endpoints	Glyphosate [mg/L] Nominal concentrations	Glyphosate [mg/L] Mean measured concentrations
EC_{50} (21 days) for parental immobility	> 100	> 108
EC_{50} (21 days) for reproduction	> 100	> 108
Overall LOEC	> 100	> 108
Overall NOEC	56	59

B. OBSERVATIONS

There was no test substance related mortality of parental daphnids at any test item concentration. The average numbers of offspring per parent at concentrations up to and including 56 mg/L were > 90 % when compared to the control. The average number at 100 mg/L ranged from 54 to 74 % when compared to the control. Statistical analysis shows significant reduction of reproductive capacity of *Daphnia magna* at 100 mg/L.

Table 8.2.5-10: Chronic toxicity of glyphosate to *Daphnia magna*

	Control	Glyphosate [mg/L]					
		5	10	18	32	56	100
Immobilisation of adults after 21 d [%]	5	20	0	0	0	10	20
Mean number offspring per day per adult from day 10 to day 21	133	145	147	151	158	160	91.7
mean living young compared to controls [%]	-	109	111	114	119	120	69

All validity criteria according to the current OECD 211 were fulfilled, as immobility of daphnids in control groups was <20 % and the mean number of live offspring produced per parent animal surviving at the end of test was ≥60.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The EC_{50} for parental immobility and reproduction were both calculated to be > 100 mg a.e./L (nominal). The overall no observed effect concentration (NOEC) was 56 mg a.e./L based on nominal concentrations.
All validity criteria according to the current OECD 211 were fulfilled .The study is therefore considered to be valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.5.1/003
Report author	[redacted]
Report year	1993
Report title	21-day Reproduction Test in Daphnia Test Article: Glyphosate isopropylamine salt
Report No	80-91-2328-05-93
Document No	-
Guidelines followed in study	OECD Guideline 202, Part I and II.
Deviations from current test guideline	Deviation from guideline OECD 211 (2012): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate isopropylamine salt on reproduction of *Daphnia magna* were evaluated in a semi-static test. Prior to the inhibition and reproduction test, a preliminary acute toxicity test was performed to determine the concentration rage for the reproduction test.

For the definite reproduction test the following concentrations were tested: 43, 94, 207, 455 and 1000 mg test item/L, equivalent to 26.49, 57.90, 127.51, 280.28 and 616.0 mg glyphosate isopropylamine salt/L or 19.63, 42.90, 94.48, 207.68 and 456.43 mg glyphosate/L, respectively. In addition, a control group was exposed to synthetic test medium only.

Daphnids were observed for immobilisation and reproduction on day 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21. The adult daphnids were observed and the young counted and removed from the test vessels. Temperature, pH-value and oxygen saturation of the test solutions were measured at the test beginning and end each renewal period.

At the highest concentration level of 1000 mg/L, all specimens were found to be immobile on day 7. At or below a concentration of 207 mg/L, no significant immobilisation was observed. Reproduction was significantly inhibited at or above a concentration of 207 mg/L.

For the number of offspring, significant reductions in reproduction rate were observed at or above a concentration level of 207 mg/L, whereas at or below a concentration of 94 mg/L, significant increases were generally observed. However, on day 19, the reproduction rate was significantly reduced at a concentration of 455 mg test item/L. Therefore, it is considered more appropriate to determine the NOEC on the basis of the average number of off-spring per adult and day over the entire reproduction period. The 21-day EC_{50} for immobilisation was 587 mg test item/L, equivalent to 361.59 mg glyphosate isopropylamine salt/L or 267.93 mg a.e./L (nominal). The NOEC for immobilization was 207 mg test item/L, equivalent to 127.51 mg glyphosate isopropylamine salt/L and 94.48 mg a.e./L (nominal), respectively. The NOEC for reproduction rate was calculated to be 94 mg test item/L equivalent to 57.90 mg glyphosate isopropylamine salt/L and 42.90 mg a.e./L (nominal), respectively. All validity criteria according to the current OECD 211 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt
Description:	viscous liquid
Lot/Batch #:	01/06/93
Purity:	61.6 % Glyphosate isopropylamine salt
Density:	1.23 g/cm^3 at 20 °C

2. Vehicle and/or positive control: none

3. Test organism:

Species:	*Daphnia magna* Strauss
Age:	neonates (< 24 h old)
Size:	Not stated
Loading:	50 mL for each animal (reproduction test)
Source:	in-house laboratory breeding
Diet/Food:	Unicellular green algae (*Scenedesmus spp.)*
Acclimation period:	Daphnids were held in groups of ca.30 organisms in 1000 mL glass at standard test conditions. They were fed once daily on green algae.

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 hours light / 8 hours dark, ~1000 lux
pH:	7.5 – 8.5
Dissolved oxygen:	> 60% of air saturation (approx. 6.0 mg O_2/L)
Conductivity:	0.049 µS/cm
Hardness:	14.5° dH

5. Experimental dates: August 27, 1993 to September 17, 1993

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The test was performed under semi-static conditions. Specimens were exposed to 43, 94, 207, 455 and 1000 mg test item/L, corresponding to 26.49, 57.90, 127.51, 280.28 and 616.0 mg glyphosate isopropylamine salt/L and 19.63, 42.90, 94.48, 207.68 and 456.43 mg glyphosate/L. In addition, a control group was exposed to the synthetic test media only. Stock solutions were prepared three times per week in which the solution was diluted with test water in a geometrical series by a factor or 2.2. Defined volumes of the stock solution were placed in a volumetric flask and filled up to the final volume of 2000 mL with synthetic test water (Elendt media). There were 8 vessels per treatment containing 5 daphnids each (500 mL glass beakers containing 50 mL test medium).

2. Observations: Daphnids were observed for immobilisation and reproduction on day 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21. The adult daphnids were observed and the young counted and removed from the test vessels. The adult daphnids were then transferred with specially prepared Pasteur pipettes. First, the young were filtered through a glass filter with 200 µL polypropylene mesh. Subsequently, the young were counted and the number of live and dead daphnids was noted. Three times a week the test medium was renewed. Subsequently, the offspring were counted and the number of live and dead animals was recorded. Temperature, pH-value and oxygen saturation were measured in line with each renewal period.

Analytical measurements were performed by HPLC analysis. Representative concentration levels of 43, 207, 455 and 1000 mg test item/L were analysed. The freshly prepared test medium was analysed on day 0, 5, 10, 14 and 19. As on day 7, no specimen survived at the highest concentration, analytical measurements were conducted on concentration levels 43, 207 and 455 mg test item/L.
The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20 % at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: The 21 d EC_{50} value was calculated according to Spearman and Karber. Fecundity was analysed using a Man-Whitney-U-test (2-tailled, corrected for ties).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The average recovery of glyphosate in test media over 21 days was 87.5 % and 93.7 %, 98.7 and 99.6 % of the nominal concentrations for 43, 207, 455 and 1000 mg test item/L, respectively. As the mean measured content of the test item always ranged between 80 and 120 % of nominal in both tests, ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 8.2.5-11: Analytical results

	[mg/L]				
Nominal concentration of test item	**Control**	**43**	**207**	**455**	**1000**
Nominal concentration of glyphosate isopropylamine salt	**Control**	**19**	**94**	**207.67**	**456.43**
Mean measured value of Glyphosate IPA salt over 21-day study	-	17.18	88.54	204.93	454.71
% of nominal	-	87.5	93.7	98.7	99.6

The NOEC value is given below are based on nominal concentrations.

Table 8.2.5-12: Endpoints

Endpoints (21-day)	**Test item [mg/L]**
EC_{50} Immobilisation	587
NOEC Immobilisation	207
NOEC Reproduction	94

B. OBSERVATIONS

Observations: At the highest concentration level of 1000 mg/L, all specimens were found to be immobile on day 7. At or below a concentration of 207, no relevant immobilisation was observed. Results of the reproduction rate revealed significant inhibitory effects at or above a concentration of 207 mg/L.

For the number of off-spring, significant reduction in reproduction rate was observed mostly at or above a concentration level of 207 mg/L, whereas at or below a concentration of 94 mg/L, significant increases were generally observed. However, the reproduction rate was significantly reduced for all concentrations on day 19. The NOEC on the basis was determined of the average number of off-spring per adult and day over the entire reproduction period. Also, all validity criteria according to the current OECD 211 were fulfilled, as immobility of daphnids in control groups was <20% and the mean number of live off-spring produced per parent animal surviving at the end of test was ≥60.

The percentage immobilisation is given below based on nominal concentrations.

Table 8.2.5-13: Chronic toxicity of glyphosate isopropylamine salt to *Daphnia magna*

		Nominal concentration of test item [mg/L]				
Parameter	**Control**	**43**	**94**	**207**	**455**	**1000**
Immobilisation of adults after 21 d [%]	0.0	7.5	0.0	2.5	10.0	100
Total number of live off-spring from day 7 to day 21	5452	4941	5111	4426	3738	0
Mean number offspring per day per adult from day 7 to day 21	8.78	8.24	7.89	7.02[1]	6.05[1]	n.d.

[1] = statistically significant when compared to control (U-test according to Mann-Whitney), α = 0.05
n.d. = not determined

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of glyphosate isopropylamine salt on *Daphnia magna* were evaluated. The 21-day EC_{50} for immobilisation was 587 mg test item/L, corresponding to 361.59 mg a.s./L and 267.93 mg a.e./L (nominal). The NOEC for immobilization was 207 mg test item/L, equivalent to 127.51 mg a.s./L and 94.48 mg a.e./L (nominal), respectively. The NOEC for reproduction rate was calculated to be 94 mg test item/L equivalent to 57.90 mg a.s./L and 42.90 mg a.e./L (nominal), respectively.
All validity criteria according to the current OECD 211 were fulfilled. The study is considered to be valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.5.1/004
Report author	[redacted]
Report year	1990
Report title	Influence of glyphosate on the reproduction of *Daphnia magna*
Report No	250795
Document No	-
Guidelines followed in study	OECD 202, Part II, Reproduction Test (1984)
Deviations from current test guideline	Deviation from guideline OECD 211 (2012): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The lethal and sub-lethal effects of glyphosate on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. The study was started with two glass beakers per test concentration, each containing 200 mL test solution. Two replicates of 10 *Daphnia* per concentration were exposed to 3.0, 9.4, 30, 94.9, and 300 mg a.s./L nominal concentrations. In addition, 2 x 10 *Daphnia* were exposed to test medium without test substance (blank control). After 7 days of exposure, 10 daphnids per test concentration and control with eggs in the brood pouch were selected and placed individually in a 100 mL beaker which contained 50 mL test solution. *Daphnia* were fed a mixture of yeast and algae (*Scenedesmus subspicatus*) at each test solution renewal.

Mortality of parent *Daphnia* and observation for the presence of alive and dead offspring were recorded three times a week at the renewal of the test media.

The pH and dissolved oxygen concentration of the test samples were measured for all treatment periods at the beginning and end of the respective periods. The temperature was measured at the renewal of the test solutions.

The concentration of glyphosate in the test solutions was determined at the first and at the last treatment period (last water renewal) directly after treatment and at the end of the respective period in the 3.0, 30, and 300 mg a.s./L test vessels.

The mean measured concentrations of glyphosate in the test solutions ranged from 82.3 to 130.1 % of nominal values. On the basis of the analytical data, the nominal concentrations were used for the calculation and reporting of all results. NOEC for survival and reproduction was 30 mg a.s./L based on nominal concentrations. All validity criteria according to the OECD guideline 211 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Lot/Batch #:	198-SI-22-1
Purity:	98.7 %

2. Vehicle and/or positive control: Vehicle: Test medium
Positive control: none

3. Test organism:

Species:	*Daphnia magna*
Age of animals:	Neonates (< 24 h old)
Loading:	First 7 days of exposure: 10 *Daphnia* in 200 mL test solution; Form day 7 to day 21: 1 *Daphnia* in 50 mL test solution
Source of organisms:	Continuous laboratory cultures

4. Environmental conditions:

Temperature:	21.5-22.5 °C
pH:	5.2-8.3 (new solutions) 5.3-8.5 (old solutions)
Dissolved oxygen:	8.6-8.8 mg O_2/L (new solutions) 8.3-8.7 mg O_2/L (old solutions)
Conductivity:	Not mentioned in the report
Hardness:	Not mentioned in the report
Photoperiod:	16 hours light /8 hours dark; 500-2000 lux

5. Experimental dates: January 17, 1990 to February 07, 1990

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The lethal and sub-lethal effects of glyphosate on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. The study was started in two glass beakers per test concentration, each containing 200 mL test solution. Two replicates of 10 *Daphnia* per concentration were exposed to 3.0, 9.4, 30, 94.9, and 300 mg a.s./L nominal concentrations. In addition, 2 x 10 *Daphnia* were exposed to test medium without test substance (blank control). After 7 days of exposure, 10 daphnids per test concentration and control with eggs in the brood pouch were selected and placed individually in a 100 mL beaker which contained 50 mL test solution. *Daphnia* were fed a mixture of yeast and algae (*Scenedesmus subspicatus*) at each test solution renewal.
A stock solution of 500 mg a.s./L was prepared on day 0 by dissolving 500 mg test item in 1000 mL of test medium. This solution was freshly prepared on days 2, 5, 7, 9, 12, 14, 16, and 19 of the exposure period. Appropriate amounts of this stock solution were diluted to prepare the test concentrations.
2. Observations: Mortality of P_0 generation of *Daphnia* and observation for the presence of alive and dead offspring were recorded three times a week at the renewal of the test media. Dead P_0 *Daphnia* and offspring were removed at the observation dates.
The pH and dissolved oxygen concentration of the test samples (controls, the lowest (3.0 mg a.s./L) and the highest (300 mg a.s./L) test concentrations of glyphosate) was measured at all treatment periods at the beginning and at the end of the respective periods. The temperature was measured at the renewal of the test solutions. The concentration of glyphosate in the test solutions was determined at the first and at the last treatment period (last water renewal) directly after treatment and at the end of the respective period in the 3.0, 30, and 300 mg a.s./L test vessels.
The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: Steel-Test.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of glyphosate in the test solutions ranged from 82.3 to 130.1 % of the nominal values for the 3.0, 30, and 300 mg a.s./L test concentrations. On the basis of the analytical data, the nominal concentrations were used for the calculation and reporting of all results.

Table 8.2.5-14: Analytical results

	[mg glyphosate/L]					
Nominal concentration	**Control**	**3.0**	**9.4**	**30.0**	**94.9**	**300**
Day 0 mean concentration	-	2.821	-	27.71	-	390.4
Day 2 mean concentration	-	3.183	-	31.40	-	365.8
Day 19 mean concentration	-	2.585	-	27.08	-	
Day 21 mean concentration	-	3.404	-	29.63	-	
Mean measured over 21 day study	-	2.99	-	28.95	-	378.1
% of nominal over 21d study	-	99.9	-	96.5	-	126

The endpoint value is given below.

Table 8.2.5-15: Endpoints

Endpoints	[mg a.s./L]
21-day NOEC for survival and reproduction	30 mg/L

B. OBSERVATIONS

Reproduction of young daphnids started on day 9 of the exposure period. No statistically significant influence of glyphosate on the reproduction rate was observed up to a concentration of 30 mg a.s./L. At the highest tested concentration of 300 mg a.s./L all daphnids were dead after 5 days of exposure.
The effects of glyphosate on *Daphnia magna* mortality and reproduction are shown below.

Table 8.2.5-16: Effects of glyphosate on Daphnia magna mortality and reproduction

Nominal concentration [mg a.s./L]	Mean adult mortality [%]	Total number of off-spring per parent animal	Total off-spring
Control	0	127±24	1266
3.0	0	123±29	1226
9.4	0	134±22	1338
30	0	102±26	1023
94.9	10	48±29[1]	476
300	100	0	0

[1] Statistically significant difference

All validity criteria according to the current OECD 211 were fulfilled, as immobility of daphnids in control groups was <20% and the mean number of live off-spring produced per parent animal surviving at the end of test was ≥60.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Lethal and sub-lethal effects of glyphosate on *Daphnia magna* were evaluated in a 21-day toxicity test. The 21-day NOEC for survival and reproduction of *D. magna* exposed to glyphosate was 30 mg a.e./L based on nominal concentrations. All validity criteria according to the current OECD 211 were fulfilled in the test.

The study is considered to be valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.5.1/005
Report author	[redacted]
Report year	1989
Report title	21-Day Prolonged Static Renewal Toxicity of Glyphosate Technical to *Daphnia magna*
Report No	AB 89-58
Document No	-
Guidelines followed in study	OECD Guideline 202 U.S. Guideline 72-4, (EPA-FIFRA, 40 CFR, Section 158.145).
Deviations from current test guideline	Deviation from guideline OECD 211 (2012): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate on the reproduction of *Daphnia magna* were evaluated in a 21-day semi-static test. The test was performed using nominal concentrations of 6.5, 13, 25, 50 and 100 mg test item/L. In addition, a control group was exposed to dilution water. The test solutions were prepared using hard blended water. The test solutions were renewed three times a week. There were four glass jars per treatment, each containing ten daphnids.

Samples for analytical confirmation were taken initially and at each renewal. Recoveries were ranging from 92.3, to 108.0% of nominal concentrations. Therefore, ecotoxicological endpoints were based on nominal concentrations of the test item.

Starting at test initiation, observations were made daily, recording the number of immobile *Daphnia magna*. Furthermore, behavioural or sublethal effects as well as any gross pathogenic or toxic response were recorded. Furthermore, survival, abnormal effects and time to first brood of daphnids were recorded daily throughout the study. Reproduction success was measured by counting and discarding the offspring produced in each concentration 3 days a week for the duration of the study.

No effects of glyphosate technical on survival, reproduction and time to first brood of *Daphnia magna* after 21-day exposure were observed in any test item treatment. No effects on behaviour were observed for the duration of the study. EC_{50} was determined to be > 100 mg a.e./L. The NOEC was determined to be ≥ 100 mg test item/L. All validity criteria according to OECD 211 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XLI-203
Purity:	97.67 %

2. Vehicle and/or positive control: None

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 specimens in 400 mL test solution
Source:	In-house culture
Diet/Food:	Once daily with a suspension of *Selenastrum capricornutum* (8×10^7 cells/400 mL), supplemented with a Tetramin®, cereal leaves and yeast suspension
Acclimation period:	None

4. Environmental conditions:

Temperature:	20 ± 2 °C
Photoperiod:	16 hours light / 8 hours dark (approx. 431 – 861 Lux), with 30-minute dawn and dusk transition periods
pH:	6.8 – 8.2 (new solutions), 7.4 – 7.9 (old solutions)
Dissolved oxygen:	New solutions: 8.3 – 9.0 mg/L (89.5 to 101 % saturation) Old solutions (2-3 days after renewal) : 4.1 – 6.8 mg/L (47 to 80 % saturation)
Conductivity:	350 µS/cm
Hardness:	174 mg $CaCO_3$/L.

5. Experimental dates: April 4, 1989 to April 25, 1989

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity of glyphosate on *Daphnia magna* was evaluated in a 21-days prolonged semi-static test, using nominal concentrations of 6.5, 13, 25, 50 and 100 mg test item/L. In addition, a control group was exposed to dilution water. The test solutions were prepared using hard blended water prepared to a total hardness of between 160 and 180 mg $CaCO_3$/L. The test solutions were renewed three times a week. There were four glass jars per treatment, each containing ten daphnids (1000 mL glass jars containing 400 mL test medium).

2. Observations: Observations were made on a daily basis to record the number of immobile *Daphnia magna*, starting from test initiation. Furthermore, behavioural or sublethal effects as well as any gross pathogenic or toxic response were recorded. Any dead individuals were immediately removed from the testing solutions. In addition, survival, effects on behaviour and observance of first brood of the organisms were recorded daily throughout the study. Reproduction success was measured by counting and discarding the offspring produced in each concentration three times a week for the duration of the study. Temperature, pH-value and oxygen saturation of the test solutions were measured on solution renewal days. In addition, total hardness and specific conductivity of the dilution water was measured weekly. Samples for analytical confirmation of the new solutions were taken initially and at each renewal days. The analytical data are reported separately (Monsanto Study No. ML-89-62).

The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20 % at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: The test parameters of survival, time to first brood (days), and total young/adult reproduction were analysed using analysis of variance. Dunnett's Test was used for mean separation. The 21-day EC_{50} values were calculated by probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements (separate report ML-89-62) were performed to determine the concentration of glyphosate in test solutions. Result showed recoveries of 92.3, 100 %, 108.0 %, 100 % and 100 % for nominal concentrations of 6.5, 13, 25, 50 and 100 mg test item/L. Therefore, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

The 21-day EC_{50} and NOEC values are given below based on nominal concentrations.

Table 8.2.5-17: Endpoints

Endpoints (21-day)	Glyphosate [mg a.e./L]
EC_{50} 21-day (95% C.I.)	> 100
NOEC 21-day	≥ 100

B. OBSERVATIONS

No effects of glyphosate technical on survival, reproduction and time to first brood of *Daphnia magna* were observed after 21-days of exposure in all test item concentrations. No effects on behaviour of adults and offspring were observed during the course of the study.

Table 8.2.5-18: Lethal effects of glyphosate to *Daphnia magna* (mean values)

Glyphosate [mg a.e./L]	Control	6.5	13	25	50	100
Survival (21-day) [%]	98	100	100	100	100	98
Reproduction (21-day) (young adult/reproduction day) (± SD)	5.2 ± 0.2	5.1 ± 0.3	5.3 ± 0.2	5.1 ± 0.1	5.1 ± 0.2	5.1 ± 0.0
Mean number of young adult/adult (21-days)	73.7	72.7	74.2	71.4	72.7	71.0
Time to fist brood (days) (± SD)	7.8 ± 0.5	7.8 ± 0.5	8.0 ± 0.0	8.0 ± 0.0	7.8 ± 0.5	8.0 ± 0.0

All validity criteria according to the current guideline OECD 211 were fulfilled, as immobility of daphnids in control groups was <20 % and the mean number of live off-spring produced per parent animal surviving at the end of test was ≥60.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In a 21-day prolonged semi-static reproduction study with *Daphnia magna*, no effects of glyphosate technical on survival, reproduction, and time to first brood of *Daphnia magna* were observed. Therefore, the 21-day EC_{50} was determined to be > 100 mg a.e./L (nominal). The NOEC was determined to be ≥ 100 mg a.e./L (nominal). All validity criteria according to the current guideline OECD 211 were fulfilled. The study is considered to be valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.5.1/006
Report author	[redacted]
Report year	1982
Report title	Chronic Toxicity of Glyphosate to *Daphnia magna* Under Flow-Through Test Conditions
Report No	AB 82-036
Document No	-
Guidelines followed in study	ASTM Committee, (Draft No. 5, September,1979, E-35.2; Draft No. 3, 1981, E-47.01; Draft No. 2, September,1979, E-35.21)
Deviations from current test guideline	Deviation from guideline OECD 211 (2012): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The effects of glyphosate on the reproduction of *Daphnia magna* were evaluated in a 21-day chronic test in flow-through conditions. The test was performed using nominal concentrations of 25, 50, 99, 199 and 397 mg test item/L. In addition, a control group was exposed to untreated water. The test solutions were permanently renewed using a one-litre proportional diluter system. There were four replicates per treatment, each containing 10 test daphnids.
The number of immobile *Daphnia magna* was recorded three times a week. Furthermore, reproductive success was measured by recording the number of off-spring produced in each treatment on every observation day for the duration of the study. In addition to survival and reproduction data, growth of adult daphnids was determined at the termination of the test.
No significant decrease in survival or length of adult daphnids was observed in organisms exposed to glyphosate for 21 days. Length of daphnids in the lowest (26 mg/L) and highest (365 mg/L) glyphosate treatment groups was significantly greater than in the control.
Reproduction significantly decreased at the three highest test item concentrations (96, 186 and 365 mg glyphosate/L). In contrast to that, at the lowest test item concentration (26 mg/L) an increase of

reproduction when compared to the control was observed. The NOEC was determined to be 50 mg test item/L (nominal). All validity criteria according to the OECD guideline 211 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate standard
Description:	White powder
Lot/Batch #:	NBP 2782610 [1992049]
Purity:	99.7 %

2. Vehicle and/or positive control: Vehicle: Deionized water
Positive control: None

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 specimens for 1000 mL test solution
Source:	In-house culture
Diet/Food:	Once daily with *Pseudokirchneriella subcapitata*
Acclimation period:	None

4. Environmental conditions:

Temperature:	20 ± 2 °C
Photoperiod:	16 hours light / 8 hours dark (approx. 538 – 753 Lux)
pH:	8.1 – 8.2 (control), 6.1 – 6.2 (highest test concentration)
Dissolved oxygen:	7.0 – 9.0 mg/L
Conductivity:	50 µS/cm
Hardness:	255 mg $CaCO_3$/L.

5. Experimental dates: March 5, 1982 to March 26, 1982

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxic effects of glyphosate on *Daphnia magna* were evaluated in a 21-day flow-through test, using nominal concentrations of 25, 50, 99, 199 and 397 mg test item/L. In addition, a control group was exposed to untreated water. The test solutions were prepared using well water at ABC's Aquatic Bioassay Laboratory, with known characteristics (hardness = 255 mg $CaCO_3$/L, pH = 8.2). The test system consisted of six sets of 1 L quadruplicate chambers, which were immersed in a circulating water bath. The test solutions were permanently renewed using a one-litre proportional diluter system, with modifications to allow intermittent delivery of large stock volumes of glyphosate and dilution water into the test chambers. The renewal rate was 200 mL/aquarium every 120 minutes, an amount sufficient to replace the 1 L test volume 3 times in a 24-hour period. There were four replicates per treatment, each containing 10 test daphnids, which were randomly placed in test chambers.

2. Observations: Observations were made three times a week (every Monday, Wednesday and Friday) to record the number of immobile *Daphnia magna*, starting from test initiation. Furthermore, the reproductive success was measured by recording and discarding the offspring produced in each concentration on every observation day for the duration of the study. Growth of adult daphnids was determined at test termination.

Temperature, pH-value and oxygen saturation of the test solutions were measured on day 0, 4, 7, 14 and 21 in control, and nominal test item treatments of 25, 99 and 397 mg glyphosate/L.
Samples for analytical confirmation of the concentration of glyphosate in test solutions were taken and analysed.
The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: Measured parameters in the quadruplicate test chambers were analysed using one-way analyses of variance (ANOVA) and as a post hoc test, Fisher's Protected Least Significant Difference (LSD), was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements were performed to determine the concentration of glyphosate in test solutions. The mean measured concentrations of glyphosate in test solutions were 26, 50, 96, 186 and 378 mg glyphosate/L for the nominal concentrations of 25, 50, 99, 199 and 397 mg test item/L respectively. Analytical recovery ranged from 93 to 104% of nominal concentrations.

Table 8.2.5-19: Analytical results

		mg glyphosate/L				
Nominal concentrations	Control	25	50	99	199	397
Day 0 measured concentrations	-	17	32	61	115	250
Day 4 measured concentrations	-	25	44	90	175	356
Day 7 measured concentrations	-	22	44	82	155	306
Day 14 measured concentrations	-	24	43	83	162	332
Day 21 measured concentrations	-	21	42	79	157	312
Mean measured concentrations over study period	-	26	50	96	186	378

The NOEC value is given below are based on nominal concentrations.

Table 8.2.5-20: Endpoints

Endpoints (21-day)	Glyphosate [mg a.e./L]
NOEC 21-day	50

B. OBSERVATIONS

No significant decreases in survival or length of adult daphnia were observed in all test item treatments. Length of daphnids in the lowest (26 mg/L) and highest (378 mg/L) glyphosate concentrations were significantly greater than control.
Reproduction significantly decreased at the three highest test item concentrations (96, 186 and 365 mg glyphosate/L). At the lowest level of glyphosate (27 mg/L) an increase of reproduction when compared to controls was observed. The highest test item concentration not resulting in decreased

reproduction was 50 mg/L. An increase of length and reproduction of daphnids observed at the lowest test item concentration is not considered to be deleterious and thus not used to estimate the NOEC.

Table 8.2.5-21: Adult length, survival and young produced per adult reproductive day of *Daphnia magna* continuously exposed to glyphosate during a 21-day life cycle study

Glyphosate [mg a.e./L] (mean measured concentrations)	Control	26	50	96	186	378
Survival (21-day) [%]	100	98	100	98	98	98
Reproduction (21-day) (young adult/reproduction day) (± SD)	4.9 ± 0.42	6.5 ± 0.15 [1]	5.1 ± 0.49	4.1 ± 0.78 [1]	3.8 ± 0.10 [1]	1.7 ± 0.32 [1]
Adult length (mm) (± SD)	3.7 ± 0.06	3.9 ± 0.05 [1]	3.7 ± 0.07	3.6 ± 0.11 [1]	3.7 ± 0.10	3.8 ± 0.03 [1]
Mean number of young adult/adult (21-days)	68.6	91.5	70.5	55.7	52.5	23.5

* Significantly different (Fishers' LSD, $\alpha = 0.05$).

All validity criteria according to the current OECD 211 were fulfilled, as immobility of daphnids in control groups was <20% and the mean number of live off-spring produced per parent animal surviving at the end of test was ≥60.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a 21-day chronic toxicity study, the exposure of *Daphnia magna* to glyphosate resulted in reduced reproduction at or above the nominal concentration of 99 mg a.e./L. No other adverse compound-related effects were observed. The NOEC was determined to be 50 mg a.e./L (nominal). All validity criteria according to the current OECD 211 were fulfilled.

The study is considered to be valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.5.1/007
Report author	[redacted]
Report year	2011
Report title	AMPA (Aminomethylphosphonic acid): A semi-static life cycle toxicity test with the Cladoceran (*Daphnia magna*)
Report No	139A-393
Document No	-
Guidelines followed in study	OECD Guideline 211 (1998), ASTM E 1193-97
Deviations from current test guideline	Deviation from guideline OECD 211 (2012): Minor: • Survival in the negative control group was slightly below the 80 % This does not affect the reliability of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of AMPA (aminomethylphosphonic acid) on the survival, growth and reproduction of *Daphnia magna* were evaluated in a 21-day reproduction test under semi-static conditions with renewal of test medium every 2 to 3 days. The reproduction test was performed using a geometric series five nominal test concentrations (7.5, 15, 30, 60 and 120 mg AMPA/L) and a dilution water control (negative control). 10 replicates with one daphnid each were prepared per test concentration and 20 replicates with one daphnid each for the control.

Parental *Daphnia magna* were observed on a daily basis for mortality, onset of reproduction and signs of toxicity. Body length and dry weights of surviving parental specimens were measured at the end of the exposure period. The number of juvenile daphnia produced in each vessel was counted three times per week and at test termination. Mean measured test concentrations were determined from samples of test media collected from each treatment and control group at test initiation, at the end of the first renewal cycle, at the beginning and end of the longest renewal cycle during the second week of the test, and at the beginning and end of the last renewal cycle (test termination).

AMPA was not detected in the control group. The mean measured concentrations of AMPA in samples collected during the test for each treatment group were 7.4, 15, 30, 57 and 120 mg AMPA/L, equivalent to 99, 100, 100, 95 and 100 % of the nominal concentrations, respectively. Therefore, the results evaluation is based on nominal test concentrations. There was no significant mortality observed during the test when compared to the control. Treatment related effects on growth were observed at 60 and 120 mg AMPA/L. There was significant decrease in mean neonate production observed in the 30, 60 and 120 mg AMPA/L treatment groups.

Survival in the negative control group was slightly below the 80% validity criterion required in the OECD 211 test guideline. However, this minor deviation is not considered to have had a significant impact on the validity of this study as the surviving daphnids in the control replicates appeared normal and healthy throughout the test suggesting that the mortality observed was most likely attributable to incidental death and not related to the health of the organisms.

Adult daphnids in the control group produced an average of 227 live young per surviving adult (CV = 11.6%), which is well above the validity criterion of ≥ 60 live young per surviving adult. Therefore, the study is considered valid according to OECD 211.
The overall no observed effect concentration (NOEC) based on reproduction (juvenile production) was determined to be 15 mg AMPA/L. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	Solid
Lot/Batch #:	GLP-0908-199984-A
Purity:	98.7 %

2. Vehicle and/or positive control: Vehicle: ASTM medium
Positive control: None

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	1 daphnid per 200 mL test medium
Source:	In-house culture
Diet/Food:	Daily: mixture of yeast, cereal grass media and trout chow (YCT) and suspension of *Pseudokirchneriella subcapitata*

4. Environmental conditions:

Temperature:	19.0 – 20.8 °C
Photoperiod:	16 hours light Light intensity = 314 lux
pH:	7.1 – 8.6
Dissolved oxygen:	6.8 – 9.1 mg O_2/L
Conductivity:	274 – 391 µS/cm
Hardness:	132 - 140 mg $CaCO_3$/L

5. Experimental dates: February 09, 2011 to March 04, 2011

B. STUDY DESIGN AND METHODS

1. Experimental treatments: A 21-day reproductive toxicity test was conducted under semi-static conditions, with renewal of test medium every 2 to 3 days. *Daphnia magna* neonates (<24 hours old) were exposed to nominal concentrations of 7.5, 15, 30, 60 and 120 mg AMPA/L in moderately hard dilution water (ASTM medium). In addition, a negative control group was prepared in parallel. Ten glass vessels (250 mL vessels containing 200 mL test medium each) were used per treatment group for the test item and 20 vessels for the control group. One daphnid (neonate < 24 hours old) was exposed per replicate (vessel).
2. Observations: The number of living, immobilised and dead parental *Daphnia magna* and the time to gravidity (presence of eggs in brood pouch) were observed on a daily basis. Body length and dry weights of surviving parental specimens were measured at the end of the exposure period (21 days).
The number of neonate daphnids was counted three days a week and their condition was recorded. The presence of unhatched eggs was recorded, when observed. Incidental mortality was also recorded, when occurred. At the end of the test, body length and dry weight of each surviving parental daphnid was measured.

The temperature, pH-values and the oxygen saturation were measured at test initiation, before and after the renewal of the test media in two replicate test chambers and at test termination. Hardness, alkalinity and specific conductance were measured in batch solutions of the negative (dilution water only) control and at the highest test item concentration at test initiation and on one renewal day each week and from pooled replicate solutions at test termination.
Analytical measurements were performed by using an HPLC method of analysis using samples taken from all test concentrations for the freshly prepared solutions, at the end of the first renewal cycle (old solution), and at the beginning and end of last renewal cycle. For the aged test media, samples were taken from 2 alternate replicates of each treatment and control group and pooled by treatment group.
The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: Data were statistically tested using Chi-square and Fisher's Exact test (discrete-variable data; $\alpha = 0.05$) and Dunnett's t-test (one-tailed, normal distributed data; $\alpha = 0.05$). The NOEC was determined by visual interpretation of the results.

II. RESULTS AND DISCUSSION

A. FINDINGS

Concentrations of AMPA in the freshly prepared test solutions, sampled on Days 0, 9 and 19 ranged from 92.5 to 106 % of the nominal concentrations. Concentrations of AMPA in the old test solutions sampled immediately prior to renewal on Days 2, 12 and at test termination on Day 21 ranged from 78.6 to 117 % of the nominal concentrations. The overall mean measured concentrations of AMPA during the test were 7.4, 15, 30, 57 and 120 mg AMPA/L, equivalent to 99, 100, 100, 95 and 100 % of the nominal concentrations, respectively. Since the mean measured test concentrations were within the 80 – 120 % of nominal test concentration, the results of the study are reported as nominal test concentrations.

Table 8.2.5-22: Analytical results

	[mg AMPA/L]					
Nominal concentration	**Control**	**7.5**	**15**	**30**	**60**	**120**
Day 0 mean concentration (fresh)	-	7.41	15.8	30.9	62.7	127
Day 2 mean concentration	-	6.21	12.8	24.5	47.2	97.9
Day 9 mean concentration (fresh)	-	7.05	13.9	29.3	56.3	112
Day 12 mean concentration	-	7.90	15.9	35.2	58.5	137
Day 19 mean concentration (fresh)		7.64	14.0	28.0	55.7	114
Day 21 mean concentration		8.04	15.7	32.4	61.4	133
Mean measured over 21-day study	-	7.4	15	30	57	120
% of nominal over 21d study	-	99	100	100	95	100

The 21-day EC_{50} and NOEC values are given below based on nominal concentrations.

Table 8.2.5-23: Endpoints

Endpoints	AMPA [mg/L]
EC_{50} (21 days) for parental survival and immobility	> 120
NOEC (21 days) for parental survival and immobility	120
EC_{50} (21 days) for reproduction (95% C.I.)	90 (84 – 94)
NOEC (21 days) for reproduction	15
EC_{50} (21 days) for growth (95% C.I.)	90 (84 – 94)
NOEC (21 days) for growth	30
Overall LOEC	30
Overall NOEC	15

B. OBSERVATIONS

Survival in the 7.5, 15, 30, 60 and 120 mg AMPA/L treatment groups at test termination was 80, 100, 70, 100 and 90%, respectively. No significant differences were detected in any treatment group in comparison to the control ($\alpha = 0.05$, Fisher's Exact test). In the 120 mg AMPA/L treatment group, all surviving parental daphnids appeared pale and smaller in comparison to the control organisms from Day 5 through test end.

The first day of brood production in the controls and in all AMPA treatments indicated no delay in the onset of egg production at any of the AMPA concentrations tested. No aborted or shed eggs were present in the control or in any of the AMPA treatments. No males or ephippia were observed during the test.

Adult daphnids in the 7.5, 15, 30, 60 and 120 mg AMPA/L treatment groups produced an average of 229, 213, 189, 169 and 59.6 live young per surviving adult, respectively. Dunnett's test indicated there was a statistically significant decrease in mean neonate production in the 30, 60 and 120 mg AMPA/L treatment groups (30, 57 and 120 mg AMPA/L as mean measured concentration) in comparison to the negative control ($\alpha = 0.05$).

In the control group , the mean body length was 5.3 mm and mean dry weight was 0.99 mg. Daphnids in the 7.5, 15, 30, 60 and 120 mg AMPA/L treatment groups had mean lengths of 5.2, 5.2, 5.1, 5.3 and 4.3 mm, respectively, and mean dry weights of 0.99, 1.0, 0.97, 0.69 and 0.45 mg, respectively. Dunnett's test indicated a significant decrease in length in the 30 and 120 mg AMPA/L (30 and 120 mg AMPA/L as mean measured concentration) treatment groups in comparison to the negative control ($\alpha = 0.05$).

However, the decreases noted in the 30 mg AMPA/L treatment group was not dose related. Dunnett's test indicated there was a statistically significant decrease in dry weight in the 60 and 120 mg AMPA/L (57 and 120 mg AMPA/L as mean measured concentration) treatment groups in comparison to the control ($\alpha = 0.05$).

Table 8.2.5-24: Chronic toxicity of AMPA to *Daphnia magna*

	Control	AMPA [mg/L]				
		7.5	15	30	60	120
Mortality of adults after 21 d [%]	25	20	0	30	0	10
Mean number offspring per adult	227±26.3	229 ±24.8	213 ±26.6	189 ±19.7[1]	169 ±22.1[1]	59.6 ±13.4[1]
Mean length of offspring	5.3 ±0.14	5.2 ±0.16	5.2 ±0.12	5.1 ±0.16[1]	5.3 ±0.18	4.3 ±0.17
Mean dry weight of offspring	0.99 ±0.24	0.99 ±0.12	1.0 ±0.22	0.97 ±0.25	0.69 ±0.20[1]	0.45 ±0.15[1]

[1]Indicates a statistically significant decrease in comparison to the negative control (Dunnett's one-tailed test, $\alpha = 0.05$).

After 21 days of exposure, survival in control group was 75 %. Although survival in the negative control group was slightly below the 80% criterion in OECD 211, this small difference is not considered to have impacted the validity of this study. The surviving daphnids in the control replicates appeared normal and healthy through until test end indicating that the mortality observed was attributed to incidental death and not the health of the organisms. Adult daphnids in the control group produced an average of 227 live young per surviving adult (CV = 11.6 %), well above the validity criterion of ≥ 60 live young per surviving adult. Therefore, the study is considered valid according to OECD 211.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of AMPA (aminomethylphosphonic acid) on the survival, growth and reproduction of *Daphnia magna* were evaluated in a 21-day reproduction test.
The nominal based EC_{50} values for reproduction, immobility and growth were 90 mg/L, ≥ 120 mg/L and 90 mg/L, respectively.
The no observed effect concentrations (NOEC) for immobility and growth were 30mg/L and ≥ 120 mg/L, respectively. The NOEC based on reproduction was determined to be 15 mg/L (nominal) for AMPA exposed daphnids.
The study is considered to be valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.5.1/008
Report author	Levine, S.L. *et al.*
Report year	2015
Report title	Aminomethylphosphonic acid has low chronic toxicity to *Daphnia magna* and *Pimephales promelas*
Document No	DOI: 10.1002/etc.2940 E-ISSN: 1552-8618
Guidelines followed in study	OECD 211 (2008), OECD 210 (1992)
Deviations from current test guideline	Deviations from current OECD guideline 211 (2012): None
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary

The purpose of the present study was to assess the potential for chronic toxicity of AMPA to fathead minnow (*Pimephales promelas*) and *Daphnia magna*. Chronic toxicity to *P. promelas* was evaluated in a fish early–life stage study. The primary endpoints were larval survival, growth, and development. The chronic toxicity to *D. magna* was evaluated in a *Daphnia* reproduction test. The primary endpoints were survival, growth, and reproduction.
The NOAEC for *P. promelas* was determined to be 12 mg/L, the highest concentration tested. The no-observed-effect concentration for *D. magna* was determined to be 15 mg/L.

Materials and methods

Test substance

Synthesis of AMPA was performed by Chemir, and it had a purity of 98.7 %. The water solubility for

AMPA is reported to be 10 500 mg/L (based on glyphosate acid solubility data [RMS Germany. 2013]); therefore, solvent (to aid the dissolution of AMPA into water) was not required for the aquatic exposures. Stock solutions for waterborne exposures were prepared in well water, appeared clear and colorless after mixing, and were stored under refrigerated conditions (~ 4 ± 1 °C).

For the *D. magna* reproduction study, primary stocks and test solutions were prepared every 2 d to 3 d during the test. A primary stock solution was prepared in ultraviolet sterilized dilution water at a nominal concentration of 120 mg AMPA/L, equivalent to the highest concentration tested. Proportional dilutions of the primary stock solution were made in dilution water to prepare test solutions at nominal concentrations of 7.5 mg AMPA/L, 15 mg AMPA/L, 30 mg AMPA/L, and 60 mg AMPA/L.

For the fish early–life stage study, stock solutions were delivered using syringe pumps into mixing vessels and mixed with diluent water in a continuous diluter system to prepare nominal test concentrations of 0.75 mg AMPA/L, 1.5 mg AMPA/L, 3.0 mg AMPA/L, 6.0 mg AMPA/L, and 12 mg AMPA/L. Delivery of the test solutions was started 7 d prior to the initiation of the test to achieve equilibrium of the test substance in the test chambers.

Daphnia magna reproduction study—Culturing, exposure, and observations
Daphnia magna are the required cladoceran test species under the Organisation for Economic Co-operation and Development (OECD) 211 guideline [OECD 2008]. *Daphnia magna* was tested because it is representative of an important group of freshwater invertebrates and has a long and successful history as a test organism in the laboratory. Neonates (juveniles) <24 h old were used to initiate the test and were obtained from established cultures. Parental daphnids were cultured in well water that was filtered with a 0.45-µm filter and passed through an ultraviolet sterilizer. The source of well water was characterized as moderately hard water with an average specific conductance of 362 µS/cm, hardness of 132 mg/L as $CaCO_3$, alkalinity of 173 mg/L as $CaCO_3$, and pH of 8.2 during the 4-wk period immediately preceding the test.

During the 2-wk period preceding the test, culture temperatures ranged from 19.6 °C to 20.8 °C, pH from 8.1 to 8.7, and dissolved oxygen from 7.6 mg/L to 9.5 mg/L. During culturing and testing, daphnids were fed daily with a mixture of yeast, cereal grass medium, and trout chow, as well as a suspension of the freshwater green alga *Pseudokirchneriella subcapitata*. During the test, organisms in each test chamber were fed 0.5 mL of yeast–cereal–trout chow and 1.0 mL of algae, which represented 0.60 mg C/daphnid/d. Although this amount of feed exceeded the OECD guideline recommended amount of 0.1 mg C/daphnid/d to 0.2 mg C/daphnid/d, an excess amount was fed to maintain sufficient feed in the system to support acceptable reproduction rates, which is an acceptable deviation from the testing guideline.

The 4 adult daphnids used to supply neonates for the test were held for 19 d prior to collection of the juveniles for testing and had each produced at least 1 previous brood. Adult daphnids in the culture had produced an average of at least 3 young per adult per day over the 7-d period prior to the test. The adults showed no signs of disease or stress, and no ephippia were produced during the holding period. To initiate the test, juvenile daphnids were collected from the cultures and indiscriminately transferred 1 or 2 at a time into the transfer chambers that were impartially assigned to a control or treatment group until each transfer chamber contained 10 daphnids. All animals were released from the transfer chambers into the assigned test chambers below the water surface (to avoid air contact) using wide-bore pipettes to not harm the neonates.

We tested AMPA in a semistatic renewal design with the renewal of test solutions every 2 d or 3 d. Concentrations of AMPA were measured on 3 occasions during the test: at the beginning and end of the first renewal cycle, at the beginning and end of the longest renewal cycle during the second wk of the test, and at the beginning and end of the last renewal cycle. Test chambers were 250-mL glass beakers that contained approximately 200 mL of test solution and were loosely covered with plastic Petri dishes. Beakers were impartially positioned in an environmental chamber that was programmed to maintain the target water temperature (20 ± 1 °C) throughout the test period. A 16:8-h light:dark photoperiod was used

with a 30-min transition period of low light intensity when lights went on and off to avoid sudden changes in lighting. Lighting was provided by fluorescent light bulbs that emit wavelengths similar to natural sunlight. At test initiation the light intensity at the water surface of 1 representative test chamber was 296 lux (measured with a SPER Scientific Model 840006C light meter).
Temperature was measured continuously in 2 replicate test chambers in each treatment group, and measurements rotated among replicates in each group. Dissolved oxygen and pH were measured in the newly prepared solutions for each treatment group at test initiation and on renewal days and in the old solutions from 2 replicate test chambers in each treatment and control group on renewal days and at test termination. When a first-generation daphnid was found dead, measurements of temperature, dissolved oxygen, and pH were taken in the replicate at that time and then discontinued. Hardness, alkalinity, and specific conductance were measured in batch solutions of the negative control, the highest test concentration at test initiation and on 1 renewal day each week (day 7 and day 14), and pooled replicate solutions at test termination. Total organic carbon (TOC) was measured in the dilution water at test initiation and termination using a Shimadzu model TOC-VCSH analyzer and following the *Standard Methods for the Examination of Water and Wastewater* [American Public Health Association]. Hardness and alkalinity were measured by titration based on procedures in the *Standard Methods for the Examination of Water and Wastewater* [American Public Health Association].

First-generation daphnids were observed daily during the test for immobility, the onset of reproduction, and clinical signs of toxicity. Following the onset of reproduction, the second- generation daphnids were counted 3 times per week and at test termination (day 21). Body lengths and dry weights of the surviving first-generation daphnids were measured at the end of the exposure period.

Fish early–life stage study—Culturing, exposure, and observations
Test methodology followed the procedure outlined in the OECD 210 test guideline for *P. promelas* with the exception of doubling the required level of replication [OECD 1992]. We selected *P. promelas* for the early–life stage study based on past use and ease of handling in the laboratory. Embryos (Chesapeake Cultures) were examined under a dissecting microscope to select healthy, viable specimens at approximately the same stage of development (<24 h). Embryos collected for use in the test were from 10 individual spawns and were <24 h old when the test initiated. Test chambers were 9-L glass aquaria filled with approximately 7 L of test solution and contained an embryo incubation cup attached to a reciprocating rocker arm (2 rpm) for water circulation during embryo incubation. To initiate the test, groups of 1 to 3 embryos were impartially distributed among incubation cups until each cup contained 20 embryos. A single incubation cup constructed from 50-mm-diameter glass cylinders with 425-µm nylon screen mesh attached to the bottom was placed into each test chamber. The incubation cup with the embryos was impartially assigned to each of the control and treatment groups.

The test was conducted in a temperature-controlled environmental chamber designed to maintain the target test temperature of 25 ± 1 °C throughout the test period. Temperature was measured in each test chamber at the beginning of the test, weekly during the test, and at the end of the test using a liquid-in-glass thermometer. Temperature also was monitored continuously in 1 negative control replicate using a Fulscope ER/C Recorder. Fluorescent light bulbs that emit wavelengths similar to natural sunlight were used on a 16:8-h light:dark photoperiod. A 30-min transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting.

The negative (dilution water) control and AMPA test concentrations were delivered in a continuous-flow diluter. Syringe pumps (Harvard Apparatus) delivered the stock solutions (at a rate of 30 µL/min) into mixing chambers and mixed with dilution water (at a rate of 125 mL/min) to achieve the target test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters that were calibrated prior to test initiation and verified at weekly intervals during the test. The flow of test water from each mixing chamber was split and directed into 4 replicate test chambers. The proportion of the test water that was split into each replicate was checked prior to the test and at approximately weekly intervals during the test to ensure that flow rates varied by no more than ± 10% of the mean for the 4 replicates. The diluter flow rate was adjusted to provide approximately 6 volume additions of test water in each test chamber per

day. The general operation of the diluter was checked visually at least 2 times/d during the test and at least once at the end of the test.

According to the OECD guideline, concentrations above the 96-h lethal concentration for 50% of the population or 10 mg/L, whichever is lower, need not be tested. To assure that a mean measured concentration ≥10 mg/L was tested, the highest nominal test concentration of 12 mg/L and the lower concentrations of 6 mg/L, 3 mg/L, 1.5 mg/L, and 0.75 mg/L were selected. Stock solutions were stored under refrigerated conditions, and fresh aliquots were placed in the syringe pumps daily during the test. Water samples were collected from 1 test chamber of each treatment and control group 4 d prior to test initiation to confirm the operation of the diluter. Water samples were collected from alternating replicate test chambers of each treatment and control group on day 0, day 7, day 14, day 21, day 28, and day 33 (test termination) to determine concentrations of the test substance in the test chambers. All samples were collected at mid-depth in the test chambers, placed in glass vials, and processed immediately for analysis. Dissolved oxygen and pH were measured in alternating replicates of each treatment and control group at the beginning of the test, weekly during the test, and at the end of the test. Hardness, alkalinity, and specific conductance were measured in alternating replicates of the negative control (dilution water) and the highest concentration treatment group at the beginning of the test, weekly during the test and at the end of the test. Hardness and alkalinity were measured by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* [American Public Health Association], and specific conductance was measured using an Acorn Series Model CON6 Conductivity-Temperature meter.

During the first day of exposure, embryos were observed twice for mortality and fungal infection. Thereafter, until hatching was complete, observations of embryo mortality and the removal of dead embryos were performed once daily. When hatching reached >90 % in the control groups on day 5 of the test, the larvae were released to their respective test chambers and the posthatch period began. During the 28-d posthatch exposure period, the larvae were observed daily to evaluate the mortality and the numbers of individuals exhibiting clinical signs of toxicity or abnormal behavior. From these observations, time to hatch, hatching success, and posthatch growth and survival were evaluated. Hatching success was calculated as the percentage of embryos that hatched successfully. Posthatch survival was calculated from the number of larvae that survived to test termination as a percentage of the number of embryos that hatched successfully.

Newly hatched larvae were fed live brine shrimp nauplii (*Artemia* sp.) 3 times/d during the first 7 d of the posthatch period. Thereafter, they were fed live brine shrimp nauplii 3 times/d on weekdays and at least 2 times/d on weekends. Fish were not fed for approximately 48 h prior to the termination of the test to allow for clearance of the digestive tract before weight measurements were made. To ensure that the feeding rate per fish remained constant, rations were adjusted at least weekly. The test chamber loading rate (the total wet wt of fish per liter of water in the tank) at the end of the test was 0.32 g fish/L.
Posthatch growth of *P. promelas* was evaluated at the conclusion of the 28-d posthatch exposure period. Total length for each surviving fish was measured to the nearest 1mm using a metric ruler, with wet and dry weights measured to the nearest 0.1 mg using an analytical balance. Fish were placed in an oven at 60 °C for up to approximately 48 h to obtain dry weight data.

Analytical method for detection of AMPA
Samples were diluted, as appropriate, with freshwater. The 2.0 mL of diluted sample and/or external calibration standards were placed into the 15-mL test tube. Then, 1.0 mL of 0.37 M aqueous potassium tetraborate was added to each test tube, followed by 2.0 mL of 0.025 M NBD-C1 (methanolic) for derivatization. Solutions were capped, mixed, and heated at approximately 80 °C for 40 min. Next, 1.0 mL of 1.2 M HCl was added to each test tube, and samples were then left undisturbed for approximately 10 min prior to analysis. Samples (25 µL injection volume) were analyzed on an Agilent Series 1100/1200 high performance liquid chromatograph equipped with an Agilent Series 1100 variable wavelength detector at 500 nm. Chromatographic separations were achieved using a YMC-Pack ODS-AM (150 mm × 4.6 mm, 3 µm particle size) analytical column at a temperature of 40 °C and eluted over a gradient of 0.1% H_3PO_4 (solvent A) and CH_3CN (solvent B). The retention time for AMPA was approximately 6.5 min to 7.3 min,

and the method limit of quantitation for these analyses was defined as 0.4 mg AMPA/L.

Statistical and power analyses

Test endpoints analyzed statistically in the *Daphnia* test for first-generation daphnids were survival, reproduction (the number of live young produced per 21-d surviving adult), and growth (length and dry wt). Neonates produced by those first-generation daphnids that did not survive the full 21 d were excluded from analysis of reproduction.

Test endpoints analyzed statistically in the fish early–life stage test were hatching success, larval survival, and growth (total length, wet wt, and dry wt). Data on time to hatch were evaluated by visual interpretation.

Discrete-variables data were analyzed using Fisher's exact test to identify treatment groups that showed a statistically significant difference ($p \leq 0.050$) from the negative control. All continuous-variable data were evaluated for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test ($p = 0.010$). When the data passed the assumptions of normality and homogeneity of variance, those treatments that were significantly different from the control means were identified using the 1-tailed Dunnett's test ($p \leq 0.050$). All statistical tests were performed using a personal computer with SAS software. The results of the statistical analyses were used to aid in the determination of the no-observed–adverse effect concentration (NOAEC), defined as the greatest test concentration that produced no significant treatment-related adverse effects on survival, reproduction, or growth.

Results

Daphnia magna survival, growth, and reproduction

Water temperatures were maintained within the targeted range of 20 ± 1 °C, dissolved oxygen concentrations remained ≥76 % of saturation (6.8 mg/L), and pH ranged from 7.1 to 8.6 during the test. Specific conductance, hardness, and alkalinity were similar between the control and treatment groups and did not appear to be influenced by AMPA. The TOC in the dilution water at test initiation and termination was <1 mg C/L.

Table 8.2.5-25: Means and ranges of water quality measurements taken during the 21-Day D. magna exposure to AMPA

Mean Measured Concentration (mg AMPA/L)	Mean ± Std. Dev. and Range of Measured Parameters					
	Temperature (°C)	Dissolved Oxygen [1] (mg/L)	pH	Hardness [2] (mg/L as $CaCO_3$)	Alkalinity [2] (mg/L as $CaCO_3$)	Conductivity [4] (µS/cm)
Negative Control	19.8 ± 0.55 (19.1 – 20.8)	8.2 ± 0.58 (7.2 – 9.1)	8.4 ± 0.10 (8.3 – 8.6)	135 ± 2 (132 - 136)	167 ± 5 (160 – 171)	339 ± 46 (280 – 391)
7.4	19.9 ± 0.48 (19.2 – 20.7)	8.0 ± 0.60 (7.1 – 9.1)	8.4 ± 0.09 (8.3 – 8.6)	--	--	--
15	19.9 ± 0.50 (19.1 – 20.8)	7.9 ± 0.65 (6.9 – 9.1)	8.4 ± 0.08 (8.2 – 8.6)	--	--	--
30	19.9 ± 0.54 (19.0 – 20.7)	7.9 ± 0.66 (6.8 – 9.1)	8.3 ± 0.10 (8.2 – 8.5)	--	--	--
57	20.0 ± 0.52 (19.1 – 20.6)	7.9 ± 0.60 (7.0 – 9.1)	8.2 ± 0.23 (7.7 – 8.5)	--	--	--
120	20.1 ± 0.54 (19.1 – 20.7)	8.0 ± 0.54 (7.1 – 9.1)	7.9 ± 0.48 (7.1 – 8.4)	139 ± 2 (136 – 140)	164 ± 6 (156 – 170)	340 ± 46 (274 – 381)

[1] A dissolved oxygen concentration of 9.1 mg/L represents 100% saturation at 20°C in freshwater. Any recorded dissolved oxygen measurement greater than 100% saturation is reported as 9.1 mg/L.

[2] -- = no measurements scheduled.

Measured concentrations of AMPA for the *D. magna* study were close to nominal concentrations throughout the renewal periods. Concentrations of AMPA in the new test solutions prepared and sampled on day 0, day 9, and day 19 ranged from 92.5% to 106% of the nominal concentrations. Concentrations of AMPA in the old test solutions sampled immediately prior to renewal on day 2, day 12, and at test termination on day 21 ranged from 78.6% to 117% of the nominal concentrations. When the measured concentrations of the samples collected during the test were averaged for each treatment group, the mean measured test concentrations were 7.4 mg AMPA/L, 15 mg AMPA/L, 30 mg AMPA/L, 57 mg AMPA/L, and 120 mg AMPA/L.

There was no significant effect of AMPA on individually exposed first-generation daphnids across the treatments, and survival was ≥80%. A summary of adult survival is presented in Figure A below. After 21 d of exposure, survival in the negative control, 7.4–mg AMPA/L, 15–mg AMPA/L, 30–mg AMPA/L, 57–mg AMPA/L, and 120–mg AMPA/L groups was 75 %, 80 %, 100 %, 70 %, 100 %, and 90 %, respectively. Although survival in the negative control group was slightly below the 80 % criterion in OECD guideline 211, it is not considered to have impacted the validity of the present study because of the small difference and with the final mortality occurring near the end of the study. In addition, there was ≥80 % survival in all treatment groups. The surviving daphnids in the control replicates appeared normal and healthy through the end of the test, indicating that the mortality observed was attributed to incidental death and not the health of the organisms. In addition, the percentage survival of the control replicates was within the control criterion of 70% as specified in the American Society for Testing and Materials standard guide E 1193-97 [ASTM International. 1997]. Survival in the 7.4–mg AMPA/L, 15–mg AMPA/L, 30–mg AMPA/L, 57–mg AMPA/L, and 120–mg AMPA/L treatment groups at test termination did not follow a concentration–response pattern and was 80 %, 100 %, 70 %, 100 %, and 90 %, respectively. No significant differences in survival were detected in any of the AMPA treatment groups in comparison with the control ($p > 0.05$, Fisher's exact test). Consequently, the no-observed-effect concentration (NOEC) for survival was 120 mg AMPA/L.

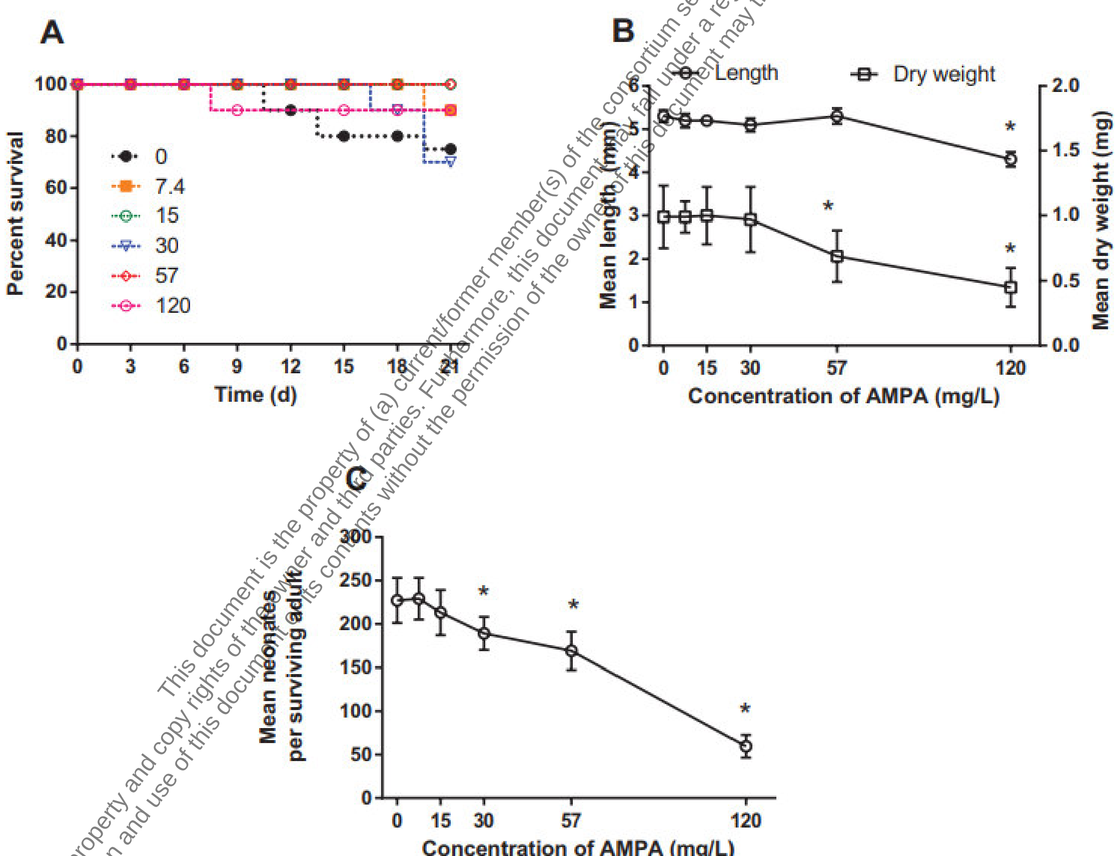


Figure 8.2.5-1: (A) Survival (percentage) of *Daphnia magna* exposed to increasing concentrations of AMPA for 21 d. (B) Sublethal endpoints in 21-d chronic *D. magna*: body length and dry weight. (C) Reproductive endpoint neonates per surviving adults. * Statistically significant difference ($p < 0.05$) from the control (0 mg/L).

Daphnids in the 7.4–mg AMPA/L, 15–mg AMPA/L, 30–mg AMPA/L, and 57–mg AMPA/L treatment groups that survived until test termination generally appeared normal. In the 120–mg AMPA/L treatment

group, all surviving first-generation daphnids appeared pale in comparison with the control organisms from day 5 through the end of the test. Daphnids in this treatment group were also observed to be smaller than the control organisms from day 7 through the end of the test. All surviving daphnids in the 7.4–mg AMPA/L, 15–mg AMPA/L, 30–mg AMPA/L, and 57–mg AMPA/L treatment groups were normal in appearance throughout the test and at test termination, with the exception of 1 daphnid in the 57–mg AMPA/L treatment group that appeared pale on day 6 of the test but appeared normal from day 7 to the end of the test.
A summary of production of neonates by surviving first-generation daphnids is presented in Figure C above. The first day of brood production in the negative controls and in all AMPA treatment replicates was day 7, day 8, or day 9 of the test, indicating that there was no apparent delay in the onset of production at any concentration of AMPA tested. Immobile neonates were noted in the control, 7.4–mg AMPA/L, and 57–mg AMPA/L treatment groups. However, the mean number of immobile neonates per surviving adult in these replicates was less than 1. No aborted brood or aborted eggs were present in the control or any of the AMPA treatment replicates. No males or ephippia were produced during the test.

Summaries of the mean lengths and dry weights of surviving first-generation daphnids are presented in Figure 8.2.5-2 'B' below. Daphnids in the negative control group averaged 5.3 mm in length and 0.99 mg in dry weight. Daphnids in the 7.4–mg AMPA/L, 15–mg AMPA/L, 30–mg AMPA/L, 57–mg AMPA/L, and 120–mg AMPA/L treatment groups had mean lengths of 5.2 mm, 5.2 mm, 5.1 mm, 5.3 mm, and 4.3 mm, respectively, and mean dry weights of 0.99 mg, 1.0 mg, 0.97 mg, 0.69 mg, and 0.45 mg, respectively. There were significant decreases in length in the 30–mg AMPA/L and 120–mg AMPA/L treatment groups in comparison with the negative control ($p \leq 0.05$) but not in the 57–mg AMPA/L treatment group. There were significant decreases in dry weight in the 57–mg AMPA/L and 120–mg AMPA/L treatment groups in comparison with the negative control ($p \leq 0.05$). Consequently, the NOEC for growth was 30 mg AMPA/L.

Adult daphnids in the negative control group produced an average of 227 live young per surviving adult (coefficient of variance of 11.6%), well above the validity criterion of ≥60 live young per surviving adult. Adult daphnids in the 7.4–mg AMPA/L, 15–mg AMPA/L, 30–mg AMPA/L, 57–mg AMPA/ L, and 120–mg AMPA/L treatment groups produced an average of 229, 213, 189, 169, and 59.6 live young per surviving adult, respectively. There was a significant decrease in mean neonate production in the 30–mg AMPA/L, 57–mg AMPA/L, and 120–mg AMPA/L treatment groups in comparison with the negative control ($p \leq 0.05$). Consequently, the NOAEC for reproduction is 15 mg AMPA/L.

Pimephales promelas embryo hatching success, growth, and survival
Samples of the test solutions collected during the test had measured concentrations that ranged from 82.5 % to 117 % of nominal concentrations. When the measured concentrations of test solution samples collected on day 0, day 7, day 14, day 21, day 28, and day 33 of the test were averaged for each treatment group, the mean measured test concentrations were 0.73 mg AMPA/L, 1.5 mg AMPA/L, 2.9 mg AMPA/L, 6.0 mg AMPA/L, and 12 mg AMPA/L, which represented 97 %, 100 %, 97 %, 100 %, and 100 % of nominal concentrations, respectively. Therefore, the results of the present study have been based on mean measured concentrations. The analytical results are summarized in Supplemental Data, Table S4.
Hatching success of the *P. promelas* embryos is summarized in Figure 8.2.5-2'A' below. Daily observations of the embryos indicated that there were no apparent differences in time to hatch between the negative control group and any of the AMPA treatment groups. All *P. promelas* embryos in the control and treatment replicates hatched by day 5 of the test. Hatching reached >90 % in the control groups on day 5 of the test, at which time the larvae were released to their respective test chambers.

Hatching success in the negative control, 0.73–mg active ingredient (a.i.)/L, 1.5–mg a.i./L, 2.9–mg a.i./L, 6.0–mg a.i./L, and 12–mg a.i./L treatment groups was 99 %, 100 %, 100 %, 100 %, 100 %, and 99 %, respectively (Figure A below). There were no statistically significant differences in hatching success in any of the AMPA treatment groups in comparison with the negative control ($p > 0.05$). Larval survival in the negative control, 0.73–mg a.i./L, 1.5–mg a.i./L, 2.9–mg a.i./L, 6.0–mg a.i./L, and 12–mg a.i./L treatment groups was 91 %, 91 %, 93 %, 90 %, 91 %, and 92 % (Figure A below), respectively; and there were no

statistically significant differences in hatching success in any of the AMPA treatment groups in comparison with the negative control ($p > 0.05$). In addition, there were no statistically significant reductions in total length, wet weight, and dry weight (Figure B below) among fish in the AMPA treatment groups in comparison with the negative control ($p > 0.05$). Based on an evaluation of each of these endpoints, the NOAEC for growth was 12 mg a.i./L.

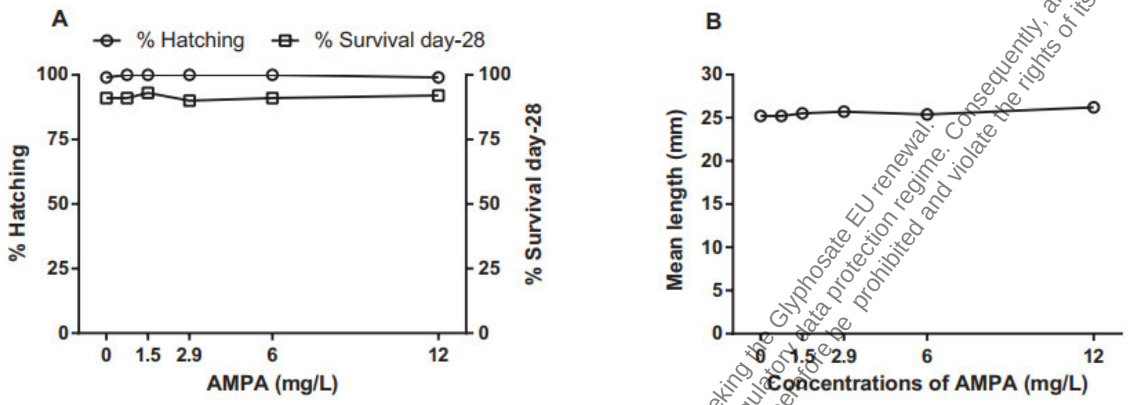


Figure 8.2.5-2: (A) Hatching success (percentage) and survival (percentage) at day 28 of *Pimephales promelas* in an early–life stage study with aminomethylphosphonic acid (AMPA). (B) Body length (millimeters) of *P. promelas* exposed to AMPA.

Table 8.2.5-26. Means and Ranges of Water Quality Measurements Taken During the 33-Day P. promelas Exposure to AMPA

Mean Measured Concentration (mg AMPA/L)	Mean ± SD and Range of Measured Parameters					
	Temperature (°C)	DO[2] (mg/L)	pH	Hardness[2] (mg/L as $CaCO_3$)	Alkalinity[2] (mg/L as $CaCO_3$)	Conductivity[2] (µS/cm)
Assay Control	24.8 ± 0.27 (24.4 – 25.4)	7.9 ± 0.40 (7.4 – 8.2)	8.1 ± 0.10 (8.0 – 8.2)	136 ± 3 (132 – 140)	172 ± 5 (166 – 178)	378 ± 11 (361 – 393)
0.73	24.7 ± 0.59 (23.7 – 25.5)	7.9 ± 0.39 (7.4 – 8.2)	8.1 ± 0.09 (8.0 – 8.2)	-- --	-- --	-- --
1.5	24.9 ± 0.66 (23.9 – 25.7)	7.9 ± 0.39 (7.3 – 8.2)	8.1 ± 0.10 (8.0 – 8.2)	-- --	-- --	-- --
2.9	25.0 ± 0.52 (24.2 – 25.6)	7.9 ± 0.33 (7.5 – 8.2)	8.1 ± 0.12 (7.9 – 8.2)	-- --	-- --	-- --
6.0	25.0 ± 0.46 (24.3 – 25.7)	7.9 ± 0.34 (7.5 – 8.2)	8.0 ± 0.12 (7.9 – 8.2)	-- --	-- --	-- --
12	25.1 ± 0.48 (24.3 – 25.7)	7.9 ± 0.37 (7.4 – 8.2)	7.9 ± 0.13 (7.8 – 8.1)	138 ± 2 (136 – 140)	174 ± 4 (170 – 180)	379 ± 11 (365 – 395)

[1] A dissolved oxygen concentration of 4.9 mg/L represents 60% saturation at 25°C in freshwater. Any recorded DO measurement greater than 100% saturation is reported as 8.2 mg/L.
[2] -- = no measurements scheduled.

Conclusion

For *D. magna* exposed to concentrations ranging from 7.4 mg AMPA/L to 120 mg AMPA/L for 21 d, reproduction was the most sensitive endpoint with significant treatment-related effects noted at 30 mg AMPA/L, 57 mg AMPA/L, and 120 mg AMPA/L. Consequently, the NOAEC based on reproduction was 15 mg AMPA/L. No impact was noted on hatching success, survival, or growth in *P. promelas* embryos exposed to concentrations ranging from 0.73 mg AMPA/L to 12 mg AMPA/L for 33 d. Consequently, the NOAEC was 12 mg AMPA/L, the greatest concentration tested. These values exceed the worst-case water concentrations from conservative modelling and surface water monitoring data by 2 to 3 orders of magnitude, indicating no unacceptable chronic risk for vertebrate and invertebrate aquatic organisms from environ mental exposure to AMPA.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Chronic toxicity tests of the glyphosate environmental metabolite aminomethylphosphonic acid (AMPA) were performed with fathead minnow (*Pimephales promelas*) and *Daphnia magna*.
During a 21-d exposure period under semi-static test conditions the effects on survival, growth, and reproduction of the cladoceran *Daphnia magna* were determined resulting in a no-observed-effect concentration (NOEC) of 15 mg AMPA/L. During a 33-d exposure period under continuous renewal test conditions the effects on time to hatch, hatching success, posthatch growth and survival of the fish *Pimephales promelas* were assessed resulting in an NOAEC of 12 mg AMPA/L, the highest tested concentration. Test methodology followed the procedure outlined in the OECD 210 test guideline for *P. promelas*. For the chronic test on *Daphnia magna* the OECD 211 guideline is mentioned in the full text.

The study is well documented and all relevant information, e.g. information on the test item, test design, application method and implementation of the study, is available. In addition, a chemical analysis of test solutions was performed. All information for evaluation of the study is given. The study is considered as reliable.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

As glyphosate is not an insecticide or insect growth regulator, studies on the reproductive and development toxicity to an additional aquatic invertebrate species are not required.

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

As glyphosate is not an insecticide or insect growth regulator, studies on the development and emergence in *Chironomus riparius* are not required. Nevertheless, the following studies are available.

1. Information on the study

Data point:	CA 8.2.5.3/001
Report author	[redacted]
Report year	2020
Report title	MON 77973: A Study on the Toxicity to the Sediment Dweller *Chironomus riparius* Using Spiked Water
Report No	20FV2ME ~~(Interim Report – no analytical report presented)~~
Document No	-
Guidelines followed in study	OECD guideline 219 (2004)
Deviations from current test guideline	Deviations from the guideline OECD 219 (2004): Minor: - Samples of sediment and pore water were not taken or analysed based on the concentrations of the test item in the overlying water measured during the range-finding test (>80 % of nominal at test start in the overlying water column at start of exposure and > 50 % of nominal for the duration of the range-finding trial). Analysis of overlying water only is therefore

considered to be sufficient and to reflect the exposure situation in this study. An impact on the integrity of this study can therefore be excluded.

- Several midges in the control emerged later than required in the guideline. Since total emergence in the control exceeded 90 % of inserted animals, and since more than 89 % of the emerged control midges had emerged by day 23, this is not considered to have any impact on the integrity of the study

Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

In a sediment-water toxicity test using spiked water first-instar larvae of freshwater dipteran *Chironomus riparius* were exposed to MON 77973 concentrations of 100 and 1000 mg a.e./L according to OECD 219 for 28 days. Exposure concentrations were based on results of a range-finding test conducted at 0.1 – 1000 mg a.e./L. The test was conducted using a limit test design at the two rates with eight replicates prepared per test item concentration and the control, with 20 organisms added per test vessel. Three times per week, the larvae were fed using a TetraMin® suspension, with the food ration increased accordingly during the test. At least three times per week the test vessels were observed in order to visually assess any behavioural differences compared with the control. Daily from day 11 the vessels were checked for emerged midges.

A concentration-response relationship of MON 77973 was not observed for emergence ratio and development rate after 28 days of exposure. A statistically significant inhibition compared to the control was not found up to and including the highest test concentration. Glyphosate was not detected in the control group. The measured concentrations of glyphosate at test initiation were 89.4 and 81.7 % of nominal for the 100 and 1000 mg a.e/L test concentrations, respectively. At day 28 the measured concentrations were 55.8 and 71.1 % of nominal for the 100 and 1000 mg a.e/L test concentrations, respectively. The biological results are expressed based on nominal concentrations in accordance with the guideline requirements. Therefore, NOEC and LOEC values were ≥ 1000 mg a.e./L and > 1000 mg a.e./L, respectively, based on nominal test concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 77973 (Glyphosate acid)
Description:	White crystalline powder
Lot/Batch #:	11493988
Purity:	97.7 wt% (acid equivalent: a.e.)

2. Vehicle and/or positive control:

Vehicle: Test medium

3. Test organism:

Species: *Chironomus riparius* (Meigen)

Age of animals:	1st instar larvae
Loading:	20 larvae/vessel
Fill volume:	570 mL
Replication:	8 replicates per test item concentration and the control
Source of animals:	House cultures, originally supplied by Aventis, D-65962 Frankfurt am Main
Diet/Food:	TetraMin® suspension three times a week Feed rate: - Day 0 – 10 = 0.25 – 0.5 mg TetraMin® per day - Day 11 until end = 0.5 – 1 mg TetraMin® per day
Acclimation period:	Egg masses and hatching larvae were maintained for at least 5 days prior to addition to the test vessels as 1st instar larvae. Animal addition occurred one day prior to spiking. Animal addition occurred after sediment had been added to test vessels and covered with test medium and acclimated under test conditions for 2 days.
4. Environmental conditions:	
Artificial Sediment:	Yes, according to OECD 219 (2004), peat content 4.8% of sediment dry weight; sediment water ratio approx. 1:4
Temperature:	19.8-21.3 °C
Photoperiod:	16 h light:8 h dark
pH range	7.6-8.3
Duration:	28 days
Dissolved oxygen range	7.1-9.1
Hardness:	254-336 mg/L $CaCO_3$
5. Dates of experimental work:	10th February to 24th March 2020

B. STUDY DESIGN AND METHODS

Experimental conditions

First-instar larvae were exposed to MON 77973 concentrations of 100 and 1000 mg a.e./L according to OECD 219 for 28 days. Eight replicates were used per test item concentration and the control designed as a limit test. Prior to application of the test item, the formulated sediment was conditioned for 7 days. For this purpose it was covered with Medium M4 (sediment:water volume ratio 1:4 (±≤ 0.5)) and was incubated under the same conditions which prevailed in the subsequent test.

A stock solution was prepared by adding 10.0 g nominal of the test item to 1000 mL of test medium. After 2 min ultrasonication and 30 min stirring, the test item had dissolved and the stock solution appeared clear. This stock solution was used undiluted as the application solution for preparation of treatment of 1000 mg/L. 100 mL of this stock solution were diluted to 1000 mL in order to prepare the application solution for treatment of 100 mg/L. The chironomid larvae were introduced into the test vessels one day prior to spiking. One day after addition of the larvae, the test item was added to the overlying water of each test vessel.

Per test vessel, an aliquot of 57 mL (nominal) of the application solutions were carefully mixed with the nominal volume of 513 mL of test medium present in each test vessel to obtain a total volume of 570 mL. Vessels were aerated daily on workdays in all test vessels. Three times per week, the larvae were fed with TetraMin®. The food ratio was 0.25–0.5 mg TetraMin® per day and larva from day 0 to day 10 and 0.5–1 mg TetraMin® per day and larva from day 11 until the end of the exposure. At least three times per

week the test vessels were observed in order to assess visually any behavioural differences compared with the control. Daily from day 11, the vessels were checked for emerged midges. Dissolved oxygen content and pH were measured in one test vessel of each concentration level and the control at start of exposure and once per week; in all test vessels at the end of the exposure. Temperature was monitored in one test vessel of each concentration level and the control at start of exposure and once per week and in all test vessels at the end of the exposure.

Analytical procedures

~~This is an ongoing study and details to analytical work are not yet available.~~

To verify the nominally applied concentrations, samples were taken from the overlying water.

Statistical calculations

To determine whether there were sex-specific effects, a Chi^2-Contingency test (one-sided greater; alpha 0.05) was performed. Since there was no significant effect on the sex ratio, the biological parameters emergence ratio and development rate were evaluated for pooled male and female emerged midges. Dunnett's multiple t-test procedure was used to evaluate whether there were significant differences between the control and the various test item concentrations (emergence ratio and development rate). Normal-distribution of data was tested with the Kologorov-Smirnov test (alpha: 0.01). Levene's test (p: 0.01) was used to test variance homogeneity. In one of the replicates, zero midges emerged during the test. The reasons are not clear, since oxygen concentrations, aeration monitoring and observation of test vessels documentation gave no hint. This replicate was therefore excluded from statistical evaluation of emergence. The statistical software package ToxRatPro® 3.3.0 (ToxRat Solutions GmbH, Naheweg 15, D-52477 Alsdorf) was used for these calculations.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data:

~~This study summary presented the biological results from an interim report, the analytical data and procedures will be presented in a final report.~~

Samples were taken from the overlying water at day 0 and day 28 to verify the nominally applied concentrations. The results are summarised below.

Table 8.2.5-27: Summary of analytical results. Concentrations of glyphosate (a.e.) measured in the overlying water.

Test period [d]	Nominal concentration [mg test item/L]	Nominal concentration [mg a.e/L]*	Measured concentration [mg a.e./L]	% of nominal concentration
0	Control	0	n.d.	n.a.
0	100	97.7	87.3	89.4
0	1000	977	798	81.7
28	Control	0	n.d.	n.a.
28	100	97.7	54.5	55.8
28	1000	977	695	71.7

*Using the test item purity of 97.7 wt% (a.e.); limit of quantification (LOQ) was 10 mg/L for water
n.d.: not detectable (< limit of detection: 3 mg/L for water)
n.a.: not applicable.

The initially measured concentrations of the test item in the overlying water represent ≥80 % of the nominal concentrations. The biological results are therefore expressed based on nominal concentrations in accordance with the guideline requirements.

Biological data:

Table 8.2.5-28: Number and emergence ratio of midges emerged per replicate of each treatment at end of exposure.

Nominal concentration [mg a.e./L]	Number of midges emerged								
Replicate	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**Mean**
Control	18	17	19	18	18	19	19	17	18.1
100	17	17	17	17	18	20	18	18	17.8
1000	0	17	18	19	20	18	19	15	15.8
	Emergence ratio								
Control	0.90	0.85	0.95	0.90	0.90	0.95	0.95	0.85	0.906
100	0.85	0.85	0.85	0.85	0.90	1.00	0.90	0.90	0.888
1000	0.00	0.85	0.90	0.95	1.00	0.90	0.95	0.75	0.788

Table 8.2.5-29: Mean development rates [1/d] of the midges (males & females, pooled) per replicate of each treatment and mean development rate per treatment.

Concentration [mg a.e./L]	Control	100	1000
Replicate			
a	0.04121	0.04916	-
b	0.05340	0.05645	0.05087
c	0.04850	0.05309	0.05002
d	0.04829	0.04557	0.04839
e	0.05157	0.05577	0.04557
f	0.05235	0.04871	0.05165
g	0.05067	0.04970	0.05188
h	0.05105	0.04944	0.04995
Mean	0.04963	0.05099	0.04976
SD	0.003826	0.003760	0.002193

B. OBSERVATIONS

No concentration-dependent observations were recorded. Across all treatments, emerged midges were occasionally observed to be unfit to fly or reluctant to leave the water surface. One dead pupa was observed at 100 mg a.e./L. A concentration-response relationship of MON 77973 was not observed for emergence ratio and development rate after 28 days of exposure. A statistically significant inhibition compared to the control was not found up to and including the highest test concentration. Therefore, NOEC and LOEC values were ≥ 1000 mg a.e./L dry sediment and > 1000 mg a.e./L, respectively.

Validity criteria

In order to consider the test to be valid according to OECD 219, the following conditions should be fulfilled:

- The emergence in the controls must be at least 70% at the end of the test
- Emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels
- At the end of the test, pH and the dissolved oxygen concentration should be measured in each vessel. The oxygen concentration should be at least 60% of the air saturation value at the temperature used, and the pH of overlying water should be in the 6-9 range in all test vessels.
- the water temperature should not differ by more than ± 1.0 °C.

Several midges in the control emerged later than required in the guideline. However, since total emergence in the control exceeded 90% of inserted animals, and since more than 89% of the emerged control midges had emerged by day 23, this is not considered to have any impact on the integrity of the study. The study is therefore considered valid.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In a sediment-water toxicity test using spiked water, *Chironomus riparius* was exposed to MON 77973 concentrations of 100 and 1000 mg a.s./L according to OECD 219. Based on nominal concentrations, the derived NOEC and LOEC were ≥ 1000 mg a.s./L and > 1000 mg a.s./L, respectively.
The study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

CA 8.2.5.4 Sediment dwelling organisms

This study is required, if the EC_{10} or NOEC of the chronic Daphnia test is below 0.1 mg/L and the test substance is considered to partition to the sediment, according to the EFSA aquatic guidance document (2015). Since the chronic Daphnia endpoint is 12.5 mg/L, this study is not considered necessary.

CA 8.2.6 Effects on algal growth

Studies on effects of the active substance glyphosate and its relevant metabolites on aquatic macrophytes to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

CA 8.2.6.1 Effects on growth of green algae

Studies considering the effects of glyphosate on algal growth were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Where appropriate position papers are available as indicated in the table below, which contain details regarding the statistical re-evaluation of the study to current requirements. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 0-1: Studies on effects of glyphosate and metabolites to green algae

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.6.1/001	[redacted] 2002	96 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	IPA salt	valid	-

Table 0-1: Studies on effects of glyphosate and metabolites to green algae

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.6.1/002	[redacted] 2002	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	Glyphosate K-salt	invalid	Coefficient of variation for section specific growth rate: > 35%
CA 8.2.6.1/003	[redacted], 2000	96 h algae inhibition	*Selenastrum caprocornutum* *(Raphidocelis subcapitata)*	Glyphosate technical	supportive	No analytical verification of test concentrations throughout the test
CA 8.2.6.1/004	[redacted] 2020	Position Paper	*Selenastrum caprocornutum* *(Raphidocelis subcapitata)*	Glyphosate	valid	-
CA 8.2.6.1/005	[redacted] 1995	120 h algae inhibition	*Selenastrum caprocornutum* *(Raphidocelis subcapitata)*	Glyphosate acid	valid	-
CA 8.2.6.1/006	[redacted] 2020	Position Paper	*Selenastrum caprocornutum* *(Raphidocelis subcapitata)*	Glyphosate acid	valid	-
CA 8.2.6.1/007	[redacted] 1995	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	Glyphosate	invalid	Coefficient of variation for section specific growth rate: > 35%
CA 8.2.6.1/008	[redacted], 1995	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	Glyphosate	invalid	Report not available
CA 8.2.6.1/009	[redacted] 1987	168 h algae inhibition	*Selenasstrum capricornutum* *(Raphidocelis subcapitata)*	Glyphosate technical	valid	-
CA 8.2.6.1/010	[redacted] 2020	Position Paper	*Selenasstrum capricornutum* *(Raphidocelis subcapitata)*	Glyphosate technical	valid	-
CA 8.2.6.1/011	[redacted] 1995	72 h algae inhibition	*Desmodesmus subspicatus*	Glyphosate acid	supportive	Report not available
CA 8.2.6.1/012	[redacted] 1994	72 h algae inhibition	*Desmodesmus subspicatus*	IPA salt	supportive	Report not available
CA 8.2.6.1/013	[redacted], 1993	72 h algae inhibition	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	IPA salt	invalid	Report not available
CA 8.2.6.1/014	[redacted], 1990	96 h algae inhibition	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	Glyphosate	invalid	Numerous deviations from guideline
CA 8.2.6.1/015	[redacted], 1990	96 h algae inhibition	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	Glyphosate	invalid	Coefficient of variation for section specific growth rate: > 35%

Table 0-1: Studies on effects of glyphosate and metabolites to green algae

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.6.1/016	[redacted], 1998	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	AMPA	invalid	Coefficient of variation for section-by-section specific growth rate: > 35%; only study currently available for algae exposed to AMPA
CA 8.2.6.1/017	[redacted] 2020	Position Paper	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	AMPA	valid	
CA 8.2.6.1/018	[redacted] 1994	72 h algae inhibition	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	AMPA	invalid	Noumerous deviations from guideline
CA 8.2.6.1/019	[redacted] 2011	72 h algae inhibition	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	HMPA	valid	-
CA 8.2.6.1/020	[redacted] 2020	Position Paper	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	HMPA	valid	-

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the effects of glyphosate or its relevant metabolites on growth of green alga. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. For discussions of literature regarding toxicity to algae, please refer to document M-CP Section 10.2.

Endpoints of studies considered valid for glyphosate are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 0-2: Endpoints: Toxicity of glyphosate to green algae

Reference*	Test item	Species	Test design	Endpoints expressed as	72h ErC50 (mg a.e./L)	72h EyC50 (mg a.e./L)	NOErC (mg a.e./L)
[redacted] 2002 CA 8.2.6.1/001	IPA salt	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	96 h algae inhibition	am	23.5	6.85	2.21

Table 0-2: Endpoints: Toxicity of glyphosate to green algae

Reference*	Test item	Species	Test design	Endpoints expressed as	72h ErC50	72h EyC50	NOErC
					(mg a.e./L)		
[redacted] 1995 CA 8.2.6.1/005	Glyphosate acid	*Selenastrum caprocornutum* *(Raphidocelis subcapitata)*	120 h algae inhibition	nom	18.9	16.4	10.0
[redacted] 1987 CA 8.2.6.1/009	Glyphosate acid	*Selenasstrum capricornutum* *(Pseudokirchneriella subcapitat)*	168 h algae inhibition	nom	27.4	12.1	< 10.0

* Endpoints for Smyth 1995 and Hughes 1987 are based on statistical re-evaluation provided in Position Papers: CA 8.2.6.1/006, CA 8.2.6.1/010;
a.e.: acid equivalents; nom: nominal; am: arithmetic mean measured

According to the provisions of the new Guidance Document on Aquatic Ecotoxicology (2013), E_rC_{50} endpoints shall be chosen for the risk assessment if available. The most sensitive 72 h algal endpoint is 18.9 mg a.e./L for the active substance.

Endpoints of studies considered valid for HMPA are shown in the table below. Since fully valid algae study with AMPA is not available (according to the current OECD test guidelines), the study by [redacted] 1998 (CA 8.2.6.1/016) is used.

Table 0-3: Endpoints: Toxicity of AMPA and HMPA to green algae

Reference[1]	Test item	Species	Test design	Endpoints expressed as	72h ErC50[2]	72h EyC50	NOErC
					(mg/L)		
[redacted] 1998 CA 8.2.6.1/016	AMPA	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	72 h algae inhibition	nom	191	110	100
[redacted] 2011 CA 8.2.6.1/019	HMPA	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	72 h algae inhibition	nom	> 120	> 120	60

[1] All endpoints are based on statistical re-evaluation provided in Position Papers: CA 8.2.6.1/017 and CA 8.2.6.1/020
[2] According to the provisions of the new Guidance Document on Aquatic Ecotoxicology (2013), ErC50 endpoints shall be chosen for the risk assessment if available.

Summary of the studies are provided below.

1. Information on the study

Data point:	CA 8.2.6.1/001
Report author	[redacted]
Report year	2002
Report title	A study on the Toxicity of Glyphosate isopropylamine salt 62.5 % to Algae (*Pseudokirchneriella subcapitata*)
Report No	A-99-02-04
Document No	-
Guidelines followed in study	OECD Guideline 201, EEC Directive 92/69 C.3
Deviations from current test guideline	Deviation from the guideline OECD 201 (2011): Minor: - The pH-values of the algal medium recommended by, "Schlösser (1982). Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen (SAG) - List of Strains", were lower than reported in OECD 201. In correlation with the slightly lower pH values measured in concentration 100.0 mg/L there could be an effect on the growth rate of the algae. - Analysis of the results were based on average recovery value instead of the geometric mean concentrations. The study is considered valid as all validity criteria were met.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate isopropylamine salt on *Pseudokirchneriella subcapitata* were evaluated in a 96-hour static toxicity test at nominal concentrations of 4.27, 9.39, 20.66, 45.45 and 100 mg test item/L. A negative control group (culture medium only) was prepared in parallel. The test vessels were 300 mL Erlenmeyer glass flasks containing 100 mL of control or test medium .The initial algal cell concentration was 1 × 104 cells/mL. At 24, 48, 72 and 96 hours, the algal cell densities in all treatment and control vessels was determined and the inhibition in cell growth, relative to the control group was determined. Cell densities were used to calculate endpoints in terms growth rate and biomass (ErC50, EbC50 and NOEC values), based on the nominal and measured glyphosate concentrations (average recovery rate was 70.1 %) derived from the chemical analysis.

At the start of the test, measured concentrations of glyphosate acid ranged between 68.9 and 80.6 % of nominal. At the end of the test, they ranged between 52.0 and 73% of nominal in the (low, mid and high) 4.27, 20.66 and 100 mg test item/L treatments. Glyphosate acid was not detected in the control group.

The validity criteria according to guideline OECD 201 are therefore fulfilled. The 72 h and 96 h ErC50 values for *Pseudokirchneriella subcapitata* exposed to glyphosate isopropylamine salt were calculated to be 31.70 and 32.01 mg/L, equivalent to 23.48 and 23.71 mg glyphosate acid/L (mean measured). The 72 h and 96 h EbC50 for *P. subcapitata* exposed to glyphosate isopropylamine salt was calculated to be 9.25 and 10.30 mg/L, equivalent to 6.85 and 7.63 mg glyphosate acid/L (mean measured). The test is considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS	
Test material:	
Test item:	Glyphosate isopropylamine salt
Description:	Light brown liquid
Lot/Batch #:	Tech L 020131
Purity:	62.66 % Glyphosate isopropylamine salt
Vehicle and/or positive control:	Vehicle: SAG medium Positive control:Potassium dichromate
Test organism:	
Species:	*Pseudokirchneriella subcapitata* (Chodat, strain: SAG 61.81)
Initial cell concentration	1 × 104 cells/mL
Source:	Pflanzenphysiologisches Institut, Göttingen, Germany
Environmental conditions:	
Temperature:	21.7 – 25.0 °C
Photoperiod:	24 h light
Light intensity	8082 lux
Light quality	Universal white light
pH:	5.7 – 6.2

B. STUDY DESIGN
Experimental dates: 12 July– 19 July 2002 (Biological work)

Experimental treatments
On the basis of the results of a range finding test, the main test was performed with five concentrations, 4.27, 9.39, 20.66, 45.45 and 100 mg test item/L and a negative control (culture medium only). A toxic reference item Potassium dichromate was performed in August 2002.
For each concentration and the control, four vessels were prepared using 300 ml Erlenmeyer flasks each containing 100 mL of control or test medium. The initial cell concentration was 1 × 104 cells/mL. The concentrations of glyphosate IPA salt in the test solutions were measured by HPLC as concentrations of glyphosate acid at the start and at the end of the test in the 4.27, 20.66 and 100 mg test item/L treatments. Endpoints were calculated using the average recovery rate of glyphosate achieved over the duration of the test, based on geometric mean measured values achieved for each of the treatment groups. A stability sample was analysed from a test vessel without algae with the highest test item concentration at the end of the exposure period.
To maintain the algae in the suspension, all flasks were shaken continuously over the entire test period (100 ± 5 oscillations/min).

Observations
After 24, 48, 72 and 96 hours of growth, the algal cell densities in the control and test concentration vessels were determined using a Thoma counting chamber with a light microscope and the % growth inhibition (biomass and rate) relative to the control group was determined. This was achieved by plotting the mean value of the cell concentration (converted in log values) against the percentage growth inhibition to generate dose-response curves for each concentration. The concentrations resulting in 50 % inhibition (ErC50, EbC50), were determined, as well as the NOEC. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured continuously with an

automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

Statistical calculations
Probit analysis was used to calculate the EC10, EC20, and EC50 values. One-way ANOVA, Cochran´s Test and subsequent Dunnett´s t-test was used to calculate whether there were significant differences between the growth of algae in the controls and the algae exposed to the various test item concentrations to establish NOErC and NOEbC values.

II. RESULTS AND DISCUSSION

A. FINDINGS

In the test, concentrations of glyphosate acid were determined. In stock solutions prepared at test start, measured concentrations were 81.9 % of nominal concentrations. In test media at the beginning of the test, mean concentrations were 75.9 % of nominal concentrations and at the end of the test (96 h), mean concentrations were 64.2 % of nominal with 45.2 % found in the stability sample without algae (see table below). The average recovery in all water samples containing algae was 70.1 % for Glyphosate isopropylamine salt. Therefore, results are based on mean measured concentrations.

Table 0-4: Analytical measurements

Test item concentration (nominal) (mg/L)	Glyphosate IPA (nominal) (mg/L)	Glyphosate acid (mean measured) (mg/L)		Glyphosate IPA salt/L (mean measured) (mg/L)		% of nominal	
		-0.5 h	96 h	-0.5 h	96 h	-0.5 h	96 h
500 (Stock solution)	313.30	190.245	-	256.7	-	81.9	-
Control	0	nd	nd	nd	nd	-	-
4.27	2.68	1.554	1.341	2.1	1.8	78.4	67.6
20.66	12.95	6.606	7.007	8.9	9.5	68.9	73.0
100	62.66	37.406	24.164	50.5	32.6	80.6	52.0
100 (stability sample without algae)	62.66	20.991	-	28.3	-	45.2	

nd = not determined

The ErCx, EbCx and NOEC values are given below based on nominal and arithmetic mean measured concentrations.

Table 0-5: Toxicity of Glyphosate IPA salt and Glyphosate acid to *Pseudokirchneriella subcapitata*

Endpoint		Glyphosate IPA salt (nominal) (mg/L)	Glyphosate IPA salt (mean measured) (mg/L)	Glyphosate acid (mean measured) 1 (mg/L)
Growth rate	72 hours			
	0 - 72 h ErC10	8.16		
	0 - 72 h ErC20	14.7		
	0 - 72 h ErC50	45.2	31.7	23.5
	0 - 72 h NOEC	4.27	2.99	2.21
	96 hours			
	0 - 96 h ErC10	13.7		
	0 - 96 h ErC20	20.8		
	0 - 96 h ErC50	45.7	32.0	23.7
	0 - 96 h NOEC	9.39	6.58	4.87
Biomass	72 hours			
	0 - 72 h EbC10	4.18		
	0 - 72 h EbC20	6.21		
	0 - 72 h EbC50	13.2	9.25	6.85
	0 - 72 h NOEC	4.27	2.99	2.21
	96 hours			
	0 - 96 h EbC20	8.06		
	0 - 96 h EbC10	5.88		
	0 - 96 h EbC50	14.7	10.3	7.63
	0 - 96 h NOEC	4.27	2.99	2.21

1 The ratio between mean measured concentration in mg glyphosate IPA salt/L and mg glyphosate acid/L is stated as 1.35 in the report.

B. OBSERVATIONS

The results of the definitive test show that for algal growth rates, after 72 hours, these were significantly inhibited at nominal concentrations of 9.39 mg test item/L and higher. After 96 hours, significant inhibition was observed at 20.66 mg test item/L and higher.

For biomass, after 72 and 96 hours, there were significant effects observed at nominal concentrations of 9.39 mg test item/L and higher.

In contrast no inhibition of the algae growth was found at or below a nominal concentration of 4.27 mg test item/L.

Table 0-6: Percentage inhibition of growth rate and biomass of to *Pseudokirchneriella subcapitata* exposed for 72 and 96 hours to glyphosate isopropylamine salt

Glyphosate isopropylamine salt formulation (nominal) (mg/L)	**Control**	**4.27**	**9.39**	**20.66**	**45.45**	**100.0**
Glyphosate isopropylamine salt (mean measured) (mg/L)1		**2.99**	**6.58**	**14.48**	**31.86**	**70.1**
Glyphosate acid (mean measured) (mg/L)2		**2.21**	**4.87**	**10.73**	**23.6**	**51.9**
Inhibition growth rate (0-72 h) (%)	-	1.6	6.6*	25.2*	64.8*	61.4*
Inhibition growth rate (0-96 h) (%)	-	-0.9	4.2	17.5*	53.4*	77.1*
Inhibition biomass (0-72 h) (%)	-	11.4	33.5*	70.7*	92.4*	91.9*
Inhibition biomass (0-96 h) (%)	-	3.2	27.1*	68.0*	94.9*	95.9*

* Significantly different from the control at $\alpha = 0.05$
1 Taken into account the average recovery of 70.1 % for Glyphosate isopropylamine salt.
2 Taken into account 1.35 ratio stated in the report.

For the toxic reference item, the 96 h EbC50 was 0.497 mg test item/L and the 96 h ErC50 was 1.721 mg test item/L. These results were in agreement with what was expected on the basis of data shown in EEC Directive 92/69 method C.3.
The biomass in the control cultures increased by a factor of ≥16 (actual value 152.9), the coefficient of variance for section-by-section specific growth rates was ≤35 % (actual values ranged between 0 and 28.0), and the coefficient of variation of average specific growth rates during the whole test period in replicate control was ≤7 % (actual value: 0.8 %). The validity criteria according to guideline OECD 201 are therefore fulfilled.

III. CONCLUSION

Assessment and conclusion by applicant:
The biomass in the control cultures increased by a factor of ≥16 (actual value 152.9), the coefficient of variance for section-by-section specific growth rates was ≤35% (actual values ranged between 0 and 28.0), and the coefficient of variation of average specific growth rates during the whole test period in replicate control was ≤7% (actual value: 0.8%). The validity criteria according to guideline OECD 201 are therefore fulfilled.
The 72 h and 96 h ErC50 values for *Pseudokirchneriella subcapitata* exposed to glyphosate isopropylamine salt were calculated to be 31.70 and 32.01 mg test item/L, corresponding to 23.5 and 23.7 mg a.e./L (arithmetic mean measured). The 72 h and 96 h EbC50 for *P. subcapitata* exposed to glyphosate isopropylamine salt was calculated to be 9.25 and 10.30 mg test item/L, equivalent to 6.85 and 7.63 mg a.e./L (arithmetic mean measured). The 72h NOErC and NOEbC value was 2.21 mg a.e./L, respectively.
The study is considered valid and 72 h NOEC, ErC50, EbC50 values of 2.21, 23.5 and 6.85 mg a.e./L (arithmetic mean measured), respectively, are reliable for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/002
Report author	[redacted]
Report year	2003
Report title	MON 78623: a 72-hour toxicity test with the freshwater alga (*Selenastrum capricornutum*)
Report No	139A-311
Document No	-
Guidelines followed in study	OECD Guideline 201 (1984) EU Directive 92/69/EEC, Method C.3. (1992) ASTM Standard Guide 1218-90E (1990)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 39.8 % instead of <35 %
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of MON 78623 (K-salt) on *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*, currently known as *Raphidocelis subcapitata*) were evaluated in a 72-hour static toxicity test. *P. subcapitata* were exposed to five nominal concentrations encompassing 7.5, 15, 30, 60 and 120 mg test item/L, and the measured concentrations were 7.1, 15, 30, 61 and 122 mg test item/L.

For each concentration, three parallel cultures in 250 ml Erlenmeyer flasks were prepared. The initial cell concentration was 104 cells/mL. For the control group, six parallel test vessels were prepared. An additional abiotic replicate at the highest test concentration was included in the experimental design for concentration verification at 72 hours.

After 24, 48 and 72 hours of growth, the numbers of viable cells for each test concentrations and control were determined and the growth inhibition was calculated. At this, concentrations resulting in 50 % inhibition (EC50, ErC50, EbC50), were determined, as well as the NOEC.

EC50, EbC50, ErC50 and the corresponding 95% confidence limits for each 24-hours exposure interval were calculated by non-linear regression.

The results of main test showed that the algae growth was inhibited at the measured concentrations of 61 and 122 mg test item/L. In contrast, no inhibition of the algae growth was found at or below a measured concentration of 30 mg test item/L.

The 72 hours-EC50, EbC50 and ErC50 for *P. subcapitata* exposed to MON 78623 was determined at 69, 74 and 114 mg test item/L. The NOEC was 30 mg test item/L. The validity criteria according to the current guideline OECD 201 were not met, therefore, this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS	
Test Material:	
Identification:	MON 78623, 47.7% Glyphosate
Lot No.:	GLP-0108-11688-F
Chemical purity:	47.7%
Physical state:	Yellow liquid
Expiration date:	October, 2003
Analytical standard:	
Identification:	Glyphosate (A.S.)
Lot No.:	GLP-9607-7215-A
Chemical purity:	99.8%
Physical state:	Powder
Expiration date:	January 31, 2003
Vehicle and/or positive control:	
Vehicle:	Dilution water
Positive control:	None
Test organism:	
Species:	*Pseudokirchneriella subcapitata,* formerly known as *Selenastrum capricornutum*
Initial cell concentration:	104 cells/mL
Source:	in-house culture, started from University of Toronto Culture Collection
Environmental conditions:	
Temperature:	22.0 – 22.3 °C
Photoperiod:	24 h light
Light intensity:	6500 – 8550 lux
Light quality:	cool-white fluorescent lighting
pH:	8.0 – 8.1 (negative control); 6.9 – 7.8 (highest test concentration)
Conductivity:	not stated
Hardness:	not stated

B. STUDY DESIGN

Experimental dates: 18 October – 21 October 2002

Experimental treatments
Three replicate cultures per test concentration of *P. subcapitata* (initial cell density in each chamber was 1 × 104 cells/mL) were exposed for 72 hours to nominal concentrations of 7.5, 15, 30, 60, and 120 mg test item/L. A negative control group with six replicate cultures was held under the same environmental conditions concurrently. An additional abiotic replicate at the highest test concentration was included in the experimental design for concentration verification at 72 hours. The methods of test solution preparation

were stock solution preparation and proportional diluting. The test flasks were shaken continuously at 100 rpm during the test.

Observations
The temperature of a container of water adjacent to the test chambers in the environmental chamber was recorded twice daily during the test using a liquid-in-glass thermometer. Light intensity was measured at five locations surrounding the test flasks on each shaker table at test initiation. The pH of the medium in each treatment and control group was measured at test initiation and at test termination.

Test medium samples were collected from each biological replicate of the treatment and control group for the determination of algal cell densities. Samples were collected at approximately 24-hours intervals during the 72-hours exposure and were held for a maximum of two days under dark, refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Prior to conducting cell counts, the linearity of the instrument response was determined at settings previously established for *P. subcapitata*.

Samples of the test solutions were collected at approximately 0 and 72 hours to measure concentrations of the test substance. At test initiation samples were collected for each treatment and control group prior to addition of the algae. At test termination, the biological replicates from each respective treatment and control group were pooled and then sampled. The 120 mg test item/L equivalent to 57.24 mg glyphosate/L abiotic replicate was sampled at test termination to determine the stability' of the test substance under the conditions of administration. All samples were collected in glass vials and processed immediately for analysis.

Statistical calculations:
Cell densities, areas under the growth curve, growth rates and percent inhibition values were calculated using SAS System for Windows (Version 8.02). Cell densities, areas under the growth curve and growth rates were analysed statistically to estimate EC50 values and the corresponding 95 % confidence limits for each 24-hours exposure interval. All EC50 values were calculated by non-linear regression.
The cell density, area under the growth curve and growth rate data were evaluated for normality and homogeneity of variance (p=0.05) using the Shapiro-Wilk's and Levene's tests, respectively. Since the data were normal with homogeneous variances, the treatment groups were compared to the negative control using ANOVA and Dunnett's test (p=0.05). The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOEC relative to each parameter at 72 hours.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50, EbC50, ErC50 and NOEC values are given below based on mean measured concentrations.

Table 0-7: Toxicity of MON 78623 to Pseudokirchneriella subcapitata

Endpoint	MON 78623 [mg test item/L]
EC50 (cell density) and 95% Confidence Limits	69 (62 – 77)
EbC50 (biomass) and 95% Confidence Limits	74 (67 – 83)
ErC50 (growth rate) and 95% Confidence Limits	114 (111 – 118)
NOEC (cell density)	30
NOEC (biomass)	30
NOEC (growth rate)	30

Concentrations of MON 78623 in the samples were determined using a HPLC (UV detector at 500nm). Calibration standards of Glyphosate, ranging in concentration from 2.00 to 20.0 mg glyphosate/L, were prepared in freshwater algal medium using a stock solution of Glyphosate in NANOpure® water. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The method limit of quantitation (LOQ) for these analyses was defined as 4.19 mg test item/L equivalent to 2.00 mg glyphosate/L. The analytical results are given below.

Table 0-8: Analytical measurements

MON 78623 nominal [mg /L]	Sampling time [hours]	MON 78623 measured [mg test item/L]	Percent of nominal [%]	MON 78623 mean measured [mg test item/L]	Mean percent of nominal [%]
-	0	< LOQ	-	-	-
	72	< LOQ	-		
7.5	0	6.20	82.7	7.1	94.7
	72	8.03	107		
15	0	14.7	98.3	15	100
	72	15.7	104		
30	0	29.5	98.3	30	100
	72	31.2	104		
60	0	59.3	98.8	61	102
	72	62.1	104		
120	0	119	99.3	122	102
	72	124	103		
120 (Abiotic)	72	125	104	-	-

Although the determined concentrations of test item in test medium always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using the mean determined concentrations of the test item.

B. OBSERVATIONS

The results of main test showed that the algae growth was inhibited at the measured concentrations of 61 and 122 mg test item/L corresponding to 28.95 and 57.96 mg glyphosate/L. In contrast, no inhibition of the algae growth was found at or below a measured concentration of 30 mg test item/L corresponding to to 14.48 mg glyphosate/L.

Table 0-9: Percentage inhibition of growth rate and biomass to P. subcapitata exposed for 72 hours to MON 78623

	Control	MON 78623 [mg test item/L]				
	-	7.1	15	30	61	122
Mean number of algae cells (10000/ml)	81.3645	92.6914	97.6039	86.9339	54.8190*	7.4236*
Inhibition growth rate (0-72 h) [%]	-	-3.1	-4.3	-1.7	9.0*	54*
Inhibition biomass (0-72 h) [%]	-	-12	-17	-6.1	26*	88*

*There were statistically significant differences (p<0.05) in comparison to the negative control replicates.

III. CONCLUSIONS

The 72 h ErC50 for *Pseudokirchneriella subcapitata* exposed to MON 78623 was determined at 114 mg test item/L. The 72 h EbC50 for *P. subcapitata* exposed to MON 78623 was 74 mg test item/L. The 72 h EC50 for *P. subcapitata* exposed to MON 78623 was 69 mg test item/L. Significant effects of MON 78623 on the growth of *P. subcapitata* were found from a concentration > 30 mg test item/L. The NOEC was 30 mg test item/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 – 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	81.4
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	39.8%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	3.4%

The biomass in the control cultures increased by a factor of ≥16 (actual: 81.4), the coefficient of variance for section specific growth rates exceeded 35% (actual: 39.8%), for the whole test period it was ≤ 7% (actual 3.4%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/003
Report author	[redacted]
Report year	2000
Report title	Acute toxicity of glifosate tecnico NUFARM to *Selenastrum capricornutum*
Report No	RF-D2.44/99
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1993)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - Analytical verification of test item only performed at the start of the test.in samples of test medium and stock solution (both >80% of nominal).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The toxicity of glyphosate technical to the green alga *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) was determined in a 96-hour, static test. The test comprised 7 nominal concentrations of glyphosate (nominal 5.6, 10, 32, 56, 100, 320, and 560 mg test item/L, corresponding to initial measured concentrations of 5.74, 9.81, 33.48, 58.55, 104.17, 325.42, and 585.52) and a control (untreated culture medium) without test item. The test vessels were 250 mL glass Erlenmeyer flasks containing 100 mL of test solution.

Three replicate vessels were prepared for each test concentration and for the control group. Each replicate test vessel was inoculated with an initial cell density of 1.6 x 104 cells/mL. After 1, 2, 3, and 4 days, samples were removed from each test and control vessel and the algal cell densities were determined by cell counting. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily. The concentrations of glyphosate technical in the test solutions were measured at the start of the test. The measured test concentration values were used for the calculation and reporting of all results.

The effective concentration of glyphosate technical causing 50 % inhibition of growth in *Pseudokirchneriella subcapitata* after 96 hours when compared to the control was 114.05 mg test item/L, the no observed effect concentration (NOEC) was 104 mg test item/L (initial measured concentrations).

The validity criteria according to the current guideline OECD 201 were met. However, analytical work was not performed throughout the test, as required per current test guidelines. Therefore, this study is considered supportive only.

I. MATERIALS AND METHODS

A. MATERIALS	
Test material:	
Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	037-919-113
Purity:	954.9 g/kg
Vehicle and/or positive control:	Vehicle: Cell growth medium Positive control: None
Test organism:	
Species:	Green algae *Pseudokirchneriella subcapitata,* UTEX 1648
Initial cell concentration	1.6 × 104 cells/mL
Source:	UTEX – The culture collection of algae at the University of Texas at Austin, Texas, USA
Acclimatisation period:	4 days
Environmental conditions:	
Temperature:	24.3-24.4 °C
Photoperiod:	Continuous illumination
Light intensity:	7933 lux
pH:	7.17 – 7.22 at 0 hour 7.46 – 9.31 at 72 hour

B. STUDY DESIGN

Experimental dates: 25 October -12 November 1999

Experimental treatments

The toxicity of glyphosate to the green alga *Pseudokirchneriella subcapitata* was determined in a 96-hour, static test. The test comprised 7 nominal concentrations of glyphosate (nominal 5.6, 10, 32, 56, 100, 320, and 560 mg test item/L, corresponding to initial measured concentrations of 5.74, 9.81, 33.48, 58.55, 104.17, 325.42, and 585.52 mg test item/L) and a control consisting of culture medium without test item. The test vessels were 250 mL glass Erlenmeyer flasks containing 100 mL of test solution.

A primary stock solution of nominal concentration of 10000 mg test item/L was prepared by dissolving 1.0 g glyphosate in 100 mL distilled and deionised water. From this initial solution, following stock solutions were prepared: 10, 100, and 1000 mg test item/L. Appropriate aliquots of these stock solutions were diluted to prepare the test concentrations. 100 mL of the appropriate test solution were dispensed to each test and blank vessel. The test comprised 3 replicates of the control (untreated culture medium) and 3 replicates of each concentration of the test item.

Each replicate test vessel was inoculated with a cell density of 1.6 × 104 cells/mL. The culture vessels were incubated at 24.3 - 24.4°C under continuous illumination for 96 hours. During incubation, the algal cells were kept in suspension by continuous shaking.

Observations

After 1, 2, 3 and 4days, samples were removed from each test and control vessel and the algal cell densities were determined by cell counting using a Neubauer improved haemacytometer and a phase-contrast microscope. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a minimum-maximum thermometer. The

concentrations of glyphosate in the test solutions were measured at the start of the test only. The effective concentration was within acceptable limits of nominal concentration (80%) for all tested concentrations.

Statistical calculations
The computer program used was STATGRAPHICS – Statistical Graphic System.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} (96 h), NOEC and LOEC values are given below based on initial measured concentrations.

Table 0-10: Toxicity of glyphosate *to Pseudokirchneriella subcapitata*

Endpoint	Glyphosate [mg test item/L]
96-h EC_{50} (95% CI)	114.05 (94.04 - 131.49)
96–h NOEC	104.17
96–h LOEC	325.42

CI = confidence interval

B. OBSERVATIONS

The effective concentration of glyphosate technical causing 50 % inhibition of growth after 96 hours when compared to the control was 114.05 mg test item/L, the no observed effect concentration (NOEC) was 104.17 mg test item/L. No morphological changes were observed after 96 hours of exposure to glyphosate technical.

Table 0-11: Mean cell densities and Percentage of inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 and 96 hours to glyphosate

	Control	Glyphosate technical [mg/L]						
Test parameters	**-**	**5.6**	**10**	**32**	**56**	**100**	**320**	**560**
Mean cell densities (0-96 h) (× 10000 cells/mL)	740	732	723	723	707	473	48.4	23.4
Mean growth rate (0-96 h) [%]		99	98	98	96	64	7	3
Mean cell densities (0-72 h) (× 10000 cells/mL)	307	290	248	215	223	173	23.4	23.4
Mean growth rate (0-72 h) [%]		94	81	70	73	57	8	8

The biomass in the control cultures increased by a factor of ≥16, the coefficient of variance for section specific growth rates was ≤35%, for the whole test period it was ≤7%. The validity criteria according to guideline OECD 201 are therefore fulfilled.

III. CONCLUSIONS

The 96 h ErC_{50} for *Pseudokirchneriella subcapitata* exposed to glyphosate technical was calculated to be 114.05 mg test item/L, the no observed effect concentration (NOEC) was 104 mg test item/L (initial measured concentrations).

3. Assessment and conclusion

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10}, EC_{20}, and EC_{50}, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	192
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	10%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	0.9%

The biomass in the control cultures increased by a factor of ≥16 (actual: 192), the coefficient of variance for section specific growth rates was ≤ 35% (actual: 10%) and the coefficient of variance for the whole test period it was ≤ 7% (actual: 0.9%). The validity criteria according to the current guideline OECD 201 were met. However, analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required per current test guidelines. As there are other studies with more sensitive endpoints available, this study is considered supportive only.

Nevertheless, endpoints were recalculated.

A statistical re-evaluation addressing EC10, EC20, EC50, NOEC and LOEC was performed (Positon Paper No. 110054-001). Endpoints are based on nominal concentrations.

Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	Glyphosate [mg a.e./L]	
	Yield	Growth rate
EC10 (95% CI)	5.54 (2.99 – 8.68)	62.6 (40.4 – 84.6)
EC20 (95% CI)	14.6 (9.40 – 20.5)	132 (100 – 161)
EC50 (95% CI)	75.9 (56.4 – 105)	469 (401 – 568)
NOEC	5.6	5.6
LOEC	10	10

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/004
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study RF-D2.44/99 on the toxicity of glifosate tecnico NUFARM to *Selenastrum capricornutum* under static conditions
Report No	110054-001
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1993)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid EC10, EC20 and EC50 as well as NOEC values was conducted for the algae study RF-D2.44/99 ([redacted], C.M., 2000) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. The calculated EC_{10}, EC_{20} and EC_{50} values are 62.6, 132, and 469 mg/L for growth rate and 5.54, 14.6, and 75.9 mg a.e./L for yield, respectively. The NOEC for growth and yield was determined to be 5.6 mg a.e./L. However, analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required as per current test guidelines. As there are other studies with more sensitive endpoints available, this study is considered supportive only and is not used for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Author:	[redacted]
Substance:	Glyphosate
Title:	Acute toxicity of glifosate tecnico NUFARM to *Selenastrum capricornutum*
Study number:	RF-D2.44/99
Completion date:	03-01-2000
Test guideline(s):	OECD 201 (1993)
GLP:	Yes
Software:	ToxRatPro Version 3.3.0
Testing facility:	BIOAGRI Laboratorios, Piracicaba, SP. Brasil
Sponsor:	NUFARM DO BRASIL Ltda., Curitiba, PR., Brasil

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC10, EC20, and EC50 as well as the NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study RF-D2.44/99 ([redacted] 2000) was statistically evaluated for the effects of glyphosate on the organism *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata,* also formerly known as *Pseudokirchneriella subcapitata*). The organisms were exposed for 96 hours to the following concentrations of glyphosate 5.6, 10, 32, 56, 100, 320, and 560 mg test item/L (nominal) and corresponding to initial measured concentrations 5.74, 9.81, 33.48, 58.55, 104.17, 325.42, and 585.52 mg test item/L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive Effect Concentrations that have 10, 20 and 50 % effects on yield and growth rate of the test subjects (EC10, EC20 and EC50), a logit analysis using linear weighted regression was performed. For growth rate, a logit analysis with linear maximum likelihood regression was used.

NOEC was determined by Welsh-t-test After Bonferroni-Holm (one-sided smaller, p = 0.05).
Analyses were performed.

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Results are provided in the table below:

Table 0-12: Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	192
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤35 %	10 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7 %.	≤7 %	0.9 %

The validity criteria according to the current guideline OECD 201 were met. However, analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required per current test guidelines.

For yield, the parameters for the logit model are estimated as slope b: 1.93240; Intercept a: -3.63371.
For growth rate, the parameters for the probit model are estimated as slope b: 2.51249; Intercept a: -6.71063.
According to the statistical parameters; Chi2(13) = 0.283521; p(Chi²): 1.000; F(1,19) = 120.416; p(F) <0.001; r²: 0.864 for yield; and Chi2(13) = 0.04958; p(Chi²): 1.000; F(1,19) = 107.785; p(F) <0.001;

r^2: 0.850 for growth rate. Based on these values the EC10, EC20 and EC50 for yield and growth rate calculations should be considered valid.

The obtained EC_{10}, EC_{20} and EC_{50}, and NOEC values for the effect of Glyphosate on growth rate and yield of *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata* or formally known as *Pseudokirchneriella subcapitata*) are presented in the table below.

Table 0-13: Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	Glyphosate technical [mg a.e./L]	
	Yield	Growth rate
EC_{10} (95% CI)	5.54 (2.99 – 8.68)	62.6 (40.4 – 84.6)
EC_{20} (95% CI)	14.6 (9.40 – 20.5)	132 (100 – 161)
EC_{50} (95% CI)	75.9 (56.4 – 105)	469 (401 – 568)
NOEC	5.6	5.6
LOEC	10	10

CI = confidence interval

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria according to the current guideline OECD 201 were met and this study is considered valid in view of parameters for increase of biomass, mean coefficient of variation for section-by section specific growth rate, and coefficient of variation of average specific growth rates. However, analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required as per current test guidelines. As there are other studies with more sensitive endpoints available, this study is considered supportive only and is not used for risk assessment.

Nevertheless, the calculated EC_{10}, EC_{20} and EC_{50} values are 62.6, 132, and 469 mg a.e./L (nominal) for growth rate and 5.54, 14.6, and 75.9 mg a.e./L (nominal) for yield, respectively. The statistical parameters showed that these values can be considered reliable. The nominal based NOEC was determined to be 5.6 mg a.e./L for yield and growth rate.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/005
Report author	[redacted]
Report year	1995
Report title	Glyphosate acid: Toxicity to the green alga *Selenastrum capricornutum*
Report No	AB0503/B
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Minor: - Initial nominal cell density of 3 × 103 cells/mL, was below the recommended density of 5 × 103 – 104 cells/mL for *P. subcapitata*, however validity criteria were met.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The toxicity of glyphosate acid to the green alga *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) was determined in a 120-hour, static test conducted at six nominal glyphosate acid concentrations (5.6, 10, 18, 32, 56, and 100 mg test item/L) and a control prepared using culture medium without test item.

Six replicate vessels were prepared for the control group with three replicate vessels prepared for each concentration of glyphosate acid. Each replicate test vessel was inoculated with 0.370 mL of the inoculum culture to give a nominal cell density of 3 × 103 cells/mL. The culture vessels were incubated at 24 ± 1 °C in an orbital incubator (vessels shaken at 100 rpm) under continuous illumination for 120 hours.

The algal cell densities were determined after 1, 2, 3, 4, and 5 days. Test and control group media pH values were determined at the beginning and end of test, with temperature measured hourly. Glyphosate acid concentrations in test solutions were measured at the start and at the end of the test. The mean measured glyphosate acid concentrations ranged from 100 to 111% of the nominal values.

The 72-hour E_bC_{50} and E_rC_{50} for *Selenastrum capricornutum* exposed to glyphosate acid were determined to be 18 and 19 mg test item/L, respectively. The 72-hour NOE_bC and NOE_rC values were 10 mg test item/L, respectively. The 120-hour E_bC_{50} and E_rC_{50} were calculated to be 17 and 21 mg test item/L. The 120-hour NOE_bC and NOE_rC were 10 mg test item/L each. The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS	
Test material:	
Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%
Vehicle and/or positive control:	Vehicle: Cell growth medium Positive control: None
Test organism:	
Species:	Green algae *Pseudokirchneriella subcapitata* Korshikov
Initial cell concentration	3 × 103 cells/mL
Source:	Brixham Environmental Laboratory culture from strain ATCC 22662
Environmental conditions:	
Temperature:	24.1 - 24.2 °C (measured by thermometer). The hourly temperature measured automatically remained within 24 ± 1°C
Photoperiod:	Continuous illumination
Light intensity:	5030 lux
pH:	3.5 – 7.5 at the start of the test 3.6 – 8.9 at the end of the test

B. STUDY DESIGN

Experimental dates: 7 August - 12 August 1995

Experimental treatments

The toxicity of glyphosate acid to the green alga *Selenastrum capricornutum* (currently known as *Raphodocelis subcapitata*) was determined in a 120-hour, static test, conducted at six nominal glyphosate acid concentrations of 5.6, 10, 18, 32, 56, and 100 mg test item/L, and a control consisting of culture medium without test item. The test vessels were 250 mL conical glass flasks containing 100 mL of test or control solution. The stock solution (nominal concentration of 100 mg a.s./L) was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 5.6, 10, 18, 32, and 56 mg test item/L. To each test and blank vessel 100 mL of the appropriate test solution were dispensed. The test was performed in six replicate cultures of the culture medium control and three replicate cultures of each concentration of glyphosate acid.

Each replicate test vessel was inoculated with 0.370 mL of the inoculum culture to give a nominal cell density of 0.3 × 104 cells/mL. The culture vessels were incubated at 24 ± 1 °C under continuous illumination for 120 h. During incubation, the algal cells were kept in suspension by continuous shaking using an orbital incubator (oscillating at 100 rpm).

Observations

The algal cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was monitored continuously with readings recording hourly with an automatic recording system. The concentrations of

glyphosate acid in the test and control solutions were measured at the start and at the end of the test.

Statistical calculations

One-way analysis of variance, and Dunnett's post-hoc test to determine the NOEC. ECx values were evaluated by linear regression against log concentration.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-14: Toxicity of glyphosate acid to *Selenastrum capricornutum*

Endpoint	Glyphosate acid [mg test item /L]	
	Growth rate	Biomass
72-h EC_{50} (95% CI)	19 (14 - 25)	18 (13 - 23)
72–h NOEC	10	10
72–h LOEC	18	18
120-h EC_{50} (95% CI)	21 (16 - 28)	17 (13 - 22)
120-h NOEC	10	10
120-h LOEC	18	17

CI= Confindence interval

The mean measured concentrations of glyphosate acid ranged from 100 to 111 % of the nominal values. On the basis of the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

B. OBSERVATIONS

Table 0-15: Mean cell densities and percentage of inhibition of cell growth of *Selenastrum capricornutum* exposed for 72, 96 and 120 hours to glyphosate

	Control	Glyphosate acid [mg test item/L]					
Test parameters	**-**	**5.6**	**10**	**18**	**32**	**56**	**100**
Mean cell densities (0-72 h) (× 10000 cells/mL)	73.4	79.1	74.5	2.05	0.143	0.021	0.033
Mean cell densities (0-96 h) (× 10000 cells/mL)	312	314	311	2.60	0.178	0.070	0.045
Mean cell densities (0-120 h) (× 10000 cells/mL)	567	605	568	4.20	0.478	0.138	0.172
Mean area under growth curve (0-72 h) [%]	-	108	104	8	-1	-1	-1
Mean area under growth curve (0-96 h) [%]	-	103	101	2	0	0	0
Mean area under growth curve (0-120 h) [%]	-	104	100	1	0	0	0
Mean growth rate (0-72 h) [%]	-	101	100	35	-13	-48	-40
Mean growth rate (0-96 h) [%]	-	100	100	31	-7	-21	-27
Mean growth rate (0-120 h) [%]	-	101	100	35	6	-10	-7

III. CONCLUSION

The 72-hour E_bC_{50} and E_rC_{50} for *Selenastrum capricornutum* exposed to glyphosate acid were determined to be 18 and 19 mg test item/L, respectively. The 72-hour NOE_bC and NOE_rC values were 10 mg test item/L, respectively. The 120-hour E_bC_{50} and E_rC_{50} were calculated to be 17 and 21 mg test item/L. The

120-hour NOE_bC and NOE_rC were 10 mg test item/L each.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10}, EC_{20}, and EC_{50}, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	245
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	9.1%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	1.6%

The biomass in the control cultures increased by a factor of ≥16 (actual: 245), the coefficient of variance for section specific growth rates was ≤ 35% (actual: 9.1%) and the coefficient of variance for the whole test period it was ≤ 7% (actual: 1.6%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC_{10}, EC_{20}, and EC_{50} was performed (Positon Paper No. CA 8.2.6.1/006).

Re-calculated EC_{10}, EC_{20} and EC_{50} values based on nominal test concentrations:

Endpoint (0 – 72 hours)	Glyphosate acid [mg a.s./L]	
	Yield	Growth rate
EC_{10} (95% CI)	4.84 (2.07 – 7.80)	5.74 (3.65 – 7.87)
EC_{20} (95% CI)	7.59 (3.93 – 11.3)	8.91 (6.25 – 11.6)
EC_{50} (95% CI)	16.4 (10.9 – 23.0)	18.9 (14.9 – 23.7)

CI = confidence interval

The 72-hour NOE_bC and NOE_rC values were provided by the study report as 10 mg a.s./L, based on glyphosate acid.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/006
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study BL5550/B on the toxicity of Glyphosate acid to *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) under static conditions
Report No	110054-002
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP was not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 72-h EC10, EC20 and EC50 values was conducted for the study BL5550/B ([redacted] 1995) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011). Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 201 (2011) were met. The calculated EC_{10}, EC_{20} and EC_{50} values are 4.84, 7.59 and 16.4 mg a.e./L (nominal), respectively for yield and 5.74, 8.91 and 18.9 mg/L (nominal), respectively for growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number: AB0503/B
Author: Smyth, D.V. *et al*.
Substance: Glyphosate acid
Title: Glyphosate acid: Toxicity to the green alga *Selenastrum capricornutum*
Completion date: 15-Aug-1995
Test guideline(s): OECD Guideline No. 201 (1984); US EPA Guideline 540/09-82-020 (1982)
Re-evaluated according to OECD 201 (2011)
GLP: Yes, conducted under GLP/Officially recognised testing facilities
Testing facility: Brixham Environmental Laboratory, Brixham Devon, UK
Sponsor: ZENECA Agrochemicals, Surrey, UK
Sponsor: ZENECA Agrochemicals, Surrey, UK

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC_{10}, EC_{20}, and EC_{50} values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study BL5550/B ([redacted] 1995) was statistically evaluated for the effects of Glyphosate acid on the organism *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*). The organisms were exposed for 120-hours to the following concentrations of Glyphosate acid: 5.6, 10, 18, 32, 56, and 100 mg test item/L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

Statistical calculations

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

The data was checked for normality using Shapiro-Wilk's Test on Normal Distribution for all time points ($p = 0.01$). Subsequently, for determination of outliers, The Dixon & Hartley outlier test was performed for parametric data (24-h and 48-h replicates), and Hampel Outlier test for non-parametric data (72-h replicates). Only if an outlier was detected repeatedly for a given replicate, it was excluded from subsequent analyses.

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50 % effects on growth rate and yield of the test subjects (EC_{10}, EC_{20} and EC_{50}), a logit analysis was performed and outlier excluded where applicable.

Furthermore, results of the original report were reviewed, which determined the NOEC.

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Results are provided in the table below:

Table 0-16: Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	245
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	9.1%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	1.6%

Outlier test:
Data sets for 24 and 48 hours follow a normal distribution, while the 72 hour dataset is not normally distributed (Annex 2, Tables 2, 3 and 4 of this report).
According to Dixon & Harley outlier test (24 and 48 hours; Annex 2, tables 5 and 6 of this report) and Hampel outlier test (72 hours, Annex 2, table 7 of this report), the following outliers were determined:

Time point	Test concentration	Replicates
24h	No outliers detected	
48h	32 mg/L 56 mg/L	Replicate 1 Replicate 2
72h	32 mg/L 100 mg/L	Replicate 1 Replicate 2

As replicate 1 in the test concentration of 32 mg/L resulted in being an outlier at 48 as well as 72 hours, this replicate is excluded from further statistical analysis.

The mean measured concentrations of glyphosate acid ranged from 100 to 111% of the nominal values. On the basis of the analytical results the nominal test concentration values were used for the calculation of all results.

For yield at 72 hours, the parameters for the logit model are estimated as slope b: 4.14430; intercept a: -5.03349.

For growth rate at 72 hours, the parameters for the logit model are estimated as slope b: 4.24735; intercept a: -5.41977.

Statistical parameters for goodness fit of the logit model are: Chi2(15) = 0.473; p(Chi2): 1.000; F(1,15) = 91.681, p(F) <0.001; R2 = 0.859 the EC10 , EC20 and EC50 for growth rate and Chi2(15) = 1.011; p(Chi2): 1.000; F(1,15) = 40.874 p(F) <0.001; R2 = 0.732 the EC10 , EC20 and EC50 for yield, calculations should therefore be considered valid.

The obtained EC_{10}, EC_{20} and EC_{50} values for *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) are presented in the table below.

Geometric mean measured test concentrations ranged from 100 to 111% of nominal. Therefore, all results are based on nominal test concentrations.

Table 0-17: Re-calculated EC_{10}, EC_{20}, EC_{50} values based on nominal test concentrations:

Endpoint (0 – 72 hours)	Glyphosate acid [mg a.e./L]	
	Yield	Growth rate
EC_{10} (95% CI)	4.84 (2.07 – 7.80)	5.74 (3.65 – 7.87)
EC_{20} (95% CI)	7.59 (3.93 – 11.3)	8.91 (6.25 – 11.6)
EC_{50} (95% CI)	16.4 (10.9 – 23.0)	18.9 (14.9 – 23.7)

CI = confidence interval

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated EC_{10}, EC_{20} and EC_{50} values are 4.84, 7.59 and 16.4 mg a.s./L (nominal), respectively for yield and 5.74, 8.91 and 18.9 mg a.s./L (nominal), respectively for growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/007
Report author	[redacted]
Report year	1995
Report title	Fresh Water Algal Growth Inhibition Test with Glyfosaat
Report No	141896
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) EEC Directive 92/69, Part C-3 (1992) ISO International Standard 8692 (1989)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 56.7% instead of <35% Validity criteria was not met.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of glyphosate on *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum,* currently known as *Raphidocelis subcapitata*) were evaluated in a 72-hour static toxicity test. After a range-finding test *Pseudokirchneriella subcapitata* were exposed to five nominal concentrations encompassing 10, 18, 32, 56 and 100 mg test item/L and a blank control.

For each test concentration and the control group, three (test concentrations) or six (control) replicates with 50 mL test solution and an initial cell density of 1 × 104 cells/mL were prepared in 100 mL vessels. Additionally, for the highest test concentration one replicate without algae was provided.

After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined

based on spectrophotometrical measurements and a linear calibration curve relating extinction and cell density.
The concentrations resulting in 50% reduction of growth rate (E_rC_{50}) and 50% inhibition of cell growth (E_bC_{50}) were determined, as well as the associated NOEC values.
Results showed glyphosate inhibited cell growth of the fresh water algae *Pseudokirchneriella subcapitata* increasingly with increasing concentrations, resulting in an almost complete inhibition at 56 and 100 mg test item/L. A significant reduction of growth rate was observed at 56 and 100 mg test item/L. Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS	
Test material:	
Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	22021
Purity:	96 %
Vehicle and/or positive control:	Vehicle: Dilution water (ISO-medium) Positive control: Potassium dichromate (K2Cr2O7)
Test organism:	
Species:	*Pseudokirchneriella subcapitata,* strain: CCAP 278/4
Initial cell concentration	1 - 104 cells/mL
Source:	In-house culture
Acclimatisation period:	Not stated
Environmental conditions:	
Temperature:	22.0° C
Photoperiod:	24 h light
Light intensity:	7000 - 8000 lux
pH:	Control (0 – 72 h): 8.1 – 8.2 10 mg/L (0 – 72 h): 7.8 – 7.9 18 mg/L (0 – 72 h): 7.3 – 7.8 32 mg/L (0 – 72 h): 6.5 – 7.6 56 mg/L (0 – 72 h): 5.9 – 6.5 100 mg/L (0 – 72 h): 4.7 – 4.9
Hardness:	24 mg CaCO3/L

B. STUDY DESIGN
Experimental dates: 28 March – 14 April 1995
Experimental treatments
Prior to the main test, a range-finding test was performed with concentrations of 0.01, 0.1, 1, 10 and 100 mg test item/L. On the basis of the preliminary test results, the main test was performed with five concentration ranges, 10, 18, 32, 56 and 100 mg test item/L. In addition, algae were exposed to test medium without test

substance or other additives (blank control). The test solutions were prepared using ISO-medium.
The culture vessels were incubated on a shaking plate over several generations for 72 hours. During the incubation, the algal cells were kept in suspension by continuous shaking. For each concentration, three parallel cultures were prepared in 100 ml all-glass vessels. To each test vessel, 50 mL of the test item preparation were added with an initial cell density adjusted to 1 × 104 cells/mL. Additionally, for the highest test concentration one replicate without algae was provided. For the control group, six parallel test vessels were prepared.

Observations
After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements and a linear calibration curve relating extinction and cell density.
The concentrations resulting in 50 % and 10 % reduction of growth rate (E_rC_{50} and E_rC_{10}) and 50 % and 10 % inhibition of cell growth (E_bC_{50} and E_bC_{10}) were determined, as well as the associated NOEC values.
The pH-values of the test solutions were measured at test initiation and test termination. The temperature was controlled daily in a temperature-control vessel.
Analytical control measurements of the actual concentration of the test item were performed by mean of HPLC analysis, using samples taken from three representative concentrations, 10, 32 and 100 mg test item/L.

Statistical calculations
The calculation of the EC_{50} and EC_{10} values was based on the percentages of growth inhibition and the percentages of growth rate reduction versus the (log) concentration using the linear regression method.

II. RESULTS AND DISCUSSION

A. FINDINGS

E_rC_{50}, E_bC_{50} and NOEC values are given below based on nominal concentrations.

Table 0-18: Toxicity of glyphosate to *Pseudokirchneriella subcapitata*

Endpoint (0 – 72 hours)	Glyphosate [mg test item/L]
E_rC_{50} (95% CI)	54 (51 - 58)
E_bC_{50} (95% CI)	48 (43 - 54)
E_rC_{10} (95% CI)	33 (upper limit of 95% CI: 36)
E_bC_{10} (95% CI)	18 (13 - 22)
NOE_rC	32
NOE_bC	10

CI = confidence interval

Analytical data: Analytical control measurements were performed on three representative concentrations. At test initiation, 106 %, 109 % and 108 % of the test item were recovered for the nominal concentrations of 10, 32 and 100 mg test item/L, respectively. At test termination, 103 %, 108 % and 111 % of the test item were recovered for the nominal concentrations of 10, 32 and 100 mg test item/L, respectively. As the mean measured content of the test item always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.
Reference item: The 72-hour E_bC_{50} was 0.69 mg reference item/L, the 72-hour E_rC_{50} was 1.32 mg reference item/L.

B. OBSERVATIONS

Glyphosate inhibited cell growth of the fresh water algae *Pseudokirchneriella subcapitata* increasingly with increasing concentrations, resulting in an almost complete inhibition at 56 and 100 mg test item/L. A significant reduction of growth rate was observed at 56 and 100 mg test item/L.

Table 0-19: Percentage reduction of growth rate and inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to glyphosate

Test parameters (0 – 72 hours)	Control	Glyphosate [mg test item/L]				
	-	10	18	32	56	100
Mean cell densities (× 10000 cells/mL)	57.4	52.3	49.3	47.8	5.3	1.2
Cell growth rate reduction [%]		2.3	3.7	4.5	58.9	96.0
Cell growth inhibition [%]		7.1	9.4	19.9	81.6	96.7

In the control the cell density increased by an average factor of 57 within three days. Analysis of samples taken from the solution without algae showed that the actual exposure concentration remained above 80 % relative to the initial concentration. Further, all test conditions remained within the ranges prescribed by the protocol.

III. CONCLUSION

Under the conditions of the present study the nominal based 72 hours E_rC_{50} and E_bC_{50} for *Pseudokirchneriella subcapitata* exposed to glyphosate were calculated to be 54 mg test item/L and 48 mg test item/L, respectively. The NOE_rC and NOE_bC were determined to be 32 mg test item/L and 10 mg test item/L, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	57.5
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	56.7%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	3.4%

The biomass in the control cultures increased by a factor of ≥16 (actual: 57.5), the coefficient of variance for section specific growth rates exceeded 35% (actual: 56.7%), for the whole test period it was ≤ 7% (actual: 3.4%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.6.1/008
Report author	[redacted]
Report year	1995
Report title	Fresh water algal growth inhibition test with glyphosate
Report No	R481
Document No	-
Guidelines followed in study	No information mentioned in the Monograph.
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Toxicity of technical glyphosate (purity >94 %) to aquatic organisms (*Pseudokirchneriella subcapitata*)
Short description of results:	No information mentioned in the Monograph
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available and no information mentioned in the Monograph 2001. However, these data were considered as not acceptable in the Monograph 2001
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a “request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	CA 8.2.6.1/009
Report author	[redacted]
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Selenastrum capricornutum*
Report No	1092-02-1100-1
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Minor: - Initial nominal cell density of 3 × 103 cells/mL was below the recommended density of 5 × 103 – 104 cells/mL for *P. subcapitata*, however validity criteria were met
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate technical on *Pseudokirchneriella subcapitata*, (formerly named *Selenastrum capricornutum,* currently named as *Raphidocelis subcapitata*) were evaluated in a 7-day static toxicity test. After a range-finding test, suspensions of *Pseudokirchneriella subcapitata* were exposed to five nominal concentrations encompassing 10, 18, 32, 56 and 100 mg test item/L. In addition, a control with the test medium (without test substance) was tested.

The test flasks were inoculated with cells from a seven-days-old pre-culture of *Pseudokirchneriella subcapitata* with an initial test cell density of 3000 cells/mL. The test concentrations and the control comprised 3 replicates. The test flasks were placed in the incubator and maintained over several generations for 7 days. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation.

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. On the basis of the mean cell count, the percentage inhibition was determined and the EC_x values calculated using the algal growth curve as determined by inverse estimation least squares linear regression.

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 9.3 % for the lowest test concentration to > 97.6% at or above the nominal test concentration of 18 mg test item/L. The 7-day EC_{50} for *Pseudokirchneriella subcapitata* exposed to glyphosate technical was calculated to be 13.8 mg test item/L.

I. MATERIALS AND METHODS

A. MATERIALS	
Test material:	
Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6 %
Water solubility:	1.2 % at 25 °C
Vehicle and/or positive control:	Vehicle: Dilution water (AAP medium) Positive control: None
Test organism:	
Species:	*Pseudokirchneriella subcapitata*
Initial cell concentration	3 × 103 cells/mL
Source:	In-house culture
Acclimatisation period:	7 days
Environmental conditions:	
Temperature:	24 ± 2 °C
Photoperiod:	24 h light
Light intensity:	4306 ± 650 Lux
pH:	7.5 ± 0.1

B. STUDY DESIGN

Experimental dates: 20 April - 27 April 1987

Experimental treatments

Prior to the main test, a range-finding test was performed with six concentrations ranging between 0.001 and 100 mg test item/L. On the basis of the preliminary test results, the main test was performed with five nominal concentrations (10, 18, 32, 56 and 100 mg test item/L) and three replicates per test item treatment group. Test concentrations were prepared by adding the required volumes of the stock solution to AAP medium in 250 mL volumetric flasks. A control with the test medium (without test substance) was tested under the same conditions as in the test groups. The test was performed in 250 mL volumetric flasks, containing each 50 mL test solution. Test algae were taken from a 7-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 3000 cells/mL. Flasks were kept in an incubator at a temperature of 24 ± 2 °C. Flasks were continuously shaken at 100 oscillations per minute.

Observations

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Three counts per replicate were made. On the basis of the mean cell count, the percentage inhibition was determined. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation. Samples of test media were taken at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analysed for active substance using HPLC.

Statistical calculations

To determine the ECx values, the log of test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50 % inhibition and the associated 95 % confidence intervals were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 value is given below based on nominal concentrations.

Table 0-20: Toxicity of glyphosate technical to *Pseudokirchneriella subcapitata*

Endpoint	Glyphosate technical [mg test item/L]
EC_{50} (7 day)	13.8

Chemical analyses were performed on samples of the test solutions to quantify glyphosate in the test solution. The mean measured concentrations were 10.6, 19.6, 35.2, 58.8 and 104 mg test item/L, corresponding to 106 %, 109 %, 110 %, 105 % and 104 % of the nominal test concentrations, respectively. As the mean measured content of the test item always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

B. OBSERVATIONS

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 9.3 % for the lowest test concentration to > 97.6% at or above the nominal test concentration of 18 mg test item/L.

Table 0-21: Percentage growth inhibition of *Pseudokirchneriella subcapitata* exposed to glyphosate for 7 days

Nominal concentrations [mg test item/L]	Control	10	18	32	56	100
Mean number of algae cells on Day 7 [× 1000 cells/mL]	7000	6347	168.333	11.0	9.333	8.333
Mean inhibition (7 days) [%]	-	9.3	97.6	99.8	99.9	99.9

III. CONCLUSIONS

The 7-day EC_{50} for *Pseudokirchneriella subcapitata* exposed to glyphosate technical was calculated to be 13.8 mg test item/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10}, EC_{20}, and EC_{50}, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	247
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	0.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤ 7%	1.7%

The biomass in the control cultures increased by a factor of ≥16 (achieved: 247), the coefficient of variance for section specific growth rates was ≤ 35% (achieved: 0.6%) and the coefficient of variance for the whole test period it was ≤ 7% (achieved: 1.7%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC was performed (Positon Paper No. CA 8.2.6.1/010). Recovery of test item concentrations ranged from 100 – 114%. Therefore, results are based on nominal concentrations.

Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC value based on nominal test concentrations

Endpoint (0 – 72 hours)	**Glyphosate technical [mg a.e./L]**	
	Yield	**Growth rate**
EC_{10} (95% CI)	< 10	< 10.0
EC_{20} (95% CI)	10.25 (9.46 – 10.9)	10.8 (< 10.0 – 15.4)
EC_{50} (95% CI)	12.11 (11.4 – 12.8)	27.4 (20.2 – 36.6)
NOEC	< 10.0	< 10.0
LOEC	10.0	10.0

CI = confidence interval

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/010
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 1092-02-1100-1 on the toxicity of Glyphosate Technical to *Selenastrum capricornutum* under static conditions
Report No	110054-003
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable None
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 72-h EC10, EC20 and EC50 as well as NOEC values for yield and growth rate was conducted for the algae study 1092-02-1100-1 ([redacted] 1987) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated EC_{10}, EC_{20} and EC_{50} values are <10, 10.8, and 27.4 mg a.e./L, respectively for growth rate and < 10.0, 10.3 and 12.1 mg/L, respectively for yield. NOEC for yield and growth rate were determined to be < 10.0 mg a.e./L. The statistical parameters showed that these values can be considered as reliable and therefore considered for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number: 1092-02-1100-1
Author: [redacted]
Substance: Glyphosate Technical
Title: The Toxicity of Glyphosate Technical to *Selenastrum capricornutum*
Completion date: 27-Apr-1987
Test guideline(s): Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) and re-evaluated according to the current test guideline OECD 201 (2011)
GLP: Yes, conducted under GLP/Officially recognised testing facilities
Testing facility: Malcolm Pirnie, Inc., mite Plains, NY 10602, USA
Sponsor: Monsanto Agricultural Company, Chesterfield, MO 63198, USA

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC_{10}, EC_{20}, and EC_{50}, and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 1092-02-1100-1 ([redacted] 1987) was statistically evaluated for the effects of glyphosate technical on the organism *Pseudokirchneriella subcapitata*, (formerly named *Selenastrum capricornutum,* currently known as *Raphidocelis subcapitata*). The organisms were exposed for 7 days to the following concentrations of Glyphosate technical: 10, 18, 32, 56 and 100 mg a.s./L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50 % effects on yield and growth rate of the test subjects (EC_{10} EC_{20} and EC_{50}), a non-linear regression analysis was performed with a 3-parametric logistic CDF (Cumulative Distribution Function) model for yield and with probit analysis for growth rate.

NOEC levels were determined by Welsh-t-test After Bonferroni-Holm Correction (one-sided smaller; p = 0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Result are provided in the table below:

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	247
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤ 35 %	0.6 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7 %.	≤ 7 %	1.7 %

Analytical recovery of test item ranged from 100 – 114 % of nominal test concentrations. Therefore, results are based on nominal concentrations.

EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

For yield, the parameters for the 3-parameter logistic CDF model are estimated as b0: 73.835; b1: 12.108; b2: 8.330.
For growth rate, the parameters for the probit model are estimated as slope b: 2.08968; Intercept a: -3.00423.

For yield, the statistical parameters are: $F(2, 3) = 1250{,}486$; p(F) = <0.001; R2 = 0.984. After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.838.

For growth rate, statistical parameters for goodness of fit test are: Chi2(13) = 1.61163; p(Chi²): 1.000; F(1,13) = 41.449; p(F) <0.001; r²: 0.761 for growth rate.
Based on these values the EC_{10}, EC_{20} and EC_{50} for yield and growth rate calculations should be considered valid.

The obtained EC_{10}, EC_{20} and EC_{50}, and NOEC values for *Raphidocelis subcapitata*, (formerly known as *Selenastrum capricornutum* or *Pseudokirchneriella subcapitata*) are presented in the table below.

Table 0-22: Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC value based on nominal test concentrations

Endpoint (0 – 72 hours)	Glyphosate technical [mg a.e./L]	
	Yield	Growth rate
EC_{10} (95% CI)	< 10.0	< 10.0
EC_{20} (95% CI)	10.3 (9.46 – 10.9)	10.8 (< 10.0 – 15.4)
EC_{50} (95% CI)	12.1 (11.4 – 12.8)	27.4 (20.2 – 36.6)
NOEC	< 10.0	< 10.0
LOEC	10.0	10.0

CI = confidence interval

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated EC_{10}, EC_{20} and EC_{50} values are <10, 10.8, and 27.4 mg a.e./L, respectively for growth rate and < 10.0, 10.3 and 12.1 mg/L, respectively for yield. NOEC for yield and growth rate were determined to be < 10.0 mg a.e./L. The statistical parameters showed that these values can be considered as reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.6.1/011
Report author	[redacted]
Report year	1995
Report title	Glyphosate: Algal inhibition test
Report No	710/12
Document No	-
Guidelines followed in study	No information mentioned in the Monograph.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Toxicity of glyphosate acid to aquatic organisms (*Desmodesmus subspicatus*) 72 hours static test.
Short description of results:	NOECb = 25 mg a.s./L NOECr = 25 mg a.s./L E_rC_{50} (24 h) = 60 mg a.s./L E_bC_{50} (72 h) = 46 mg a.s./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available. However, these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Acceptability/Reliability	Supportive. The study report is not available to the applicant. Data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015. Validity cannot be checked. Other valid studies with more sensitive endpoints are available.
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 8.2.6.1/012
Report author	[redacted]
Report year	1994
Report title	Testing of toxic effects of aminomethylphosphonic acid (AMPA) on the single cell green alga *Scenedesmus subspicatus*.
Report No	XX-93-271
Document No	-
Guidelines followed in study	Information mentioned in the Monograph: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Acute and chronic toxicity of glyphosate isopropylamin-salt to aquatic organisms (purity 61-65 %) 72 hours static test.
Short description of results:	NOECb = 4.8 mg a.s./L NOECr = 24.0 mg a.s./L E_rC_{50} (72 h) = 166 mg a.s./L E_bC_{50} (72 h) = 72.9 mg a.s./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available. However, these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Acceptability/Reliability	Supportive. The study report is not available to the applicant. Data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015. Other valid studies with more sensitive endpoints are available.
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point	CA 8.2.6.1/013
Report author	[redacted]
Report year	1993
Report title	Algae growth inhibition test – Test article: "Glyphosate isopropylamine salt"
Report No	80-91-2328-01-93
Document No	-
Guidelines followed in study	OECD Guideline 201(1984) and in compliance with "Hemmung der Zellvermehrung bei Grünalge *Scenedesmus subspicatus* – Verfahrensvorschlag der ad hoc Arbeitsgruppe des Umweltbundesamtes Berlin"
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 47.5%, instead of ≤ 35%, and the coefficient for the whole period was 7.8% instead of ≤ 7%
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of glyphosate isopropylamine salt on *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) were evaluated in a 72-hour static toxicity test. After a range-finding test *D. subspicatus* were exposed to six nominal concentrations encompassing 1.6, 5.0, 15.8, 50.0, 158 and 500 mg test item/L.

For each concentration, four parallel cultures in 250 ml Erlenmeyer flasks were prepared. The initial cell concentration was 104 cells/mL. For the control group, six parallel test vessels were prepared.

After 24, 48, and 72 hours of growth, the numbers of viable cells for each test concentrations and control were determined and the growth inhibition was calculated. At this, concentrations resulting in 50 % inhibition (E_rC_{50}, EbC_{50}), were determined, as well as the NOEC.

The EbC and ErC values were calculated by the mean of dose response curve in regression analysis. The EC50 and EC10 values calculated on the basis of the area under the curve are designated as EbC and the EC values based on the calculation of the growth rate are designated as E_rC.

The 72 h E_rC_{50} for *Desmodesmus subspicatus* was determined to be 241 mg glyphosate isopropylamine salt/L. The 72 h E_bC_{50} for *D. subspicatus* was 41.1 mg glyphosate isopropylamine salt/L. Significant effects of glyphosate isopropylamine salt on the growth of *D. subspicatus* were found at a concentration ≥ 15.8 mg test item/L. The NOEC was 15.8 mg test item/L. The validity criteria according to the current guideline OECD 201 were not met. Therefore, this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS	
Test Material:	
Test item:	Glyphosate isopropylamine salt
Lot No.:	01/06/93
Chemical purity:	61.6%
Physical state:	viscous liquid
Density:	1.23 g/cm3 at 20 °C
Vehicle and/or positive control:	
Vehicle:	None
Positive control:	None
Test organism:	
Species:	*Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*)
Initial cell concentration:	104 cells/mL
Source:	Pflanzenphysiologisches Institut, Göttingen, Germany (Stock No. 8681 SAG)
Environmental conditions:	
Temperature:	21 – 23 °C
Photoperiod:	24 h light
Light intensity:	10900 – 11200 lux
Light quality:	Universal white light (8 × 25 W)
pH:	6.69 – 10.59
Conductivity:	not stated
Hardness:	not stated

B. STUDY DESIGN

Experimental dates: 26 July – 29 July 1993

Experimental treatments:
On the basis of the results of a range finding test, the main test was performed with six concentrations, 1.6, 5, 15.8, 50, 158 and 500 mg test item/L.
To maintain the algae in the suspension and to facilitate transfer of CO_2 during the test, the flasks were rotated continuously over the entire test period. For each concentration, four parallel cultures in 250 ml Erlenmeyer flasks were prepared. To each Erlenmeyer flask, 100 mL of the test item preparation were added. The initial cell concentration was 104 cells/mL. For the control group, six parallel test vessels were prepared.

Observations:
After 24, 48, and 72 hours of growth, the numbers of viable cells for each test concentrations and control were and the growth inhibition was calculated. At this, the mean value of the cell concentration (converted

in log values) was plotted versus percentage growth inhibition to generate dose-response curves for each concentration. The concentrations resulting in 50% inhibition (ErC50, EbC50), were determined, as well as the NOEC.

Statistical calculations:
The area under the growth curves, the percentage inhibition of the cell growth at each test concentration, the average specific growth rate for exponentially growing cultures were calculated according to formulas in OECD 201 (1984). The EC50 and EC10 values calculated on the basis of the area under the curve are designated as E_bC, and the EC values based on the calculation of the growth rate are designated as E_rC. The E_bC and E_rC values on the basis of nominal concentrations were calculated by regression analysis after log transformation of the concentration values.

II. RESULTS AND DISCUSSION

A. FINDINGS

The ErC10, EbC10, ErC50, EbC50 and NOEC values are given below based on nominal concentrations.

Table 0-23: Toxicity of Glyphosate isopropylamine salt to *Desmodesmus subspicatus*

Endpoint (72 h)	Glyphosate isopropylamine salt [mg test item/L]
ErC10	18.9
EbC10	6.3
ErC50	241
EbC50	41.1
NOEC	15.8

Analytical measurements were performed by HPLC on four representative concentration levels of glyphosate isopropylamine salt, at 15.8 mg test item/L, equivalent to 7.21 mg glyphosate/L, 50 mg test item/L, equivalent to 22.82 mg glyphosate/L, 158 mg test item/L, equivalent to 72.12 mg glyphosate/L and at the highest concentration tested, 500 mg test item/L, equivalent to 228.22 mg glyphosate/L. The analytical results of the determination of glyphosate isopropylamine salt on the basis of glyphosate are given below.

Table 0-24: Measured concentration and recoveries of glyphosate isopropylamine salt based on glyphosate

Nominal concentration		Measured concentration [mg glyphosate/L]		Recovery [%]	
[mg glyphosate isopropylamine salt/L]	[mg glyphosate/L]	0 h	72 h	0 h	72 h
500	228.216	198.901	197.598	87.2	86.6
158	72.116	74.271	72.599	103.0	100.7
50	22.822	25.318	24.479	110.9	107.3
15.8	7.212	7.834	7.607	108.6	105.5

As the measured contents of glyphosate ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

B. OBSERVATIONS

The results of the definite test show that algae growth was completely inhibited at a nominal concentration of 500 mg test item/L. In contrast, no inhibition of the algae growth was found at or below a nominal concentration of 15.8 mg test item/L.

Table 0-25: Percentage inhibition of growth rate, yield and biomass of to *Desmodesmus subspicatus* exposed for 72 hours to glyphosate isopropylamine salt

Glyphosate isopropylamine salt [mg test item/L]	Mean number of algae cells [10000/ml]	Inhibition growth rate (0-72 h) [%]	Inhibition biomass (0-72 h) [%]
Control	119.1	-	-
1.6	107.4	-5.3	-9.5
5	123.9	-15.0	-4.4
15.8	112.2	17.9	12.4
50	26.1	16.9	69.8
158	15.8	34.2	86.3
500	1.9	84.7	96.9

The required minimum of a 16-fold cell multiplication in the control cultures during the test period was achieved.

III. CONCLUSIONS

The 72 h E_rC_{50} for *Desmodesmus subspicatus* was determined to be 241 mg glyphosate isopropylamine salt/L. The 72 h E_bC_{50} for *D. subspicatus* was 41.1 mg glyphosate isopropylamine salt/L. Significant effects of glyphosate isopropylamine salt on the growth of *D. subspicatus* were found at a concentration >15.8 mg test item/L. The NOEC was 15.8 mg test item/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	66.2
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	47.5%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	7.8%

The biomass in the control cultures increased by a factor of ≥ 16 (actual: 66.2), the coefficient of variance for section specific growth rates exceeded 35% (actual: 47.5%), for the whole test period it exceeded 7% (actual: 7.8%). Because the coefficient of variation for the section specific growth rate was > 35%, and the coefficient for the whole period was > 7%, the validity criteria according to the current guideline

OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.6.1/014
Report author	[redacted]
Report year	1990
Report title	Algal growth inhibition test with compound glyphosate TCN
Report No	1-7-46-90
Document No	-
Guidelines followed in study	OECD 201
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	The toxicity of Glyphosate TCN to *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) was determined in a 96 - hour static test. The test incorporated 5 nominal concentrations at 20, 50 100, 200 and 400 mg a.s./L (Glyphosate TCN: sample No. 16/03/90 with 95% purity) and an untreated control. The test comprised three replicate cultures of each test concentration and the control. The initial nominal cell density was 1.00 × 104 cells/mL. The cell densities were determined microscopically with the help of the Neubauer counting chamber. Cell numbers were counted at test start, after 72 and 96 hours. The pH-values and O2 values were determined in the test media at the beginning and at the end of the test. The room temperature was 22 ± 2°C and light intensity was approx. 8000 lux. The algae were illuminate continuously with fluorescent lamp (Universalweiß Typ L 25, Osram)
Short description of results:	**Glyphosate TCN (mg a.s./L)** / **Inhibition in algal growth (%)** Control - / 20 0 / 50 6.7 / 100 33.3 / 200 55.4 / 400 84.6 **Endpoints (96 h)** / **Glyphosate TCN (mg a.s./L)** LC10 56 ± 26 / LC50 136 ± 64 There were no differences in parameters oxygen and temperature between the test item treatments and the control. The pH values decreased very clearly with increasing dosage. At 400 mg a.s./L 50% of the cells were damaged. This was not observed in the other test item concentrations.
Reasons for why the study is not considered	The study design is not in line anymore with the current guideline OECD 201 requirements (*eg*. control biomass and section specific

relevant/reliable or not considered as key study:	growth rates were not determined, no analytical measurement performed). The validity criteria according to the current guideline could not be concluded. Therefore, no consistent conclusions could be drawn from the study. The study is considered as not relevant according to various shortcomings.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Acceptability/Reliability	Invalid.
Category study in AIR 5 dossier (L docs)	Category 3b

1. Information on the study

Data point	CA 8.2.6.1/015
Report author	[redacted]
Report year	1990
Report title	Acute Toxicity of Glyphosate to *Scenedesmus subspicatus* (OECD – Algae Growth Inhibition Test)
Report No	250773
Document No	
Guidelines followed in study	OECD Guideline 201 (1984)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 101.6%, instead of ≤ 35%
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of glyphosate on *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) were evaluated in a 96-hour static toxicity test. Based on the results of a range finding test, *Desmodesmus subspicatus* were exposed to five nominal concentrations encompassing 1.6, 8.0, 40, 200 and 1000 mg test item/L and a control.

For each test concentration and control treatment three replicates with 30 mL test solution and an initial cell density of 104 cells/mL were prepared in 50 mL Erlenmeyer flasks. Additionally, for the highest test concentration one replicate without algae was provided. The culture vessels were incubated in a shaking water bath at 24°C for 96 hours.

After 24, 48, 72 and 96 hours, the number of algae was estimated microscopically after 24 and 48 hours and after 72 and 96 hours by spectrophotometer.

Test item concentrations were verified by HPLC in the 1.6, 40 and 1000 mg test item/L test item treatments and the 1000 mg/L stability control at the beginning and the end of the test (after 96 hours). During the test period test item concentrations were in the range from 56.9 to 66.6 % of the nominal values. Therefore, all

reported results are related to mean measured concentrations of the test item.
Glyphosate inhibited cell growth of the fresh water algae *Desmodesmus subspicatus* after 72 hours at mean measured concentrations of 200 and 1000 mg test item/L and after 96 hours at mean measured concentrations of 8.0, 40, 200 and 1000 mg test item/L.
The 72 hours EbC_{50} for *Desmodesmus subspicatus* exposed to glyphosate was 326.9 mg/L (300.2 – 354.3 mg test item/L), the 96 hours E_bC_{50} was 117.8 mg/L (107.3 - 129.5 mg test item/L). The NOEC and LOEC for *D. subspicatus* after 96 hours of exposure were 40 and 200 mg test item/L, respectively.
Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	
Test item:	Glyphosate
Description:	Solid
Lot/Batch #:	198-SI-22-1
Purity:	98.7%
Solubility:	Aqueous: 12000 mg/L at 25 °C
Vehicle and/or positive control:	Vehicle: Test medium Positive control: Potassium dichromate (K2Cr2O7)
Test organism:	
Species:	Algae (*Desmodesmus subspicatus)*
Initial cell concentration	104 cells/mL
Source:	Umweltbundesamt, Berlin, Germany
Environmental conditions:	
Temperature:	24.0°C
Photoperiod:	24 h light
Light intensity	8000 lux
pH:	7.7 (adjusted at test start), 6.3 (control), 7.3 (mean of all test concentrations)

B. STUDY DESIGN
Experimental dates: 9 to 13 October 1989

Experimental treatments
Based on the results of a range-finding test the definitive study encompassed five nominal concentrations: 1.6, 8.0, 40, 200 and 1000 mg test item/L. In addition, algae (*Desmodesmus subspicatus)* were exposed to test medium without test substance or other additives (control).
The culture vessels were incubated on a shaking plate in a water bath at 24 °C for 96 hours. During incubation, the algal cells were kept in suspension by continuous shaking. For each concentration and the control, three replicates were prepared in 50 ml Erlenmeyer flasks. To each test vessel, 30 mL of the test item preparation were added with an initial cell density adjusted to 104 cells/mL. Additionally, for the highest test concentration one replicate without algae was provided.

Observations
After 24 and 48 hours, the number of algae was estimated microscopically and spectrophotometrically after 72 and 96 hours. The concentrations resulting in 50 % reduction of growth rate (E_bC_{50}), 100 % reduction of growth rate (E_bC_{100}) and no growth rate reduction (E_bC_0) were determined as area under the growth curve. The pH-values of the test solutions were adjusted at test initiation and measured at test termination. Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis, using duplicate samples of 5 mL taken from the low (1.6 mg/L), medium (40 mg/L) and high (1000 mg/L) test concentration at test termination. From the additional test vessel containing 1000 mg/L and no algae samples of 100 mL and 10 mL were taken after 0 and 96 hours.

Statistical calculations
Inhibition of cell growth was determined from the area under the growth curve. The NOEC and LOEC after 96 hours were statistically determined with the Dunnett´s test.

II. RESULTS AND DISCUSSION

A. FINDINGS
The E_bC_{50} (0 - 72, 0 - 96 hours), NOEC and LOEC values are given below based on nominal concentrations.

Table 0-26: Toxicity of glyphosate to *Desmodesmus subspicatus*

Endpoint	Glyphosate [mg test item/L]
0 - 72 hours E_bC_{50} (95 % CI)	326.9 (300.2 - 354.3)
0 - 96 hours E_bC_{50} (95 % CI)	117.8 (107.3 - 129.5)
NOE_bC	40
LOE_bC	200

CL = confidence limit

Analytical control measurements were performed in the test solutions with nominal values of 1.6, 40 and 1000 mg test item/ and at 1000 mg test item/L without algae. At test initiation and test termination the test concentrations were in a range of 56.9 to 66.6 % of nominal. In the 1000 mg/L stability test the concentration was 117.3 % of nominal at test initiation and 92.9 % of nominal at test termination.
As the mean measured content of the test item was not in the range between 80 and 120 % of nominal, the endpoints are given as nominal concentrations.
Reference item: The 96-hour E_bC_{50} was 1.514 mg/L (95% CI: 1.488 – 1.542 mg/L). These results were in agreement with what was expected on the basis of historical data.

B. OBSERVATIONS
Glyphosate inhibited cell growth of the fresh water algae *Desmodesmus subspicatus* after 72 hours at test concentrations of 200 and 1000 mg test item/L and after 96 hours at test concentrations of 8.0, 40, 200 and 1000 mg test item/L.

Table 0-27: Mean cell densities and percentage of inhibition of cell growth *of Desmodesmus subspicatus* exposed for 72 and 96 hours to glyphosate

	Control	Glyphosate [mg test item/L]				
Test parameters	**-**	**1.6**	**8.0**	**40**	**200**	**1000**
Mean cell densities (0 - 72 h) (× 10000 cells/mL)	35.6	38.0	32.8	36.7	18.2	6.5
Mean cell densities (0 - 96 h) (× 10000 cells/mL)	363.7	348.4	291.5	311.2	107.4	0
Cell growth inhibition (0 - 72 h) [%]	-	-2.2	12.1	8.2	62.0	94.6
Cell growth inhibition (0 - 96 h) [%]	-	-21.3	-12.4	-8.5	36.6	78.9

III. CONCLUSIONS

The 72 hours E_bC_{50} for *Desmodesmus subspicatus* exposed to glyphosate was 326.9 mg/L (300.2 – 354.3 mg test item/L), the 96 hours E_bC_{50} was 117.8 mg/L (107.3 - 129.5 mg test item/L). The NOEC and LOEC for *D. subspicatus* after 96 hours of exposure were 40 and 200 mg test item/L, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	35.6
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	101.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	4.9%

The biomass in the control cultures increased by a factor of ≥16 (actual: 35.6), the coefficient of variance for section specific growth rates exceeded 35% (actual: 101.6%), for the whole test period it was ≤ 7% (actual: 4.9%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/016
Report author	[redacted]
Report year	1998
Report title	Fresh Water Algal Growth Inhibition Test with (Aminomethyl)Phosphonic Acid
Report No	232458
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) EEC Directive 92/69, Part C-3 (1992) ISO International Standard 8692 (1989)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 58.5% instead of ≤35%
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes, conducted under GLP officially recognised testing facilities
Acceptability/Reliability	Invalid (however, study is used for risk assessment, as this is the most reliable algae study with AMPA)
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of (Aminomethyl)phosphonic acid (AMPA) on *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum,* currently known as *Raphidocelis subcapitata*) were evaluated in a 72-hour static toxicity test. After a range-finding test *Pseudokirchneriella subcapitata* were exposed to five nominal concentrations encompassing 10, 22, 46, 100 and 220 mg test item/L and a blank control.

For each test concentration and the control group, three (test concentrations) or six (control) replicates with 50 mL test solution and an initial cell density of 104 cells/mL were prepared in 100 mL vessels. The culture vessels were incubated on a shaking plate for 72 h. After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements.

The concentrations resulting in 50% reduction of growth rate (ErC50) and 50 % inhibition of cell growth (EbC50) were determined, as well as the associated NOEC values.

Results showed that the cell densities were comparable to those of the control at nominal concentrations up to 46 mg test item/L while cell densities at 100 mg test item/L and 220 mg test item/L were increasingly reduced. At 220 mg test item/L almost no increase in cell densities were observed during the test period. Statistically significant inhibition of cell growth was found at test concentrations of 100 mg test item/L and higher.

Growth rates were in the range of the control at concentrations from 10 to 46 mg test item/L during the 72-hour test period, whereas the growth rate of algae exposed to 100 and 220 mg test item/L were increasingly reduced. Statistically significant reduction of growth rate was found at test concentrations of 100 mg/L and higher.

The 72 h ErC50 and 72 h EbC50 values for *Pseudokirchneriella subcapitata* exposed to AMPA were calculated to be 200 mg test item/L and 110 mg test item/L, respectively. NOErC and NOEbC were both determined to be 46 mg test item/L. Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met. Therefore, this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	(Aminomethyl)phosphonic acid (AMPA)
Description:	White powder
Lot/Batch #:	A010047101
Purity:	99 %
Vehicle and/or positive control:	Vehicle: Dilution water (ISO-medium) Positive control: Potassium dichromate ($K_2Cr_2O_7$)
Test organism:	
Species:	*Pseudokirchneriella subcapitata*, strain: CCAP 278/4
Initial cell concentration:	1 × 104 cells/mL
Source:	In-house culture
Acclimatisation period:	4 days
Environmental conditions:	
Temperature:	22.5 – 23.0 °C
Photoperiod:	24 h light
Light intensity:	6000 - 7500 lux
Light quality:	TLD-lamps of 18 Watt
pH:	Blank-control (0 – 72 h): 8.5 10 mg/L (0 – 72 h): 7.7 – 8.0 22 mg/L (0 – 72 h): 7.5 – 8.0 46 mg/L (0 – 72 h): 7.1 – 7.8 100 mg/L (0 – 72 h): 6.2 – 7.0 220 mg/L (0 – 72 h): 6.0 – 6.8
Hardness:	24 mg CaCO3/L

B. STUDY DESIGN

Experimental dates of work: 19 May to 29 May 1998

Experimental treatments

Prior to the main test, a range-finding test was performed with concentrations of 0.1, 1, 10 and 100 mg test item/L. On the basis of these preliminary test results, the main test was performed with five concentrations: 10, 22, 46, 100 and 220 mg test item/L. In addition, algae were exposed to test medium without test substance or other additives (blank control). The test solutions were prepared using ISO-medium.

The culture vessels were incubated on a shaking plate over several generations for 72 h. For each concentration, three parallel cultures were prepared in 100 ml all-glass vessels. To each test vessel, 50 mL of the test item preparation were added, with an initial cell density adjusted to 104 cells/mL. Additionally, for the highest test concentration one replicate without algae was provided. For the control group, six parallel test vessels were prepared.

Observations

After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements and a linear calibration curve relating extinction and cell density.

The concentrations resulting in 50 % reduction of growth rate (E_rC_{50}) and 50 % inhibition of cell growth (E_bC_{50}) were determined, as well as the associated NOEC values.
The pH values of the test solutions were measured at test initiation and test termination. Temperature was controlled daily in a temperature-control vessel.
Analytical control measurements of the actual concentration of the test item were performed by HPLC analysis using samples taken from three representative concentrations, 10, 46 and 220 mg test item/L.

Statistical calculations

The calculation of the EC_{50} values was based on linear regression analysis of the percentages of growth inhibition and the percentages of growth rate reduction versus the logarithms of the corresponding nominal concentrations of the test substance.

II. RESULTS AND DISCUSSION

A. FINDINGS

The ErC50, EbC50 and NOEC values are given below, based on nominal concentrations.

Table 0-28: Toxicity of AMPA to *Pseudokirchneriella subcapitata*

Endpoint (0 – 72 hours)	AMPA [mg test item/L]
E_rC_{50} (95% CI)	200 (98 - 410)
E_bC_{50} (95% CI)	110 (72 - 180)
E_rC_{10} (95% CI)	68 (34 - 140)
E_bC_{10} (95% CI)	53 (33 - 86)
NOE_rC	46
NOE_bC	46

CI = confidence interval

Analytical data: Analytical control measurements were performed on three representative concentrations. At test initiation, 99 %, 100 % and 102 % of the test item were recovered for the nominal concentrations of 10, 46 and 220 mg test item/L, respectively. At test termination, 98 %, 98 % and 96 % of the test item were recovered for the nominal concentrations of 10, 46 and 220 mg test item/L, respectively.
As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Reference item: The 72-hour E_bC_{50} was 1.3 mg reference item/L (95 % CI: 0.34 - 4.6 mg reference item/L), the 72-hour E_rC_{50} was 1.7 mg reference item/L (95 % CI: 1.1 - 2.8 mg reference item/L).

B. OBSERVATIONS

Mean cell densities: Cell densities were comparable to blank at nominal concentrations up to 46 mg test item/L while cell densities at 100 mg test item/L and 220 mg test item/L were increasingly reduced. At 220 mg test item/L almost no increase in cell densities were observed during the 72 hour test period.

Inhibition of cell growth: Inhibition of cell growth increased with increasing concentration of AMPA from a nominal concentration of 22 mg test item/L upwards. Statistically significant inhibition of cell growth was found at test concentrations of 100 mg test item/L and higher.

Reduction of growth rate: Growth rates were in the range of the controls at the concentrations from 10 to 46 mg test item/L during the 72-hour test period, whereas the growth rate of algae exposed to 100 and 220 mg test item/L were increasingly reduced. Statistically significant reduction of growth rate was found at test concentrations of 100 mg test item/L and higher.

Table 0-29: Percentage reduction of growth rate and inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to AMPA

Test parameters (0 – 72 hours)	Control	AMPA [mg test item/L]				
	-	10	22	46	100	220
Mean cell densities (× 10000 cells/mL)	67.8	73.0	67.6	64.5	41.5	5.4
Cell growth rate reduction [%]		-1.7	0.1	1.2	12.0	59.8
Cell growth inhibition [%]		-3.5	3.0	6.6	35.4	87.8

In the controls, cell density increased by an average factor of > 16 within 3 days. Analysis of samples taken from the solution without algae showed that the actual exposure concentration remained above 80% relative to the initial concentration. Further, all test conditions remained within the ranges prescribed by the protocol.

III. CONCLUSION

Under the conditions of the present study the nominal based 72 h E_rC_{50} and the 72 h E_bC_{50} for *Pseudokirchneriella subcapitata* exposed to AMPA were calculated to be 200 mg test item/L and 110 mg test item/L, respectively. The NOE_rC and NOE_bC were both determined to be 46 mg test item/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	67.9
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	58.5%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤ 7%	0.6%

The biomass in the control cultures increased by a factor of ≥16 (actual: 67.9), the coefficient of variance for section specific growth rates exceeded 35% (actual: 58.5%), for the whole test period it was ≤ 7% (actual: 0.6%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.
However, due to the more severe shortcomings of the algae study with *Desmodesmus* exposed to AMPA (CA 8.2.6.1/018, Dengler D. 1994), this study is used in risk assessment.

A statistical re-evaluation addressing EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC was performed (Positon Paper No. 110054-004).

Since analytical recoveries of the test item ranged from 96 to 102%, results are based on nominal test concentrations.

Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	AMPA [mg/L]	
	Yield	Growth rate
EC_{10} (95% CI)	58.2 (45.3 – 74.8)	92.8 (84.6– 102)
EC_{20} (95% CI)	72.5 (57.4– 91.8)	119 (109– 130)
EC_{50} (95% CI)	110 (82.2– 147)	191 (171 – 213)
NOEC	100	100
LOEC	220	220

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/017
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 232458 on the toxicity of (Aminomethyl) phosphonic acid (AMPA) to *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*) under static conditions
Report No	110054-004
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 72-h EC10 EC20 and EC50 as well as NOEC values was conducted for the algae study 232458 [redacted] 1998) to fulfil the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011). The validity criteria according to the current guideline OECD 201 were met for increase of biomass and for coefficient of variation of average specific growth rates in the controls. However, mean coefficient of variation for section-by-section specific growth rate was 58.5 % and exceeds the required 35 %.

As this is the only study currently available for algae exposed to AMPA, the data was further analysed to obtain the required effect concentrations.
The calculated EC10, EC20 and EC50 values are 58.2, 72.5 and 110 mg/L for yield, respectively and 92.8, 119 and 191 mg/L, respectively for growth rate. The statistical parameters presented showed that these values can be considered reliable/valid and therefore considered for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details
Study number: 232458
Author: [redacted]
Substance: (Aminomethyl) phosphonic acid (AMPA)
Title: Fresh Water Algal Growth Inhibition Test with (Aminomethyl)Phosphonic Acid
Completion date: 29 June 1998
Test guideline(s): OECD Guideline No. 201 (1984)
EEC Directive 92/69, Part C-3 (1992)
ISO International Standard 8692 (1989)
GLP: Yes
Testing facility: NOTOX B.V., DD 's-Hertogenbosch, The Netherlands
Sponsor: AgriChem BV, AG OOSTERHOUT, The Netherlands

B. STUDY DESIGN

Dates of work: April 2020
Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC10, EC20, and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 232458 [redacted]) was statistically evaluated for the effects of (Aminomethyl) phosphonic acid (AMPA) on the organism *Pseudokirchneriella subcapitata,* strain: CCAP 278/4 (currently known as *Raphidocelis subcapitata*). The organisms were exposed for 72 h to the following concentrations of (Aminomethyl) phosphonic acid (AMPA): 10, 22, 46, 100 and 220 mg test item/L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50 % effects on growth rate and yield of the test subjects (EC10, EC20 and EC50), the 3-parametric normal CDF (Cumulative Distribution Function) model was used for growth rate and yield.

NOEC for growth rate and yield was determined by Welsh-t-test After Bonferroni-Holm Correction (one-sided smaller, p = 0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met for increase of biomass and for coefficient of variation of average specific growth rates in the controls. However, mean coefficient of variation for section-by-section specific growth rate was 58.5% and exceeds the required 35%.
Results are provided in the table below:

Table 0-30: Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	68
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤ 35 %	58.5 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7 %.	≤ 7 %	0.6 %

As this is the only study currently available for algae exposed to AMPA, the data was further analysed to obtain the required effect concentrations.

The mean measured content of the test item always ranged between 80 and 120% of nominal, therefore, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

For yield, the parameters for the 3-parameter normal CDF model are estimated as b0: 68.412; b1: 1.765; b2: 0.216.
For growth rate, the parameters for the 3-parameter normal CDF model are estimated as b0: 1.411; b1: 1.968; b2: 0.244.

For yield, the statistical parameters are: F (2, 3) = 225.575; p(F) = <0.001; R2 = 0.939. After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.396.
For growth rate, the statistical parameters are: F (2, 3) = 901.363; p(F) = <0.001; R2 = 0.990. After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.637.
Based on these values the EC10, EC20 and EC50 for yield and growth rate calculations should be considered valid.

The obtained EC10, EC20 and EC50 values for *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*) are presented in the table below.

Table 0-31: Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	AMPA [mg test item/L]	
	Yield	Growth rate
EC10 (95% CI)	58.2 (45.3 – 74.8)	92.8 (84.6– 102)
EC20 (95% CI)	72.5 (57.4 – 91.8)	119 (109– 130)
EC50 (95% CI)	110 (82.2 – 147)	191 (171 – 213)
NOEC	100	100
LOEC	220	220

CI = confidence interval

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria according to the current guideline OECD 201 were met for increase of biomass and for coefficient of variation of average specific growth rates in the controls. However, mean coefficient of variation for section-by-section specific growth rate was 58.5% and exceeds the required 35%.

As this is the only study currently available for algae exposed to AMPA, the data was further analysed to obtain the required effect concentrations.

The calculated EC10, EC20 and EC50 values are 58.2, 72.5 and 110 mg/L for yield, respectively and 92.8, 119 and 191 mg/L, respectively for growth rate. NOEC for yield and growth rate were determined to be 100 mg/L.

The statistical parameters presented showed that these values can be considered reliable/valid and therefore considered for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.6.1/018
Report author	[redacted]
Report year	1994
Report title	Testing of toxic effects of aminomethyl phosphonic acid (AMPA) on the single cell green alga *Scenedesmus subspicatus*
Report No	IFU93006/01-Ss
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - Raw data is provided as optical density, however a correlation with biomass is not provided. - Test was conducted in three runs (not replicates). No replicates for each concentration. - In the 2nd and 3rd run, a test substance was used not originally purchased from sponsor, rendering lower absolute growth densities. - Control biomass was not determined and section specific growth rates are not reproducible. - The measured concentrations of AMPA were reported only for one test concentration at the start and at the end of the test.
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The toxicity of AMPA to the green algae *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) was determined in a 72-hour, static test. The test incorporated six nominal concentrations of AMPA (0.192, 0.96, 4.8, 24, 120, and 600 mg a.s./L) and a dilution water control without test item. The test was performed in 3 replicates per test concentration and control. At the start of the test, 50 mL test solutions (or test medium without AMPA for the controls) was inoculated with 104 algae cells/mL. The culture vessels were incubated at 23±2°C under continuous illumination for 72 h. The cell number was determined by photometric measurements at 0, 15, 24, 39, 48, 63, and 72 hours of exposure. The pH-values were determined in the test media at the beginning and at the end of the test.

The nominal concentration in the analysed dilution step was 0.96 mg AMPA/L; the analytical values were 0.99 mg /L at the start of the test and 1.06 mg/L at the end of the test. For that reason AMPA can be regarded as stable under test conditions. Due to various deviations from the current OECD 201 guideline, this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item::	Aminomethyl phosphonic acid (AMPA)
Description:	not stated
Lot/Batch #:	A) PIT-8912-1385A B) 09203L7
Purity:	A) 99.1% B) 99%
Vehicle and/or positive control:	Vehicle:Cell growth medium Positive control: None
Test organism:	
Species:	Algae *Desmodesmus subspicatus* CHODAT
Initial cell concentration:	104 cells/mL
Source:	Collection of algae cultures, Pflanzenphysiologisches Institut der Universitaet, 37073 Goettingen, Germany
Acclimatisation period:	3 days
Environmental conditions:	
Temperature:	23 ± 2 °C (no measured data reported)
Photoperiod:	Continuous illumination
Light intensity:	approximately 8000 lux
pH:	4.32 – 6.39 at the start of the test 4.34 – 7.34 at the end of the test

B. STUDY DESIGN

Experimental dates: 5 November – 10 December 1993

Experimental treatments

The toxicity of AMPA to the green algae *Desmodesmus subspicatus* was determined in a 72-hour, static test. The test incorporated six nominal concentrations of AMPA (0.192, 0.96, 4.8, 24, 120, and 600 mg test item/L) and a dilution water control without test item. The six test concentrations were prepared by appropriate dilutions of a stock solution. The test was performed in three runs per test concentration and control. At the start of the test, 50 mL test solution (or test medium without AMPA for the controls) was inoculated with 104 algae cells/mL. The culture vessels were incubated at 23 ± 2 °C under continuous illumination for 72 hours.

Observations

The cell number was determined by photometric measurements at 0, 15, 24, 39, 48, 63, and 72 hours of exposure. The pH-values were determined in the test media at the beginning and at the end of the test.

Statistical calculations

Graphical determination of endpoints.

II. RESULTS AND DISCUSSION

A. FINDINGS

The ErC50, EbC50 and NOEC values are given below, based on nominal concentrations.

Table 0-32: Toxicity of AMPA to *Desmodesmus subspicatus* (nominal values)

Endpoints (72 hours)	AMPA [mg test item/L]
NOErC	8.3
ErC10	18.5
ErC50	452
NOEbC	7.9
EbC10	12.9
EbC50	89.8

The nominal concentration in the analysed dilution step was 0.96 mg AMPA/L; the analytical values were 0.99 mg AMPA/L at the start of the test and 1.06 mg/L at the end of the test. For that reason, AMPA can be regarded as stable under test conditions.

B. OBSERVATIONS

AMPA inhibited cell growth of the fresh water algae *Desmodesmus subspicatus* after 72 hours within a test item concentration of 0.1 to 600 mg test item./L (nominal).

Table 0-33: Calculation of the percentage of inhibition for the determination of the EbC value (0-72 h)

Nominal concentration (mg AMPA/L)	Parallel 1		Parallel 2		Parallel 3	
	Area (A)	% inhibition	Area (A)	% inhibition	Area (A)	% inhibition
Control	0.9085	0	0.478	0	0.495	0
0.192	0.980	-7.87*	0.506	-5.85*	0.546	-10.3*
0.96	1.0415	-14.63*	0.5565	-16.42*	0.598	-20.8*
4.8	0.9875	-8.69*	0.515	-7.74*	0.513	-3.63*
24	0.897	1.26	0.446	6.69	0.444	10.3
120	0.6725	25.97*	0.094	80.33	0.116	76.65
600	0.231	74.5	0.080	83.26	0.092	81.41

* Not taken for the calculation

Table 0-34: Calculation of the percentage of inhibition for the determination of the ErC value (0-72 h)

	Parallel 1		Parallel 2		Parallel 3	
Nominal concentration (mg AMPA/L)	**μ (1/h)**	**% inhibition**	**μ (1/h)**	**% inhibition**	**μ (1/h)**	**% inhibition**
Control	0.0871	0	0.0890	0	0.0894	0
0.192	0.0823	5.51*	0.0702	21.12*	0.0810	9.39*
0.96	0.0742	14.81*	0.0712	20.00*	0.0827	7.49*
4.8	0.0785	9.87*	0.0700	21.34*	0.0742	17.00*
24	0.0762	12.51	0.0605	32.02*	0.0716	19.91
120	0.0717	17.68	0.0598	32.80	0.0609	31.88
600	0.0377	56.71	0.0349	60.78	0.0429	52.01

* Not taken for the calculation

III. CONCLUSION

The 72 h EbC50 for *Desmodesmus subspicatus* exposed to AMPA was 89.8 mg test item/L (nominal). The 72 h ErC50 was 452 mg test item/L (nominal).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study shows various deficiencies.

- Raw data is provided as optical density, however a correlation with biomass is not provided.
- Test was conducted in three runs instead of simultaneous replication of each test concentration.
- For the 2nd and 3rd run, a test substance was used with a different source and lot number compared to the first run, rendering lower absolute growth densities.
- Control biomass was not determined and section specific growth rates are not reproducible.
- The measured concentrations of AMPA were reported only for one test concentration at the start and at the end of the test.

Therefore, the study is not considered valid. However, an additional study with AMPA is available.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.6.1/019
Report author	[redacted]
Report year	2011
Report title	HMPA (hydroxymethylphosphonic acid): A 72-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*)
Report No	139A-396A
Document No	-
Guidelines followed in study	OECD Guideline 201 (2006) EU Directive 92/69/EEC, Method C.3. (1992)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of HMPA on *Pseudokirchneriella subcapitata* were evaluated in a 72-hour static toxicity test. *P. subcapitata* were exposed to five nominal concentrations encompassing 7.5, 15, 30, 60 and 120 mg HMPA/L, and the measured concentrations were 7.3, 14, 29, 60 and 115 mg HMPA/L respectively.
For each concentration, three parallel cultures in 250 ml Erlenmeyer flasks were prepared. The initial cell concentration was 1 x 104 cells/mL. For the control group, six parallel test vessels were prepared. After 24, 48, and 72 hours of growth, the numbers of viable cells for each test concentrations and control were determined and the growth inhibition was calculated. Exposure concentrations resulting in 50 % inhibition (ErC50, EC50), were determined, as well as the NOAEC. EC50, ErC50 and the corresponding 95 % confidence limits for each 24-hour exposure interval were calculated by non-linear regression.
The results of main test showed that the algal growth was not inhibited at the measured test item concentrations of 7.3, 14, 29 and 60 mg HMPA/L, and was inhibited slightly at the measured test item concentration of 115 mg HMPA/L.
The 72 h-ErC50 and EC50 for *P. subcapitata* exposed to HMPA was determined both >115 mg HMPA/L. The NOAEC was 60 mg HMPA/L. The validity criteria according to the current guideline OECD 201 were met. Therefore, this study is considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS	
Test Material:	
Identification:	Hydroxymethyl phosphonic acid (HMPA)
Lot No.:	GLP-1003-20448-A
Chemical purity:	97 %
Physical state:	White powder
Storage condition:	Ambient desiccated
Expiration date:	30 April 2012

Vehicle and/or positive control:	
Vehicle:	None
Positive control:	None
Test organism:	
Species:	*Pseudokirchneriella subcapitata*
Initial cell concentration:	104 cells/mL
Source:	in-house culture, started from University of Toronto Culture Collection
Environmental conditions:	
Temperature:	23.0 – 24.8 °C
Photoperiod:	24 h light
Light intensity:	6030 – 7040 lux
Light quality:	cool-white fluorescent lighting
pH:	7.0 – 7.2 (test start); 7.5 – 9.3 (test termination)
Conductivity:	not stated
Hardness:	not stated

B. STUDY DESIGN

Experimental dates: 13 June - 16 June 2010

Experimental treatments
Three replicate cultures per test concentration of *P. subcapitata* (initial cell density in each chamber was 1 × 104 cells/mL) were exposed for 72 hours to nominal concentrations of 7.5, 15, 30, 60, and 120 mg HMPA/L. A negative control group with six replicate cultures was held under the same environmental conditions concurrently.
A primary stock solution with a nominal concentration of 120 mg HMPA/L was prepared, and the pH of mixed sufficiently stock solution was determined as 3.0. The pH of the stock solution was adjusted to 7.0 ± 0.1 with 0.1 N NaOH, then another four test solutions with the nominal concentrations of 7.5, 15, 30 and 60 HMPA/L were prepared through proportionally diluting of stirred stock solution.

Observations
Test medium samples were collected from each biological replicate of the treatment and control group for the determination of algal cell densities. Samples were collected at approximately 24-hour intervals during the 72-hour exposure and were held for a maximum of two days under dark, refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Cell counts. Prior to conducting cell counts, the linearity of the instrument response was determined at settings previously established for *P. subcapitata*.

Samples of test solution were collected from each of the replicates per treatment and control group at the end of the test. These samples were pooled within their respective treatments, and subsamples were removed and examined microscopically for atypical cell morphology (e.g., changes in cell shape, size or color). Cells in the replicate test chambers also were assessed for aggregations or flocculation of cells, and adherence of the cells to the test chamber.
Samples of the test solutions were collected at approximately 0 and 72 hours to measure concentrations of the test substance. At test initiation, samples were collected for each treatment and control group prior to

distribution of test solution into test chambers. At 72 hours, samples were collected from the pooled biological replicates from each respective treatment and control group.

The temperature was recorded twice daily during the test using a liquid-in-glass thermometer. Light intensity was measured at test initiation. The pH of the medium in each treatment and control group was measured at test initiation and at test termination

Statistical calculations
Cell densities, growth rates and percent inhibition values were calculated according to formulas in OECD 201 (2006) using SAS System for Windows (Version 8.2). EC50, ErC50 and the corresponding 95 % confidence intervals for each 24-hour exposure interval were calculated by non-linear regression.

The 72-hour cell density and growth rate data were evaluated for normality and homogeneity of variance (p=0.01) using the Shapiro-Wilk's and Levene's tests, respectively. All data met the assumptions for normality and homogeneity of variance; therefore, the treatment groups were compared to the negative control using Dunnett's test (p=0.05). The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOAEC relative to each parameter at 72 hours.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50, ErC50 and NOAEC values are given below based on mean determined concentrations.

Table 0-35: Toxicity of HMPA to *Pseudokirchneriella subcapitata* exposed for 72 hours to HMPA

Endpoint	HMPA [mg test item/L]
EC50 (cell density)	> 115
ErC50 (growth rate)	> 115
NOAEC (cell density)	60
NOAEC (growth rate)	60

Concentrations of HMPA in the samples were determined using a HPLC/MS. Calibration standards of HMPA, ranging in concentration from 1.00 to 10.0 mg HMPA/L, were prepared in freshwater algal medium using a stock solution of HMPA in methanol. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The method limit of quantitation (LOQ) for these analyses was defined as 1.00 mg HMPA/L. The analytical results are given below.

Table 0-36: Measured concentrations of HMPA in freshwater algal medium samples

Nominal concentration [mg HMPA/L]	Sampling time [hours]	Measured concentration [mg HMPA/L]	Percent of nominal [%]	Mean measured concentration [mg HMPA/L]	Mean percent of nominal [%]
-	0	< LOQ	-	-	-
	72	< LOQ	-		
7.5	0	7.92	106	7.3	97
	72	6.60	88.0		

Table 0-36: Measured concentrations of HMPA in freshwater algal medium samples

Nominal concentration [mg HMPA/L]	Sampling time [hours]	Measured concentration [mg HMPA/L]	Percent of nominal [%]	Mean measured concentration [mg HMPA/L]	Mean percent of nominal [%]
15	0	14.1	94.1	14	93
	72	13.9	92.8		
30	0	29.8	99.4	29	97
	72	27.7	92.4		
60	0	62.5	104	60	100
	72	57.5	95.8		
120	0	110	91.7	115	96
	72	120	100		

Although the measured concentrations of test item in test medium always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using the mean measured concentrations of the test item.

B. OBSERVATIONS

At test initiation, algal cells appeared normal. After 72-hours of exposure there were no noticeable changes in cell morphology in any of the tested concentrations when compared to the control. No flocculation or aggregation of cells or adherence of cells to test chambers were observed.

The results showed that the algal growth was not inhibited at the measured test item concentrations of 7.3, 14, 29 and 60 mg HMPA/L, and was inhibited slightly at the measured test item concentration of 115 mg HMPA/L

Table 0-37: Percentage inhibition of growth rate and cell density to *P. subcapitata* exposed for 72 hours to HMPA (mean measured)

	Control	HMPA [mg test item/L]				
	-	7.3	14	29	60	115
Mean number of algae cells (10000/ml)	298.8	319.2	294.9	286.5	273.1	186.4[1]
Inhibition growth rate (0-72 h) [%]	-	-1	0	1	2	8[1]
Inhibition cell density (0-72 h) [%]	-	-7	1	4	9	38[1]

[1] There were statistically significant differences (p<0.05) in comparison to the negative control replicates.

The mean cell density in the control flasks increased by a factor greater than 16 within three days, and the factor was 299. The coefficient of variation of average specific growth rate in the control replicates during the whole test period did not exceed 7 %, and it was 0.96 %. The mean percent coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) did not exceed 35 %, and it was 23.4 %.

III. CONCLUSIONS

The 72 h ErC50 for *Pseudokirchneriella subcapitata* exposed to HMPA was determined >115 mg HMPA/L. The 72 h EC50 for *P. subcapitata* exposed to HMPA was also >115 mg HMPA/L. Slight effect of HMPA on the growth of *P. subcapitata* were found at the measured concentration of 115 mg HMPA/L. The NOAEC was 60 mg HMPA/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	299
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	23.4%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	1.0%

The biomass in the control cultures increased by a factor of ≥16 (actual: 299), the coefficient of variance for section specific growth rates was ≤ 35% (actual: 23.4%) and the coefficient of variance for the whole test period it was ≤ 7% (actual: 1.0%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC10 and EC20 was performed (Positon Paper No. CA 8.2.6.1/020).

Re-calculated EC10 and EC20 values based on nominal test concentrations

Endpoint (0 – 72 hours)	HMPA [mg/L]	
	Yield	Growth rate
EC10 (95% CI)	57.8 (40.7 – 82.1)	> 120
EC20 (95% CI)	80.4 (56.1 – 116)	> 120

CI = confidence interval

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.1/020
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 139A-396A on the toxicity of Hydroxymethyl phosphonic acid (HMPA) to *Pseudokirchneriella subcapitata* under static conditions
Report No	110054-005
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 72 h EC10 and EC20 values was conducted for the study 139A-396A ([redacted] 2011) to fulfil the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number: 139A-396A
Author: [redacted]
Substance: HMPA (hydroxymethylphosphonic acid)
Title: HMPA (hydroxymethylphosphonic acid): A 72-Hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata*)
Completion date: 11-Oct-2011
Test guideline(s): EU Directive 92/69/EEC, Method C.3., OECD 201 (2011)
GLP: yes
Testing facility: Wildlife International, Ltd., Easton, Maryland 21601 USA
Sponsor: Monsanto Company, St. Louis, Missouri 63167; USA

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72 h EC10 and EC20 values were calculated to fulfil the data requirements according to regulation EU 283/2013. The study 139A-396A ([redacted] 2011) was statistically evaluated for the effects of HMPA (hydroxymethylphosphonic acid) on the organism *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*). The organisms were exposed for 72 hours to the following concentrations of HMPA: 7.5, 15, 30, 60 and 120 mg HMPA/L, and the measured concentrations were 7.3, 14, 29, 60 and 115 mg HMPA/L respectively. Additionally, a control was tested in parallel.

The report states the 72-h EC50 for yield and growth rate to be > 115 mg HMPA/L based on mean measured concentrations, corresponding to > 120 mg HMPA/L based on nominal concentrations. The NOEC was determined to be 60 mg HMPBA/L for growth rate and cell density.
The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.
The data used to derive the 72-h EC10 and EC20 were obtained from the original study report.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive Effect Concentrations that have 10 and 20% effects on growth rate and yield of the test subjects (EC10 and EC20), a non-linear 3-parameter normal CDF (Cumulative Distribution Function) model for growth rate and yield and regression analysis was performed.
All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Result are provided in the table below:

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	299
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤35 %	23.4 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7 %.	≤7 %	1.0 %

For yield, the parameters for the 3 parameter normal CDF model are estimated as b0: 300.355, b1: 1.762, and b2: 0.326.

For growth rate, the parameters for the 3 parameter normal CDF model are estimated as b0: 1.902, b1: 2.122, and b2: 0.438.

According to the statistical parameters; F (2, 3) = 46.773; p(F) = <0.001; R2 = 0.817 the EC10 and EC20 for yield and F (2, 3) = 65.380; p(F) = <0.001; R2 = 0.865 the EC10 and EC20 for growth rate, calculations should be considered valid.

After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.237 for yield and 0.321 for growth rate as shown in Appendix 2 of this report.

The obtained EC10 and EC20 effect of HMPA on growth rate and yield on *Pseudokirchneriella subcapitata* values are presented in the table below.

Recovery of test concentrations ranged from 94.1 to 106% for fresh solutions and from 88.0 to 100% for spent solutions. Therefore, endpoints are given based on nominal concentrations.

Table 0-38: Re-calculated EC10 and EC20 values based on nominal test concentrations

Endpoint (0 – 72 hours)	HMPA [mg/L]	
	Yield	Growth rate
EC10 (95% CI)	57.8 (40.7 – 82.1)	> 120
EC20 (95% CI)	80.4 (56.1 – 116)	> 120

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid

The calculated EC10 and EC20 values are 57.8 and 80.4 mg/L, respectively for yield and > 120 and > 120 mg/L for growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:

CA 8.2.6.2 Effects on growth of an additional algal species

Studies considering the effects of glyphosate on additional algal species were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Where appropriate position papers are available as indicated in the table below, which contain details regarding the statistical re-evaluation of the study to current requirements. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below

Table 0-1: Studies on effects of glyphosate and metabolites to additional algal species

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.6.2/001	[redacted] 1996	96 h algae inhibition	*Anabaena flos-aquae*	Glyphosate acid	Supportive	Correlation between biomass and optical density cannot be demonstrated.
CA 8.2.6.2/002	[redacted] 1987	168 h algae inhibition	*Anabaena flos-aquae*	Glyphosate technical	valid	-
CA 8.2.6.2/003	[redacted] 2020	Position Paper	*Anabaena flos-aquae*	Glyphosate technical	valid	-
CA 8.2.6.2/004	[redacted] 1996	120 h algae inhibition	*Navicula pelliculosa*	Glyphosate acid	invalid	Coefficient of variation for section specific growth rate: > 35%

Table 0-1: Studies on effects of glyphosate and metabolites to additional algal species

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.6.2/005	[redacted], 1987	168 h algae inhibition	*Navicula pelliculosa*	Glyphosate technical	invalid	Biomass increase in control cultures: <16, coefficient of variation for the whole period: >10%,
CA 8.2.6.2/006	[redacted] 1996	96 h algae inhibition	*Skeletonema costatum*	Glyphosate acid	valid	-
CA 8.2.6.2/007	[redacted] 2020	Position Paper	*Skeletonema costatum*	Glyphosate acid	valid	-
CA 8.2.6.2/008	[redacted], 1987	168 h algae inhibition	*Skeletonema costatum*	Glyphosate technical	invalid	Biomass increase in control cultures: <16 and coefficient of variation for section specific growth rate: > 35%
CA 8.2.6.2/009	[redacted] 1978	96 h algae inhibition	*Skeletonema costatum*	Glyphosate intermediate	invalid	No information on validity criteria
CA 8.2.6.2/010	[redacted] 1996	96 h algae inhibition	*Nitzschia palea*	Glyphosate technical	invalid	Numerous validity criteria not met

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the effects of glyphosate or its relevant metabolites on growth of additional algal species. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to algae, please refer to document M-CP Section 10.2.

Endpoints of studies considered valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 0-2: Endpoints: Toxicity of glyphosate to additional algae species

Reference*	Test item	Species	Test design	Endpoints expressed as	72h ErC50[1]	72h EyC50	NOErC
					(mg a.e./L)		
[redacted] 1987 CA 8.2.6.2/002	Glyphosate acid	Algae *Anabaena flos-aquae*	168 h algae inhibition*	nom	33.4	16.4	10.0
[redacted] 1996 CA 8.2.6.2/006	Glyphosate acid	marine alga *Skeletonema costatum*	96 h algae inhibition*	nom	13.5	9.00	5.6

* All endpoints are based on statistical re-evaluation provided in Position Papers: CA 8.2.6.2/003, CA 8.2.6/007
a.e.: acid equivalents; nom: nominal; Endpoint in **bold is** used for risk assessment.
[1] According to the provisions of the new Guidance Document on Aquatic Ecotoxicology (2013), ErC50 endpoints shall be chosen for the risk assessment if available

Study summaries are provided below.

1. Information on the study

Data point	CA 8.2.6.2/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid; Toxicity to blue-green alga *Anabaena flos-aquae*
Report No	AB0503/J
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982)
Deviations from current test guideline	Deviations to OECD 201 (2011): Major: - Raw data is provided as optical density, however a correlation with biomass is not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary
Executive Summary
The toxicity of glyphosate acid to the blue-green alga *Anabaena flos-aquae* was determined in a 120-hour, static test. Algae were exposed to glyphosate acid at nominal concentrations of 0.75, 1.5, 3.0, 6.0, 12, 24, 48, 96 mg test item/L. A control group consisting of culture medium without test item was also prepared in parallel.
The test vessels were 250 mL conical flask containing 100 mL of test or control medium. Six vessels were prepared for the control, and three replicate vessels at each concentration of glyphosate acid. Each replicate test vessel was inoculated with a nominal cell density of 2.05 × 104 cells/mL. All vessels were incubated at 24 ± 1 °C under continuous illumination for 120 hours.

After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel and algal cell densities were determined by spectrophotometrically. The pH-values in the test and control media, were determined at the beginning and at the end of the test. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.
The mean measured concentrations of glyphosate acid ranged from 98 to 110 % of the nominal values.
The 72 h EbC50 (based on nominal concentrations) for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg test item/L, the 72 h ErC50 was 22 mg/L and the 72-hour NOEbC and NOErC values were both 12 mg test item/L. The 120 h EbC50 for *Anabaena flos-aquae* exposed to glyphosate acid was 15 mg test item/L. The 120 h ErC50 was 38 mg/L.
A satisfactory correlation between optical density and biomass cannot be made as the report does not provide a calibration curve. Therefore, this study is considered supportive.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	
Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Vehicle and/or positive control:	Vehicle: Cell growth medium Positive control: None
Test organism:	
Species:	Blue-green alga *Anabaena flos-aquae*
Initial cell concentration:	2.05× 104 cells/mL
Source:	Brixham Environmental Laboratory culture from strain CCAP 1403/13A, Culture Centre of Algae and Protozoa, Institute of Freshwater Ecology. Windermere Laboratory, Far Sawrey, Ambleside, Cumbria, UK
Environmental conditions:	
Temperature:	24.1-24.2 °C (measured by thermometer) The hourly temperature measured automatically remained within 24 ± 1°C
Photoperiod:	Continuous illumination
Light intensity:	3600 lux
pH:	3.5 – 7.2 at the start of the test 3.6 – 8.2 at the end of the test

B. STUDY DESIGN

Experimental dates: 4 March – 9 March 1996
Experimental treatments
The toxicity of glyphosate acid to the blue-green alga *Anabaena flos-aquae* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (0.75, 1.5, 3.0, 6.0, 12, 24, 48, 96 mg test item/L) and a control consisting of culture medium without test item.
The stock solution of nominal concentration of 96 mg test item/L was prepared by adding 192 mg of

glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 0.75, 1.5, 3.0, 6.0, 12, 24, and 48 mg test item/L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel.
The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution, with six replicate vessels prepared for the control group with culture medium only and three replicate vessels prepared for each concentration of glyphosate acid. Each replicate test vessel was inoculated with 1.120 mL of the inoculum culture to give a nominal cell density of 2.05 × 104 cells/mL. The culture vessels were incubated at 24 ± 1°C under continuous illumination for 120 hours. A blank vessel (without algal inoculum) containing control medium and single blank vessels for each test concentration were also incubated concurrently.

Observations
The algal cell densities were determined by spectrophotometric adsorbance, using a Uvikon 860 UV/visible spectrophotometer. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank solution absorbance was subtracted from that of the test culture to obtain the algal absorbance reading. At the start of the test, the absorbance of a range of dilutions of the inoculum culture was used to determine the relationship between absorbance and cell density. The pH-values were measured in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

Statistical calculations
One-way analysis of variance, and Dunnett's procedure.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of glyphosate acid ranged from 98 to 110 % of the nominal values. On the basis of the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

Table 0-3: Toxicity of glyphosate acid to *Anabaena flos-aquae* (nominal values)

Endpoint	Glyphosate acid [mg a.s./L]	
	0 – 72 hours	0 -120 hours
ErC50 (95% CI)	22 (8.8 – >96)	38 (20 – >96)
EbC50 (95% CI)	8.5 (2.6 – 28)	15 (9.7 – 27)
NOErC	12	12
LOErC	24	24
NOEbC	12	12
LOEbC	24	24

B. OBSERVATIONS

Glyphosate acid inhibited cell growth of the fresh water algae *Anabaena flos-aquae* after 120 hours at test concentrations of 24, 48 and 96 mg test item/L (nominal).

Table 0-4: Mean areas under the growth curve

Nominal concentration [mg a.s./L]	0-3 day		0-4 day		0-5 day	
	Mean area under growth curve	% of control	Mean area under growth curve	% of control	Mean area under growth curve	% of control
Control	0.4	-	1.5	-	3.5	-
0.75	0.4	91	1.5	103	3.6	105
1.5	0.3	85	1.5	99	3.6	102
3.0	0.3	80	1.4	94	3.5	99
6.0	0.3	82	1.4	94	3.5	100
12	0.3	76	1.3	87	3.3	93
24	0.0*	6	0.0*	2	0.0*	1
48	0.0*	5	0.0*	2	0.0*	1
96	0.0*	5	0.0*	2	0.0*	1

* Significant difference from the culture control (P=0.05)

Table 0-5: Mean growth rates

Nominal concentration [mg a.s./L]	0-3 day		0-4 day		0-5 day	
	Mean growth rate	% of control	Mean growth rate	% of control	Mean growth rate	% of control
Control	1.392	-	1.331	-	1.139	-
0.75	1.365	98	1.357	102	1.145	101
1.5	1.336	96	1.355	102	1.139	100
3.0	1.328	95	1.344	101	1.141	100
6.0	1.321	95	1.342	101	1.144	100
12	1.299	93	1.321	99	1.138	100
24	0.231*	17	0.216*	16	0.251*	22
48	0.231*	17	0.173*	13	0.139*	12
96	0.231*	17	0.173*	13	0.139*	12

* Significant difference from the culture control (P=0.05)

III. CONCLUSIONS

The 72 h EbC50 for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg test item/L, the 72 h ErC50 was 22 mg/L and the 72-hour NOEbC and NOErC values were 12 mg test item/L, respectively. The 120 h EbC50 for *Anabaena flos-aquae* exposed to glyphosate acid was 15 mg test item/L. The 120 h ErC50 was 38 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The nominal based 72 h EbC50 for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg a.s/L, the 72 h ErC50 was 22 mg a.s./L and the 72-hour NOEbC and NOErC values were 12 mg a.s./L, respectively. Raw data of the study is given in optical density. A satisfactory correlation between optical density and biomass cannot be made as the report does not provide a calibration curve.
Therefore, this study is considered supportive. Another valid study with *Anabaena flos-aquae* is available.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.2/002
Report author	[redacted]
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Anabaena flos-aquae*
Report No	1092-02-1100-4
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Minor: - Initial cell density of 3 × 103 cells/mL was below the recommended density of 104 cells/mL for *Anabaena flos-aquae*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate technical on *Anabaena flos-aquae* were evaluated in a 7-day static toxicity test. The test comprised five nominal test concentrations of 10, 18, 32, 56 and 100 mg test item/L (mean measured test concentration: 9.7, 18.1, 32.6, 55.1 and 102.2 mg test item/L). In addition, a control (untreated culture medium) was tested.

The test flasks were inoculated with cells from a seven-days-old pre-culture of *Anabaena flos-aquae* with an initial test cell density of 3000 cells/mL. The test was performed in 500 mL volumetric flasks, containing each 100 mL test solution. The test concentrations and the control were prepared in three replicates. The test flasks were placed in an incubator and maintained over several generations for 7 days. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation.

Cells were counted on test days 2, 3, 4, and 7 after test initiation by using a Coulter counter. On the basis of the mean cell count, the percentage inhibition was determined and the ECx values calculated using of the algal growth curve as determined by inverse estimation least squares linear regression.

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 79.8% for the nominal test concentration of 18 mg test item/L to 99.5% for the highest nominal test concentration of 100 mg test item/L. At the lowest nominal concentration of 10 mg test item/L, however, a slight algal growth increase of 5.4% relative to control was observed.

The 7-day EC50 for *Anabaena flos-aquae* exposed to glyphosate technical was calculated to be 4.4 mg test item/L. The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6%
Water solubility	1.2 % at 25°C
Vehicle and/or positive control:	Vehicle: Dilution water (AAP medium) Positive control: None
Test organism:	
Species:	*Anabaena flos-aquae*
Initial cell concentration:	3000 cells/mL
Source:	In-house culture
Environmental conditions:	
Temperature:	24 ± 2°C
Photoperiod:	24 h light
Light intensity	2153 ± 323 Lux
pH:	7.5 ± 01
Conductivity:	Not stated
Hardness:	Not stated

B. STUDY DESIGN

Experimental dates of work: 20 April to 27 April 1987

Experimental treatments

Prior to the main test, a range-finding test was performed with six concentrations ranging between 0.001 and 100 mg test item/L. On the basis of the preliminary test results, the main test was performed with five nominal concentrations (10, 18, 32, 56 and 100 mg test item/L) and three replicates per test item treatment group. Test concentrations were prepared by adding the required volumes of the stock solution to AAP medium. A control with the test medium (without test substance) was tested under the same conditions as in the test groups. The test was performed in 500 mL volumetric flasks, containing each 100 mL test solution. Test algae were taken from a 7-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 3000 cells/mL. Flasks were kept in an incubator at a temperature of 24 ± 2°C. Flasks were manually shaken on every working day.

Observations

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Based on the mean cell count, the percentage inhibition was determined. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation. Samples of test media were taken at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analysed for active substance using HPLC.

Statistical calculations
To determine the ECx values, the log of test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50 % inhibition and the associated 95 % confidence intervals were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 value is given below based on mean measured concentrations.

Table 0-6: Toxicity of glyphosate technical to *Anabaena flos-aquae*

Endpoint	Glyphosate technical [mg a.e./L]
EC50 (7 day)	4.4

Chemical analyses were performed on samples of the test solutions to quantify glyphosate technical in the test solution. The mean measured concentrations were 9.7, 18.1, 32.6, 55.1 and 102.2 mg test item/L, corresponding to 97.0 %, 100.6 %, 101.9 %, 98.4 % and 102.2 % of the nominal test concentrations of 10, 18, 32, 56 and 100 mg test item/L respectively.

B. OBSERVATIONS
The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 79.8 % for the nominal test concentration of 18 mg test item/L to 99.5 % for the highest nominal test concentration of 100 mg test item/L. At the lowest nominal concentration of 10 mg test item/L, however a slight algal growth increase of 5.4 % relative to control was observed.
As the mean measured content of the test item always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table 0-7: Percentage of growth inhibition of *Anabaena flos-aquae* exposed to glyphosate technical for 7 days

Nominal concentrations [mg test item/L]	**Control**	**10**	**18**	**32**	**56**	**100**
Measured concentrations [mg a.e./L]	**-**	**9.7**	**18.1**	**32.6**	**55.1**	**102.2**
Mean number of algae cells on Day 7 [× 1000 cells/mL]	1486.66	1566.667	300.0	10.0	8.333	7.667
Mean inhibition (7 days) [%]	-	-5.4	79.8	99.3	99.4	99.5

III. CONCLUSIONS

The 7-day EC50 for *Anabaena flos-aquae* exposed to glyphosate technical was calculated to be 4.4 mg test item/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	27
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	20.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤10%	6.4%

The biomass in the control cultures increased by a factor of ≥16 (achieved: 27), the coefficient of variance for section specific growth rates was ≤ 35% (achieved: 20.6%) and the coefficient of variance for the whole test period it was ≤ 10% (achieved: 3.4%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC10, EC20, EC50, NOEC and LOEC was performed (Positon Paper No. CA 8.2.6.2/003).

Recovery of mean measured concentrations ranged from 91 to 108%. Therefore, endpoints are based on nominal test concentrations.

Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal test concentrations

Endpoint (0 – 72 hours)	Glyphosate technical [mg a.e./L]	
	Yield	Growth rate
EC10 (95% CI)	9.97 (7.21 – 11.7)	7.63 (3.08 – 11.9)
EC20 (95% CI)	11.8 (9.35 – 13.4)	12.7 (6.71 – 17.7)
EC50 (95% CI)	16.4 (14.7 – 18.1)	33.4 (25.7 – 43.7)
NOEC	10	10
LOEC	18	18

CI = confidence interval

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.2/003
Report author	
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 1092-02-1100-4 on the toxicity of Glyphosate technical to *Anabaena flos-aquae* under static conditions
Report No	110054-006
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 72-h EC10, EC20 and EC50 as well as the NOEC values was conducted for the algae study 1092-02-1100-4 (1987) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated EC10, EC20 and EC50 values are 9.97, 11.8 and 16.4 mg/L for yield and 7.63, 12.7 and 33.4 mg a.e./L for growth rate, respectively. The NOEC was determined to be 10 mg a.e./L for yield and growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number: 1092-02-1100-4
Author:
Substance: Glyphosate
Title: The toxicity of glyphosate technical to *Anabaena flos-aquae*
Completion date: 20-Apr-1987
Test guideline(s): Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
GLP: Yes, conducted under GLP/Officially recognised testing facilities
Testing facility: Malcolm Pirnie, Inc., White Plains, NY, USA
Sponsor: Monsanto Agricultural Company, Chesterfield, MO, USA

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and the 72-h EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 1092-02-1100-4 (1987) was statistically evaluated for the effects of Glyphosate technical on the organism *Anabaena flos-aquae.* The organisms were exposed for 7 days to the following concentrations of Glyphosate technical: 10, 18, 32, 56 and 100 mg a.s. /L (nominal concentrations, units equivalent to mg a.e./L). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50% effects on growth rate and yield of the test subjects (EC10 EC20 and EC50), for yield and growth rate probit analysis using linear maximum likelihood regression was used.

NOEC for yield and growth rate was estimated by Welsh-t-test After Bonferroni-Holm Correction (one-sided smaller, p = 0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Results are provided in the table below:

Table 0-8: Validity Criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	27
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	20.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤10%	6.4%

Recovery of mean measured concentrations ranged from 91 to 108 % of nominal. Therefore, endpoints are based on nominal test concentrations.

For yield, the parameters for the probit model are estimated as slope b: 5.95612; Intercept a: -7.22883.

For growth rate, the parameters for the probit model are estimated as slope b: 1.99737; Intercept a: -3.04435.

According to the statistical parameters; Chi2(13) = 0.59361; p(Chi²): 1.000; F(1,13) = 34.365; p(F) <0.001; r²: 0.726 for yield; and Chi2(13) = 1.26237; p(Chi²): 1.000; F(1,13) = 34.400; p(F) <0.001; r²: 0.726 for

growth rate. Based on these values the EC10, EC20 and EC50 for yield and growth rate calculations should be considered valid.

The obtained EC10 EC20 and EC50 values on the effect of Glyphosate technical on growth rate and yield of *Anabaena flos-aquae* are presented in the table below.

Table 0-9: Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal test concentrations

Endpoint (0 – 72 hours)	Glyphosate technical [mg a.e./L]	
	Yield	Growth rate
EC10 (95% CI)	9.97 (7.21 – 11.7)	7.63 (3.08 – 11.9)
EC20 (95% CI)	11.8 (9.35 – 13.4)	12.7 (6.71 – 17.7)
EC50 (95% CI)	16.4 (14.7 – 18.1)	33.4 (25.7 – 43.7)
NOEC	10	10
LOEC	18	18

CI = confidence interval

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated EC10, EC20 and EC50 values are 9.97, 11.8 and 16.4 mg/L for yield and 7.63, 12.7 and 33.4 mg a.e./L for growth rate, respectively. The NOEC was determined to be 10 mg a.e./L for yield and growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.2/004
Report author	
Report year	1996
Report title	Glyphosate acid: Toxicity to freshwater diatom *Navicula pelliculosa*
Report No	AB0503/K
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982)

Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 135.5 %, instead of ≤ 35%. Minor: - Initial cell density of 3 × 103 cells/mL, which is below the recommended density of 104 cells/mL for *Navicula pelliculosa*.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The toxicity of glyphosate acid to the freshwater diatom *Navicula pelliculosa* was determined in a 120-hours static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.8, 3.2, 5.6, 10, 18, 32, 56, and 100 mg test item/L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution.

The test was performed in 6 replicate cultures of the culture medium control and 3 replicate cultures of each concentration of glyphosate acid. The initial cell density was 0.300 × 104 cells/mL. The cell densities were determined by electronic particle counting, using a Coulter counter after 1, 2, 3, 4, and 5 days. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

The mean measured concentrations of glyphosate acid ranged from 106 to 111 % of the nominal values. Based on the analytical results the nominal test concentration values were used for the calculation and reporting of all results. Glyphosate acid inhibited cell growth of the fresh water diatom *Navicula pelliculosa* after 120 hours at test concentrations of 32, 56 and 100 mg test item/L in terms of area under growth curve and growth rates.

The 72 hours EbC50 for *Navicula pelliculosa* exposed to glyphosate acid was 16 mg test item/L; the 72 hours ErC50 was 17 mg test item/L. The 120 hours EbC50 and ErC50 were both 17 mg test item/L.

The NOErC and LOErC for *Navicula pelliculosa* after 72 hours and 120 hours of exposure were both 18 mg test item/L, respectively. The NOEbC and LOEbC for *Navicula pelliculosa* after 72 hours of exposure were 3.2 and 5.6 mg test item/L, respectively. The NOEbC and LOEbC for *Navicula pelliculosa* after 120 hours of exposure were <1.8 and 1.8 mg test item/L, respectively. The validity criteria according to current guideline OECD 201 were not met. Therefore, this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
2. Vehicle and/or positive control:	Vehicle: Cell growth medium Positive control: None

3. Test organism:	
Species:	Freshwater diatom *Navicula pelliculosa*
Initial cell concentration:	3 × 103 cells/mL
Source:	Brixham Environmental Laboratory culture from strain UTEX 667
4. Environmental conditions:	
Temperature:	24.0-24.1°C (measured by thermometer). The hourly temperature measured automatically remained within 24 ± 1°C
Photoperiod:	Continuous illumination
Light intensity:	4560 lux
pH:	3.7 – 8.3 at the start of the test 3.7 – 8.7 at the end of the test

B. STUDY DESIGN

Experimental dates: 29 January - 3 February 1996

Experimental treatments

The toxicity of glyphosate acid to the freshwater diatom *Navicula pelliculosa* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.8, 3.2, 5.6, 10, 18, 32, 56, and 100 mg test item/L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution.

The stock solution of nominal concentration of 100 mg test item/L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg test item/L. To each test and blank vessel 100 mL of the appropriate test solution were dispensed.

The test was performed in 6 replicate cultures of the culture medium control and 3 replicate cultures of each concentration of glyphosate acid. Each replicate test vessel was inoculated with 0.915 mL of the inoculum culture to give a nominal cell density of 0.300 × 104 cells/mL. The culture vessels were incubated at 24 ± 1°C under continuous illumination for 120 hours. During incubation, the cells were kept in suspension by continuous shaking.

Observations

The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

Statistical calculations

One-way analysis of variance, and Dunnett's post-hoc test.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EbC50 and ErC50 (72 hours and 120 hours), corresponding NOEC and LOEC values are given below based on nominal concentrations.

Table 0-10: Toxicity of glyphosate acid to *Navicula pelliculosa*

Endpoint	Glyphosate acid [mg test item/L]	
	0 – 72 hours	**0 -120 hours**
ErC50 (95% CI)	17 (13 - 24)	17 (12 - 24)
EbC50 (95% CI)	16 (12 - 22)	17 (13 - 24)
NOErC	18	18 A
LOErC	32	32
NOEbC	3.2	<1.8 B
LOEbC	5.6	1.8

A Effects observed in the 5.6 mg test item/L test concentrations were due to growth enhancement. No inhibitory effects were observed below the nominal 32 mg test item/L test concentration.
B Effects observed in the 1.8, 3.2, 5.6, and 10 mg test item/L test concentrations were due to growth enhancement. No inhibitory effects were observed below the nominal 32 mg test item/L test concentration.

Analytical data: The mean measured concentrations of glyphosate acid ranged from 106 to 111 % of the nominal values. Based on the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

B. OBSERVATIONS

Glyphosate inhibited cell growth of the fresh water diatom *Navicula pelliculosa* after 120 hours at test concentrations of 32, 56 and 100 mg glyphosate acid/L in terms of area under growth curve and growth rates.

Table 0-11: Mean algal densities of *Navicula pelliculosa* after treatment with glyphosate acid

Glyphosate acid [mg test item/L]	Algal cell density [× 104 cells ml-1]				
	Day 0	**Day 1**	**Day 3**	**Day 4**	**Day 5**
Control	0.321	0.169	18.2	93.2	170
1.8	0.321	0.109	22.0	165	197
3.2	0.321	0.271	3.43	171	156
5.6	0.321	3.38	32.0	190	166
10	0.321	0.347	29.8	177	160
18	0.321	0.136	10.9	74.2	187
32	0.321	0.060	0.071	0.181	0.237
56	0.321	0.008	0.005	0.035	0.212
100	0.321	0.001*	0.006	0.001*	0.147

*Algal density measurement for replicate was lower than the blank solution

Table 0-12: Mean area under growth curve and mean growth rates of *Navicula pelliculosa* exposed for 72 hours and 120 hours to glyphosate acid

	Control	Glyphosate acid [mg test item/L]							
Test parameters	**-**	**1.8**	**3.2**	**5.6**	**10**	**18**	**32**	**56**	**100**
Mean areas under the growth curve (0 – 72 h)	11.0	12.1	16.7	22.6*	17.9*	5.8	-0.7*	-0.8*	-0.8*
Mean areas under the growth curve (0 – 72 h) % of control	-	111	153	206	163	53	-6	-7	-7
Mean growth rates (0 – 72 h)	1.346	1.409	1.485	1.534	1.510	1.175	-0.504*	-1.366*	-1.309*
Mean growth rates (0 – 72 h) % of control	-	105	110	114	112	87	-37	-102	-97
Mean areas under growth curve (0 – 120 h)	197.7	285.8*	278.6*	311.3*	288.9*	178.4	-1.0*	-1.3*	-1.4*
Mean areas under growth curve (0 – 120 h) [%] of control	-	145	141	157	146	90	0	-1	-1
Mean growth rates (0 – 120 h)	1.255	1.284	1.237	1.250	1.243	1.274	-0.061*	-0.083*	-0.156*
Mean growth rates (0 – 120 h) [%] of control	-	102	99	100	99	102	-5	-7	-12

* Significant difference from the control (p=0.05)

III. CONCLUSIONS

The NOErC and LOErC for *Navicula pelliculosa* after 72 hours and 120 hours of exposure were both 18 mg test item/L, respectively. The NOEbC and LOEbC for *Navicula pelliculosa* after 72 hours of exposure were 3.2 and 5.6 mg test item/L, respectively. The NOEbC and LOEbC for *Navicula pelliculosa* after 120 hours of exposure were <1.8 and 1.8 mg test item/L, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	56.6
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤35 %	135.5 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10 %.	≤10 %	4.4 %

The biomass in the control cultures increased by a factor of ≥16 (actual: 56.6), the coefficient of variance for section specific growth rates exceeded 35% (actual: 135.5%), for the whole test period it was ≤ 10% (actual: 4.4%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met. Therefore, this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. **Information on the study**

Data point	CA 8.2.6.2/005
Report author	
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Navicula pelliculosa*
Report No	1092-02-1100-2
Document No	
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The biomass in the control cultures increased by a factor of 9 instead of ≥16, and the coefficient for the whole period was 10.1% instead of ≤ 10% Minor: - Nominal cell density of 3 × 103 cells/mL was below the recommended density of 104 cells/mL for *Navicula pelliculosa*,
Previous evaluation	Yes, accepted in RAR (2015)

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

2. Full summary

Executive Summary

The effects of glyphosate technical on *Navicula pelliculosa* were evaluated in a 7-day static toxicity test. After a range-finding test, suspensions of *Navicula pelliculosa* were exposed to five nominal concentrations encompassing 10, 18, 32, 56 and 100 mg test item/L (measured: 10.6, 19.1, 33.6, 56.1 and 103 mg glyphosate technical/L). In addition, a control with the test medium (without test substance) was tested.

The test flasks were inoculated with cells from a 7-days-old pre-culture of *Navicula pelliculosa* with an initial test cell density of 1000 cells/mL. The test was performed in 250 mL volumetric flasks, containing each 50 mL test solution. The test concentrations and the control were prepared in 3 replicates. The test flasks were placed in the incubator and maintained over several generations for 7 days. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation.

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Three counts per replicate were made. On the basis of the mean cell count, the percentage inhibition was determined and the ECx values calculated using of the algal growth curve as determined by inverse estimation least squares linear regression.

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 97.9 to 99.7 % for the nominal test concentrations of 56 mg test item/L and 100 mg test item/L respectively. At or below the nominal test concentration of 32 mg test item/L no algal growth inhibition was observed. Rather slight algal growth increases of 2.0 % and 7.7 % were observed for the nominal concentrations of 18 mg test item/L and 32 mg test item/L respectively. Because the biomass in the control cultures increased by a factor of <16, and the coefficient of variation for the whole period > 10%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	
Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6 %
Water solubility	1.2 % at 25 °C
Vehicle and/or positive control:	Vehicle: Dilution water Positive control: None
Test organism:	
Species:	*Navicula pelliculosa*
Initial cell concentration:	3 × 103 cells/mL
Source:	In-house culture

Environmental conditions:	
Temperature:	20 ± 2 °C
Photoperiod:	24 h light, 4306 ± 650 Lux
pH:	7.5 ± 0.1
Conductivity:	Not stated
Hardness:	Not stated

B. STUDY DESIGN

Experimental dates of work: 13 April to 20 April 1987

Experimental treatments

Prior to the main test, a range-finding test was performed with six concentrations ranging between 0.001 and 100 mg test item/L. Based on the preliminary test results, the main test was performed with five nominal concentrations (10, 18, 32, 56 and 100 mg test item/L). Test concentrations were prepared by adding the required volumes of the stock solution to AAP/Si (medium with silicon) medium. A control with the test medium (without test substance) was tested under the same conditions as in the test groups. The test was performed in 250 mL volumetric flasks, containing each 50 ml test solution. Test algae were taken from a 7-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 1.11 × 106 cells/mL. Flasks were kept in an incubator at a temperature of 20 ± 2° C and were continuously shaken at 100 oscillations per minute.

Observations

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Three counts per replicate were made. All counts were multiplied by the appropriate conversion factors (for sample dilution and volume counted) to yield cells/mL. Samples ranging in volume from 0.1 to 2.0 mL, depending upon the expected population density, were collected aseptically using an automatic micropipette with sterile tips. Based on the mean cell count, the percentage inhibition was determined and the ECx values calculated using the algal growth curve as determined by inverse estimation least squares linear regression. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation. Samples of test media were made at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analysed for active substance using HPLC.

Statistical calculations

To determine the ECx values, the log of test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50 percent inhibition and the associated 95 % confidence limits were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 value is given below based on mean measured concentrations.

Table 0-13: Toxicity of glyphosate technical to *Navicula pelliculosa*

Endpoint	Glyphosate technical [mg test item/L]
EC50 (7 day)	24.9

Chemical analyses were performed on samples of the test solutions to quantify glyphosate in the test

solution. The mean measured concentrations were 10.6, 19.1, 33.6, 56.1 and 103 mg glyphosate technical/L, corresponding to 106.0, 106.1, 105.0, 100.2 and 103.0 % of the nominal test concentrations of 10, 18, 32, 56 and 100 mg glyphosate technical/L respectively. The ecotoxicological endpoints were evaluated using measured concentrations of the test item.

B. OBSERVATIONS

Observations:

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 97.9 to 99.7 % for the nominal test concentrations of 56 mg test item/L and 100 mg test item/L respectively. At or below the nominal test concentration of 32 mg test item/L no algal growth inhibition was observed. Rather slight algal growth increases of 2.0 % and 7.7 % were observed for the nominal concentrations of 18 mg test item/L and 32 mg test item/L respectively.

Table 0-14: Percentage growth inhibition of *Navicula pelliculosa* exposed to glyphosate technical for 7 days

Nominal concentrations [mg test item/L]	Measured concentrations [mg test item/L]	Mean number of algae cells (day7) [× 1000 cells/mL]	Mean inhibition (7 days) [%]
Control	Control	3020	-
10	10.6	2933	2.9
18	19.1	3080	-2.0
32	33.6	3253	-7.7
56	56.1	63.5	97.9
100	103	8	99.7

III. CONCLUSIONS

The 7-day EC50 for *Navicula pelliculosa* exposed to glyphosate technical was calculated to be 24.9 mg test item/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	9
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤ 35 %	29.1 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10 %.	≤ 10 %	10.1 %

The biomass in the control cultures increased by a factor of <16 (actual: 9), the coefficient of variance for section specific growth rates was ≤ 35 % (actual: 29.1 %), for the whole test period it exceeded 10 % actual: 10.1 %). Because the biomass in the control cultures increased by a factor of <16, and the coefficient of variation for the whole period > 10%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.2/006
Report author	
Report year	1996
Report title	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*
Report No	AB0503/I
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982)
Deviations from current test guideline	Deviation from the guideline 201 (2011): None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The toxicity of glyphosate acid to the marine alga *Skeletonema costatum* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.e./L) and a control consisting of culture medium without test item. The test comprised six replicate cultures of the culture medium control and three replicate cultures of each concentration of glyphosate acid. The initial nominal cell density was 1.00 × 104 cells/mL. The culture vessels were incubated at 20 ± 1°C for 120 hours.

The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

The mean measured concentrations of glyphosate acid ranged from 94 to 106 % of the nominal values. Based on the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

The 72 h EbC50 for *Skeletonema costatum* exposed to glyphosate acid was 11 mg/L; the 72 h ErC50 was 18 mg test item/L. The 120 h EbC50 was 12 mg test item/L; the 120 h ErC50 was 24 mg test item/L. The 72-hour NOEbC and NOErC values were 1.8 mg test item/L, respectively. The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Vehicle and/or positive control:	Cell growth medium
Test organism:	
Species:	Marine alga *Skeletonema costatum*, strain CCAP 1077/1C
Initial cell concentration:	1.00 × 104 cells/mL
Source:	Culture centre of algae and protozoa, Dunstaffnage Marine Laboratory, Oban, Argyll, UK
Environmental conditions:	
Temperature:	20.0 - 20.1°C (measured by thermometer). The hourly temperature measured automatically remained within 20 ± 1°C
Photoperiod:	16 h light / 8 h dark
Light intensity:	4340 lux
pH:	7.1 – 8.1 at the start of the test 8.1 – 8.8 at the end of the test

B. STUDY DESIGN

Experimental dates: 5 February – 10 February 1996

Experimental treatments

The toxicity of glyphosate acid to the marine alga *Skeletonema costatum* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg test item/L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. The stock solution of nominal concentration of 56 mg test item/L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, and 32 mg test item/L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel.

The test was performed in six replicate cultures of the culture medium control and three replicate cultures of each concentration of glyphosate acid. The initial nominal cell density was 1.00 × 104 cells/mL. The culture vessels were incubated at 20 ± 1°C for 120 hours. During incubation, the cells were kept in suspension by continuous shaking.

Observations

The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system.

The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

Statistical calculations
One-way analysis of variance, and Dunnett's post-hoc test.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of glyphosate acid ranged from 94 to 106 % of the nominal values. Based on the analytical results the nominal test concentration values were used for the calculation and reporting of all results.
The EC50 (72 h and 120 h), NOEC and LOEC values are given below based on nominal concentrations.

Table 0-15: Toxicity of glyphosate acid to *Skeletonema costatum* (nominal values)

Endpoint	Glyphosate acid [mg test item/L]	
	0 – 72 hours	0 -120 hours
ErC50 (95% CI)	18 (10 – 42)	24 (12 – >56)
EbC50 (95% CI)	11 (7.1 – 20)	12 (7.6 – 19)
NOErC	1.8	10
LOErC	3.2	18
NOEbC	1.8	1.8
LOEbC	3.2	3.2

B. OBSERVATIONS

Glyphosate inhibited cell growth of the marine algae *Skeletonema costatum* after 120 hours at test concentrations of 18, 32 and 56 mg glyphosate acid/L; mean area under growth curve was affected at 10, 18, 32 and 56 mg glyphosate acid/L.

Table 0-16: Mean cell densities and percentage of inhibition of cell growth of *Skeletonema costatum* exposed for 120 hours to glyphosate acid

	Control	Glyphosate acid [mg test item/L]							
Test parameters	**-**	**1.0**	**1.8**	**3.2**	**5.6**	**10**	**18**	**32**	**56**
Mean areas under the growth curve (0 - 72 h)	37.4	38.0	38.9	29.5*	34.2	17.9*	2.8*	2.3*	1.5*
Mean areas under the growth curve (0 - 72 h) % of control	-	102	104	79	92	48	8	6	4
Mean growth rates (0 - 72 h)	1.423	1.433	1.443	1.322*	1.387	1.111*	0.362*	0.295*	0.188*
Mean growth rates (0 - 72 h) % of control	-	101	101	93	97	78	25	21	13
Mean areas under growth curve (0 - 120 h)	162.2	162.7	163.3	149.5*	156.9	132.1*	7.1*	4.0*	2.2*
Mean areas under growth curve (0 - 120 h) [%] of control	-	100	101	92	97	81	4	2	1
Mean growth rates (0 - 120 h)	0.882	0.879	0.869	0.873	0.875	0.905	0.315*	0.115*	0.055*
Mean growth rates (0 - 120 h) [%] of control	-	100	99	99	99	103	36	13	6

* Significant difference from the culture control (p=0.05)

III. CONCLUSIONS

The 72 h EbC50 for *Skeletonema costatum* exposed to glyphosate acid was 11 mg test item/L; the 72 h ErC50 was 18 mg/L (nominal). The 120 h EbC50 was 12 mg test item/L; the 120 h ErC50 was 24 mg test item/L. The 72-hour NOEbC and NOErC values were 1.8 mg/L, respectively. All endpoints are based on nominal test concentrations.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	72
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤35 %	33.1 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10 %.	≤10 %	4.3 %

The biomass in the control cultures increased by a factor of ≥16 (achieved: 72), the coefficient of variance for section specific growth rates was ≤ 35% (achieved: 33.1 %) and the coefficient of variance for the whole test period it was ≤ 10% (achieved: 4.3 %). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC10, EC20, EC50, NOEC and LOEC was performed (Positon Paper No. CA 8.2.6.2/007).
Recovery of test concentrations ranged from 94 to 106%. Therefore endpoints are based on nominal.

Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	Glyphosate acid [mg a.e./L]	
	Yield	Growth rate
EC10 (95% CI)	5.22 (2.44 – 6.70)	1.87 (1.18 – 2.62)
EC20 (95% CI)	6.38 (2.90 – 7.73)	2.98 (2.86 – 5.26)
EC50 (95% CI)	9.00 (7.58 – 10.4)	13.5 (10.8 – 20.7)
NOEC	5.6	5.6
LOEC	10.0	10.0

CI = confidence interval
n.d. = not determined

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.2/007
Report author	
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study BL5684/B on the toxicity of Glyphosate acid to *Skeletonema costatum* under static conditions
Report No	110054-007
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Deviations to current guideline OECD 201 (2011): None
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 72 h EC10, EC20 and EC50 values was conducted for the study BL5684/B (Smyth, et al., 1996) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated 72 h- EC10, EC20 and EC50 values are 5.22, 6.38 and 8.99 mg a.s./L, respectively for yield and 1.87, 2.98, and 13.5 mg a.s./L for growth rate, respectively, based on glyphosate acid. NOEC was determined to be 5.6 mg for yield as well as for growth rate. The statistical parameters showed that these values can be considered reliable for use in the risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number:	AB0503/I
Author:	
Substance:	Glyphosate acid
Title:	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*
Completion date:	10-Feb-1996
Test guideline(s):	OECD Guideline No. 201 (1984); US EPA Guideline 540/09-82-020 (1982) Re-evaluated according OECD 201 (2011)
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Testing facility:	Brixham Environmental Laboratory, Brixham Devon, UK
Sponsor:	ZENECA Agrochemicals, Surrey, UK

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study BL5684/B (1996) was statistically evaluated for the effects of Glyphosate acid on the marine alga *Skeletonema costatum.* The organisms were exposed for 120-hours to the following concentrations of glyphosate acid: 1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.s./L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50% effects on growth rate and yield of the test subjects, a 3-parameter logistic CDF (Cumulative Distribution Function) model was used for yield and for growth rate and a non-linear regression analysis was performed.

NOEC levels were determined by Welsh-t-test After Bonferroni-Holm Correction for yield, and Williams Multiple Sequential t-test for growth rate (one-sided smaller, p= 0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10} and EC_{20} and EC_{50} values were calculated to fulfil the data requirements according to regulation EU 283/2013.Validity parameters are provided in the table below:

Table 0-17: Validity Criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	72
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤35 %	33.1 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10 %.	≤10 %	4.3 %

For yield, the parameters for the 3 parameter logistic CDF model are estimated as: b0: 53.393, b1: 8.991; b2: 4.038.
For growth rate, the parameters for the logit analysis are estimated as slope b: 2.46938; intercept a: -2.86713.

According to the statistical parameters; $F(2, 6) = 147.118$; $p(F) < 0.001$; $R^2 = 0.910$ for yield.
After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.069

For growth rate, statistical parameters for goodness of fit are: $Chi^2(22) = 0.54119$; $p(Chi^2)$: 1.000; $F(1,22) = 97.922$, $p(F) < 0.001$; $R^2 = 0.817$.

Therefore, the obtained EC_{10}, EC_{20} and EC_{50} calculations are considered valid. The values are presented in the table below.

Considering yield, there is a statistically significant difference to control at test concentrations 3.2, 10.0, 18, 23, and 56 mg/L. No statistically significant effect is determined for the intermediate test concentration of 5.6 mg/L. As this does not follow a dose response scenario and continuous effects are observed from 10.0 mg/L and all higher concentration levels, the NOEC is set to 5.6 mg/L for yield.

For growth rate, % inhibition at 72 hours was -0.7, -1.5, 6.9, 2.4, 21.7, 74.8, 79.2, and 86.8% compared to the control for test concentrations 1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.s./L, respectively.
However, statistically significant effects have been determined for all test concentrations, except for the two lowest levels. Even inhibition of 6.9 and 2.4% at 3.2 and 5.6 mg/L are statistically determined to show an effect. Based on the fact that the inhibition values at these test item concentrations were below 10% these significances were considered to be not scientifically relevant according to Heger et al (1998).

Recovery of mean measured test concentrations ranged from 94 to 106% of nominal. Therefore, endpoints are based on nominal.

Table 0-18: Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	Glyphosate acid [mg a.s./L]	
	Yield	Growth rate
EC_{10} (95% CI)	5.22 (2.44 – 6.70)	1.87 (1.18 – 2.62)
EC_{20} (95% CI)	6.38 (3.90 – 7.73)	2.98 (2.86 – 5.26)
EC_{50} (95% CI)	8.99 (7.58 – 10.4)	13.5 (10.8 – 20.7)
NOEC	5.6	5.6
LOEC	10.0	10.0

CI = confidence interval

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated 72 h- EC_{10}, EC_{20} and EC_{50} values are 5.22, 6.38 and 8.99 mg a.s./L, respectively for yield and 1.87, 2.98, and 13.5 mg a.s./L for growth rate, respectively, based on glyphosate acid. NOEC was determined to be 5.6 mg a.s./L for yield as well as for growth rate. The statistical parameters showed that these values can be considered reliable for use in the risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.2/008
Report author	
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Skeletonema costatum*
Report No	1092-02-1100-3
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The biomass in the control cultures increased by a factor of 3.6 instead of ≥16, and the mean coefficient of variation for section-by-section specific growth rates in the control cultures was 78.4% instead of ≤35%
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of glyphosate technical on *Skeletonema costatum* were evaluated in a 7-day static toxicity test. After a range-finding test, suspensions of *Skeletonema costatum* were exposed to six nominal concentrations encompassing 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg test item/L (measured: 0.24, 0.28, 0.48, 1.79 and 3.42 mg glyphosate technical/L). In addition, a control with the test medium (without test substance) was tested. The test flasks were inoculated with cells from a 7-days-old pre-culture of *Skeletonema costatum* with an initial test cell density of 104 cells/mL. The test was performed in 250 mL volumetric flasks, containing each 50 mL test solution. The test concentrations and the control were prepared in 3 replicates. The test flasks were placed in the incubator and maintained over several generations for 7 days. The temperature was measured daily and the salinity was adjusted to 30 ‰ at test initiation. Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. On the basis of the mean cell count, the percentage inhibition was determined and the ECx values calculated using of the algal growth curve as determined by inverse estimation least squares linear regression. The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 9.2 % for the lowest mean measured test concentration of 0.24 mg test item/L to 95.7 % for the highest test concentration of 3.42 mg test item/L. At the mean measured concentration of 0.28 mg test item/L, a sporadic growth increase of 13.6 % relative to control was observed.

The 7-day EC50 for *Skeletonema costatum* exposed to glyphosate technical was calculated to be 0.64 mg test item/L.

Because the factor of exponential increase in biomass in the control cultures was <16 and the coefficient of variation for the section specific growth rate was >35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6 %
Water solubility	1.2 % at 25 °C
Vehicle and/or positive control:	Vehicle: filter-sterilized distilled deionized water Positive control: None
Test organism:	
Species:	*Skeletonema costatum*
Initial cell concentration:	104 cells/mL
Source:	In-house culture
Environmental conditions:	
Temperature:	20 ± 2° C
Photoperiod:	14 h light / 10 h dark
Light intensity:	4306 ± 650 Lux
Salinity:	30 ‰
Conductivity:	Not stated
Hardness:	Not stated

B. STUDY DESIGN

Experimental dates: 20 April 1987 to 27 April 1987

Experimental treatments

Prior to the main test, a range-finding test was performed with six concentrations ranging between 0.001 and 100 mg test item/L. On the basis of the preliminary test results, the main test was performed with six nominal concentrations (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg test item/L) and three replicates per test item treatment group. Test concentrations were prepared by adding the required volumes of the stock solution to synthetic seawater (prepared by adding approximately 30 grams of a commercial salt mix to 1 L of distilled deionised water). A control with the test medium (without test substance) was tested under the same conditions as in the test groups. The test was performed in 250 mL volumetric flasks, containing each 50 mL test solution. Test algae were taken from a 7-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 104 cells/mL. Flasks were kept in an incubator at a temperature of 20 ± 2°C. Flasks were manually shaken each working day.

Observations

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Based on the mean cell count, the percentage inhibition was determined and the ECx values calculated using the algal growth curve as determined by inverse estimation least squares linear regression. The temperature was measured daily and the salinity was adjusted to 30‰ at test initiation. Samples of test media were made at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analysed for active substance using HPLC.

Statistical calculations

To determine the ECx values, the log of test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and

the concentrations corresponding to 25 and 50 % inhibition and the associated 95 % confidence limits were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 value is given below based on mean measured concentrations.

Table 0-19: Toxicity of glyphosate technical to *Skeletonema costatum*

Endpoint	Glyphosate technical [mg test item/L]
EC50 (7 day) (95% confidence limits)	0.64 (0.21 – 1.70)

Analytical data:
Chemical analyses were performed on samples of the test solutions to quantify glyphosate in the test solution. The mean measured concentrations were 0.24, 0.28, 0.48, 0.94, 1.79 and 3.42 mg glyphosate/L, corresponding to 240.0 %, 140.0 %, 120.0 %, 117.5 %, 111.9 % and 106.9 % of the nominal test concentrations of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg glyphosate/L, respectively. Therefore, ecotoxicological endpoints were evaluated using measured concentrations of the test item.

B. OBSERVATIONS

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 9.2 % for the lowest mean measured test concentration of 0.24 mg test item/L to 95.7 % for the highest test concentration of 3.42 mg test item/L. At the mean measured concentration of 0.28 mg test item/L, a sporadic growth increase of 13.6 % relative to control was observed.

Table 0-20: Percentage growth inhibition of *Skeletonema costatum* exposed to glyphosate technical for 7 days

Nominal concentrations [mg test item/L]	Measured concentrations [mg test item/L]	Mean number of algae cells (day 7) [× 1000 cells/mL]	Mean inhibition (7 days) [%]
Control	Control	360.667	-
0.1	0.24	327.333	9.2
0.2	0.28	410.667	-13.6
0.4	0.48	250.667	30.5
0.8	0.94	76.333	78.8
1.6	1.79	24.000	93.3
3.2	3.42	15.667	95.7

III. CONCLUSIONS

The 7-day EC50 for *Skeletonema costatum* exposed to glyphosate technical was calculated to be 0.64 mg test item/L, based on mean measured concentrations.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	3.6
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤ 35 %	78.4 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10 %.	≤ 10 %	2.5 %

The biomass in the control cultures increased by a factor of <16 (actual: 3.6), the coefficient of variance for section specific growth rates exceeded 35% (actual: 78.4%), for the whole test period it was ≤10 % (actual: 2.5 %). Because the factor of exponential increase in biomass in the control cultures was <16 and the coefficient of variation for the section specific growth rate was >35 %, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.2/009
Report author	
Report year	1978
Report title	Toxicity of seven test materials to the marine alga, *Skeletonema costatum*
Report No	BP-78-4-031
Document No	-
Guidelines followed in study	Environmental Protection Agency: Bioassay procedures for the ocean disposal permit program (1976)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011) Major: - Report does not provide sufficient information
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of seven test items, two solid test items (Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45) and five liquid test items (Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and Comp. 5A) on *Skeletonema costatum,* were evaluated in a 96-hour static toxicity test. The test was performed using nominal concentration encompassing 0.6, 1.0, 1.8, 3.2 and 5.6 mg test item/L for Glyphosate, BN-78-44 and 3.2, 10, 32, 100, 320 and 560 mg test item/L for Glyphosate intermediate, BN-78-45). For the liquid test item (Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.) the nominal concentration used were 0.6, 1.0, 3.2, 10, 32 and 56 % effluent. Duplicate cultures were employed for each of the test concentrations and control, except in the test with Comp. 5A, in which all test concentrations and the control were triplicate. The test solutions were prepared using deionised water. The initial cell concentration was 2 × 104 cells/mL. Cell cultures were incubated for 96 hours at 20 ± 1 °C.

Measurements of *in vivo* chlorophyll α in cultures were performed and cell counts were made at 24, 48, 72 and 96 hours after the test initiation. Due to the nature of two of the test materials, Comp. #2, BN-78-47 and Comp. #3, BN-78-48, *in vivo* chlorophyll α could not be accurately measured. Cell counts were the only growth measurement for both test items. The EC50 values were calculated in terms of chlorophyll α measurements and cell counts.

For the test item Glyphosate, BN-78-44, all test concentrations led to a reduction in both chlorophyll α content and the cell number, varying from 12% to 98%. For the test item Glyphosate intermediate BN-78-45, a reduction in chlorophyll α content and the cell number were observed from the nominal concentrations of 320 mg test item/L (for chlorophyll α) and 10 mg test item/L (for cell number), respectively. At the highest test concentrations, the reductions in both chlorophyll α content and the cell number varied from 95 % to 98 % for both solid test items.

For the liquid test items Comp. #3A, BN-78-48, Comp. #4, BN-78-49, and Comp. 5A, reductions in chlorophyll α content and/or cell number were observed from the lowest test concentration (0.6 % effluent), except for Comp. 5A, for which the reduction in chlorophyll α content was observed only at or above the concentration of 10% effluent. At the highest test concentration (56 % effluent), reductions in both chlorophyll α content and the cell number varied from 88 % to 100 % for all liquid test items.
Validity of the study could not be checked due to lack of information given in the report. The study is therefore not used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	
Test item (Description):	Glyphosate, BN-78-44 (white, crystalline solid) Glyphosate intermediate, BN-78-45 (fine, white powder) Comp. #1, BN-78-46 (clear liquid) Comp. #2, BN-78-47 (clear liquid) Comp. #3A, BN-78-48 (murky liquid) Comp. #4, BN-78-49 (clear liquid)

	Comp. 5A. (clear liquid)
Vehicle and/or positive control:	Dodecyl sodium sulphate (DSS)
Test organism:	
Species:	*Skeletonema costatum*
Initial cell concentration	2 × 104 cells/mL
Source:	Environmental Protection Agency's Protection Agency's'Environmental Research Laboratory, Narragansett, Rhode Island, USA
Environmental conditions:	
Temperature:	20 ± 1 °C
Photoperiod:	Not stated
Light intensity:	2000 Lux
pH:	Glyphosate, BN-78-44, (8.2 – 8.5) Glyphosate intermediate, BN-78-45 (6.1 – 8.4) Comp. #1, BN-78-46 (7.6 – 8.4) Comp. #2, BN-78-47 (7.1 – 8.4) Comp. #3A, BN-78-48 (8.1 – 8.5 Comp. #4, BN-78-49 (8.0 – 8.9) Comp. 5A (8.2 – 8.5)
Dissolved oxygen:	Not stated
Conductivity:	Not stated
Hardness:	Not stated

B. STUDY DESIGN

Experimental dates: Not stated

Experimental treatments

Toxicity tests for the seven test materials were performed using nominal concentration encompassing 0.6, 1.0, 1.8, 3.2 and 5.6 mg test item/L for Glyphosate, BN-78-44, and 3.2, 10, 32, 100, 320 and 560 mg test item/L for Glyphosate intermediate, BN-78-45).For the liquid test item (Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.) the nominal concentration used were 0.6, 1.0, 3.2, 10, 32 and 56% effluent. Duplicate cultures were employed for each of the test concentrations and control, except in the test with Comp. 5A, in which all test concentrations and the control were triplicate. For solid test materials, appropriate amounts were added to deionised water; the pH was adjusted to 8.0, and the materials were finally added test containers to obtain appropriate concentrations. For liquid materials, the effluents were directly added into the test containers. To the prepared tests concentrations, the algal suspension was added to obtain an initial cell concentration of 2 × 104 cells/mL. Cell cultures were incubated for 96 hours at 20 ± 1 °C.

Observations

Measurements of *in vivo* chlorophyll α in cultures were performed by using a fluorometer and cell counts were made by a means of a haemocytometer and a standard microscope at 24, 48, 72 and 96 hours after the test initiation. Due to the nature of two of the test materials, Comp. #2, BN-78-47, and Comp. #3, BN-78-48, *in vivo* chlorophyll α could not be accurately measured. Cell counts were the only growth measurement for both test items. The EC50 values were calculated in terms of chlorophyll α measurements and cell counts. A separate test was conducted, in which cultures of the alga were exposed to the reference toxicant dodecyl sodium sulphate under the same test conditions stated above.

Statistical calculations

The EC50 values were calculated by linear regression in a Probit data analysis. The salinity growth data

were analysed by Student's t-test at $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 values are given below based on nominal concentrations.

Table 0-21: Toxicity to *Skeletonema costatum*

Test materials		EC50 (96 h) (95% confidence interval) [% effluent or mg test item/L]
Glyphosate, BN-78-44 (CL)	chlorophyll α	1.2 (0.6 - 2.3)
	cell counts	1.3 (0.7 - 2.5)
Glyphosate intermediate BN-78-45 (CL)	chlorophyll α	>100 <320
	cell counts	140 (51 - 379)
Comp. #1, BN-78-46	chlorophyll α	13 (6.1 - 27)
	cell counts	15 (6.8 - 33)-
Comp. #2, BN-78-47	chlorophyll α	n.d.
	cell counts	> 1<10
Comp. #3A, BN-78-48	chlorophyll α	n.d.
	cell counts	> 3.2 < 10-
Comp. #4, BN-78-49	chlorophyll α	12 (6.8 - 23)
	cell counts	19 (7.8 - 48)
Comp. 5A.	chlorophyll α	14 (7.6 - 25)
	cell counts	4.5 (2.2 - 9.1)

n.d.= not determined

B. OBSERVATIONS

For the test item Glyphosate, BN-78-44, all test concentrations led to a reduction in both chlorophyll α content and the cell number, varying from 12 % to 98 %. For the test item Glyphosate intermediate, BN-78-45, a reduction in chlorophyll α content and the cell number were observed from the nominal concentrations of 320 mg test item/L (for chlorophyll α) and 10 mg test item/L (for cell number). At the highest test concentrations, the reductions in both chlorophyll α content and the cell number varied from 95 % to 98 % for both solid test items. For the liquid test items Comp. #3A, BN-78-48, Comp. #4, BN-78-49, and Comp. 5A, reductions in chlorophyll α content and/or cell number were observed from the lowest test concentration (0.6 % effluent), except for Comp. 5A, for which the reduction in chlorophyll α content was observed only at or above the concentration of 10% effluent. At the highest test concentration (56 % effluent), reductions in both chlorophyll α content and the cell number varied from 88 % to 100 % for all liquid test items.

Table 0-22: Lethal effects of Glyphosate, BN-78-44, on *Skeletonema costatum*

Glyphosate, BN-78-44 [mg test item/L]	Control	0.6	1.0	1.8	3.2	5.6
Chlorophyll α (96 h) [%]	-	-12	-42	-84	-93	-98
Cell number [%] (96 h) [%]	-	-12	-35	-69	-90	-97

Table 0-23: Lethal effects of Glyphosate intermediate, BN-78-45, on *Skeletonema costatum*

Glyphosate intermediate, BN-78-45 [mg test item/L]	Control	3.2	10	32	100	320	560
Chlorophyll α (96 h) [%]	-	+10	+7	+19	+10	-90	-95
Cell number [%] (96 h) [%]	-	+44	-3	-7	-14	-89	-90

Table 0-24: Lethal effects of Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49, and Comp. 5A on *Skeletonema costatum*

Test items [% effluent]	Control	0.6	1.0	3.2	10	32	56	Sal. b
Comp. #1, BN-78-46								
Chlorophyll α (96 h) [%]	-	+2	-5	+10	-33	-95	-100	-83 a
Cell number [%] (96 h) [%]	-	+5	-21	+25	-18	-97	-99	-85 a
Comp. #2, BN-78-47								
Chlorophyll α (96 h) [%]	-	-	-	-	-	-	-	-
Cell number [%] (96 h) [%]	-	+7	-1	-50	-79	-80	-88 a	-
Comp. #3A, BN-78-48								
Chlorophyll α (96 h) [%]	-	-	-	-	-	-	-	-
Cell number [%] (96 h) [%]	-	-20	-21	+11	-94	-99	-98	-48 a
Comp. #4, BN-78-49								
Chlorophyll α (96 h) [%]	-	-10	+14	-10	-5	-74	-100	-
Cell number [%] (96 h) [%]	-	-24	+24	-16	-1	-68	-97	-
Comp. 5A								
Chlorophyll α (96 h) [%]	-	+8	+3	+5	-24	-97	-100	-
Cell number [%] (96 h) [%]	-	-10	-15	-3	-56	-96	-100	-

a significantly different (α=0.05) from the control
b Salinity control

Table 0-25: Lethal effects of the toxic reference Dodecyl sodium sulfate on *Skeletonema costatum*

Dodecyl sodium sulfate [mg test item/L]	Control	1	2	3
Chlorophyll α (96 h) [%]	-	0	-57	-81
Cell number [%] (96 h) [%]	-	-4	-55	-79

III. CONCLUSION

The effects of seven glyphosate-related test items on *Skeletonema costatum* were studied in an acute toxicity test. For the solid test items (Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45), the EC50 values varied from 1.2 mg test item/L to 320 mg test item/L. For the liquid test items (Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and Comp. 5A.), the EC50 values varied between 1 % effluent to 19 % effluent.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Validity of the study could not be checked due to lack of information given in the report. The study is not used for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.6.2/010
Report author	
Report year	1996
Report title	Alga, Growth Inhibition Test to *Nitzschia palea*
Report No	960606FH
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - the coefficient of variance for section specific growth rates exceeded 35 % (actual: 72.7 %).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of glyphosate on *Nitzschia palea* were evaluated in a 96-hour static toxicity test, at seven nominal concentrations of 0.32, 1.0, 3.2, 10, 32, 100 and 320 mg test item/L and a control. Three replicate vessels were prepared per concentration level and control. The flask containing 10 mL of test or control medium were inoculated with algal cells to obtain an initial cell density of 1.0 - 1.4 × 104 cells/mL. The temperature was measured continuously, and the pH was determined at the beginning and end of the test.
At test start (0 h) and after 24, 48, 72 and 96 hours cell density was determined by chlorophyll-fluorescence and growth inhibition was calculated. EC10 and EC50 value for biomass (EbC) and growth rate (ErC) inhibition were calculated using Probit analysis whereas the EC0 (NOEC) values were deducted from the dose-response-relationship.
The 96 h ErC50 for *Nitzschia palea* exposed to glyphosate was calculated to be 11.90 mg test item/L. The 96 h EbC50 was 4.47 mg test item/L. The NOEC (biomass & growth rate) were both determined to be 1.0 mg test item/L.
The validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White/crystalline
Lot/Batch #:	01/06/96
Purity:	96.7 %
Density:	Not specified
Water solubility:	12g/L at 20 °C
Vehicle and/or positive control:	Vehicle: Cell growth medium Positive control: None
Test organism:	
Species:	*Nitzschia palea* (Kützing)
Initial cell concentration:	1.0 – 1.4 × 104 cells/mL
Source:	Pflanzenphysiologisches Institut der Universität Göttingen, Göttingen, Germany
Environmental conditions:	
Temperature:	21.5 – 23.8 °C
Photoperiod:	24 h light
Light intensity:	70 – 90 µE/m2 s
Light quality:	Fluorescent tube, radium NL 58w/31, Spectralux Warmton
pH:	7.78 – 8.72 (control replicates) 7.71 – 8.58 at 0.32, 1.0, 3.2 and 10 mg/L 6.43 – 7.74 at 32 mg/L 5.81 – 6.74 at 100 mg/L 3.20 – 3.22 at 320 mg/L
Conductivity:	not stated
Hardness:	not stated

B. STUDY DESIGN

Experimental dates: 14 October – 18 October 1996

Experimental treatments

Prior to the main test, a range-finding test was performed using concentrations of 0.01, 0.1, 1.0, and 10 mg test item/L. The test flasks were inoculated with cells from a three-day-old pre-culture of *Nitzschia palea* to obtain an initial cell density of 1.0 -1.4 × 104 cells/mL.

On the basis of the preliminary test results, the main test was performed with seven test item treatment rates, 0.32, 1.0, 3.2, 10, 32, 100 and 320 mg test item/L. A control with the test medium (without test substance) was tested under the same conditions. The test was performed in 20 mL plastic cuvettes containing 10 mL test medium in static conditions. The test concentrations and the control were prepared in three replicates. The test cultures were mixed every 2 h for 15 min at 70 rpm with shaker. The temperature was measured continuously, and the pH was determined at the beginning and end of the toxicity test.

Observations

At test start (0 h) and after 24, 48, 72 and 96 hours growth of cell density was determined by chlorophyll-fluorescence and algal growth inhibition was calculated.

At test start and test termination, samples of test media were taken for analysis of the active ingredient from 0.32, 1.0, 3.2, 10, 32, 100 and 320 mg test item/L treatments. All samples were analysed for active substance using a validated HPLC.

Measured pH values were as follows:

Nominal conc. [mg/L]	Start	End
Control	7.78	8.72
0.32	7.84	8.58
1.0	7.81	8.58
3.2	7.77	8.24
10	7.71	7.97
32	6.43	7.74
100	5.81	6.74
320	3.22	3.20
320 – pH adjusted	7.97	8.09

Statistical calculations
The EC10 and EC50 value for biomass (EbC) and growth rate (ErC) inhibition were calculated using Probit analysis whereas NOEC values were deducted from the dose-response-relationship.

II. RESULTS AND DISCUSSION

A. FINDINGS
The ErC50, EbC50 and NOEC values are given below based on nominal concentrations.

Table 0-26: Toxicity of glyphosate to *Nitzschia palea*

Endpoint	Glyphosate technical [mg test item/L]
0 - 96 h ErC50	11.90
0 – 96 h ErC10	3.11
0 - 96 h EbC50	4.47
0 - 96 h EbC10	2.12
NOEC (growth rate)	1.0
NOEC (biomass)	1.0

The analytical recovery rates at the beginning of the test were in the range of 78 % and 108 % of the active substance. At the end of the test, recovery rates were in the range of 68 % and 98 %. Low recoveries of 68 % and 71 % respectively were found in the lowest test concentration and 76 % to 77 % recoveries were found at the test end for 10 mg test item/L. As the overall recovery rates were >80 %, the report presents data based on nominal concentrations.

B. OBSERVATIONS
The results of the main test showed that the algal growth was completely inhibited at a nominal test item concentration of 320 mg test item/L No inhibition effects were observed at and below a concentration of 1 mg test item/L. The effects on growth rate and biomass are below.

Table 0-27: Percentage inhibition of growth rate and biomass of *Nitzschia palea* exposed for 96 hours to glyphosate

Glyphosate technical [mg test item/L]	C pH	C	0.32	1.0	3.2	10	32	100	320
Biomass integral	4.86	213.76	219.03	215.39	90.75	17.88	-6.26	-11.76	-36.67
Inh. biomass (0-96 h) [%]	97.73	-	-2.47	-0.76	57.55	91.63	100	100	100
Growth rate (0-96 h)	0.14	0.75	0.74	0.76	0.56	0.29	0.09	0.04	0.00
Rate related inhibition (0-96 h) [%]	81.89	-	1.07	-1.03	25.43	60.72	88.09	94.42	100

C = control; C pH = control pH (320 mg glyphosate/L pH adjusted); Inh. = inhibition

III. CONCLUSION

The 96 h ErC50 for *Nitzschia palea* exposed to glyphosate was calculated to be 11.90 mg test item/L. The 96 h EbC50 for *Nitzschia palea* was 4.47 mg test item/L. The NOEC (biomass) and NOEC (growth rate) were both determined to be 1.0 mg test item/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013. Due to the slow growth of test species, the study was extended to 96 hours, according to the guideline OECD 201. Therefore the validity criteria are applied to the time point 96 hours.

Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required (0 - 96 h)	Obtained (0 - 96 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	19.9
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %.	≤ 35 %	72.7 %
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10 %.	< 10 %	2.0 %

The study was considered valid when compared to the guideline used at the time of study conduct. However, compared with the current control validity criteria, the biomass in the control cultures increased by a factor of > 16 (actual: 19.9), the coefficient of variance for section specific growth rates exceeded 35 % (actual: 72.7 %) and for the whole test period it was ≤ 10 % (actual: 2 %). Because the coefficient of variation for the section specific growth rate was > 35 %, the validity criteria according to guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

CA 8.2.7 Effects on aquatic macrophytes

Studies on effects of the active substance glyphosate and its relevant metabolites on aquatic macrophytes to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

Studies considering the effects of glyphosate on aquatic macrophytes were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Where appropriate position papers are available as indicated in the table below, which contain details regarding the statistical re-evaluation of the study to current requirements. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 8.2.7-1: Studies on toxicity of glyphosate to aquatic macrophytes

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.2.7/001	2002	7-day, static	*Lemna minor*	IPA salt	Valid	-
CA 8.2.7/002	2020	Position Paper	*Lemna minor*	IPA salt	Valid	-
CA 8.2.7/003	, 1999	14-d, semi static	*Lemna gibba*	IPA salt	Valid	-
CA 8.2.7/004	2020	Position Paper	*Lemna gibba*	IPA salt	Valid	-
CA 8.2.7/005	1996	14-d, semi static	*Lemna gibba*	Glyphosate acid	Valid	-
CA 8.2.7/006	2020	Position Paper	*Lemna gibba*	Glyphosate acid	Valid	-
CA 8.2.7/007	1987	14-d, static	*Lemna gibba*	Glyphosate Technical	Valid	-
CA 8.2.7/008	2020	Position Paper	*Lemna gibba*	Glyphosate Technical	Valid	-
CA 8.2.7/009	s, 1987	Toxicity to Lemna gibba	*Lemna gibba*	Glyphosate Technical	Invalid	Report not available
CA 8.2.7/010	, 2012	14-d, static	*Myriophyllum aquaticum*	Glyphosate acid	Valid	-
CA 8.2.7/011	, 2012	14-d static	*Myriophyllum aquaticum*	AMPA	Valid	-
CA 8.2.7/012	2011	7-d, semi-static	*Lemna gibba*	HMPA	Valid	-

Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate on aquatic macrophytes are summarised in the table below. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to aquatic macrophytes, please refer to document M-CP Section 10.2.

Table 8.2.7-2: Literature on toxicity of glyphosate to aquatic macrophytes

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.7/013	Yanhui *et al.*, 2015	OECD 221 7-d semi-static	Glyphosate	Reliable with restrictions	no analytical test verifications

Endpoints of studies considered valid for glyphosate are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.2.7-3: Endpoints: Toxicity of glyphosate to aquatic macrophytes

Reference	Test item	Species	Test design/ GLP	Endpoint based on	EC_{50} [2] (mg a.e./L)	NOEC (mg a.e./L)
, 2002 CA 8.2.7/001[1]	IPA salt	*Lemna minor*	7-d static Recalculated endpoint	nom	fronds GR: 30.3 Y: 16.5 Change in Biomass: 32.1	fronds GR: 8.65 Y: 18.0 Change in Biomass: 8.65
1999 CA 8.2.7/003[1]	IPA salt	*Lemna gibba*	14-d semi static Recalculated endpoint based on 7-d exposure	gm	Fronds: GR: 34.8 Y: 28.1	Fronds: GR: 14.7 Y: 14.7
, 1996 CA 8.2.7/005[1]	Glyphosate acid	*Lemna gibba*	14-d semi static Recalculated endpoint based on 7-d exposure	nom	Fronds: GR: 36.0 Y:24.0	Fronds: GR:12.0 Y: 6.0
, 1987 CA 8.2.7/007[1]	Glyphosate Technical	*Lemna gibba*	14-d static Recalculated endpoint based on 7-d exposure	gm	Fronds: GR: 66.2 Y:25.0	Fronds: GR:16.6 Y: 16.6
, 2012 CA 8.2.7/010	Glyphosate acid	*Myriophyllum aquaticum*	14-d static	nom	Relative increase: TSL: 78.7 FW: 12.3 DW: 25.2 RL: 18.0 Growth rate: TSL: 276 **FW: 23.4** DW: 30.2 RL: > 500	Relative increase: TSL: 5.0 FW: <5.0 DW: 50.0 RL: <5.0 Growth rate: TSL: 5.0 FW: <5.0 DW: 50.0 RL: <5.0

[1] All endpoints are based on statistical re-evaluation provided in Position Papers: CA 8.2.7/002, CA 8.2.7/004, CA 8.2.7/006 and CA 8.2.7/008. Endpoint in **bold** used for risk assessment. a.e.: acid equivalents; nom: nominal; gm: geometric mean measured, GR: growth rate; Y: yield; TSL: total shoot length; FW: fresh weight; DW: dry weight; RL: root length.

Table 8.2.7-3: Endpoints: Toxicity of glyphosate to aquatic macrophytes

Reference	Test item	Species	Test design/ GLP	Endpoint based on	EC_{50} [2] (mg a.e./L)	NOEC (mg a.e./L)

[2] According to the provisions of the new Guidance Document on Aquatic Ecotoxicology (2013), growth rate endpoints (ErC_{50}) shall be chosen for the risk assessment if available.

Endpoints of studies considered valid for AMPA and HMPA are shown in the table below.

Table 8.2.7-4: Endpoints: Toxicity of AMPA and HMPA to aquatic macrophytes

Reference	Test item	Species	Test design/ GLP	Endpoint based on	EC_{50} [1] (mg/L)	NOEC (mg/L)
, 2012 CA 8.2.7/011	AMPA	*Myriophyllum aquaticum*	14-d static	gm	Relative increase: TSL: 103.3 FW: 70.8 DW: 63.2 RL: 31.1 Growth rate: TSL: >94.6 FW: 97.3 **DW: 72.0** RL: 150.1	Relative increase: TSL: 14.3 FW: 14.3 DW: 37.1 RL: 5.4 Growth rate: TSL: 14.3 FW: 14.3 DW: 37.1 RL: 5.4
2011 CA 8.2.7/012	HMPA	*Lemna gibba*	7-d semi-static	am	Fronds: **GR:>123** Y:>123 Biomass: GR: >123 Y: >123	≥123

am: arithmetic mean measured, gm: geometric mean measured; GR: growth rate; Y: yield; TSL: total shoot length; FW: fresh weight; DW: dry weight; RL: root length
[1] According to the provisions of the new Guidance Document on Aquatic Ecotoxicology (2013), ErC_{50} endpoints shall be chosen for the risk assessment if available
Endpoint in **bold** is used for risk assessment.

Study summaries are provided below.

1. Information on the study

Data point:	CA 8.2.7/001
Report author	
Report year	2002
Report title	IPA Salt of Glyphosate: Effects on *Lemna minor*
Report No	CEMR-1873
Document No	-
Guidelines followed in study	OECD Guideline 221
Deviations from current test guideline	Deviation from guideline OECD 221 (2006): none.
Previous evaluation	Yes, accepted in RAR (2015)

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

2. Full summary

Executive Summary

The effect of isopropylamine (IPA) salt of glyphosate on the growth of the duckweed *Lemna minor* was evaluated in a 7 day semi-static toxicity test at nominal concentrations of IPA salt of glyphosate of 2.92, 5.83, 11.7, 24.3, 48.6 and 97.2 mg/L, equivalent to 2.16, 4.32, 8.64, 18.0, 36.0 and 72.0 mg glyphosate acid/L. Furthermore, a negative control group with *Lemna minor* exposed to test medium without test substance (negative control) was prepared in parallel.

The test vessels were 250mL glass beakers containing 150mL of the test or control medium. The vessels were continuously illuminated. The medium in each of the test vessels was renewed twice; day 2 and 4. Growth in each vessel was determined by counting the numbers of plants and fronds on three occasions during the definitive test and measuring the dry weights of the fronds after seven days. Some visible effects (chlorosis and dark frond) were noted for all concentrations ≥ 11.7 mg/L. Analytical samples for analysis of glyphosate were collected from the three highest samples at the start and end of the test and following each media renewal (fresh and old media). Glyphosate isopropylamine salt was not detected in the control group. The mean measured content of the IPA salt ranged between 96 and 104% of nominal, the results are therefore based on nominal concentrations. Based on nominal concentrations of IPA salt of glyphosate, growth of *L. minor* was significantly inhibited at 24.3mg/L, but not affected at 11.7 mg/L.
All validity criteria according to the OECD guideline 221 were fulfilled.

The lowest 7-day $EC_{50}$for *Lemna minor* exposed to glyphosate IPA salt was calculated to be 25.5 mg/L, equivalent to 18.9 mg glyphosate acid/L. The 7-day NOEC for *Lemna minor* exposed to glyphosate IPA salt was determined to be 11.7 mg/L, equivalent to 8.64 mg glyphosate acid/L. The lowest observed effect concentration (LOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7 day exposure period was 24.3 mg/L, equivalent to 18.0 mg glyphosate acid/L.
According to the statistical reanalysis, the 7 day ErC_{50} was 30.3 mg a.e./L based on frond numbers at 7 days. The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* over a 7-day exposure period was 8.65 mg a.e./L. This study is considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine (IPA) salt
Description:	White powder
Lot/Batch #:	1002B
Purity:	97.1 % as IPA salt
2. Vehicle and/or positive control:	Positive control: none
3. Test organism:	
Species:	*Lemna minor*
Source:	Pond in Marlow, Buckinghamshire, UK

4. Environmental conditions:	
Temperature:	20.5 – 22.8 °C
Photoperiod:	24 h fluorescence light
Light intensity	6600 - 8100 Lux
pH:	6.06 – 6.96
5. Dates of experimental work	Sept 30th to Nov 28th 2002

B. STUDY DESIGN AND METHODS

1. Experimental treatments: On the basis of the results of a range finding test, the definitive test was performed with six concentration levels, 2.92, 5.83, 11.7, 24.3, 48.6 and 97.2 mg glyphosate IPA salt/L, equivalent to 2.16, 4.32, 8.65, 18.0, 36.0 and 72.0 mg glyphosate/L. Furthermore, a negative control group with *Lemna minor* exposed to culture medium (SIS) only was run in parallel. The medium in each of the test vessels was renewed on day 2 and 4. Three replicates were prepared with 9-10 fronds (in 3-4 colonies) were used for each test concentration and control. Temperatures and pH values were measured in the test media were measured at the start of tests and at the end. In addition, temperature was monitored continuously. Analytical samples for analysis of glyphosate were collected at the start of the tests and at the end and following each media renewal. Samples were analysed using HPLC with fluorescence detection.

2. Observations: The numbers of fronds and colonies were counted on days 0 (start), 2, 4 and 7 during the definitive test. Dry weights of the fronds were determined at the end of the tests. The fronds from each vessel were collected, rinsed with de-ionised water and dried at 60 °C to a constant weight. The dry weights of fronds from each vessel were measured to ±0.1 mg.

3. Statistical calculations: EC_{50} values were calculated using the LC_{50} program of Stephan *et al.*, 1986. The no-observed-effect concentration (NOEC) and the lowest- observed-effect concentration (LOEC) were based on statistical analysis of *L. minor* final frond numbers, growth rate and area under growth curve values, as well as the final biomass, for the definitive test. Data were first tested for compliance with the assumptions of ANOVA in terms of normality of distribution and homogeneity. The treatment means were tested for significant difference from the control mean at α=0.05 using the Dunnett's test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Chemical analyses were performed on samples of the test media to quantify glyphosate in the test solution. The mean measured content of the test item always ranged between 80 and 120 % of nominal.

Table 8.2.7-5: Analytical results

Nominal concentration of IPA salt [mg/L]	0	2.92	5.93	11.7	24.3	48.6	97.2
Nominal concentration of glyphosate [mg/L]	0	2.16	4.32	8.65	18.0	36.0	72.0
Day 0 concentration (fresh)	< 0.26	2.14	4.23	8.44	17.9	36.5	74.1
Day 3 concentration (old)	-	2.08	4.18	8.26	17.0	31.5	69.5
Day 3 concentration (fresh)	< 0.26	2.31	4.33	8.81	17.7	36.6	85.2
Day 7 concentration (old)	< 0.26	1.85	3.94	8.32	17.4	34.6	70.8
Mean measured [mg/L]	<0.26	2.10	4.17	8.46	17.5	34.7	74.9
% of nominal	-	97	96	98	97	96	104

Table 8.2.7-6: *L. minor* colony and frond numbers

Nominal concentration of IPA salt of glyphosate (mg/L)	Replicate number	Numbers of Lemna colonies				Numbers of Lemna fronds			
		Day 0	Day 2	Day 4	Day 7	Day 0	Day 2	Day 4	Day 7
0	1	3	3	4	23	9	17	35	128
	2	3	3	8	36	9	22	47	177
	3	3	3	5	24	9	18	36	138
2.92	1	3	3	4	19	9	17	29	114
	2	3	3	5	23	10	17	37	137
	3	3	3	6	24	9	20	44	154
5.83	1	3	4	8	28	9	17	39	147
	2	3	3	6	37	9	20	45	157
	3	3	3	7	26	9	22	43	148
11.7	1	3	3	6	27	9	20	36	112
	2	4	4	9	47	10	28	51	172
	3	3	4	7	29	10	23	38	114
24.3	1	3	3	7	18	10	16	28	55
	2	3	3	8	23	9	22	32	78
	3	3	3	7	18	10	20	31	61
48.6	1	3	3	4	9	9	14	19	24
	2	3	4	6	11	9	22	26	34
	3	3	3	5	8	9	15	18	24
97.2	1	3	3	5	8	10	15	17	20
	2	3	3	5	6	9	16	18	19
	3	3	3	4	4	9	12	15	17

Table 8.2.7-7: *L. minor* growth rates

Nominal concentration of IPA salt of glyphosate (mg/L)	Replicate Number	Growth rate (0-7 days)	Frond doubling time (days)	Average growth rate (0-7 days)	Percent inhibition in growth rate relative to controls
0	1	0.379	1.83	0.398	
	2	0.426	1.63		
	3	0.389	1.78		
2.92	1	0.363	1.91	0.381	4%
	2	0.374	1.85		
	3	0.406	1.71		
5.83	1	0.399	1.74	0.402	-1%
	2	0.408	1.70		
	3	0.400	1.73		
11.7	1	0.360	1.92	0.371	7%
	2	0.406	1.71		
	3	0.348	1.99		
24.3	1	0.244	2.85	0.270	32%
	2	0.308	2.25		
	3	0.258	2.68		
48.6	1	0.140	4.95	0.157	61%
	2	0.190	3.65		
	3	0.140	4.95		
97.2	1	0.099	7.00	0.099	75%
	2	0.107	6.49		
	3	0.091	7.63		

Table 8.2.7-8: *L. minor* area under growth curves

Nominal concentration of IPA salt of glyphosate (mg/L)	Replicate Number	Area under growth curves (0-7 days)	Average a.u.g.c. (0-7 days)	Percent inhibition in area under growth curve relative to controls
0	1 2 3	27.65 30.87 28.19	28.90	-
2.92	1 2 3	26.36 27.50 29.84	27.90	3
5.83	1 2 3	28.61 30.01 29.89	29.50	-2
11.7	1 2 3	27.88 31.28 27.99	29.05	-1
24.3	1 2 3	23.19 26.49 24.67	24.78	14
48.6	1 2 3	18.99 22.96 18.93	20.29	30
97.2	1 2 3	17.46 18.42 16.31	17.40	40

Table 8.2.7-9: *L. minor* change in biomass

Nominal concentration of IPA salt of glyphosate (mg/L)	Replicate Number	Total increase in dry weights of fronds over 7 days (g)	Average increase in biomass (0-7 days) (g)	Percent inhibition in biomass increase relative to controls
0	1 2 3	0.0342 0.0387 0.0343	0.0357	-
2.92	1 2 3	0.0264 0.0302 0.0362	0.0309	13%
5.83	1 2 3	0.0331 0.0334 0.0361	0.0342	4%
11.7	1 2 3	0.0343 0.0393 0.0298	0.0345	3%
24.3	1 2 3	0.0178 0.0233 0.0218	0.0210	41%
48.6	1 2 3	0.0147 0.0167 0.0122	0.0145	59%
97.2	1 2 3	0.0125 0.0122 0.0121	0.0123	66%

Visible effects were noted and described below for each concentration:
- 97.2 mg/L: some chlorosis and elongation of fronds, some fronds became very dark, algal growth apparent in all vessels on surface.
- 48.6 mg/L and 24.3 mg/L: some cholrosis and elongation of fronds, some very dark fronds.
- 11.7 mg/L: slight chlorosis and slight elongation of fronds.
- 5.83 mg/L and 2.92 mg/L: no visible effect in comparison with controls.

B. OBSERVATIONS

The results of the definitive test showed no effect on frond growth at 11.7 mg IPA salt/L and partial and statistically significant inhibition at 24.3 mg IPA salt/L. At 48.6 and 97.2 mg IPA salt/L the inhibition of frond growth was greater at 81% and 87% inhibition for final frond numbers. The validity criteria according to guideline OECD 221 are fulfilled.

The endpoints given below are based on nominal concentrations of IPA salt of glyphosate and glyphosate acid.

Table 8.2.7-10: Toxicity of glyphosate IPA salt and glyphosate acid to *Lemna minor*

Endpoint	Glyphosate IPA-salt [mg/L]	Glyphosate acid [mg/L]
$EC_{50,\ frond\ number}$ (7 day)	25.5 (C.I.: 11.1 – 73.4)	18.9 (C.I.: 8.2 – 54.4)
$NOEC_{frond\ number}$ (7 day)	11.7	8.65
$EC_{50,\ biomass}$ (7 day)	46.2 (C.I.: 18.6 – 1673)	34.2 (C.I.: 13.8 – 1239)
$NOEC_{biomass}$ (7 day)	11.7	8.65
$EC_{50,\ area\ under\ growth\ curve}$ (7 day)	Not calculable	Not calculable
$NOEC_{area\ under\ growth\ curve}$ (7 day)	11.7	8.65
$EC_{50,\ growth\ rate}$ (7 day)	42.6 (C.I.: 26.3 – 87.8)	31.6 (C.I.: 19.5 – 65.0)
$NOEC_{growth\ rate}$ (7 day)	11.7	8.65

C.I.: confidence interval

The lowest observed effect concentration (LOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7 day exposure period was 24.3 mg IPA salt/L, equivalent to 18.0 mg glyphosate acid/L. The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7-day exposure period was 11.7 mg/L, equivalent to 8.65 mg glyphosate acid/L. The lowest 7 day EC_{50} was 25.5 mg/L with 95 % confidence limits of 11.1 to 73.4 mg/L measured from final frond numbers at 7 days, equivalent to 18.9 mg glyphosate acid/L (8.22 – 54.37 mg a.s./L).

III. CONCLUSIONS

Assessment and conclusion by applicant:

The lowest 7 day EC_{50} was 25.5 mg/L with 95% confidence limits of 11.1 to 73.4 mg/L measured from final frond numbers at 7 days, equivalent to 18.9 mg glyphosate acid/L (8.22 – 54.37 mg a.s./L).

The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7 day exposure period was 11.7 mg/L, equivalent to 8.65 mg glyphosate acid/L.

Statistical re-analysis of endpoints has been performed to comply with Commission Regulation (EU) 283/2013 to determine 7-day EC_{10}, EC_{20} and EC_{50} endpoints.
The percent recovery nominal test concentrations are presented below.

Analytical verification of test item

Parameter	Nominal concentration of glyphosate acid equivalent [mg/L]					
	2.16	4.32	8.65	18.0	36.0	72.0
	Measured concentration of glyphosate acid equivalent [mg/L] (% of nominal)					
Day 0	2.14 (99)	4.23 (98)	8.44 (98)	17.0 (94)	36.5 (101)	74.1 (103)
Day 2 aged	2.08 (96)	4.18 (97)	8.26 (95)	17.7 (98)	31.5 (88)	69.5 (97)
Day 2 fresh	2.31 (107)	4.33 (100)	8.81 (102)	17.4 (97)	36.6 (102)	85.2 (118)
Day 7 fresh	1.85 (86)	3.94 (91)	8.32 (96)	17.5 (97)	34.6 (96)	70.8 (98)
Geometric mean	2.088	4.167	8.455	17.398	34.737	74.657
	Equivalence in IPA salt nominal concentration [mg/L]*					
	2.92	5.83	11.67	24.29	48.58	97.17

* conversion factor from IPA salt to acid equivalent has been stated as 0.741 by RMS.

Analytical recovery of the test item ranged from 86 to 118% throughout the study. Therefore, calculated endpoints will be based on nominal concentrations.

Details of statistical re-evaluation are given in the position paper CA 8.2.7/002.

The 7 day ECx values for yield and growth rate based on frond numbers has been calculated based on the nominal concentrations and are provided the table below:

7-d ECx values for Yield, Growth Rate

7-day endpoints	Nominal concentration of glyphosate acid equivalent [mg/L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (Frond number)	18.0	7.80 (3.21 – 10.7)	10.3 (5.77 – 13.2)	16.5 (13.1 – 19.9)
Growth rate (Frond number)	8.65	8.16 (5.38 – 12.4)	12.8 (8.65 – 18.9)	30.3 (18.7 – 48.6)
Growth rate (Biomass)	8.65	5.72 (0.09 – 12.54)	10.3 (0.71 – 19.1)	32.1 (16.6 – 94.3)

According to the statistical reanalysis, the 7 day ErC_{50} was 30.3 mg a.e./L based on frond numbers at 7 days.
The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* over a 7-day exposure period was 8.65 mg a.e./L.

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.7/002
Report author	
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study CEMS-1873 on the toxicity of Glyphosate isopropylamine (IPA) salt to Lemna minor under static conditions
Report No	110054-008
Document No	-
Guidelines followed in study	OECD Guideline 221
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	No, not conducted under GLP (GLP is not compulsory for statistical evaluation)
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 7 day NOEC, EC_{10}, EC_{20} and EC_{50} values was conducted for the study CEMS-1873 (, 2002) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 221 (2006).

Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 221 (2006) were met (doubling time: 1.7 days, mean growth rate: 0.398/d) this study is considered valid for risk assessment purposes.

Based on the nominal concentration of glyphosate the 7-day endpoints EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 7.80, 10.3, and 16.5 mg a.e./L for yield (frond number), respectively; 8.16, 12.8, and 30.3 mg a.e./L for growth rate (frond number), and 5.72, 10.3, and 32.1 mg a.e./L for change in biomass. The NOEC was determined to be 8.65 mg a.e./L.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number: CEMR-1873
Author:
Substance: Glyphosate isopropylamine (IPA) salt
Title: IPA Salt of Glyphosate: Effects on *Lemna minor*
Completion date: 05-Dec-2002
Test guideline(s): OECD Guideline 221 (Draft version, 2002), re-evaluated according to OECD 221 (2006)
GLP: Yes
Testing facility: CEM Analytical Services Limited (CEMAS), Berkshire, UK
Sponsor: Sinon Corporation, Taichung, Taiwan, R.O.C.

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was evaluated according to the current test guideline OECD 221 (2006) and 7 days EC_{10}, EC_{20}, and EC_{50} values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study CEMS-1873 (2002) was statistically evaluated for the effects of Glyphosate isopropylamine (IPA) salt on the organism *Lemna minor.* The organisms were exposed for 7 days to the following concentrations of Glyphosate isopropylamine (IPA) salt: 2.92, 5.83, 11.7, 24.3, 48.6 and 97.2 mg glyphosate IPA salt/L, equivalent to 2.16, 4.32, 8.65, 18.0, 36.0 and 72.0 mg glyphosate acid/L. Additionally, a control was tested in parallel. The frond count data as well as change in biomass data for the individual control and treatment group replicates will be used to calculate the ECx values.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:

In order to derive the 7-day Effect Concentrations that have 10, 20 and 50% effects on yield and growth rate for frond number, a 3-parameter logistic CDF (Cumulative Distribution Function) model and a 3 parameter normal CDF model was used, respectively.

To estimate the effects on yield for change in biomass, probit analysis with linear maximum likelihood regression was used.

For yield and growth rate, the NOEC was determined by Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm Correction (one sided smaller, $p > 0.01$).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The percent recovery nominal test concentrations between 4 and 7 days are presented below.

Table 8.2.7-11: Analytical verification of test item

Parameter	Nominal concentration of glyphosate acid equivalent [mg a.e./L]					
	2.16	4.32	8.65	18.0	36.0	72.0
	Measured concentration of glyphosate acid equivalent [mg/L] (% of nominal)					
Day 0	2.14 (99)	4.23 (98)	8.44 (98)	17.0 (94)	36.5 (101)	74.1 (103)
Day 2 aged	2.08 (96)	4.18 (97)	8.26 (95)	17.7 (98)	31.5 (88)	69.5 (97)
Day 2 fresh	2.31 (107)	4.33 (100)	8.81 (102)	17.4 (97)	36.6 (102)	85.2 (118)
Day 7 fresh	1.85 (86)	3.94 (91)	8.32 (96)	17.5 (97)	34.6 (96)	70.8 (98)
Geometric mean	2.088	4.167	8.455	17.398	34.737	74.657

Analytical recovery of the test item ranged from 86 to 118 % of nominal throughout the study. Therefore, calculated endpoints will be based on nominal concentrations.

The mean measured content of the test item always ranged between 80 and 120 % of nominal. Therefore, the endpoints given below are based on nominal concentrations of glyphosate IPA salt, expressed as glyphosate acid equivalent.

Considering frond numbers:
For yield, the parameters for the 3 parameter logistic CDF model are estimated as b0: 136.975, b1: 16.476, and b2: 2.937.
According to the statistical parameters; $F(2, 4) = 101.777$; p(F) = <0.001; $R^2 = 0.997$ the EC_{10} and EC_{20}, and EC_{50} calculations should be considered valid.
After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.632.
For growth rate, parameters for the 3 parameter normal CDF model are estimated as b0: 0.400, b1: 0.911, and b2: 0.445.
According to the statistical parameters; $F(2, 4) = 101.205.117$; p(F) = <0.001; $R^2 = 0.0.989$ the EC_{10} and EC_{20}, calculations should be considered valid.
After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.177.

Considering change in biomass:
The parameters for the logit model are estimated as slope b: 1.71104; Intercept a: -2.57759.
Statistical parameters for goodness fit are: $Chi^2(15) = 0.27989$; p(Chi^2): 1.000; F(1,15) = 14.751, p(F) <0.001; $R^2 = 0.787$ the EC_{10}, EC_{20} and EC_{50}, calculations should therefore be considered valid.
The obtained 7-day EC_{10}, EC_{20} and EC_{50} values for the effect of Glyphosate isopropylamine (IPA) salt on yield and growth rate, considering frond numbers for *Lemna minor* is presented in the table below.

Table 8.2.7-12: 7-day ECx values for Yield, Growth Rate

7-day endpoints	Nominal concentration of glyphosate acid equivalent [mg a.e./L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (Frond number)	18.0	7.80 (3.21 – 10.7)	10.3 (5.77 – 13.2)	16.5 (13.1 – 19.9)
Growth rate (Frond number)	8.65	8.16 (5.38 – 12.4)	12.8 (8.65 – 18.9)	30.3 (18.7 – 48.6)
Change in biomass	8.65	5.72 (0.09 – 12.54)	10.3 (0.71 – 19.1)	32.1 (16.6 – 94.3)

CI = confidence interval

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid.

III. CONCLUSION

Assessment and conclusion by applicant:
Based on the nominal concentration of glyphosate the 7-day endpoints EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 7.80, 10.3, and 16.5 mg a.e./L for yield (frond number), respectively; 8.16, 12.8, and 30.3 mg a.e./L for growth rate (frond number), and 5.72, 10.3, and 32.1 mg a.e./L for change in biomass. The NOEC was determined to be 8.65 mg a.e./L.

The statistical parameters demonstrate that these values can be considered reliable/valid and therefore considered for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.7/003
Report author	
Report year	1999
Report title	Glyphosate 62% IPA-Salt, aquatic plant toxicity test using *Lemna gibba*
Report No	980909FH
Document No	-
Guidelines followed in study	Guideline ASTM E 1415- 91 (June 1991)
Deviations from current test guideline	Deviations from guideline OECD 221 (2006): Minor: - The study was performed for 14 days instead of 7.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate isopropylamine salt on growth of *Lemna gibba* were evaluated in a 14 day semi-static toxicity test. Based on the results of a range finding test, the definitive test was performed with five concentration levels of glyphosate IPA-salt, 6.25, 12.5, 25, 50 and 100 mg test item /L and a control with 3 replicates per test item treatment using three plants per replicate (four fronds each). Renewal of the test media was performed on day 2, 4, 7, 9 and 11. A reference substance (zinc chloride) was equally tested at 1.0, 3.2 and 10 mg/L. The number of fronds affected was determined on day 0, 7 and 14. Observation of change in colour, break-up of plants and destruction of roots was conducted on day 7 and 14. Dry biomass weight was determined on day 14 (end of the test).

Analysis of the test concentration was carried out on day 4 and day 11(freshly prepared media) and on day 7 and 14 (3 day old test media). All test concentrations and control replicates were analysed. Result showed an increase of growth of *Lemna gibba* at nominal concentrations of 6.25, 12.5 and 25 mg test item/L. Glyphosate isopropylamine salt was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above concentrations of 50 mg IPA salt /L.

The EC_{50} values for inhibition of front number and dry weight after 14 days were 53.56 mg IPA salt/L (equivalent to 33.42 mg glyphosate/L) and 62.59 mg IPA salt /L (equivalent to 39.06 mg glyphosate/L) respectively. The NOEC was determined to be 25 mg IPA salt /L equivalent to 15.60 mg glyphosate/L.

Analytical recovery of the test item ranged from 78 to 113 % from 4 to 7 days. Therefore, calculated endpoints will be based on geometric mean concentrations.
According to the statistical reanalysis, the 7 day ErC_{50} is 34.8 mg a.e./L with 95% confidence limits of 29.7 to 41.3 mg a.e./L for frond number parameter at 7 days.
The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna gibba* over a 7-day exposure period was 14.7 mg a.e./L.
The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate 62 % IPA-Salt
name	Glyphosate Isopropylamine Salt
Description:	Clear, liquid, yellowish
Lot/Batch #:	22-9754
Purity:	62.4 % glyphosate acid
Density:	1.2355 g/mL
2. Vehicle and/or positive control:	Positive control: Zinc chloride
3. Test organism:	
Species:	*Lemna gibba*
Source:	Bundesanstalt für Gewässerkunde, Koblenz, Germany
4. Environmental conditions:	
Temperature:	25 ± 2 °C
Photoperiod:	24 h florescence light
Light intensity	around 4200 – 6700 lux
pH:	7.5 ± 0.1
Conductivity:	not stated
Hardness:	not stated
5. Experimental dates of work:	Sept 30th 1989 to Feb 3rd 1999

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, the definitive test was performed with five concentration levels, 6.25, 12.5, 25, 50 and 100 mg test item/L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions. Three plants per replicate were used. The plants were placed in 500 mL test vessels, which already contained the 300 mL 20X-AAP test media prepared according to the guideline. The pH of the test medium was adjusted prior to the test. Three uniformly healthy-looking plants with 4 fronds each were used in each test vessel. The test was conducted under semi-static conditions with renewal of test media on day 2, 4, 7, 9 and 11. The reference substance (zinc chloride) was equally tested at 1.0, 3.2 and 10 mg/L.

2. Observations:

Biological data: The amount of the plants and fronds affected were determined on day 0, 7 and 14. Every frond that visibly projected beyond the edge of a parent frond was counted as separate frond. Observation of change in colour, break-up of plants and destruction of roots were made on day 7 and 14. Dry biomass weight was determined on day 14.

Physical data: The pH values were measured on day 0, 2, 4, 7, 9, 11 and 14. The room temperature in the test chamber was measured and recorded continuously. Sampling and analysis of the test concentration were carried out on day 4 and day 11(freshly prepared media) and on day 7 and 14 (3 day old test media). All test concentrations and control replicates were analysed.

3. Statistical calculations: EC_{50} and EC_{90} values of frond number inhibition after day 7 and 14 were calculated by Probit analysis. The NOEC values were determined by calculation of statistical significance

using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dray weight, respectively, at $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 14d EC_{50} and NOEC values are given below based on nominal concentrations.

Table 8.2.7-13: Toxicity of glyphosate isopropylamine salt to *Lemna gibba*

Endpoint	IPA salt [mg/L]		Glyphosate [mg/L]	
	Frond number	Biomass dry weigh	Frond number	Biomass dry weight
7d				
EC_{50}	56.26			
95% confidence limit	45.53 - 69.53			
NOEC	25			
14d				
EC_{50}	53.56	62.59	33.42	39.06
95% confidence limit	42.91 - 66.85	47.94 - 81.73	26.78 - 41.71	29.91.- 51.00
NOEC	25	25	15.60	15.60

Analytical data: In freshly prepared test media the recoveries of the glyphosate varied between 78 % and 86 % for day 4 and 94 % to 113 % for day 11. In the aged test media (3 days old), 106 % to 113 % of the glyphosate were recovered for day 7 and 87 % to 104 % for day 14. As the mean measured content of the glyphosate always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the IPA salt.

Table 8.2.7-14: Analytical results

Test substance nominal [mg IPA salt/L]	Glyphosate nominal [mg/L]	Day 4 (new media)		Day 7 (old media)		Day 11 (new media		Day 14 (old media)	
		Measured conc. [mg/L]	% of nominal	Measured conc. [mg/L]	% of nominal	Measured conc. [mg/L]	% of nominal	Measured conc. [mg/L]	% of nominal
Control	-	< LOD		< LOD		< LOD		< LOD	
100	62.4	48.67	78	67.08	108	62.25	100	54.34	87
		53.12	85	66.29	106	58.88	94	57.09	91
50	31.2	26.87	86	33.44	107	31.34	100	29.32	94
		26.33	84	33.91	108	31.8	102	29.17	93
25	15.6	12.76	82	17.00	108	15.90	102	15.10	97
		12.54	80	17.12	110	15.32	98	14.64	94
12.5	7.8	6.72	86	8.29	106	8.26	106	8.01	103
		6.57	84	8.49	109	8.20	105	7.75	99
6.25	3.9	3.37	86	4.20	108	4.39	113	3.93	101
		3.21	82	4.42	113	4.10	105	4.06	104

Limit of detection of glyphosate: new media = 0.90 mg/L, old media = 0.81 mg/L.

B. OBSERVATIONS

Observations: Increase of growth was found at nominal concentrations of 6.25, 12.5 and 25 mg IPA salt/L. Glyphosate isopropylamine salt was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a concentration of 50 mg test item/L. Front number inhibition values after day 14 as well as biomass dry weight inhibition are presented below.

Table 8.2.7-15: Frond numbers and inhibition values (day 0/14)

			Control	Test item [mg/L]				
Test item (IPA salt)				**6.25**	**12.5**	**25**	**50**	**100**
Glyphosate				**3.90**	**7.80**	**15.60**	**31.20**	**62.40**
Frond number	Mean	Day 0	12.0	12.0	12.0	12.0	12.0	12.0
		Day 14	535.0	776.7	757.3	875.7	119.3	20.7
Increase of frond number	Mean	Day 14	523.0	764.7	745.3	863.7	107.3	8.7
Inhibition	Mean±SD	[%]	-	-46 ± 14.0	-43± 12.7	-65 ± 15.4	79 ± 7.5	98 ± 1.1

Table 8.2.7-16: Dry weight after 14 days and inhibition values

			Control	Test item [mg/L]				
Test item (IPA salt)				**6.25**	**12.5**	**25**	**50**	**100**
Glyphosate				**3.90**	**7.80**	**15.60**	**31.20**	**62.40**
Biomass dry weight [mg]	Mean	Day 14	48.9	65.2	66.0	69.0	18.7	6.6
Inhibition	Mean±SD	[%]	-	-33± 10.8	-35± 9.8	-43± 12.8	62± 15.4	86± 2.7

The doubling time of frond numbers in the control was less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days. The validity criteria according to the current guideline OECD 221 are therefore fulfilled. The EC_{50} values for inhibition of front number and dry weight after 14 days were 53.56 mg IPA salt/L (equivalent to 33.42 mg glyphosate/L) and 62.59 mg IPA salt/L (equivalent to 39.06 mg glyphosate/L) respectively. The NOEC was determined to be 25 mg IPA salt/L, equivalent to 15.60 mg glyphosate/L.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Glyphosate isopropylamine salt was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 50 mg IPA salt/L. The EC_{50} values for inhibition of frond number and dry weight after 14 days were 53.56 mg IPA salt/L (equivalent to 33.42 mg glyphosate/L) and 62.59 mg IPA salt/L (equivalent to 39.06 mg glyphosate/L) respectively.

Statistical re-analysis of endpoints has been performed to comply with Commission Regulation (EU) 283/2013 to determine 7-day EC_{10}, EC_{20} and EC_{50} endpoints.

The percent recovery nominal test concentrations between 4 and 7 days are presented below.

Table 8.2.7-17: Analytical verification of test item between 4 and 7 days

Parameter	Nominal concentration of glyphosate acid equivalent [mg/L]				
	3.9	7.8	15.6	31.2	62.4
	Measured concentration of glyphosate acid equivalent [mg/L] (% of nominal)				
Day 4 new	3.37 (86)	6.72 (86)	12.76 (82)	26.87 (86)	48.67 (78)
	3.21 (82)	6.57 (84)	12.54 (80)	26.33 (84)	53.12 (85)
Day 7 aged	4.2 (108)	8.29 (106)	17 (109)	33.44 (107)	67.08 (108)
	4.42 (113)	8.49 (109)	17.12 (110)	33.91 (109)	66.29 (106)
4 - 7 days Geometric mean	**3.8**	**7.5**	**14.7**	**29.9**	**58.2**

Analytical recovery of the test item ranged from 78 to 113 % from 4 to 7 days. Therefore, calculated endpoints will be based on geometric mean concentrations.

Details of statistical re-evaluation are given in the position paper CA 8.2.7/004.
The 7-day endpoints for yield and growth rate based on frond numbers have been calculated based on the geometric mean concentrations and are provided in the table below:

Table 8.2.7-18: 7-d endpoints for Yield frond number, Growth Rate frond number based on geometric mean measured concentrations

7-day endpoints	glyphosate [mg a.e./L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (Frond number)	14.7	6.42 (3.38 – 9.45)	11.1 (7.16 – 15.9)	28.1 (19.3 – 52.0)
Growth rate (Frond number)	14.7	12.8 (9.59 – 15.8)	19.1 (15.4 – 22.6)	34.8 (29.7 – 41.3)

According to the statistical reanalysis, the 7 day ErC_{50} is 34.8 mg a.e./L with 95% confidence limits of 29.7 to 41.3 mg a.e./L for frond number parameter at 7 days.
The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna gibba* over a 7-day exposure period was 14.7 mg a.e./L.
The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

2. Information on the study

Data point	CA 8.2.7/004
Report author	
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study TLA60871 on the toxicity of Glyphosate 62% IPA-Salt to *Lemna gibba* under static conditions.
Report No	110054-009
Document No	-
Guidelines followed in study	Guideline ASTM E 1415- 91 (June 1991)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values was conducted for the study TLA60871 (1999) to fulfill the data requirements according to regulation EU 283/2013. Futhermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 221 (2006).

Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 221 (2006) were met, this study is considered valid for risk assessment purposes.

The 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values are calculated for yield (frond number) and growth rate (frond number) based on the geometric mean measured concentrations of glyphosate acid equivalents.

The 7-day endpoints of EC_{10}, EC_{20} and EC_{50} values were estimated to be 6.42, 11.1, and 28.1 mg a.e./L for yield (frond number), and 12.8, 19.1, and 34.8 mg a.e./L for growth rate (frond number), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number: 980909FH

Author:

Substance: Glyphosate 62% IPA-Salt

Title: Glyphosate 62% IPA-Salt, aquatic plant toxicity test using *Lemna gibba*

Completion date: 12-Feb-1999

Test guideline(s): Guideline ASTM E 1415- 91 (June 1991)

GLP: Yes

Testing facility: DR. U. NOACK-LABORATORIUM, Sarstedt, Germany

Sponsor: Feinchemie Schwebda GmbH, Köln, Germany

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was evaluated according to the current test guideline OECD 221 (2006) and 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study TLA60871 (1999) was statistically evaluated for the effects of Glyphosate 62 % IPA-Salt on the organism *Lemna gibba*, as the report only provides 14-day endpoints. According to current test guidelines and EFSA Aquatic Guidance (2013), this study type requires a 7-day endpoint.

The organisms were exposed for 14 days to the following concentrations of Glyphosate 62 % IPA-Salt: 6.25, 12.5, 25, 50 and 100 mg test item/L, corresponding to 3.9, 7.8, 15.6, 31.2 and 62.4 mg glyphosate/L. Additionally, a control was tested in parallel. The frond count data for the individual control and treatment group replicates will be used to calculate the ECx values.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 7-day Effect Concentrations that have 10, 20 and 50% effect on yield (frond number), and growth rate (frond number) of the test subjects (EC_{10}, EC_{20} and EC_{50} values), a Probit analysis using linear maximum likelihood regression for yield and growth rate (frond number) analysis was performed. For determination of the no-observed-effect concentration (NOEC), Williams Multiple Sequential t-test Procedure was used (one-sided smaller; p=0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

Endpoints based on biomass cannot be determined, as no data for day 7 is available.

II. RESULTS AND DISCUSSION

A. FINDINGS

The doubling time of frond numbers in the control was less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days. The validity criteria according to the current guideline OECD 221 (2006) are therefore fulfilled.

The percent recovery of nominal test concentrations between 4 and 7 days are presented below.

Table 8.2.7-19: Analytical verification of test item between 4 and 7 days

Parameter	Nominal concentration of glyphosate[1] [mg a.e./L]				
	3.9	7.8	15.6	31.2	62.4
	Measured concentration of glyphosate acid equivalent [mg/L] (% of nominal)				
Day 4 new	3.37 (86)	6.72 (86)	12.76 (82)	26.87 (86)	48.67 (78)
	3.21 (82)	6.57 (84)	12.54 (80)	26.33 (84)	53.12 (85)
Day 7 aged	4.2 (108)	8.29 (106)	17 (109)	33.44 (107)	67.08 (108)
	4.42 (113)	8.49 (109)	17.12 (110)	33.91 (109)	66.29 (106)
4 - 7 days Geometric mean	**3.8**	**7.5**	**14.7**	**29.9**	**58.2**

[1] Test concentrations based on active ingredient glyphosate as stated in the study report.

Analytical recovery of the test item ranged from 78 to 113% of nominal from 4 to 7 days duration. Therefore, calculated endpoints will be based on geometric mean measured concentrations.

The parameters for the logit model are estimated as slope b: 3.42368; intercept a: -4.96199 for yield (frond numbers).

The parameters for the Weibull analysis using linear maximum likelihood regression are estimated as slope b: 4.34953; intercept a: -7.06993 for growth rate (frond numbers).

Statistical parameters for goodness of fit are: $Chi^2(13) = 0.61737$; $p(Chi^2)$: 1.000; $F(1,13) = 32.754$, $p(F) < 0.001$; $R^2 = 0.716$ the EC_{10}, EC_{20} and EC_{50} for yield and $Chi^2(13) = 0.23866$; $p(Chi^2)$: 1.000; $F(1,13) = 101.124$; $p(F) < 0.001$; $R^2 = 0.886$ the EC_{10}, EC_{20} and EC_{50} for growth rate, calculations should therefore be considered valid.

The obtained 7-day EC_{10}, EC_{20} and EC_{50} and NOEC values are presented in the table below. The dose response curve obtained from the analysis of the effect of Glyphosate 62% IPA-Salt on the parameters being analysed of *Lemna gibba* is presented below.

Table 8.2.7-20: 7-day endpoints for Yield (frond number) and Growth Rate (frond number) based on geometric mean measured concentrations.

7-day endpoints	Glyphosate [mg a.e./L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (Frond number)	14.7	6.42 (3.38 – 9.45)	11.1 (7.16 – 15.9)	28.1 (19.3 – 52.0)
Growth rate (Frond number)	14.7	12.8 (9.59 – 15.8)	19.1 (15.4 – 22.6)	34.8 (29.7 – 41.3)

CI: confidence interval

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid.

III. CONCLUSION

Assessment and conclusion by applicant:
The 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values are calculated for yield (frond number) and growth rate (frond number) based on the geometric mean measured concentrations of glyphosate acid equivalents.

The 7-day endpoints of EC_{10}, EC_{20} and EC_{50} values were estimated to be 6.42, 11.1, and 28.1 mg a.e./L for yield (frond number), and 12.8, 19.1, and 34.8 mg a.e./L for growth rate (frond number), respectively.

The statistical parameters presented showed that these values can be considered reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.7/005
Report author	
Report year	1996
Report title	Glyphosate acid: Toxicity to duckweed (*Lemna gibba*)
Report No	AB0503/L
Document No	-
Guidelines followed in study	EPA FIFRA Subdivision J Guideline 123-2
Deviations from current test guideline	Deviations from the guideline OECD 221 (2006): Minor: - The study was performed for 14 days instead of 7.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of Glyphosate acid on growth of *Lemna gibba* were evaluated in a 14 day semi-static toxicity test. The test was performed with eight concentration levels, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.e./L and a control with 3 replicates per test concentration using three plants per replicate (four fronds each). The number of fronds affected was determined after 2, 5, 7, 9, 12 and 14 days. Observation of toxicity symptoms were recorded on these dates, too. Sampling and analysis of the test concentration were carried out at test start and on day 5, 9 and 14.

Result showed a significant inhibition of frond number growth of *Lemna gibba* at nominal concentrations of 6.00 mg a.e./L and significant tissue weight inhibition at 12.0 mg a.e./L.

In conclusion, Glyphosate acid was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 6 mg a.e./L. The 14-d EC_{50} value for inhibition of front number was 12 mg a.e./L (95% CL= 11- 14) and for tissue dry weight 20 mg a.e./L (95% CL= 18 – 22). The NOEC was determined to be 3.0 and 6.0 mg a.e./L for frond number and weight increase, respectively.

Statistical re-analysis of endpoints has been performed. Based on the mean measured concentration of glyphosate acid the endpoints for 7-day EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 10.5, 14.2 and 24.0 mg a.e./L for yield (frond number), respectively; 13.3, 18.7 and 36.0 mg a.e./L for growth rate (frond number), respectively.

The statistical parameters presented above showed that these values can be considered reliable and therefore considered for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
2. Vehicle and/or positive control:	Positive control: none
3. Test organism:	
Species:	*Lemna gibba*, Strain G3
Source:	In-house culture originally obtained from University of Waterloo, Canada
4. Environmental conditions:	
Temperature:	24.6 – 25.0 °C
Photoperiod:	24 h illumination
Light intensity	5000 lux
pH:	Freshly prepared test media: 3.6 – 4.7 Old test media: 3.6 – 5.8
5. Dates of experimental work:	17th Jan to 31st Jan 1996

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test on *Lemna gibba* was performed with eight concentration levels, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.e./L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions as the test groups.
The plants were placed in 400 mL test vessels, which already contained 160 mL of Hoagland's M-medium prepared according to Hillman (1961). The test was conducted under semi-static conditions with renewal of the test medium after 5 and 9 days. Three uniform healthy-looking plants with 4 fronds each were used in each test vessel.

2. Observations: The number of plants and fronds were counted after 2, 5, 7, 9, 12 and 14 days. Also symptoms of toxicity (*eg.* pale frond colouration, emergence of stunted new frond growth, reduced root growth and unnatural floating on the solution surface) were recorded on these dates. At test end the weight of the dried plant tissue (at 60 °C) was recorded. The pH was measured in the old and the new test medium (new= day 0, 5 and 9, old = day 5, 9 and 14). Temperature in the test chamber was recorded daily and light intensity once a week.
Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis at test start and after 5 and 9 d (after test medium renewal).

3. Statistical calculations: The EC_{50} and its 95% confidence interval were calculated by moving average angle method using Stephan's method. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dry weight, respectively, at $p = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 14-d EC_{50}, NOEC and LOEC values are given below based on nominal concentrations.

Table 8.2.7-21: Toxicity of Glyphosate acid to *Lemna gibba*

Endpoint	Glyphosate acid [mg/L]		
	Frond number	**Biomass dry weigh**	**Visual observed effects**
14-d EC_{50} (95% CL)	12 (11 – 14)	20 (18 – 22)	-
NOEC	3.0	6.0	1.5
LOEC	6.0	12	-

Analytical data: Analytical control measurements were performed in the freshly prepared (day 0, 5 and 9) and the old (day 5, 9 and 14) test media. The measured concentrations of glyphosate acid in the fresh media ranged from 90 – 108% of nominal and in the old media from 87 – 102% of nominal (overall mean measured: 93 – 100% of nominal). As the mean measured content of the glyphosate acid always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the glyphosate acid.

Table 8.2.7-22: Analytical results

Day sample taken	Nominal concentration of glyphosate acid [mg/L]							
	0.75	**1.5**	**3.0**	**6.0**	**12**	**24**	**48**	**96**
	Measured concentration of glyphosate acid [mg/L]							
0 (fresh)	0.68	1.4	2.9	5.6	12	23	46	92
5 (spent)	0.65	1.3	2.8	5.5	12	24	49	96
5 (fresh)	0.65	1.4	2.8	5.4	12	22	48	92
9 (fresh)	0.75	1.5	3.0	6.0	13	25	50	100
14 (spent)	0.75	1.4	2.9	5.6	12	23	47	98
Mean measured [mg/L]	0.70	1.4	2.9	5.6	12	23	48	96
% of nominal	93	93	97	93	100	96	100	100

B. OBSERVATIONS

The increase in frond number was significantly inhibited starting with a nominal test concentration of 6.0 mg a.e./L when compared to the control. The growth of the plant tissues dry weight was significantly reduced at 12 mg a.e./L. At 24, 48 and 96 mg a.e./L dose related symptoms like pale frond colouration, emergence of stunted new frond growth, reduced root growth and unnatural floating on the solution surface were observed from day 2 onwards. Visually observed effects were apparent at concentrations of 3.0 mg/L and above. Therefore, overall NOEC is 1.5 mg a.e./L.

Table 8.2.7-23: Frond numbers, increase in frond numbers and inhibition compared to the control

Test item rate [mg/L]	Number of fronds						Increase in frond numbers	Inhibition [%]
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	(Day 0 – 14)	
Control	21	48	85	134	222	327	315	-
0.75	23	47	79	125	232	343	331	0
1.5	23	45	78	113	220	323	311	1
3.0	21	48	78	120	206	300	288	9
6.0	21	49	81	116	198	269	257	18[1]
12	20	44	74	105	148	173	161	49[1]
24	16	28	44	59	82	91	79	75[1]
48	15	21	24	28	28	30	18	94[1]
96	13	14	15	16	18	17	5	98[1]

[1]significant inhibition compared to the control medium

Table 8.2.7-24: Mean dry weight of plant tissue after 14 d, main increase in dry weight and inhibition compared to the control

Test item rate [mg/L]	Mean tissue dry weight after 14 day [mg]	Mean increase [mg]	Inhibition [%]
Control	40.7	39.2	-
0.75	51.3	49.8	0
1.5	49.8	48.3	0
3.0	44.0	42.5	0
6.0	40.3	38.8	1
12	29.8	28.3	28[1]
24	16.5	15.0	62[1]
48	6.0	4.5	89[1]
96	1.4	< 0.1	100[1]

[1]significant inhibition compared to the control medium

All validity criteria according to OECD 221 were fulfilled, as the doubling time of frond number in the control were less than 2.4 d.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Glyphosate acid was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 6 mg a.e./L. The 14-d EC_{50} value for inhibition of front number was 12 mg a.e./L (95% CL= 11- 14 mg test item/L) and for tissue dry weight 20 mg a.e./L (95% CL= 18 – 22 mg a.e./L). The 14-d NOEC was determined to be 3.0 and 6.0 mg a.e./L for frond number and weight increase, respectively.

Statistical re-analysis of endpoints has been performed to comply with Commission Regulation (EU) 283/2013 to determine 7-day EC_{10}, EC_{20} and EC_{50} endpoints. Details of statistical re-evaluation are given in the position paper CA 8.2.7/006.

The 7 day ECx values for yield and growth rate based on frond numbers has been calculated based on nominal concentrations and are provided the table below.

Table 8.2.7-25: 7-d ECx values for Yield and Growth Rate

7-day endpoints	Concentration of glyphosate acid [mg/L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield Frond number	6.0	10.5 (6.76-13.4)	14.2 (10.5-17.1)	24.0 (20.6-27.5)
Growth rate	12.0	13.3 (10.6-16.7)	18.7 (15.1-23.3)	36.0 (27.5-46.8)

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.7/006
Report author	
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study BL5662/B on the toxicity of Glyphosate acid to *Lemna gibba* under static conditions
Report No	110054-010
Document No	-
Guidelines followed in study	EPA FIFRA Subdivision J Guideline 123-2
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 7-day EC_{10}, EC_{20}, EC_{50}, and NOEC values was conducted for the study BL5662/B (1996) to fulfill the data requirements according to regulation EU 283/2013. Futhermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 221 (2006).
Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 221 (2006) were met, this study is considered valid for risk assessment purposes.
Based on the mean measured concentration of glyphosate acid the endpoints for 7-day EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 10.5, 14.2 and 24.0 mg a.e./L for yield (frond number), respectively; 13.3, 18.7 and 36.0 mg a.e./L for growth rate (frond number), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

Software:	ToxRatPro Version 3.3.0
Original report details	
Study number:	AB0503/L
Author:	
Substance:	Glyphosate acid
Title:	Glyphosate acid: Toxicity to duckweed (*Lemna gibba*)
Completion date:	31-Jan-1996
Test guideline(s):	EPA FIFRA Subdivision J Guideline 123-2
GLP:	Yes
Testing facility:	Brixham Environmental Laboratory, Zeneca Limited, Brixham Devon, UK
Sponsor:	Zeneca Agrochemicals, Surrey, UK

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was evaluated according to the current test guideline OECD 221 (2006) and 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study BL5662/B (1996) was statistically evaluated for the effects of Glyphosate on the organism *Lemna gibba* G3 as the report only provides 14 day endpoints. According to current test guidelines and EFSA Aquatic Guidance (2013), this study type requires a 7-day endpoint.

The organisms were exposed for 14 days to the following concentrations of Glyphosate acid: 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.e./L. Additionally, a control was tested in parallel. The frond count data for the individual control and treatment group replicates will be used to calculate the ECx values.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 7-day Effect Concentrations that have 10, 20 and 50% effects on yield (frond number), growth rate (frond number), growth rate (frond area), and growth rate (biomass) of the test subjects (EC_{10}, EC_{20} and EC_{50} values), a non-linear regression model the 3-parameter logistic CDF analysis for yield and the 3-parameter normal CDF growth rate (frond number) analysis was performed.

For determination of the no-observed-effect concentration (NOEC), Williams Multiple Sequential t-test Procedure was used (one-sided smaller; α=0.05).
All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

Endpoints based on biomass cannot be determined, as no data for day 7 is available.

II. RESULTS AND DISCUSSION

A. FINDINGS

Results

The doubling time of frond numbers in the control was less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days. The validity criteria according to the current guideline OECD 221 (2006) are therefore fulfilled.

The percent recovery of nominal test concentrations between 4 and 7 days are presented below.

Table 8.2.7-26: Analytical results

Day sample taken	Nominal concentration of glyphosate acid [mg/L]							
	0.75	**1.5**	**3.0**	**6.0**	**12**	**24**	**48**	**96**
	Measured concentration of glyphosate acid [mg/L]							
0 (fresh)	0.68	1.4	2.9	5.6	12	23	46	92
5 (spent)	0.65	1.3	2.8	5.5	12	24	49	96
5 (fresh)	0.65	1.4	2.8	5.4	12	22	48	92
9 (fresh)	0.75	1.5	3.0	6.0	13	25	50	100
14 (spent)	0.75	1.4	2.9	5.6	12	23	47	98
Mean measured [mg/L]	0.70	1.4	2.9	5.6	12	23	48	96
% of nominal	93	93	97	93	100	96	100	100

The measured concentrations of glyphosate acid in the fresh media ranged from 90 – 108 % of nominal and in the old media from 87 – 102 % of nominal (overall mean measured: 93 – 100 % of nominal). As the mean measured content of glyphosate acid always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations.

The parameters for the 3 parameter logistic CDF model are estimated as b0: 68.792, b1: 23.999 and b2: 2.653 for yield. According to the statistical parameters F (2, 6) = 218.135; p(F) = <0.001; R^2 = 0.986 the EC_{10}, EC_{20} and EC_{50} for yield (frond number) calculations should be considered valid.
The parameters for the 3 parameter normal CDF model are estimated as b0: 0.272, b1: 1.124, and b2: 0.338 for growth rate. According to the statistical parameters; F (2, 6) = 456.502; p(F) = <0.001; R^2 = 0.985 for growth rate the EC_{10} EC_{20} and EC_{50} calculations should be considered valid.
After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.605 for yield and 0.799 for growth rate.
The 7-day EC_{10}, EC_{20} and EC_{50} values obtained from the analysis of the effect of Glyphosate acid on the parameters being analysed of *Lemna gibba* are presented in the table below.

Table 8.2.7-27: 7-day EC_x values for Yield and Growth Rate

7-day endpoints	Concentration of glyphosate acid [mg/L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (frond number)	6.0	10.5 (6.76-13.4)	14.2 (10.5-17.1)	24.0 (20.6-27.5)
Growth rate	12.0	13.3 (10.6-16.7)	18.7 (15.1-23.3)	36.0 (27.5-46.8)

CI: confidence interval

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid.

III. CONCLUSION

Assessment and conclusion by applicant:
The 7-day EC_{10}, EC_{20} and EC_{50} values are calculated for yield (frond number) and growth rate (frond number) based on the nominal concentration of glyphosate acid.

Based on the mean measured concentration of glyphosate acid the endpoints for 7-day EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 10.5, 14.2 and 24.0 mg a.e./L for yield (frond number), respectively; 13.3, 18.7 and 36.0 mg a.e./L for growth rate (frond number), respectively.

The statistical parameters presented above showed that these values can be considered reliable and therefore considered for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.7/007
Report author	
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Lemna gibba*
Report No	1092-02-1100-5
Document No	
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from guideline OECD 221 (2006): Minor: - The study was performed for 14 days instead of 7. - Dry weights are not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of glyphosate technical on growth of *Lemna gibba* were evaluated in a 14 day static toxicity test. The definitive test was performed with five concentration levels, encompassing 5, 9, 16, 28 and 50 mg glyphosate/L (measured: 4.28, 9.02, 16.6, 29.0 and 49.4 mg glyphosate/L) in triplicates. Furthermore, a

control group with *Lemna gibba* exposed to test medium, without test substance (negative control) was tested.

Three 4-frond colonies and one 3-frond colony, taken from 7-day old stock cultures were aseptically added to 200 mL test medium for a total of 15 fronds per vessel. The pH of the test medium was adjusted prior to the test. Frond counts were made on day 0, 2, 4, 7, 9, 11 and 14 after test initiation. Every frond visibly projecting beyond the edge of the parent frond was counted. The temperature was measured daily and the pH was adjusted to 7.5 ±0.1 at test initiation.

As results, the effects of the test item on frond growth inhibition on day 14, relative to the control, ranged from 14.2 % for the measured test concentration of 16.6 mg glyphosate/L to 85.6% for the highest measured test concentration of 49.4 mg glyphosate/L. At or below the measured test concentration of 9.02 mg glyphosate/L, no inhibition effects of the test item on frond's development were observed. All validity criteria according to the OECD guideline 221 were fulfilled.

Analytical recovery of the test item ranged from 99 to 104% on day 0 and from 71 to 104% on day 14. Therefore, calculated endpoints will be based on geometric mean measured concentrations. Statistical re-analysis of endpoints has been performed. The calculated EC_{10}, EC_{20} and EC_{50} values are 18.2, 20.3, and 25.0 mg a.e./L, respectively for yield (frond number) and 20.8, 31.9, and 66.2 mg a.e./L for growth rate (frond number).
The statistical parameters presented showed that these values can be considered reliable and therefore considered for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6 %
Water solubility	1.2 % at 25 °C
2. Vehicle and/or positive control:	none
3. Test organism:	
Species:	*Lemna gibba* G3
Source:	In-house culture
4. Environmental conditions:	
Temperature:	25 ± 2 °C
Photoperiod:	24 h florescence light
Light intensity	4198 - 5813 Lux
pH:	7.5 ± 0.1
Conductivity:	Not stated
Hardness:	Not stated
5. Dates of experimental works:	March 30th to April 13th 1987

B. STUDY DESIGN AND METHODS

1. Experimental treatments: On the basis of the results of a range finding test, the definitive test was performed with five concentration levels, 5, 9, 16, 28 and 50 mg glyphosate/L (prepared using 20X-AAP

medium), with 3 replicates per test concentration. Furthermore, a control group with *Lemna gibba* exposed to test medium (without test substance) was tested in three replicates under the same conditions as the test groups. Three 4-frond colonies and one 3-frond colony, taken from 7-day old stock cultures were aseptically added to each test vessel, for a total of 15 fronds per vessel. The plants were placed in 1000 mL test vessels, which already contained the 200 mL test media. The pH of the test medium was adjusted prior to the test. The test was conducted under static conditions.

2. Observations: Frond counts were made on day 0, 2, 4, 7, 9, 11 and 14 after test initiation. In order to eliminate subjective decisions on frond maturity, every frond visibly projecting beyond the edge of the parent frond was counted. Fronds were not removed from the test vessels for counting. For each nominal test concentration, the mean measured value on day 0 and day 14 was calculated, based on mean measured test concentrations. Mean frond count values at test termination for each test concentration were expressed as a percent relative to that in the control. On the basis of the mean frond count values, the percentage inhibition was determined and the EC_x values calculated by inverse estimation least squares linear regression. The temperature was measured daily and the pH was adjusted to 7.5 ±0.1 at test initiation. Samples of test media were made at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analyzed for active substance using HPLC.

3. Statistical calculations: To determine the EC_x values, the log of measured test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50 percent inhibition and the associated 95 % confidence limits were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} value is given below based on mean measured concentrations.

Table 8.2.7-28: Toxicity of glyphosate technical to *Lemna gibba*

Endpoint	mg glyphosate/L
EC_{25} (14 day)	18.0
EC_{50} (14 day)	25.5

Analytical data: Chemical analyses were performed on samples of the test solutions to quantify glyphosate in the test solution. The mean measured concentrations were 4.28, 9.02, 16.6, 29.0 and 49.4 mg glyphosate/L, corresponding to 85.6 %, 100.2 %, 103.8 %, 103.6 % and 98.8 % of the nominal test concentrations, respectively. The mean measured content of the test item always ranged between 80 and 120 % of nominal. Nevertheless, the ecotoxicological endpoints were evaluated using mean measured concentrations of the test item.

Table 8.2.7-29: Analytical results

Parameter	Nominal concentration of glyphosate [mg/L]					
	0	5	9	16	28	50

	Measured concentration of glyphosate [mg/L]					
Day 0 Concentration	< 0.05	5.01	9.35	16.8	28.8	49.5
Day 14 Concentration	< 0.05	3.54	8.69	16.5	29.1	49.4
Mean measured [mg/L]	< 0.05	4.28	9.02	16.6	29.0	49.4
% of nominal	-	85.6	100.2	103.7	103.6	98.8

Table 8.2.7-30: Frond counts during assay

Mean Measured Concentration, mg/L[1]		Day 2 4-1-87	Day 4 4-3-87	Day 7 4-6-87	Day 9 4-8-87	Day 11 4-10-87	Day 14 4-13-87
<0.05	A	32	61	170	309	425	656
(0)	B	31	62	172	267	485	689
	C	30	71	164	282	416	649
	Mean	31	65	169	286	442	665
	SD[2]	1.00E+00	5.51E+00	4.16E+00	2.13E+01	3.75E+01	2.14E+01
	Var[3]	1.00E+00	3.03E+01	1.73E+01	4.53E+02	1.41E+03	4.56E+02
4.28	A	31	66	195	333	521	681
(5)	B	29	65	173	268	499	655
	C	28	61	176	331	631	693
	Mean	29	64	181	311	550	676
	SD	1.53E+00	2.65E+00	1.19E+01	3.70E+01	7.07E+01	1.94E+01
	Var	2.33E+00	7.00E+00	1.42E+02	1.37E+03	5.00E+03	3.77E+02
9.02	A	27	57	192	299	525	728
(9)	B	33	73	187	375	569	688
	C	25	54	168	301	513	648
	Mean	28	61	182	325	536	688
	SD	4.16E+00	1.02E+01	1.27E+01	4.33E+01	2.95E+01	4.00E+01
	Var	1.73E+01	1.04E+02	1.60E+02	1.88E+03	8.69E+02	1.60E+03
16.6	A	27	60	167	298	450	549
(16)	B	31	61	173	271	479	586
	C	27	62	177	255	510	582
	Mean	28	61	172	275	480	572
	SD	2.31E+00	1.00E+00	5.03E+00	2.17E+01	3.00E+01	2.03E+01
	Var	5.33E+00	1.00E+00	2.53E+01	4.72E+02	9.00E+02	4.12E+02
29.0	A	25	48	107	143	175	170
(28)	B	26	44	98	145	175	165
	C	23	46	110	153	186	189
	Mean	25	46	105	147	179	175
	SD	1.53E+00	2.00E+00	6.24E+00	5.29E+00	6.35E+00	1.27E+01
	Var	2.33E+00	4.00E+00	3.90E+01	2.80E+01	4.03E+01	1.60E+02
49.4	A	21	36	83	113	115	110
(50)	B	24	40	82	118	125	108
	C	24	33	66	100	100	107
	Mean	23	36	77	110	113	108
	SD	1.73E+00	3.51E+00	9.54E+00	9.29E+00	1.26E+01	1.53E+00
	Var	3.00E+00	1.23E+01	9.10E+01	8.63E+01	1.58E+02	2.33E+00

B. OBSERVATIONS

The effects of the test item on frond growth inhibition on day 14, relative to the control, ranged from 14.2 % for the measured test concentration of 16.6 mg glyphosate/L to 85.6 % for the highest measured test concentration of 49.4 mg glyphosate/L. At or below the measured test concentration of 9.02 mg glyphosate/L, no inhibition effects of the test item on frond's development were observed.

Table 8.2.7-31: Percentage growth inhibition of *Lemna gibba* exposed to glyphosate for 14 days

Nominal concentrations [mg glyphosate/L]	**Control**	**5**	**9**	**16**	**28**	**50**
Measured concentrations [mg glyphosate/L]	**-**	**4.28**	**9.02**	**16.6**	**29.0**	**49.4**
Mean number of fronds on Day 7	169	181	182	172	105	77
Mean number of fronds on Day 14	665	676	688	572	175	108
Mean inhibition (14 days) [%]	-	-1.8	-3.6	14.2	75.4	85.6

The doubling time of frond number in the control was less than 2.5 days (2.1 fold in 2 days in the test), and the frond number in the control was more than seven-fold after seven days (approx. 11.3 folds in 7 days in the test). The validity criteria according to guideline OECD 221 are therefore fulfilled.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The 14-day EC_{50} for *Lemna gibba* exposed to glyphosate technical was calculated to be 25.5 mg/L.

Statistical re-analysis of endpoints has been performed to comply with Commission Regulation (EU) 283/2013 to determine 7-day EC_{10}, EC_{20} and EC_{50} endpoints.

The percent recovery nominal test concentrations are presented below.

Table 8.2.7-32: Analytical verification of test item

Parameter	**Nominal concentration of glyphosate [mg/L]**					
	0	5	9	16	28	50
	Measured concentration of glyphosate [mg/L]					
Day 0 Concentration	< 0.05	5.01	9.35	16.8	28.8	49.5
Day 0 % of nominal	**-**	**100**	**104**	**105**	**103**	**99**
Day 14 Concentration	< 0.05	3.54	8.69	16.5	29.1	49.4
Day 14 % of nominal	**-**	**71**	**97**	**103**	**104**	**99**
Geometric mean [mg/L]	**-**	**4.2**	**9.0**	**16.6**	**28.9**	**49.4**

Analytical recovery of the test item ranged from 99 to 104% on day 0 and from 71 to 104% on day 14. Therefore, calculated endpoints will be based on geometric mean measured concentrations.

Details of statistical re-evaluation are given in the position paper CA 8.2.7/008
The 7 day ECx values for yield and growth rate based on frond numbers has been calculated based on the geometric mean concentrations and are provided in the table below:

Table 8.2.7-33: 7-d ECx values for Yield and Growth Rate

7-day endpoints	**Geometric mean concentration of glyphosate acid [mg/L]**			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield Frond number	16.6	18.2 (15.3 – 21.5)	20.3 (17.3 – 23.7)	25.0 (20.7 – 30.2)

Growth rate	16.6	20.8 (10.9 – 28.9)	31.9 (21.0 – 40.4)	66.2 (55.0 – 77.7)

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

Information on the study

Data point	CA 8.2.7/008
Report author	
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 1092-02-1100-5 on the toxicity of Glyphosate to *Lemna gibba* under static conditions
Report No	110054-011
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary

A statistical evaluation addressing the calculation of valid 7-day EC_{10}, EC_{20}, EC_{50}, and NOEC values was conducted for the study 1092-02-1100-5 (1987) to fulfil the data requirements according to regulation EU 283/2013. Futhermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 221 (2006).

Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 221 (2006) were met, this study is considered valid for risk assessment purposes. The calculated EC_{10}, EC_{20} and EC_{50} values are 18.2, 20.3, and 25.0 mg a.e./L, respectively for yield (frond number) and 20.8, 31.9, and 66.2 mg a.e./L for growth rate (frond number).

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details
Study number: 1092-02-1100-5

Author:	
Substance:	Glyphosate
Title:	The Toxicity of Glyphosate Technical to *Lemna gibba*
Completion date:	13-Apr-1987
Test guideline(s):	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
GLP:	Yes
Testing facility:	Malcolm Pirnie, Inc, White Plains, NY 10602, USA
Sponsor:	Monsanto Agricultural Company, Chesterfield, MO 63198, USA

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was evaluated according to the current test guideline OECD 221 (2006) and 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 1092-02-1100-5 (1987) was statistically evaluated for the effects of Glyphosate on the organism *Lemna gibba* G3 as the report only provides 14-day endpoints. According to current test guidelines and EFSA Aquatic Guidance (2013), this study type requires a 7-day endpoint.

The organisms were exposed for 14 days to the following concentrations of Glyphosate: 5, 9, 16, 28 and 50 mg glyphosate/L (mean measured: 4.28, 9.02, 16.6, 29.0 and 49.4 mg glyphosate/L). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 7-day Effect Concentrations that have 10, 20 and 50% effects on growth rate and yield of the test subjects (EC10, EC20 and EC50), a Probit analysis using linear maximum likelihood regression for yield (frond number) and a non-linear regression analysis of 3-parameter normal CDF (Cumulative Distribution Function) for growth rate (frond number) was performed. For determination of the no-observed-effect concentration, Williams Multiple Sequential t-test Procedure was used (one-sided smaller; p=0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

Endpoints based on biomass cannot be determined, as no data for day 7 is available.

II. RESULTS AND DISCUSSION

A. FINDINGS

Results

The doubling time of frond number in the control was less than 2.5 days (2.1 fold in 2 days in the test), and the frond number in the control was more than seven-fold after 7 days (approx. 11.3 folds in 7 days in the test). The validity criteria according to guideline OECD 221 are therefore fulfilled.

The percent recovery nominal test concentrations are presented below.

Table 8.2.7-34: Analytical verification of test item

Parameter	Nominal concentration of glyphosate [mg a.e./L]					
	0	5	9	16	28	50
	Measured concentration of glyphosate [mg a.e./L]					
Day 0 Concentration	< 0.05	5.01	9.35	16.8	28.8	49.5
Day 0 % of nominal	**-**	**100**	**104**	**105**	**103**	**99**
Day 14 Concentration	< 0.05	3.54	8.69	16.5	29.1	49.4
Day 14 % of nominal	**-**	**71**	**97**	**103**	**104**	**99**
Geometric mean [mg/L]	**-**	**4.2**	**9.0**	**16.6**	**28.9**	**49.4**

Analytical recovery of the test item ranged from 99 to 104% on day 0 and from 71 to 104% on day 14. Therefore, calculated endpoints will be based on geometric mean measured concentrations.

The parameters for the 4 parameter normal CDF model are b0: 162.4, b1: 1.259, b2: 0.109, b3: 61.678 for yield. According to the statistical parameters $F(3,2) = 108.669$; $p(F) < 0.001$; $R^2 = 0.950$ the EC_{10}, EC_{20} and EC_{50} calculations for yield (frond number) should be considered valid.
For growth rate, the parameters for the 3 parametric logistic CDF model are estimated as b0: 0.357, b1: 66.209, and b2: 1.895. According to the statistical parameters $F(3,2) = 79.795$; $p(F) < 0.001$; $R^2 = 0.919$ the EC_{10}, EC_{20} and EC_{50} calculations for growth rate (frond number) should be considered valid. After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.004 for growth rate (frond number).

The obtained EC_{10}, EC_{20} and EC_{50} values are presented in the table below. The dose response curve obtained from the analysis of the effect of Glyphosate on yield (frond number) being analysed of *Lemna gibba* G3 is presented below.

Table 8.2.7-35: 7-day ECx values for Yield and Growth Rate

7-day endpoints	Geometric mean concentration of glyphosate acid [mg/L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (frond number)	16.6	18.2 (15.3 – 21.5)	20.3 (17.3 – 23.7)	25.0 (20.7 – 30.2)
Growth rate	16.6	20.8 (10.9 – 28.9)	31.9 (21.0 – 40.4)	66.2 (55.0 – 77.7)

CI: confidence interval

III. CONCLUSION

Assessment and conclusion by applicant:
The calculated EC_{10}, EC_{20} and EC_{50} values are 18.2, 20.3, and 25.0 mg a.e./L, respectively for yield (frond number) and 20.8, 31.9, and 66.2 mg a.e./L for growth rate (frond number).

The statistical parameters presented showed that these values can be considered reliable and therefore considered for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.2.7/009
Report author	
Report year	1987
Report title	The toxicity of glyphosate technical to *Lemna gibba*.
Report No	XX-88-416
Document No	-
Guidelines followed in study	No information mentioned in the Monograph 2001.
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations	Toxicity of technical glyphosate (purity >94 %) to aquatic plants (*Lemna gibba*).
Short description of results	No information mentioned in the Monograph 2001.
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available and no information mentioned in the Monograph 2001, so these data were considered as not acceptable in the Monograph 2001.
Reasons why the study report is not available for submission	The notifier does not have access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 8.2.7/010
Report author	
Report year	2012
Report title	Effect of MON77973 (Glyphosate acid) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment. Test with a subsequent Recovery Period.
Report No	CHE-015/4-80/A
Document No	-
Guidelines followed in study	(2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP
Deviations from current test guideline	Deviations from guideline: none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The toxicity of Glyphosate acid on growth of *Myriophyllum aquaticum* was evaluated in a 14 day static toxicity test, with subsequent 7 day recovery test, performed at concentrations of 5.0, 15.8, 50, 158 and 500 mg glyphosate/L, equivalent to 5.87, 18.5, 58.7, 185.4 and 587 mg glyphosate acid/L. A negative control (Smart & Bako medium) was prepared in parallel.

Two sets of vessels (exposure and recovery set) were prepared, with each set comprising three replicates for each test concentration and six replicates for the controls. Test vessels were 2-L beakers, each containing five individual plants potted in individual pots containing artificial sediment. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days and after 21 days (recovery vessels). At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants. At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. After 14 days, all plants in recovery vessels were transferred to vessels containing dilution water only to assess recovery following exposure.

Test media were analysed for Glyphosate acid content at test start and end of exposure and recovery periods. The measured concentrations ranged from 92.0 – 100.6% of nominal. Glyphosate acid was not detected in the control group.

Relative to the control group, at the highest treatment rate (500 mg glyphosate acid/L) there was 100 % growth inhibition based on fresh weight. At 500 mg Glyphosate acid/L fresh weight increase was inhibited by 100 %, shoot length increase by 70.8 % and growth rate by 57.1 %. The recovery period demonstrated that *Myriophyllum aquaticum* pre-exposed to up to 50.0 mg Glyphosate acid/L were able to recover to control levels of growth, in untreated culture medium within 7 days of transfer.

The study fulfilled the validity criteria of achieving at least 50% increase in control plant growth in terms of length within 7 days of test initiation. The test was therefore considered to be valid.

Glyphosate acid significantly inhibited the fresh weight of *Myriophyllum aquaticum* after 14 days at a nominal concentration of <5.0 mg glyphosate acid/L. Shoot length was inhibited at or above nominal concentrations of 5.0 mg glyphosate acid/L. The 14-d EC_{50} value for fresh weight inhibition was 12.3 mg glyphosate acid/L and for shoot length it was 78.7 mg glyphosate acid/L. *Myriophyllum aquaticum* pre-exposed for 14 days to up to 50.0 mg glyphosate acid/L were able to recover in untreated culture medium after a 7 day recovery period.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid (MON77973)
Description:	White crystalline powder
Lot/Batch #:	GLP-0807-19475-T
Purity:	85.2% Glyphosate
2. Vehicle and/or positive control:	Positive control: none
3. Test organism:	
Species:	*Myriophyllum aquaticum*
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, D-35315 Homberg (Ohm), Germany
4. Environmental conditions:	
Growth medium:	Smart & Bako medium
Artificial sediment:	4-5% peat 20% kaolin clay 75-76% quartz sand $CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5) Based on artificial soil used in OECD guideline 219 Moistening of sediment up to 30% with deionised water or nutrient medium (ammonium chloride and sodium phosphate)
Temperature:	18.0-20.5 °C
Photoperiod:	16 h light/ 8 h dark
Light intensity	6541-7097 lux
pH:	<u>Values recorded at test start and end (in brackets) of 14 day exposure period</u>: Controls = 7.99 (8.14-9.06) 5 mg/L = 8.06 (8.77-10.0) 15.8 mg/L: = 7.99 (8.96-9.96) 50.0 mg/L = 7.36 (7.35-9.13) 158 mg/L = 3.84 (4.88-5.28) 500 mg/L = 2.80 (3.29-3.43) <u>Values at start and end of 7 day recovery period:</u> Recovery period start = 7.95 Recovery period end = 8.17 – 9.48
Oxygen saturation	<u>14 day exposure period:</u> 92 – 94% at the start of the test 114 – 193% at the end of the test <u>7 day recovery period:</u> 96% at the start of the test 95 – 131% at the end of the test
5. Dates of experimental work:	Sept 27th to Oct 11th 2010

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test on *Myriophyllum aquaticum* was performed with six concentration levels of 5.0, 15.8, 50, 158 and 500 mg glyphosate/L, equivalent to 5.87, 18.5, 58.7, 185.4 and 587 mg Glyphosate acid/L, with 3 replicates per test concentration. Six control replicates (without test substance) were tested under the same conditions as the test groups. Two sets of vessels (exposure and recovery) were prepared at the start of the test
The plants were planted in small plastic plant pots into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate.
After 14 days exposure plants in the recovery set of *Myriophyllum aquaticum* replicates, exposed to the same concentration levels, were transferred into freshly prepared test medium without test item to determine the potential recovery after an exposure event.

2. Observations: Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was recorded at test start and after 14 days.
Analytical control measurements of the actual concentration of the glyphosate acid were performed by means of LC/MS-MS analysis at test start, after 14 (after exposure phase) and 21 days (after recovery phase).
3. Statistical calculations: The EC_{10}, EC_{20} and EC_{50} and its 95 % confidence interval were calculated by Probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Williams' t-test, Dunnett's t-test or Welch's t-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements of the actual concentration of the glyphosate acid were performed at test start and after 14 days. The measured concentrations ranged from 92.0 – 100.6 % of nominal. As the mean measured content of the test item always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.
Additional analytical measurements were made at the end of the 7 d recovery period (at day 21). The measured concentration in the test media were < LOQ at the lowest test concentrations and between 1.7 and 2.1 % of the test media concentrations at the end of the growth test.

Table 8.2.7-36: Analytical results

Nominal [mg/L]	Test start 14 d growth test		End of test 14 d growth test		End of 7 d recovery test	
	Measured [mg/L]	% of nominal	Measured [mg/L]	% of nominal	Measured [mg/L]	% of nominal
Control	< LOQ -	-	<LOQ	-	< LOQ	-
5.0	4.95	99.1	4.86	97.2	< LOQ	-
15.8	15.4	97.4	14.5	92.0	0.32	2.1
50.0	49.8	99.6	49.6	99.3	1.03	2.1
158	149	94.3	157	99.2	2.73	1.7
500	488	97.6	503	100.6	8.70	1.7
Pore water 500 mg/L	-	-	95.1	19.0	28.8	5.8

LOQ= 0.25 mg/L

The EC_{50}, EC_{20} and NOEC values after 14 day growth inhibition test are given below based on nominal concentrations.

Table 8.2.7-37: 14-day endpoints

Endpoint	Glyphosate acid [mg/L]			
	14 Day EC_{10}	14 Day EC_{20}	14 Day EC_{50}	14 Day NOEC
Shoot length/relative increase	n.d.	4.05 (0.82 - 9.35) [1]	78.7 (46.1 - 146)	5.0
Shoot length/growth rate	2.40[1] (0.31-6.76)	12.1 (3.55-24.2)	276 (159 - 664)	5.0
Fresh weight/relative increase	n.d.	1.72 (0.88 - 2.75) [1]	12.3 (9.19 - 15.8)	<5.0
Fresh weight/ growth rate	n.d.	3.60 (1.85 - 5.69) [1]	23.4 (17.2 - 30.9)	<5.0
Dry weight/relative increase	3.06[1] (0-10.7)	6.31 (0 - 17.6)	25.2 (2.61 - 151)	50.0
Dry weight/ growth rate	3.68[1] (0-12.8)	7.58 (0 - 21.1)	30.2 mg/L (3.54-191)	50.0
Root length/relative increase	n.d.	3.26 [1]	18.0 (5.19 - 43.0)	<5.0
Root length/growth rate	n.d.	n.d.	>500	<5.0

CI = 95% confidence interval
[1] extrapolated, lowest test concentration was 5.0 mg/L.
n.d. not determined

The EC_{50}, EC_{20} and NOEC values after 7 day recovery period are given below based on nominal concentrations.

Table 8.2.7-38: 7-day endpoints

Endpoint	Glyphosate acid [mg/L]			
	7 Day EC_{10}	7 Day EC_{20}	7 Day EC_{50}	7 Day NOEC
Shoot length/relative increase	26.0 (14.0-37.1)	41.2 (26.5-54.2)	99.5 (79.7-125)	50
Shoot length/growth rate	29.5 (14.6-43.3)	46.9 (28.5-63.0)	114 (89.5-147)	50
Fresh weight/relative increase	n.d.	n.d	n.d.	158
Fresh weight/ growth rate	n.d.	n.d	n.d.	158
Dry weight/relative increase	n.d.	n.d	n.d.	≥500
Dry weight/ growth rate	n.d.	n.d	n.d.	≥500
Root length/relative increase	>500	>500	>500	≥500
Root length/growth rate	>500	>500	>500	≥500

n.d.: not determined due to mathematical reasons or inappropriate data

B. OBSERVATIONS

There was a concentration dependent effect on growth, root length, fresh and dry weight of *Myriophyllum aquaticum.* Growth was significantly reduced at 5.00 mg glyphosate/L, fresh weight at <50 mg Glyphosate acid/L, dry weight at 50.0 mg Glyphosate acid/L and root length at <50 mg Glyphosate acid/L during the 14 day exposure test. In the subsequent recovery test; it was shown that *Myriophyllum aquaticum*, pre-exposed to up to 50.0 mg Glyphosate acid/L were able to recover to control levels of growth in untreated culture medium within 7 days of the exposure period.

Table 8.2.7-39: Percentage of inhibition of shoot length of *Myriophyllum aquaticum* exposed for 14 days to glyphosate acid

Test parameters	Glyphosate acid [mg/L]				
	5.0	**15.8**	**50.0**	**158**	**500**
Inhibition of shoot length increase (%)	19.2	29.9	55.9	50.3	70.8
Inhibition of shoot length growth rate (%)	11.8	19.5	41.9	36.7	57.9
Inhibition of fresh weight increase (%)	34.2	57.5	69.2	83.7	109
Inhibition of fresh weight growth rate (%)	24.6	46.5	59.0	76.7	115
Inhibition of dry weight increase (%)	-11.8	46.5	26.8	92.7	108
Inhibition of dry weight growth rate (%)	-10.2	40.8	40.4	92.4	114
Inhibition of root length increase (%)	19.4	52.3	76.0	79.7	88.8
Inhibition of root length growth rate (%)	2.0	7.0	13.5	15.1	21.1

The study fulfils the validity criteria as stated in the study plan which follows the criteria established by the AMRAP working group, with an increase of biomass (shoot length) in controls was > 50 %, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The mean measured content of the test item always ranged between 80 and 120% of nominal so the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.
The 14-d ErC50 value for fresh weight was 23.4 mg a.e./L and for shoot length it was 276 mg a.e./L.
The study is considered valid and reliable for risk assessment purposes.

Endpoint in glyphosate acid	14 Day EC_{50} [mg/L]	14 Day NOEC [mg/L]
Shoot length/relative increase	78.7	5.0
Shoot length/growth rate	276	5.0
Fresh weight/relative increase	12.3	<5.0
Fresh weight/ growth rate	**23.4**	<5.0
Dry weight/relative increase	25.2	50.0
Dry weight/ growth rate	30.2	50.0
Root length/relative increase	18.0	<5.0
Root length/growth rate	>500	<5.0

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.7/011
Report author	
Report year	2012
Report title	Effect of AMPA (Aminomethylphosphonic acid) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment, with a subsequent Recovery Period
Report No	CHE-022/4-80/A
Document No	-
Guidelines followed in study	Maltby, L., et al. (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP
Deviations from current test guideline	Deviations from guideline: none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The toxicity of Glyphosate acid on growth of *Myriophyllum aquaticum* was evaluated in a 14 day static toxicity test, with subsequent 7 day recovery test, performed at concentrations of 1.0, 2.6, 6.4, 16, 40 and 100 mg AMPA/L. A negative control (Smart & Bako medium) was prepared in parallel.

Two sets of vessels (exposure and recovery set) were prepared, with each set comprising three replicates for each test concentration and six replicates for controls were used. Test vessels were 2-L beakers, each containing five individual plants potted in individual pots containing artificial sediment. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days and after 21 days (recovery vessels). At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants. At the end of the test all plants were harvested, and the root length was assessed semi-quantitatively in terms of length of the main root. After 14 days, all plants in recovery vessels were transferred to vessels containing dilution water only to assess recovery following exposure.

Test media were analysed for AMPA content at test start, test end and at the end of the recovery period. The measured concentrations ranged from 75.5 - 102% of nominal. AMPA was not detected in the control group. Therefore, the test was evaluated using the geometric mean measured concentrations.

Result showed a significant inhibition of fresh weight and shoot length at the lowest test concentration of >14.3 mg AMPA/L. The following recovery test demonstrated that *Myriophyllum aquaticum* pre-exposed to up to 5.4 mg AMPA/L were able to recover in untreated culture medium after a 7 day recovery period.

The study fulfilled the validity criteria of achieving at least 50% increase in control plant growth in terms of length within 7 days of test initiation. The test was therefore considered to be valid.

AMPA significantly inhibited the fresh weight and shoot length of *Myriophyllum aquaticum* after 14 days at a nominal concentration of >14.3 mg AMPA/L. The 14-d EC_{50} value for fresh weight inhibition was 70.8 mg AMPA/L and for shoot length > 94.6 mg AMPA/L. *Myriophyllum aquaticum* pre-exposed for 14 day to up to 5.4 mg AMPA/L were able to recover in untreated culture medium after a 7 day recovery period.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	White crystalline solids
Lot/Batch #:	GLP-0905-19864A (recertified as GLP-110521446-A)
Purity:	98.5 %
2. Vehicle and/or positive control:	Positive control: none
3. Test organism:	
Species:	*Myriophyllum aquaticum*
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, D-35315 Homberg (Ohm), Germany
4. Environmental conditions:	
Growth medium:	Smart & Bako medium
Artificial sediment:	4-5 % peat 20 % kaolin clay 75-76 % quartz sand $CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5) Based on artificial soil used in OECD guideline 219 Moistening of sediment up to 30 % with deionised water or nutrient medium (ammonium chloride and sodium phosphate)
Temperature:	20.5 – 21.0 °C
Photoperiod:	16 h light/ 8 h dark
Light intensity	7571 - 7903 lux
pH:	<u>Values recorded at test start and end (in brackets) of 14 day exposure period</u>: Controls = 7.91 (8.54–8.91) 0.88 mg/L = 8.06 (8.04-8.08) 2.23 mg/L: = 7.99 (8.05-8.11) 5.43 mg/L = 7.36 (8.05-8.07) 14.3 mg/L = 3.84 (7.90-7.99) 37.1 mg/L = 2.80 (7.75-7.79) 94.6 mg/l = 6.60 (7.23-7.33) <u>Values at start and end of 7 day recovery period:</u> Recovery period start = 7.97-9.04 Recovery period end = 8.18 – 9.28
Oxygen saturation	<u>14 day exposure period:</u> 95 – 97 % at the start of the test 101 – 138 % at the end of the test <u>7 day recovery period:</u> 96 – 138 % at the start of the test 90 – 114 % at the end of the test
5. Dates of experimental work:	Aug 18th to Sept 8th 2011

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test on *Myriophyllum aquaticum* was performed with six concentration levels of 1.0, 2.6, 6.4, 16, 40 and 100 mg AMPA/L with 3 replicates per test concentration. Six control replicates (without test substance) were tested under the same conditions as the test groups.
The plants were planted in small plastic plant pots into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate. After 14 days exposure another set of *Myriophyllum aquaticum* replicates, exposed to the same concentration levels, was transferred into freshly prepared test medium without test item to determine the potential recovery after an exposure event.

2. Observations: Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 5, 8 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was at test start and after 14 days.
Analytical control measurements of the actual concentration of AMPA were performed by means of LC/MS-MS analysis at test start, after 14 and 21 days (after recovery phase).

3. Statistical calculations: The EC_{10}, EC_{20} and EC_{50} and its 95 % confidence interval were calculated by Probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Dunnett's t-test or Welch's t-test (p = 0.05).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements of the actual concentration of AMPA were performed at test start, after 14 and 21 days (after recovery phase). The measured concentrations ranged from 75.5 – 102 % of nominal. Therefore the test was evaluated using the geometric mean measured concentrations.
Measured concentrations of AMPA in the macrophyte growth inhibition test are depicted below.

Table 8.2.7-40: Analytical results

Nominal [mg/L]	Test start 14 d growth test		End of test 14 d growth test		Mean measured [mg/L]
	Measured [mg/L]	% of nominal	Measured [mg/L]	% of nominal	
Control	< LOQ	-	<LOQ	-	< LOQ
1.0	1.02	101.7	0.76	76.4	0.88
2.6	2.49	95.8	1.99	76.6	2.23
6.4	6.09	95.2	4.85	75.7	5.43
16	15.5	96.6	13.2	82.2	14.26
40	40.0	100.0	34.4	86.1	37.13
100	98.3	98.3	91.1	91.1	94.61

LOQ = limit of quantification = 0.5 mg/L

The EC_{50} and NOEC values after 14-day growth inhibition test are given below based on geometric mean measured concentrations.

Table 8.2.7-41: 14-day endpoints

Endpoint	AMPA [mg/L][2]			
	14 Day EC_{10}	14 Day EC_{20}	14 Day EC_{50}	14 Day NOEC
Shoot length/relative increase	1.3 (0.2-3.2)	5.8 (2.1-10.4)	103.3[1] (54.8-337)	14.3
Shoot length/growth rate	6.1 (2.2-10.6)	22.5 (13.7-33.1)	> 94.6	14.3
Fresh weight/relative increase	19.7 (11.3-26.9)	30.6 (21.0-38.3)	**70.8** (59.4-87.7)	14.3
Fresh weight/ growth rate	24.2 (14.5-32.2)	39.0 (28.4-47.5)	97.3 (81.8-126)	14.3
Dry weight/relative increase	33.9 (17.7-44.9)	42.0 (25.7-53.2)	63.2 (49.0-79.2)	37.1
Dry weight/ growth rate	38.4 (22.2-49.1)	47.6 (31.6-58.1)	72.0 (59.4-83.6)	37.1
Root length/relative increase	5.1 (4.0-6.2)	9.5 (7.9-11.0)	31.1 (28.1-34.6)	5.4
Root length/growth rate	17.0 (14.9-19.0)	35.9 (33.2-38.5)	150.1[1] (136.1-168.1)	5.4

[1] extrapolated, highest test concentration was 94.6 mg AMPA/L
[2] 95% confidence intervals presented in brackets.

The EC_{50} and NOEC values after 7 day recovery period are given below based on geometric mean measured concentrations.

Table 8.2.7-42: 7-day recovery endpoints

Endpoint	AMPA [mg/L][1]			
	7 Day EC_{10}	7 Day EC_{20}	7 Day EC_{50}	7 Day NOEC
Shoot length/relative increase	5.4 (0-15.7)	13.5 (0.1-31.1)	78.2 (34.2-6082.1)	37.1
Shoot length/growth rate	6.4 (0-17.6)	16.0 (0.2-35.3)	92.8 (41.9-8310.6)	37.1
Fresh weight/relative increase	1.4 (0-4.8)	3.0 (0-8.1)	12.6 (2.5-79.7)	5.4
Fresh weight/ growth rate	1.5 (0-5.1)	3.2 (0-8.7)	13.6 (2.8-87.3)	5.4
Dry weight/relative increase	n.d.	n.d.	≥ n.d.	≥ 94.6
Dry weight/ growth rate	n.d.	n.d.	n.d.	≥ 94.6
Root length/relative increase	n.d.	n.d.	≥ n.d.	≥ 94.6
Root length/growth rate	n.d.	n.d.	n.d.	≥ 94.6

[1] 95% confidence intervals presented in brackets.
n.d.: not determined due to mathematical reasons or inappropriate data

B. OBSERVATIONS

There was a concentration dependent effect on growth, fresh and dry weight of *Myriophyllum aquaticum.* Growth and fresh weight was significantly reduced at >14.3 mg AMPA/L. In the subsequent recovery test it was shown that *Myriophyllum aquaticum*, pre-exposed to up to 5.4 mg AMPA/L were able to recover in untreated culture medium after a 7 day recovery period.

Table 8.2.7-43: Percentage of inhibition of shoot length of *Myriophyllum aquaticum* exposed for 14 days to AMPA

Test parameters	AMPA [mg/L]					
	0.88	**2.23**	**5.43**	**14.26**	**37.13**	**94.61**
Inhibition of shoot length increase (%)	20.8	16.8	12.5	16.7	40.8	54.3
Inhibition of shoot length growth rate (%)	11.7	9.2	6.4	9.0	26.4	38.0
Inhibition of fresh weight increase (%)	-14.1	-15.2	-7.0	-10.9	29.0	60.2
Inhibition of fresh weight growth rate (%)	-9.0	-9.4	-3.9	-6.9	20.8	48.3
Inhibition of dry weight increase (%)	-47.5	-45.6	-7.1	1.1	-4.6	79.9
Inhibition of dry weight growth rate (%)	-28.9	-26.5	-4.9	1.6	-2.1	71.2
Inhibition of root length increase (%)	-13.1	-8.8	15.7	26.4	55.0	79.3
Inhibition of root length growth rate (%)	-3.5	-2.5	4.2	7.7	20.4	39.5

The study fulfils the validity criteria as stated in the study plan which follows the criteria established by the AMRAP working group; with an increase of biomass (shoot length) in controls was > 50 %, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The EC50 and NOEC values after 14-day growth inhibition test are given below based on geometric mean measured concentrations.

The 14-d ErC50 value for dry weight was 72.0 mg AMPA/L, fresh weight was 97.3 mg AMPA/L and for shoot length > 94.6 mg AMPA/L.

The study is considered valid so the following EC50 and NOEC can be used for risk assessment purposes:

Endpoint in AMPA	14 Day EC_{50} [mg/L]	14 Day NOEC [mg/L]
Shoot length/relative increase	103.3[1]	14.3
Shoot length/growth rate	> 94.6	14.3
Fresh weight/relative increase	70.8	14.3
Fresh weight/ growth rate	97.3	14.3
Dry weight/relative increase	63.2	37.1
Dry weight/ growth rate	**72.0**	37.1
Root length/relative increase	31.1	5.4
Root length/growth rate	150.1*	5.4

[1] extrapolated, highest test concentration was 94.6 mg AMPA/L

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.7/012
Report author	
Report year	2011
Report title	HMPA (hydroxymethylphosphonic acid): A 7-Day Static-Renewal Toxicity Test with Duckweed (*Lemna gibba* G3)
Report No	139A-397
Document No	-
Guidelines followed in study	OPPTS 850.4400, ASTM Standard Guide 1415-91 E (1991) OECD Guideline 221 (2006)
Deviations from current test guideline	Deviation from guideline OECD 221 (2006): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of HMPA (hydroxymethylphosphonic acid) on growth of *Lemna gibba* G3 were evaluated in a 7-day static-renewal toxicity test at nominal concentrations of 7.5, 15, 30, 60, and 120 mg HMPA/L, corresponding to mean measured concentrations of 7.4, 15, 30, 60 and 123 mg HMPA/L, respectively. A negative control was prepared in parallel. Three replicates were prepared per control and test item treatment using four plants (totalling 12 fronds) per replicate, each. The pH of the 20X AAP test medium was adjusted to 7.6 with 0.1 N NaOH. Renewal of the test media was performed on day 3 after test initiation. Direct counts of number of fronds were conducted on day 3, 5 and 7. Observations of chlorosis, necrosis, break-up of duckweed colonies, root destruction, death and any other abnormalities in plant or frond appearance were also performed at those times. Dry weight was determined at the beginning (representative sample) and at the end of the test (each vessel). EC_{50} values were calculated based on replicate frond counts, biomass and growth rates based on frond counts and biomass on day 7 of the test. Analysis of the test concentration was carried out at test initiation, on day 3 and at test termination on day 7. The mean measured content of the test item ranged between 99 and 103% of nominal concentrations. HMPA was not detected in the control group.

Percent inhibition of frond growth in the 7.4, 15, 30, 60 and 123 mg HMPA/L treatment groups at test termination was -9, -15, -1, -7 and -20 %, respectively. Percent inhibition of growth rate based on frond number in the 7.4, 15, 30, 60 and 123 mg HMPA/L treatment groups at test termination was -4, -6, -1, -4, and -8 %, respectively. Percent inhibition biomass in the 7.4, 15, 30, 60 and 123 mg HMPA/L treatment groups at test termination was -13, -25, -15, -20 and -33 %, respectively. Percent inhibition of growth rate based on biomass in the 7.4, 15, 30, 60 and 123 mg HMPA/L treatment groups at test termination was -5, -9, -6, -8 and -12 %, respectively.

Based on these results, the EC_{50} for frond number, biomass and growth rates based on frond number and biomass for HMPA was determined to be >123 mg HMPA/L. After 7 days of exposure, there were no apparent treatment-related effects upon growth at any of the concentrations tested. The validity criteria according to guideline OECD 221 are fulfilled.

Since no inhibition effects of HMPA were observed on frond number, frond number growth rate, biomass and biomass growth rate of *Lemna gibba* after 7 days at all concentrations tested, the EC_{50} values after 7 days of exposure were all >123 mg HMPA/L, the highest concentration tested. The NOEC was determined to be ≥123 mg HMPA/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Test item:	HMPA (hydroxymethylphosphonic acid)
Description:	Solid
Lot/Batch #:	GLP-1003-20448-A
Purity:	97.0%
2. Vehicle and/or positive control:	Positive control: none
3. Test organism:	
Species:	*Lemna gibba* G3, up to 7 days old
Source:	In-house culture
4. Environmental conditions:	
Temperature:	23.7 – 25.4 °C
Light intensity:	Continuous illumination, 4410 - 5250 lux
pH:	7.1 – 8.0 at test start; 8.8 – 9.0 at test termination
Hardness:	20.88 mg (K_2HPO_4/L)
5. Dates of experimental work	June 10th to June 19th 2010

B. STUDY DESIGN AND METHODS

1. Experimental treatments: On the basis of the results of a range finding test, the definitive test was performed at five concentration levels, 7.5, 15, 30, 60, and 120 mg HMPA/L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions. Four plants totalling 12 fronds were added to each replicate test chamber. The plants were placed in 250 mL test vessels containing 100 mL 20X-AAP test media. The pH of the test medium was adjusted with 0.1N NaOH prior to the test. The test was conducted under a 7-day static-renewal test conditions. The renewal of the test media was performed on day 3 after test initiation.

2. Observations:
Biological data: The toxicity of HMPA to duckweed was determined by direct counts of frond numbers and observations for chlorosis, necrosis, dead fronds and frond appearance were made on Days 3, 5 and 7. Dry weight was measured at the beginning of the test on a representative sample from the culture used to initiate the test. At the end of the test, dry weight was determined from each test vessel.
Physical data: The pH values were measured on day 0, 3, and 7. Temperature was measured continuously and recorded twice daily. Samples of the test solutions were collected from new solution of each experimental group at the beginning of the test, from new solutions and pooled old solutions at the end of the renewal period on Day 3, and from pooled test solutions at test termination to determine test substance concentrations. Samples were processed immediately for analysis. All test concentrations and control replicates were analysed using HPLC with mass selective detection.

3. Statistical calculations: The 7-day EC_{50} value for frond counts; biomass and growth rates based on frond counts and biomass are based on descriptive analysis of the data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dry weight, respectively, at $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: In freshly prepared test media the recovery of the active substance ranged between 92.5 % and 103 %. In the aged test media (7 days old), 104 % to 110 % of the active substance was recovered. Samples from new and old test solution at Day 3 renewal ranged from 90.1 to 101 % and 96.9 to 107 %, respectively. The overall mean measured concentrations were within the range of 80 to 120 % of nominal however, the results were based on mean measured concentrations.

Table 8.2.7-44: Analytical results

Nominal concentration [mg HMPA/L]	7.5	15	30	60	120
Day 0 concentration (fresh)	7.61	15.3	30.8	56.2	111
Day 3 concentration (spent)	6.89	14.3	27.0	55.3	121
Day 3 concentration (fresh)	7.36	14.5	30.0	64.3	126
Day 7 concentration (spent)	7.84	16.0	32.5	64.8	132
Mean measured [mg HMPA/L]	7.4	15	30	60	123
% of nominal	99	100	100	100	103

The overall mean measured concentrations were within the range of 80 to 120 % of nominal however, the results were based on mean measured concentrations.
The EC_{50} and NOEC values are given below based on mean measured concentrations.

Table 8.2.7-45: Endpoints

Endpoint	mg HMPA/L
$EC_{50,\ frond\ number}$ (7 day)	>123
$NOEC_{frond\ number}$ (7 day)	≥123
$EC_{50,\ biomass}$ (7 day)	>123
$NOEC_{biomass}$ (7 day)	≥123
$EC_{50,\ growth\ rate\ (frond\ number)}$ (7 day)	>123
$NOEC_{growth\ rate\ (frond\ number)}$ (7 day)	≥123
$EC_{50,\ growth\ rate\ (biomass)}$ (7 day)	>123
$NOEC_{growth\ rate\ (biomass)}$ (7 day)	≥123

B. OBSERVATIONS

Observations: None of the parameters recorded, i.e. frond number, biomass, growth rate based on front number and growth rate based on biomass was found to be significantly different from the control (Dunnett's t-test [$\alpha = 0.05$]); see the table below.

Table 8.2.7-46: Frond numbers and inhibition values of *Lemna gibba* G3 after 7 days of exposure to HMPA

Test item	Control	HMPA [mg/L]				
Nominal concentrations [mg HMPA/L]	-	7.5	15	30	60	120
Mean measured concentrations [mg HMPA/L]	-	7.4	15	30	60	123
Mean frond number	145	158	166	147	156	174
Mean inhibition [%]	-	-9	-15	-1	-7	-20
Mean biomass [mg]	16.73	18.90	20.93	19.17	20.10	22.20
Mean inhibition [%]		-13	-25	-15	-20	-33
Mean growth rate based on frond number	0.3531	0.3681	0.3751	0.3564	0.3656	0.3818
Mean inhibition [%]	-	-4	-6	-1	-4	-8
Mean growth rate based on biomass	0.3494	0.3679	0.3821	0.3699	0.3763	0.3909
Mean inhibition [%]	-	-5	-9	-6	-8	-12

The doubling time of frond numbers in the control was less than 2.5 days (1.96 days), corresponding to approximately a twelve-fold increase after seven days. The validity criteria according to the current guideline OECD 221 are therefore fulfilled.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The EC50 and NOEC values are given below based on mean measured concentrations.

Since no inhibition effects of HMPA was observed on the frond number, frond number growth rate, biomass and biomass growth rate of Lemna gibba G3 after 7 days at all concentrations tested, the EC50 values for frond number, frond number growth rate, biomass and biomass growth rate were all >123 mg HMPA/L, the highest concentration tested. The NOEC was determined to be ≥123 mg HMPA/L.
The EC50 values for frond number, frond number growth rate, biomass and biomass growth rate were all >123 mg HMPA/L, the highest concentration tested. The NOEC was determined to be ≥123 mg HMPA/L.

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.2.7/013
Report author	Yanhui, T.. *et al.*
Report year	2015
Report title	Growth inhibition of two herbicides on *Spirodela polyrhiza*
Document No	ISSN: 1002-5480

Guidelines followed in study	OECD 221
Deviations from current test guideline	Not reported
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary

The inhibitory activities of glyphosate on the aquatic macrophyte *Spirodela polyrhiza,* were studied in the laboratory by using quantity of the thallus as test indicator. The effects of glyphosate were tested in a semi-static exposure of 7 days at concentrations between 8.4 and 20.902 mg/L. The results showed that glyphosate had remarkable effects on the growth inhibition of *Spirodela polyrhiza,* and the inhibitory rate increased with higher concentrations. The 168 hour-EC_{50} value was determined to be 12.817 mg/L.

Materials and Methods

Test materials and culture

The tested organism, *Spirodela polyrhiza*, was introduced from Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

The *Spirodela polyrhiza* was placed in a crystal dish with the volume of 300 mL (10 × 5cm). The Swiss standard (SIS) culture medium was added (see table below). The light was 9000 - 10 000 lx incandescent light, the temperature was 24 ± 2 ℃, and the culture medium was replaced every 7 days to maintain the stability of the concentration of the nutritional ingredients in the solution. It can only be used in the experiment after 14 days of continuous pre-culture. Before the experiment, enough 4-leaf *Spirodela polyrhiza* with good shape and similar shape and size were selected to carry out the experiment. The above experimental operations should be carried out in an ultra-clean work table to prevent culture medium pollution.

Main instruments and test reagents for the test

Main instruments and reagents

Intelligent artificial climate box PRX-350B (Ningbo Saifu Experimental instrument Co., Ltd.), super clean worktable VS-1300L-U (Sujing Antai), biosafety cabinet BHC-1300 II A/B3 (Suzhou Antai); Glyphosate 96.8 % original drug (provided by Ministry of Agriculture Pesticide Inspection Institute), Dimethylformamide (Analytical Reagent, Beijing Chemical Plant), Twin 80 (Analytical Reagent, Beijing Chemical Plant)

Table 8.2.7-47: (SIS) culture medium component[1]

Storage solution serial No.	Reagent	Storage solution concentration (g/L)	Concentration of culture medium (mg/L)
A	$NaNO_3$	8.5	85
	KH_2PO_4	1.34	13.4
B	$MgSO_4\ 7H_2O$	15	75
C	$CaCl_2{\cdot}2H_2O$	7.2	36
D	Na_2CO_3	4	20
E	$Na_2EDTA{\cdot}2H_2O$	0.28	1.4
	$FeCl_3{\cdot}6H_2O$	0.17	0.84
F	H_3BO_3	1	1
	$CuSO_4\ 5H_2O$	0.005	0.005
	$ZnSO_4\ 7H_2O$	0.05	0.05
	$MnCl_2\ 4H_2O$	0.2	0.2
	$Na_2MoO_4\ 2H_2O$	0.01	0.01
	$Co(NO_3)_2\ 6H_2O$	0.01	0.01

[1] All storage solutions shall be kept in refrigerated and dark conditions, and the storage solution AE can be kept for 6 months, while the reserve liquid F can only be kept for 1 month. Prepare 1LSIS medium, take 10 mL stock solution A, 5 mL storage

solution B~E, 1 mL stock solution F into volumetric flask, add 900 mL distilled water, adjust pH to 6~7 with 1 mol/L HCl, and then use distilled water to 1L.

Experimental Methods

Allocation of test mother liquid: 0.1 g glyphosate was obtained by dissolving it in aseptic water, and the volume was fixed to 100 mL capacity bottle. And then 1 000 mg/L glyphosate mother liquid could be obtained. After sealing the above liquid with sealing film, put it in the refrigerator at 4°C for further test.

Experimental Design

On the basis of the pre-test, a series of concentration gradients are set according to the equal ratio difference. The concentrations of glyphosate were 8.4, 10.08, 12.096, 14.515, 17.418, 20.902 mg/L and solvent control group and blank control group. 200 mL (height > 2cm) culture solution containing different concentrations of glyphosate was added to the crystal dish with diameter 10 cm. Three selected *Spirodela polyrhizas* were put into the above toxic solution, sealed with an aseptic culture container ligated with a rubber band. 3 repeats were set up in each treatment, and finally they were randomly placed in an artificial climate box. The experimental conditions were consistent with the pre-culture conditions. In order to maintain the concentration of the test solution, semi-static culture was used in this experiment. PH was measured before replacing the culture test solution on the 3rd and 5th day, respectively. All the above operations should be operated under aseptic conditions to prevent culture medium pollution. The test period was seven days. After the experiment was over, the average specific growth rate μ of the blank control was calculated, and the growth inhibition percentage of each treatment group was also calculated.

Test Index

The number and growth condition of *Spirodela polyrhiza* in each treatment group were recorded every 2 days, and whether the culture medium was normal or not was also recorded. All clearly visible leaves should be counted. The increase of the number of *Spirodela polyrhiza* leaves indicated its growth, and the difference between each concentration group and the control group indicated the toxic effect.

Data Processing

The average specific growth rate (μ)

The average specific growth rate in a specific period is to calculate the growth variables (leaf number, total leaf area) during the logarithmic growth period, and the following formula is used to calculate each repetition of the control and treatment.

$$I = \frac{\mu_c - \mu_t}{\mu_c} \times 100\%$$

In this: I - Average specific growth inhibition rate, %;

μ_c - control group μ mean value;

μ_t - control group μ mean value

Results

In the process of effectiveness analysis, the solvent control group grew well and the solvent content was less than 100 μL/L. In addition, the pH variation range (0.6 - 1.2) was not more than 1.5 before and after the replacement of the *Spirodela polyrhiza* culture solution. The average specific growth rates of leaf bodies in each blank treatment group were calculated to be 0.294 d^{-1} and 0.317 d^{-1}, respectively, both > 0.275 d^{-1}. The average specific growth rate of leaves in each blank treatment group was 0.294 d^{-1} and 0.317 d^{-1}, respectively. The above test results meet the requirements of *Spirodela polyrhiza* growth inhibition test in OECD, and the test system is effective.

Effect of glyphosate on the growth of *Spirodela polyrhiza* can be seen from the Figure below. Within a certain range, the herbicide can inhibit the growth of *Spirodela polyrhiza*, and with the increase of the concentration of the test solutions, the inhibition effect is strengthened.

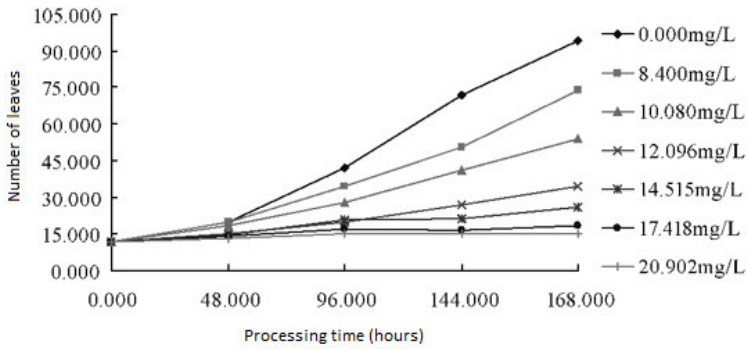


Figure 8.2.7-1: Inhibition of different concentrations of glyphosate on the growth of *Spirodela polyrhiza*

The growth inhibition rates of glyphosate on the leaves of *Spirodela polyrhiza* can be seen from the table below. The coefficient of variation of each treatment group changed a bit, and the growth inhibition rates on the leaves of *Spirodela polyrhiza* showed significant differences at different concentrations of the test solution. Within a certain range, the growth inhibition rates on the leaves of the *Spirodela polyrhiza* increased with the increase of the concentration of the test solution.

Table 8.2.7-48: Inhibition rate of different concentrations of glyphosate on the growth of Spirodela polyrhiza

Treatment concentration (mg/L)	Coefficient of variation (%)	Inhibition rate of growth* I (%)
0.000	1.359	0.000 ± 0.231g
8.400	2.707	11.650 ± 0.406f
10.080	1.231	26.926 ± 0.153e
12.096	5.600	48.512 ± 0.489d
14.515	4.980	62.456 ± 0.317c
17.418	7.070	78.548 ± 0.257b
20.902	15.230	88.113 ± 0.307a

* indicates growth inhibition rate ± standard error. In the same column of data, the same letter indicates that there is no significant difference at 0.05 level (P = 0.05).

The EC_{50} of glyphosate on the leaves of duckweed was calculated by using "SPSS Statistics 17.0" software. The EC_{50}, 95% confidence interval and linear equation of glyphosate for *Spirodela polyrhiza* were calculated (see table below). It can be seen from the correlation coefficient of the linear equation that the growth inhibition rate of the two herbicides on the *Spirodela polyrhiza* is a good linear relationship with the concentration of the test solution. The EC_{50} of glyphosate to the *Spirodela polyrhiza* was 12.817 mg/L.

Table 8.2.7-49: Inhibitory medium concentration of glyphosate

Test solution	EC_{50} (mg/L)	EC_{50} 95% confidence interval	Linear equation
Glyphosate	12.817	12.256 - 13.388	$y = 5.928x – 6.567$ $R^2 = 0.993$

Conclusion

The results showed that glyphosate had remarkable effects on the growth inhibition of *Spirodela polyrhiza*, and the inhibitory rate increased with higher concentrations. The 7 day-EC_{50} value was determined to be 12.817 mg/L.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The effects of glyphosate to the aquatic macrophyte *Spirodela polyrhiza* was tested in a semi-static exposure of 7 days at concentrations between 8.4 and 20.902 mg/L. The 7 day-EC_{50} value was determined to be 12.817 mg/L.

This study was conducted to guideline but not to GLP. The test concentrations were not analytically verified and thus the exact exposure concentrations of the aquatic macrophyte are unknown. Therefore, the study should considered as reliable with restrictions.

CA 8.2.8 Further testing on aquatic organisms

Additional testing is not required considering the studies provided above.
Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate on aquatic organisms are summarised in the table below. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to amphibians, please refer to document M-CP Section 10.1 and 10.2.

Table 8.2.88-1 Literature on aquatic organisms

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.2.8/001	Daam *et al.*, 2019. Lethal toxicity of the herbicides acetochlor, ametryn, glyphosate and metribuzin to tropical frog larvae	OECD 241. ASTM E1439-12.	Glyphosate	Reliable with restrictions	Acute toxicity of glyphosate to larvae of *Physalaemus cuvieri* and *Hypsiboas pardalis.* The LC_{50} for *Physalaemus cuvieri* and *Hypsiboas pardali* was determined to be 115 mg a.s./L and 106 mg a.s./L, respectively.

A summary is provided below.

1. Information on the study

Data point:	CA 8.2.8/001
Report author	Daam, M.A. *et al.*

Report year	2019
Report title	Lethal toxicity of the herbicides acetochlor, ametryn, glyphosate and metribuzin to tropical frog larvae
Document No	doi.org/10.1007/s10646-019-02067-5 ISSN: 0963-9292
Guidelines followed in study	OECD (2015) Test No. 241: the larval amphibian growth and development assay ASTM (2013) Standard guide for conducting the frog embryo teratogenesis assay-Xenopus (FETAX). ASTM E1439-12
Deviations from current test guideline	Not reported
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes / Reliable with restrictions

2. Full summary of the study according to OECD format

The aim of this study was to evaluate the acute toxicity of the active ingredient glyphosate to tadpoles of two tropical frog species: Physalaemus cuvieri and Hypsiboas pardalis. The calculated 96 h LC50 (median lethal concentration; in mg a.s./L) values for P. cuvieri and H. pardalis were 115 and 106 mg a.s./L, respectively.

Materials and methods

Test species

Three or more egg masses from different parents of *Physalaemus cuvieri* and *Hypsiboas pardalis* were collected from ponds at the Estação Biológica de Boracéia in Salesópolis, South-East Brazil (23°37′59”S, 45°31′59”W), which is located within a non-polluted, protected watershed. Egg masses were transported in sealed plastic bags containing water from the collection site to the laboratory of the School of Arts, Sciences and Humanities in the University of São Paulo. Hatched larvae were kept in 50 L plastic tanks filled with tap water filtered through an activated carbon granular filter. Tank water was renewed every other day. The temperature in the laboratory was controlled at 25 ± 2 °C with natural photoperiod. Larvae were fed daily with a 3:1 ground mixture of rabbit chow (Purina Mills, LLC, USA; ~16% protein) and Tetra Min Fish Flakes (Tetra Werke, Melle, Germany; ~45% protein) ad libitum until the beginning of the experiments. The bioassays were conducted with Gosner stage 25 tadpoles. Only healthy individuals, as judged by external morphology and behavior, were selected for the experiments.

Lethality tests

Acute (96 h) bioassays were conducted to evaluate the sensitivity of *P. cuvieri* and *H. pardalis* to the pure active ingredients glyphosate (CAS Number 1071-83-6; Purity 99.2 %; Sigma-Aldrich). A semi-static design was adopted, in which test solutions were renewed 48 h after the start of the experiment.
The tests were conducted under the same conditions as those described above, except that animals were not fed during the test. Based on the results of range-finding tests, five logarithmically-spaced test concentrations (all in mg a.i./L) were determined: Glyphosate: 84; 97; 112; 130; 150.

Test concentrations were prepared with stock solutions. Each treatment was conducted in quadruplicate, in which each replicate consisted of a glass jar containing 10 tadpoles in 1 L test solution. Every 24 h, water quality parameters (pH, temperature, conductivity, DO) were recorded using a multi-parameter meter (YSI 556), and dead individuals counted and removed.

Data analysis

The 96 h LC_{50}, LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) were calculated based on the % mortality rates in the different treatments using the statistical programs PROBIT 1.5 and TSK 1.5. In all cases, the most appropriate statistical test was defined depending on the experimental design and the nature of the available data, following the recommendations of EPA. To test for interspecies differences in sensitivity, LC50 values for each compound and species were compared with

a Z test using the formula proposed by EPA. Analyses of Variance (ANOVA) followed by post hoc tests were employed to test for treatment effects on physical-chemical variables (mean values for each treatment over the experimental period) using the software PAST.

Results

Survival was 100 % in all control treatments. Water quality parameters were comparable in control replicates with a cofficient of variation of less than 4% for all para- meters (pH, temperature, conductivity and DO).

The 96 h LC_{50} values generated and is presented in the table below, whereas the mortality levels of the individual treatments for *P. cuvieri* and *H. pardalis* are visualized in the Figures below.

Table 8.2.8-2: LC_{50} (median lethal concentration; in mg/L) and the 95% confidence interval as determined for larval *Physalaemus cuvieri* and *Hypsiboas pardalis* after 96-h exposure to glyphosate.

	Physalaemus cuvieri	Figure	*Hypsiboas pardalis*	Figure
Glyphosate	115 (112–119)[b]	1	106 (103–109)[b]	2

[b]Probit test

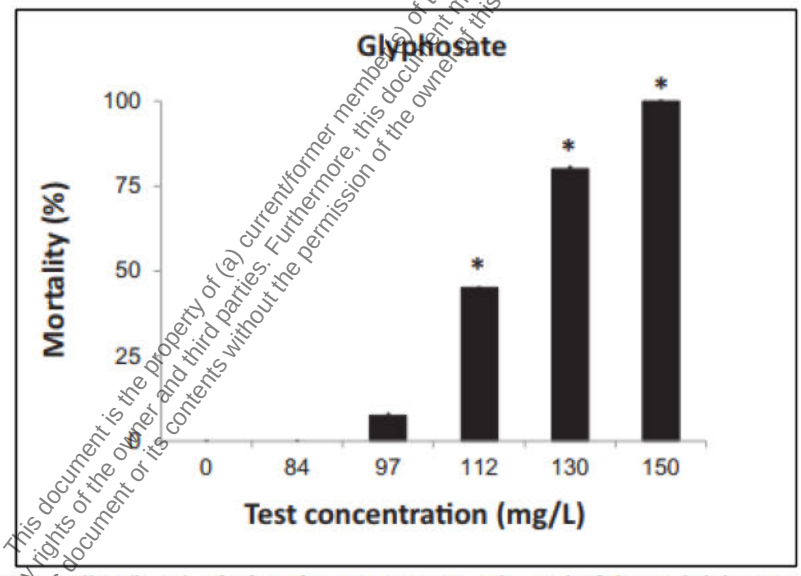


Figure 8.2.8-01: Mortality (in %) of *Physalaemus cuvieri* at the end of the 96 h laboratory tests evaluating the toxicity of glyphosate. Bars represent mean ± 1 SE of four replicates. Asterisks represent significant differences ($p < 0.05$) relative to the control.

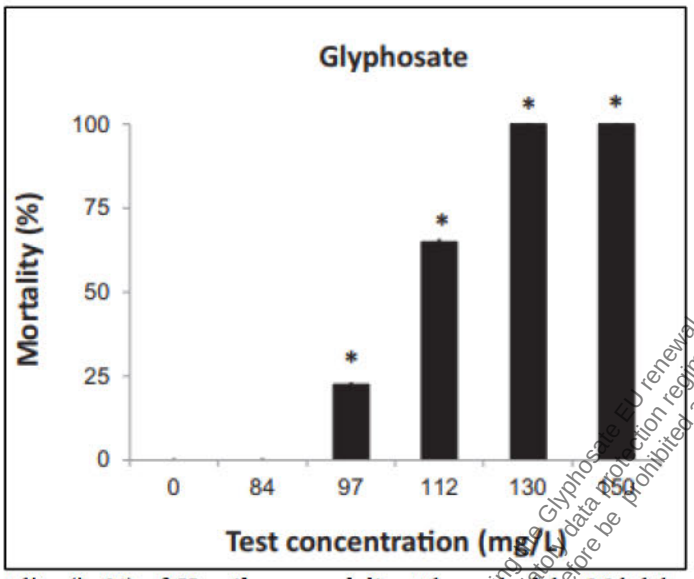


Figure 8.2.8-02: Mortality (in %) of *Hypsiboas pardalis* at the end of the 96 h laboratory tests evaluating the toxicity of glyphosate. Bars represent mean ± 1 SE of four replicates. Asterisks represent significant differences ($p < 0.05$) relative to the control.

Conclusion
The LC_{50} for *Physalaemus cuvieri* and *Hypsiboas pardali* was determined to be 115 mg a.s./L and 106 mg a.s./L, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study investigated the acute toxicity of glyphosate to larvae of *Physalaemus cuvieri* and *Hypsiboas pardalis*. The LC_{50} for *Physalaemus cuvieri* and *Hypsiboas pardali* was determined to be 115 mg a.s./L and 106 mg a.s./L, respectively.

The study was conducted according to portions of OECD 241. However, validity criteria were not reported. It is unknown if the larvae were exposed to any other chemicals as no analysis of watershed water was provided.There was no analytical verification of test concentrations reported. The study is considered as reliable with restrictions.

CA 8.3 Effects on Arthropods

CA 8.3.1 Effects on bees

Studies on effects of the active substance glyphosate on pollinators to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

An extensive regulatory pollinator toxicology database has been summarised to evaluate acute and long-term toxicity of glyphosate and glyphosate salts. The results of these studies demonstrate that glyphosate and glyphosate salts are of low acute and long-term toxicity to honeybees and other pollinator species.

CA 8.3.1.1 Acute toxicity to bees

Studies considering the effects of glyphosate on pollinators were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 0-1: Studies on acute oral and contact toxicity of glyphosate to pollinators

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.3.1.1.1/001	, 2003	Acute oral	*Apis mellifera L.*	Glyphosate K-salt	Valid	-
CA 8.3.1.1.1/002	1998	Acute oral	*Apis mellifera L.*	Glyphosate acid	Valid	-
CA 8.3.1.1.1/003	' 1996	Acute oral	*Apis mellifera L.*	Glyphosate	Valid	-
CA 8.3.1.1.1/004	, 1995	Acute oral	*Apis mellifera L.*	Glyphosate acid	Valid	-
CA 8.3.1.1.1/005	' 1995	Acute oral	*Apis mellifera L.*	Glyphosate	Valid	-
CA 8.3.1.1.1/006	,1972	Acute oral	*Apis mellifera L.*	Glyphosate technical and IPA-salt	Invalid	control mortality >10%
CA 8.3.1.1.1/007	, 2017a	Acute oral	*Bombus terrestris*	Glyphosate IPA-salt	Valid	-
CA 8.3.1.1.2/001	, 2003	Acute contact	*Apis mellifera L.*	Glyphosate K-salt	Valid	-
CA 8.3.1.1.2/002	, 2000	Acute contact	*Apis mellifera L.*	Glyphosate isopropylamine salt	Valid	-
CA 8.3.1.1.2/003	' 1998	Acute contact	*Apis mellifera L.*	Glyphosate acid	Valid	-
CA 8.3.1.1.2/004	1996	Acute contact	*Apis mellifera L.*	Glyphosate	Valid	-
CA 8.3.1.1.2/005	1995	Acute contact	*Apis mellifera L.*	Glyphosate acid	Valid	-
CA 8.3.1.1.2/006	1995 '	Acute contact	*Apis mellifera L.*	Glyphosate	Valid	-
CA 8.3.1.1.2/007	, 1972	Acute contact	*Apis mellifera L.*	Glyphosate technical and IPA-salt	Invalid	control mortality >10%
CA 8.3.1.1.2/008	, 2017a	Acute contact	*Bombus terrestris*	Glyphosate IPA-salt	Valid	-
CA 8.3.1.1.2/009	, 2017b	Acute contact	*Osmia bicornis*	Glyphosate IPA-salt	Valid	-

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the acute impact of glyphosate or its relevant metabolites on pollinator species. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. For discussions of literature regarding toxicity to pollinator species, please refer to document M-CP Section 10.3.

Endpoints of studies considered valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid

equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 0-2: Endpoints: Acute oral and contact toxicity of glyphosate to pollinators

Reference	Test item	Species	Test design/ GLP	LD_{50} (µg a.e./bee)	NOED (µg a.e./bee)
2003 CA 8.3.1.1.1/001	**Glyphosate K-salt**	***Apis mellifera L.***	**Acute oral, 48 h**	**>104**	**-**
, 1998 CA 8.3.1.1.1/002	Glyphosate acid	*Apis mellifera L.*	Acute oral, 48 h	≥182	≥182
, 1996 CA 8.3.1.1.1/003	Glyphosate	*Apis mellifera L.*	Acute oral, 48 h	>40	-
, 1995 CA 8.3.1.1.1/004	Glyphosate technical	*Apis mellifera L.*	Acute oral, 48 h	>200	-
, 1995 CA 8.3.1.1.1/005	Glyphosate	*Apis mellifera L.*	Acute oral, 72 h	116.67	-
r, 2017 CA 8.3.1.1.1/007	Glyphosate IPA-salt	*Bombus terrestris*	Acute oral, 48 h	>412	≥412
2003 CA 8.3.1.1.2/001	**Glyphosate K-salt**	***Apis mellifera L.***	**Acute contact, 48 h**	**>100**	**-**
, 2000 CA 8.3.1.1.2/002	Glyphosate IPA-salt	*Apis mellifera L.*	Acute contact, 48 h	>61.3	-
, 1998 CA 8.3.1.1.2/003	Glyphosate acid	*Apis mellifera L.*	Acute contact, 48 h	>103	-
1996 CA 8.3.1.1.2/004	Glyphosate	*Apis mellifera L.*	Acute contact, 48 h	>20	-
, 1995 CA 8.3.1.1.2/005	Glyphosate technical	*Apis mellifera L.*	Acute contact, 48 h	>200	-
, 1995 CA 8.3.1.1.2/006	Glyphosate	*Apis mellifera L.*	Acute oral, 72 h	100	-
2017 CA 8.3.1.1.2/008	Glyphosate IPA-salt	*Bombus terrestris*	Acute contact, 48 h	>461	≥461
, 2017 CA 8.3.1.1.2/009	Glyphosate IPA-salt	*Osmia bicornis*	Acute contact, 48 h	>461	≥461

a.e.: acid equivalents
Endpoints in **bold** is used for risk assessment

Study summaries are provided below.

CA 8.3.1.1/1 Acute oral toxicity

1. Information on the study

Data point	CA 8.3.1.1.1/001
Report author	
Report year	2003
Report title	Laboratory bioassays to determine acute oral and contact toxicity of MON 78623 to the honeybee, *Apis mellifera*
Report No	MON-02-10
Document No	-
Guidelines followed in study	EPPO guideline 170 (1992)
Deviations from current test guideline	Deviations according to guideline OECD 213(1998): Minor: - Relative humidity was slightly above the recommended range - No mortality assessment at 4 hours
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a laboratory study, the acute oral toxicity of glyphosate K-salt to the honey bee, *Apis mellifera* L., was established. Following a range finding test, a definitive test was conducted exposing worker bees to nominal doses of 100 µg glyphosate acid equivalent/bee.

Five replicate cages each containing 10 bees (50 bees per control or test group) were prepared for the test item treatment and for the control (50% sucrose only- no test substance). There were three replicates for each of the five reference item treatment groups also prepared. Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation.

At 24 hours, there was a single bee mortality in the control group, with two bee mortalities in the 100 µg a.e./bee test group. At 48 hours, there were a further two bee mortalities in the control with a three additional mortalities in the 100 µg a.e./bee group. The overall control corrected mortality for oral toxicity was 4 %. There were no sub-lethal effects observed. All validity criteria according to OECD 213 were fulfilled.

In conclusion, the toxicity of glyphosate K-salt was tested in an acute oral toxicity test on honey bees. The LD_{50} (48 h) was > 104 µg glyphosate acid equivalent/bee.

The study is considered valid so LD_{50} >104 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 78623
Description:	Amber liquid
Lot/Batch #:	GLP-0108-11688-F
Purity:	58 % K salt of glyphosate, equivalent to 47.3 %.w/w glyphosate a.e.
Vehicle and/or positive control:	Vehicle for test item: Farmon Blue (87.3% w/w alkyl phenol ethylene oxide) / Positive control: Dimethoate technical grade

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Roselea Apiaries, East Wellow, Hampshire
Diet/Food:	50 % w/v aqueous sucrose solution

Environmental conditions:

Temperature:	25 – 26 °C
Humidity:	64 – 79 %
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	22 July – 27 July 2002

B. STUDY DESIGN

Experimental treatments

A range finding test was conducted using two replicate vessels – each containing 10 bees, at 0.1, 1, 10 and 100 µg a.e//bee and a 50 % w/v sucrose control group.

The definitive test was conducted at a single rate (100 µg test item/bee) and included a single control group (50 % w/v aqueous sucrose solution).

A toxic reference item (dimethoate) test was conducted in parallel at five test rates (0.200, 0.175, 0.150, 0.125 and 0.100 µg a.s./bee and included a 50 % w/v sucrose control group.

Bees were exposed to the test item dispersed in 50% w/v sucrose solution, presented in in narrow glass vials, which were weighed before and after introduction into the three cages per treatment. In the definitive test with MON 78623, at the highest treatment level, the mean dose consumed was 104 µg a.e./bee.

Observations

Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation.

Statistical calculations

Corrected mortality was calculated according to Abbott (1925). LC_{50} values were determined by Probit analysis and the 95% confidence interval by Chi-square goodness of fit test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-1: Toxicity of glyphosate K-salt to honey bees (*Apis mellifera* L.) in the oral toxicity test

Dose [µg a.e./bee]	Mean intake of test item [µg a.e./bee]	Mortality [%]			
		1	3	24 h	48 h
Sucrose control	-	0	0	2	6
100	104	0	0	4	10 (4)

In brackets the Abbot corrected mortality is given

B. OBSERVATIONS

No sublethal effects of bees were observed during the 48 hour test period for the test concentration of 104 µg glyphosate acid equivalent/bee and in the sucrose control.

The corrected mortality after 48 h was 4%. The determined contact 48h LD_{50} for the reference item dimethoate was 0.126 µg/bee for oral toxicity. These results are in line with published values, indicating that the test insects were of suitable sensitivity.

Deviations according to guideline OECD 213(1998):
- Relative humidity was slightly above the recommended range
- No mortality assessment at 4 hours

These deviations are not expected to have a negative impact on the validity of the study.

All validity criteria according to OECD 213 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of glyphosate acid was tested in an acute oral toxicity test on honey bees. The LD_{50} (48 h) was >104 µg glyphosate acid equivalent/bee.

The study is considered valid so LD_{50} >104 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.1/002
Report author	
Report year	1998
Report title	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*)
Report No	FN9700
Document No	-
Guidelines followed in study	EPPO guidelines (1992) OPPTS 850.3020 Draft OECD 213 (1997)
Deviations from current test guideline	Deviations from guideline OECD 213 (1998): Minor: - The starvation of bees before test initiation was 2 h and 10 min, instead of 1-2 h.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute oral toxicity of glyphosate acid to the honey bee *Apis mellifera* L., was determined in a definitive laboratory test with worker bees exposed to nominal doses of 0.0984, 0.984, 9.84, 103 and 206 µg glyphosate acid /bee, presented in 50 w/v sucrose syrup. A reference treatment (dimethoate) group was also included.

Three replicate cages, each containing 10 bees, were prepared for the control and for each test item group and for the reference group. Mortality and sub-lethal effects were assessed 24 and 48 h after test initiation for oral toxicity.

No sub-lethal effects nor mortality of bees was observed after 48 hours of exposure, in the test item and the control groups. All validity criteria according to OECD 213 were fulfilled.

In conclusion, the 48 hour LD_{50} toxicity value for oral exposure of honeybees to glyphosate acid was determined to be >182 µg test item/bee in the oral toxicity test, with a corresponding NOEL of ≥182 µg test item/bee.

The study is considered valid so LD_{50} >182 µg a.s./bee and NOEL of ≥182 µg a.s./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Technical Glyphosate acid
Description:	White powder
Lot/Batch #:	TSC 0521/05148
Purity:	97.6 %

2. Vehicle and/or positive control:

Vehicle for positive control: Triton X100
Positive control: Dimethoate (BASF 40 lot 083.10/96)

3. Test organisms:

Species: Honey bee (*Apis mellifera* L.)

Age:	Adult worker bees
Source:	Own colony
Diet/Food:	Not stated
Environmental conditions:	
Temperature:	25 ± 1 °C
Humidity:	65 ± 5 %
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	24 August to 04 September 1998

B. STUDY DESIGN

Experimental treatments

The definitive test was conducted with 0.0984, 0.984, 9.84, 103 and 206 µg glyphosate acid/bee, dispersed in 50 % w/v aqueous sucrose solution. All test solutions were prepared using an initial stock solution prepared at 103 mg a.s./mL, using deionised water containing 500 mg/L Agral 90. In turn a stock solution at 9.84 mg a.s./mL was prepared and then serially diluted to achieve the required test concentrations. An aliquot of each test concentration (0.5 mL) was diluted to a 10 mL final volume using 50 % w/v sucrose solution. The control group received 50% w/v sucrose solution containing 0.5 mL of the 500 mg/L Agral 90.

In the toxic reference group, dimethoate was added to deionised containing 1 g Triton X100/L to achieve 3.5 mg a.s./mL stock solution from which a dilution series was prepared. With a control group of bees receiving 50 % w/v sucrose solution containing 0.5 mL Triton X100.

The bees collected from a local hive, were anaesthetised with carbon dioxide immediately before dosing and counted into the mesh covered petri dishes. Each group of 10 bees were offered control, test item or reference item containing feed solutions (0.2 mL) in a glass feeder attached to the mesh cage. The feeders were weighed before and after introduction into the cages. The test was conducted in the dark, with bees held in an incubator at 25 ± 1 ºC and 65 ± 5 % relative humidity. Duration of uptake was 4 hours for the test item treatments, with all feeders being replaced with fresh feeders containing only 50 % sucrose solution.

Observations

Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation.

Statistical calculations

Doses and LD_{50} calculations were based on the analysed content of glyphosate acid. The mortality results were analysed using a probit programme.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-2:Toxicity of glyphosate acid to honey bees (*Apis mellifera*) in the oral toxicity test

Dose [µg test item/bee]	Mean intake of glyphosate acid [µg a.s./bee]	Mortality [%]		
		24 h	48 h	72 h
Control	-	0	0	0
0.0984	0.0947	0	0	0
0.984	0.937	0	0	0
9.84	9.7	0	0	0
103	81	0	0	0
206	182	0	0	0

B. OBSERVATIONS

There were no sub-lethal effects nor mortality of bees observed in the 48 hour test period. In the oral toxicity test the maximum nominal test level of 206 µg test item/bee) corresponded to an actual intake of 182 µg a.s./bee.

Deviations according to the current guideline OECD 213:

- The starvation of bees before test initiation was 2 h and 10 min, instead of 1-2 h.

This does not affect the reliability of the study.

All validity criteria according to OECD 213 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate acid was tested in an acute oral toxicity test on honey bees. The LD_{50} (48 h) was > 182 µg a.s./bee, with a corresponding NOEL of ≥182 µg a.s./bee.

The study is considered valid so LD_{50} >182 µg a.s./bee and NOEL of ≥182 µg a.s./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.1/003
Report author	
Report year	1996
Report title	Glyphosate: Acute contact and oral toxicity to honeybees
Report No	1413/3-1018
Document No	-
Guidelines followed in study	EPPO Guideline No. 170: Test methods for evaluating the side-effects of plant protection products on honeybee (1992)
Deviations from current test guideline	Deviations from guideline OECD 213 (1998): Minor: - Mortality observation was not assessed at 4 hours - Relative humidity exceeded the recommended values
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
In an acute laboratory study the oral toxicity of glyphosate to honeybee, *Apis mellifera* was tested. After a preliminary dose range-finding test, adult worker bees were treated with 1.25, 2.5, 5.0, 10, 20 and 40 µg glyphosate/bee in the oral test. Three replicate cages, containing 10 bees each, were used. Mortalities and sub-lethal effects were made 1, 4, 24 and 48 h after treatment. No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period. The validity criteria according to current OECD guideline 213 are fulfilled.
The study is considered valid and the 24 and 48-hour oral LD_{50} values for glyphosate were >40 µg a.s./bee for oral exposure (nominal).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	H95 D161A
Purity:	95.3 %
Vehicle and/or positive control:	Vehicle: reverse-osmosis water Poistive control: formulated Dimethoate (BASF Dimethoate 40 EC)

Test organisms:

Species:	Honey bee (*Apis mellifera*)
Age:	Adult worker bees
Source:	The Bee Farm, Wetherby, West Yorkshire, UK
Diet/Food:	50 % sucrose solution *ad libitum*
Acclimatisation:	Not stated

Environmental conditions:

Temperature:	24.5 - 25.8 °C
Relative humidity:	49.1 - 86.0 %
Photoperiod:	darkness
Experimental dates:	27 June – 06 July 1996

B. STUDY DESIGN

Experimental treatments
To determine the test concentrations for the definitive study a range-finding test was performed. The nominal doses of glyphosate used for the range-finding test were 0, 0.04, 0.4, 4 and 40 µg a.s./bee for oral dosing.
The nominal doses of glyphosate used for the definitive oral test were 0, 1.25, 2.5, 5.0, 10, 20 and 40 µg a.s./bee. Three replicate cages, containing 10 bees each, were used. The reference substance was prepared and dosed in the same media and manner as the test substance doses. The toxic standard test was run in concurrently with the range-finding test and shared the controls. The nominal doses of dimethoate were 0, 0.2, 0.4 and 0.8 µg a.s./bee in the contact test and 0, 0.1, 0.15 and 0.2 µg a.s./bee in the oral test. There were three replicate cages of 10 bees each at each dose level of the reference substance.

Observations
Assessments of mortality and sub-lethal effects were conducted 1, 4, 24 and 48 hours after treatment.

Statistical calculations
Descriptive Statistics; the LD_{50} values of the toxic standard, dimethoate, were calculated by Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS
No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period. The 48 h LD_{50}-value for dimethoate was calculated to be 0.146 µg a.s./bee (95% confidence limits: 0.131 to 0.161) for oral exposure.

Deviations according to the current guideline OECD 213:
- Mortality observation was not assessed at 4 hours
- Relative humidity exceeded the recommended values

These deviations are not expected to have a negative impact on the validity of the study which was valid at the time of conduct.

The test is considered to be valid according to OECD guideline 213 as mortality in the negative control did not exceed 10% after 48 hours. In addition, the LD_{50} for the reference item met the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of glyphosate was tested in an acute oral toxicity test on honey bees. The oral LD_{50} (24 h/48 h) values for glyphosate were >40 µg a.s./bee for oral exposure (nominal).
The study is considered valid so LD_{50} >40 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.1/004
Report author	
Report year	1995
Report title	Testing Toxicity to Honeybee - *Apis mellifera* L. (laboratory) according to EPPO Guideline No 170. Glyphosate (tec.)
Report No	95 10 48 065
Document No	-
Guidelines followed in study	EPPO Guideline No 170
Deviations from current test guideline	Deviations from guideline OECD 213 (1998): Minor: - Mortality observation was not assessed at 4 hours.
Previous evaluation	Yes, accepted in RAR 2015
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
In a laboratory study, the acute oral toxicity of technical glyphosate to the honey bee, *Apis mellifera* L. was tested. Adult worker bees were exposed to two nominal test doses of 100 and 200 µg test item/bee.
In the test, three replicate cages, each containing 10 bees were used for the test item treatment, control and reference treatment. Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after treatment initiation.
Results showed a single bee mortality in the 100 µg a.s./bee treatment group at 24 hours, with no further mortality recorded at 48 hours at both the 100 and 200 µg a.s./bee treatment groups. In addition, no behavioural abnormalities were observed in test item groups and control groups during the whole test period. All validity criteria according to the OECD guideline 213 was fulfilled.
The study is considered valid so LD_{50} >200 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	Not stated
Lot/Batch #:	01/07/95
Purity:	98.2 % a.s.
Vehicle and/or positive control:	Dimethoate EC 400, containing 411,14 g a.s./L Extravon (surfactant)

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Purchase from the bee-keeper Mr Weimann / Gottscheina
Diet/Food:	50% aqueous sucrose solution *ad libitum* (except for 1 – 2 hours prior to oral test initiation)

Environmental conditions:

Temperature:	25 – 26 °C
Humidity:	53 – 70 %
Photoperiod:	8 hours diffuse light/16 hours darkness
Experimental dates:	21 August – 01 September 1995

B. STUDY DESIGN

Experimental treatments

The oral toxicity test was conducted with two nominal test doses of 100 and 200 µg a.s./bee. In addition, a control group was fed with 50% sucrose solution. Dimethoate was used a toxic reference, at test doses ranging from 0.20 to 0.40 µg/bee. The oral toxicity test was conducted in triplicate using 10 bees per replicate (30 bees), with the test item or reference item delivered to the bees in 50 % sucrose solution in feeding tubes, attached to the bee cages. The bees were fed with 50 % aqueous sucrose solutions, containing appropriate concentrations of the test item.

Observations

Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after test start.

Statistical calculations

Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LD_{50} value is given below based on nominal concentrations.

Table 0-5: Toxicity of technical glyphosate to honey bees in an oral toxicity tests

Endpoints (48 h)	Technical glyphosate [µg a.s./bee]
Oral LD_{50}	>200

B. OBSERVATIONS

No biologically relevant mortality of bees was observed during the 48-hour test period for test concentrations of up to 200 µg a.s./bee, which was the highest concentration tested. In addition, no behavioural abnormalities were observed at any test item concentration and in the control groups.
For the toxic reference dimethoate, the highest test doses caused 83 % and 97 % mortalities for oral and contact test respectively.

Table 0-3: Mortality of honey bees in an oral toxicity tests

Test	Time [h]	Mortality [%]			
		Control	Technical glyphosate [µg a.s./bee]		Toxic reference
		-	100	200	Highest test dose
Oral	24	0	3	0	83
	48	0	3	0	83

Deviations according to the current guideline OECD 213:
- Mortality observation was not assessed at 4 hours.

This deviation is not expected to have a negative impact on the validity of the study.

The validity criteria according to the OECD guideline 213 were fulfilled as the mortality in the control was <10 % at test termination.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of technical glyphosate was tested in an acute oral toxicity test on honey bees. The LD_{50} (48 h) was >200 µg a.s/bee.
The study is considered valid so LD_{50} > 200 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.1/005
Report author	
Report year	1995
Report title	Honey Bees (*Apis mellifera* L.), oral toxicity study in the laboratory with Glyphosate
Report No	141907
Document No	-
Guidelines followed in study	EPPO guidelines 22, 203 – 215 (1992)
Deviations from current test guideline	Deviations from guideline OECD 213 (1998): Minor: - Mortality observation was not assessed at 4 hours - Humidity was lower than the expected range: 34-37 % instead of 50-70 %
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a laboratory study the acute oral toxicity of glyphosate technical material (96 % purity) to the honey bee, *Apis mellifera* L., was tested. Following a range finding test, a definitive test was conducted exposing worker bees to a single nominal dose of 121 µg a.s./bee.

In the test, three replicate cages, each containing 10 bees, were used for the test item treatment, control and reference treatment. Mortality and paralysis effects were recorded at least at the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment.

No mortality of bees was observed during the 72 hours of exposure. In addition, no paralysis was observed in the test item and the control groups during the 72 hours test period. The validity criteria according to guideline OECD 213 are fulfilled.

In an oral toxicity test, glyphosate had no effects on mortality of honey bees at concentrations of up to and including 116.67 µg a.s./bee (mean (df = 3) actual consumed dose). Therefore, the oral LD_{50} of glyphosate was determined to be >116.67 µg a.s./bee.

The study is considered valid so LD_{50} >116.7 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	GLYFOSAAT (Spelling for report: GLYPHOSATE)
Description:	White powder
Lot/Batch #:	22021
Purity:	96%

Vehicle and/or positive control: Vehicle: Tap water
Positive control: Parathion 25 % liquid

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Research Centre for Insect Pollination and Beekeeping, "Ambrosiushoeve"
Diet/Food:	50% aqueous sucrose solution *ad libitum* (except during oral dosing and prior starvation)

Environmental conditions:

Temperature:	24 – 25 °C
Humidity:	34 – 37 %
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	March 08 to March 16 1995

B. STUDY DESIGN

Experimental treatments
Prior to the main test, a range-finding test was performed exposing bees to nominal concentrations of 1.0, 10, 51 and 101 µg a.s./10 µL sucrose solution. The definitive test was conducted as a limit test with a single nominal concentration of 121 µg a.s./10 µL sucrose solution. All test solutions were prepared in a 50 % sucrose solution. In addition, a water-treated control and a reference substance (Parathion 25 % liquid) were tested. Food was withheld from the bees for about one to two hours prior to the test. For the test, 10 bees per cage were exposed in triplicate and fed with the test substance suspension. Per group of 10 bees 100 µL test substance suspension was administered (10 µL test solution/bee).

Observations
Mortality, paralysis and any other abnormalities were recorded at least at the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment start.

Validity criteria
For a test to be valid the following conditions apply:

- The average mortality for the total number of controls must not exceed 10 % at the end of the test.
- The LD_{50} of the toxic standard meets the specified range.

Statistical calculations
Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

The bees were offered sugar solution containing a concentration of 121 µg a.s./bee. The mean (df = 3) amount of glyphosate consumed by the bees over 72 hours was 116.67 µg a.s./bee. A summary of the mortality is provided below.

Table 0-4: Toxicity of glyphosate to honey bees (*Apis mellifera* L.) in an oral toxicity test

Dose [µg a.s./bee]	Intake of test item [µg a.s./bee]	Mortality [%]		
		24 h*	48 h*	72 h*
Control (sugar solution)	-	0.00	3.33	3.33
121	116.67	0	0	0

* Corrected for mortality in the negative control

B. OBSERVATIONS

No mortality of bees was observed at the in the 72 hour limit test at the test concentration of 121 µg a.s./bee. In addition, no paralysis was observed in the test item group and the control group during the 72 hours test period.

Deviations according to the current guideline OECD 213 (1998):

- Mortality observation was not assessed at 4 hours
- Humidity was lower than the expected range: 34-37% instead of 50-70 %

This deviation is not expected to have a negative impact on the validity of the study.

All validity criteria according to OECD 213 were fulfilled, since the average mortality in the control group did not exceed 10 % (actual value: 3.33 %) and the 24-hour LD_{50} of the toxic standard meets the standard of less than 1.0 µg a.s./bee based on historical data (actual value: 0.4 µg a.s./bee).

In an oral toxicity test, glyphosate had no effects on mortality of honey bees at concentrations of up to and including 116.67 µg a.s./bee.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate was tested in an acute oral toxicity test on honey bees. The LD_{50} (72 h) was >116.67 µg a.s./bee.

The study is considered valid so LD_{50} >116.7 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.1/006
Report author	
Report year	1972
Report title	The acute contact and oral toxicities of CP67573 and MON2139 to worker honey bees
Report No	HU85X094
Document No	-
Guidelines followed in study	Working Document 13 produced by the UK Pesticide Safety Precautions Scheme
Deviations from current test guideline	Deviations from guideline OECD 213 (1998): Major: - Mortality in the control was >10% at test termination Minor: - Only 2 replicates (10 replicates only for the highest concentration tested) per treatment group, - No additional solvent control was tested, - Mortality observation was not assessed at 4 hours
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed.
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

* Two test materials were assessed in this study; namely CP67573 and MON2139 (a 36% w/v formulation). MON2139 contains a surfactant that is not present in the representative formulation for the Annex I renewal. This summary therefore only contains information on CP67573 (glyphosate technical).

2. Full summary

Executive Summary

The acute oral toxicity of CP67573 (glyphosate technical) to young adult worker bees (*Apis mellifera* L.) determined in a limit tests performed at a nominal dose of 100 µg a.s./bee. The test comprised 10 replicate mesh cages, each containing 10 bees. In a parallel test, honey bees were exposed to a reference item in a dose response test using dimethoate at concentrations ranging from 0.048 to 0.117 µg dimethoate/bee. In both tests, the test substance was suspended in 20% sucrose and 0.2 mL was fed to each replicate of 10 bees. Control groups consisting of 2 cages of 10 bees were included alongside each of the tests.
Assessments of mortality were conducted after 24 and 48 hours. The validity criteria according to OECD guideline 213 were not fulfilled as mortality in the control was >10% at test termination.
In the 100 µg CP67573/bee treatment group, at 24 and 48 hours, there was 46 % and 56 % mortality, with corresponding mortality in the control group of 10 % and 15 %, respectively.
This resulted in overall control corrected mortality levels of 40 and 48 % achieving a 48 hour LD_{50} of 100 µg a.s./bee. The study is considered invalid as mortality in the control was >10 % at test termination.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: CP67573 (technical active ingredient)
Description: Not stated
Lot/Batch #: No batch details presented in report
Purity: Not stated

Density:	Not stated
Vehicle and/or positive control:	Vehicle: 50 % acetone Positive control: Dimethoate
Test organisms:	
Species:	Honey bee (*Apis mellifera*)
Age:	Young adult worker bees
Source:	Experienced apiarist in Huntingdonshire, U.K.
Diet/Food:	Bees were fed with 20 % sucrose
Acclimatisation:	Not reported
Environmental conditions:	
Temperature:	26 – 27 °C
Relative humidity:	Not reported
Photoperiod:	Not reported
Experimental dates:	Not reported

B. STUDY DESIGN

Experimental treatments
Honey bees were exposed orally to CP67573 in a limit test conducted at 100 µg a.s./bee, in nylon coated 2 mm wire mesh tubes, with 11.5 cm high and 3.5 cm in diameter, closed by corks at both ends. Bees were placed in each cage and were fed with 20 % sucrose. For the oral toxicity tests, compounds were suspended in 20 % sucrose and 0.2 mL was fed to each replicate of 10 bees.
There were 10 cages per test item treatment, with two control cages containing 10 worker bees each. A reference item dose-response test (dimethoate) was conducted in parallel, at five test rates between 0.048 and 0.117 µg test item/bee, with two cages of ten bees per treatment and control group.

Mortality in the test or reference item treatment groups, were corrected for control mortalities using Abbot's correction, to give overall control corrected levels of mortality, on which the endpoint LD_{50} values were based.

Observations
Mortality was recorded 24 and 72 hours after test initiation.

Statistical calculations
Descriptive statistics; LD_{50} for dimethoate were obtained by graphical interpolation on probability/log paper, confidence limits were calculated according to Litchfield & Wilson (1949).

II. RESULTS AND DISCUSSION

A. FINDINGS
A summary of the mortality results is provided below.

Table 0-5: Toxicity of glyphosate to honey bees (Apis mellifera L.) in an oral toxicity test

Endpoints (48 h)	CP67573 [µg a.s./bee]
LD_{50} oral	100

Table 0-6: Oral toxicity of CP67573 to honey bees (*Apis mellifera* L.)

Exposure	Mortality [%]		Corrected mortality [%]
	Control	100 µg/bee	
oral (24 h)	10	46	40
oral (48 h)	15	56	48

B. OBSERVATIONS

In the test with CP67573, the corrected bee mortality did not reach or exceed 50 % (max mortality was 48 %), resulting in overall control corrected mortality levels of 40 and 48% at 24 and 48 hour respectively, achieving a 48 hour LD_{50} of 100 µg a.s./bee.
In the reference item test with dimethoate, a 48 hour oral exposure LD_{50} value of 0.056 µg dimethoate/bee (95 % C.I. of 0.045 - 0.070 µg dimethoate/bee) was observed.

Deviations according to the current guideline OECD 213:

- Only 2 replicates (10 replicates only for the highest concentration tested) per treatment group,
- No additional solvent control was tested,
- Mortality observation was not assessed at 4 hours

These deviations are not expected to have a negative impact on the validity of the study.

- Mortality in the control was >10 % at test termination.

This deviation has a negative impact on the validity of the study.
The validity criteria according to the OECD guidelines 213 were not fulfilled as mortality in the control was >10 % at test termination.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of CP67573 was tested in an acute oral toxicity test on honey bees. The oral LD_{50} (48 h) were 100 µg a.s./bee.
The study is considered invalid as mortality in the control was >10% at test termination.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.1/007
Report author	
Report year	2017
Report title	MON 0139: Acute Oral and Contact Toxicity to the Bumble Bee, *Bombus terrestris* L. under Laboratory Conditions
Report No	S16-06634
Document No	-
Guidelines followed in study	Based on the proposal for new OECD Guidelines: Bumblebee, acute oral toxicity test (2016) and Bumblebee, acute contact toxicity test (2016)
Deviations from current test guideline	Deviation from OECD guideline 247 (2017): none
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The acute oral toxicity of MON 0139 to bumblebees (*Bombus terrestris*) was established in a 48 hour laboratory toxicity test, with bees exposed at five test rates (62.5, 125, 250, 500 and 1000 µg product/bumble bee, equivalent to 28.8, 57.6, 115, 231 and 461 µg a.e./bumble bee, via oral ingestion in aqueous sucrose solution. In the main test, for the control and test group, there were 35 individually housed bumblebees, with application solutions (50 % w/v sucrose solution) presented in plastic feeder syringes.

A reference item test was conducted in parallel with bumble bees exposed to dimethoate at 1.5 µg a.s./bumble bee, with exposure of 32 individually housed bumblebees via 50 % w/v sucrose solution in syringe feeders.

Mortality assessments were made at 4, 24 and 48 hours after application (after start of feeding in the oral toxicity test). Observations for sublethal effects were recorded at each observation interval.

There was 100 % mortality in the reference item test demonstrating the test system as being appropriate and the bumblebees were sensitive.

In the main study, the 48 hours oral LD_{50} (Lethal Dose causing 50 % mortality) for MON 0139 was determined to be >894 µg product/bumble bee (equivalent to >412 µg a.e./bumble bee). The NOED for mortality after 48 hours was determined to be ≥894 µg product/bumble bee (equivalent to ≥412 µg a.e./bumble bee).

The validity criteria for the control group in the main test and reference item mortality were met and thus, the test was considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Test item:	MON 0139
Lot/Batch #:	GLP-1503-23921-T
Actual content of active ingredients:	Glyphosate: 46.1% (a.e.); 574.4 g/ml
Description:	liquid / slightly yellow
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	February 13, 2018
Density:	1.2460 g/cm^3

Treatments

Test rates:	Oral toxicity test: Target doses: 62.5, 125, 250, 500 and 1000 µg prod./bumble bee, equivalent to 28.8, 57.6, 115, 231 and 461 µg a.e./bumble bee Actual uptake: 56.9, 113, 226, 453 and 894 µg prod./bumble bee, equivalent to 26.2, 52.1, 104, 209 and 412 µg a.e./bumble bee
Control:	Pure 50% (w/v) aqueous sucrose solution
Toxic standard:	BAS 152 11 I (dimethoate, analysed 405.2 g a.s./L) 1.5 µg a.s./bumble bee (target doses) 1.36 µg a.s./bumble bee (actual uptake)
Administration:	Oral: ingestion in 50% w/v aqueous sucrose solution.

Test organisms

Species:	*Bombus terrestris* L. (Hymenoptera: Apidae)
Source:	From healthy colony owned and maintained by Biobest Belgium, Ilse Velden 18, 2260 Westerlo, Belgium.
Food:	50% w/v aqueous sucrose solution

Test design

Test cage description:	Nicot cages with plastic syringe feeders attached.
Replication:	35
No. of bees/arena:	1
Duration of test:	48 hours

Environmental conditions

Temperature:	24.8 – 25.3°C
Humidity:	50.9 ± 60.4%
Photoperiod:	Darkness (except during application and observations)
Experimental dates:	10 April to 13 April 2017

B. STUDY DESIGN

Experimental treatments

Adult worker bumblebees (*Bombus terrestris*) were exposed to MON 0139 via oral ingestion in aqueous sucrose solution. To immobilise the bumblebees during the course of treatment, they were anaesthetised using CO_2. Bumblebees were starved for 2 hours until treatment, to ensure that the bees were equal in terms of their gut contents at the start of the test. Each bumblebee was offered 40 µL of the test material or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose was contained in this 40 µL. The doses were measured into the feeding tubes and the weights of these were recorded before the doses were made available to the bumblebees. After four hours, the feeding tubes were

replaced with similar tubes containing untreated 50 % w/v aqueous sucrose solution supplied *ad libitum*. All feeding tubes with test solutions were weighed in order to calculate actual mean consumption per bee for each treatment.

Assessments
Mortality was recorded 4 and 24 hours after application (after start of feeding in the oral toxicity test) and thereafter at 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. In the reference item group, behavioural assessments were not conducted as it was assumed that moribund and affected bumble bees of the reference item group would die by the end of the test.

Statistics
For the statistical evaluation the statistics program ToxRat professional, Version 3.2.1 was used. Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment groups in the oral toxicity test and to determine the NOED based on mortality.

The LD_{50} with 95% confidence limits could not be calculated in the oral toxicity test since the observed mortalities were below 50% in all test item groups. Statistical evaluation was not necessary in the oral toxicity test, since no mortality occurred in any test item treatment group or the control group.

II. RESULTS AND DISCUSSION

A. FINDINGS

In the control group fed with pure 50 % (w/v) aqueous sucrose solution, no mortality was observed at the final assessment after 48 hours. In the test item treatment group, no mortality was observed at any target dose 48 hours after start of feeding. No treatment related behavioural abnormalities were recorded during the 48 hour testing period at any target dose.

Table 0-7: Summary of oral acute toxicity of MON 0139 to the bumblebee

MON 0139	Oral toxicity test	
	[µg product/bumble bee]	[µg a.e./bumble bee]
LD_{50} (24 h)	>894	>412
LD_{50} (48 h)	>894	>412
NOED (48 h)	≥894	≥412

Validity criteria
The study is considered valid since the control and reference item validity criteria were met:
The mean control mortality was ≤10 % at the end of the test;
The mean reference item mortality was ≥50 % at the end of the test

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 hours oral LD_{50} for MON 0139 was determined to be >894 µg product/bumble bee, equivalent to >412 µg a.e./bumble bee. The NOED for mortality after 48 hours was determined to be ≥894 µg product/bumble bee, equivalent to ≥412 µg a.e./bumble bee.
The study is considered valid so LD_{50} >412 µg a.e./bumble bee and NOED ≥412 µg a.e./bumble bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

CA 8.3.1.1/2 Acute contact toxicity

1. Information on the study

Data point:	CA 8.3.1.1.2/001
Report author	
Report year	2003
Report title	Laboratory bioassays to determine acute oral and contact toxicity of MON 78623 to the honeybee, *Apis mellifera*
Report No	MON-02-10
Document No	-
Guidelines followed in study	EPPO guideline 170 (1992)
Deviations from current test guideline	Deviations from guideline OECD 214 (1998): Minor: - Relative humidity was slightly above the recommended range - No mortality assessed at 4 hours.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a laboratory study the acute contact toxicity of glyphosate K-salt to the honey bee, *Apis mellifera* L., were tested. Following a range finding test, a definitive test was conducted exposing worker bees to nominal doses of 100 µg glyphosate acid equivalent/bee.

Five replicate cages, each containing 10 bees, were used for the test item treatments, controls and three for the reference treatments. Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation. Corrected mortality for contact toxicity was 0%. No sublethal effects were observed except for one bee one hour after test item application. All validity criteria according to OECD 214 were fulfilled.

In conclusion, the toxicity of glyphosate K-salt was tested in an acute contact toxicity test on honey bees. The study is considered valid so LD_{50} >100 µg a.e./bee can be used for risk assessment purposes

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 78623
Description:	Amber liquid
Lot/Batch #:	GLP-0108-11688-F
Purity:	58 % K salt of glyphosate, equivalent to 47.3 % w/w glyphosate a.e.
Vehicle and/or positive control:	Vehicle for test item: Farmon Blue (87.3% w/w alkyl phenol ethylene oxide) / Positive control: Dimethoate technical grade
Test organisms:	
Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Roselea Apiaries, East Wellow, Hampshire
Diet/Food:	50 % w/v aqueous sucrose solution
Environmental conditions:	
Temperature:	25 – 26 °C
Humidity:	64 – 79 %
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	22 July – 27 July 2002

B. STUDY DESIGN

Experimental treatments
Following an initial range-finding test, the definitive test was conducted as a limit test with 100 µg glyphosate acid equivalent/bee, prepared in an appropriate carrier (0.05 % solution of the wetting agent Farmon Blue) and administered as a 1.0 µL droplet per bee (dorsal thorax) to each of ten bees in each of five cages per treatment. A vehicle control containing 0.05 w/v solution of Farmon Blue and deionised water and a toxic reference solution containing dimethoate were run in parallel. During the observation method a 50 % w/v aqueous sucrose solution was provided.

Observations
Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation.

Statistical calculations
Corrected mortality was calculated according to Abbott (1925). LC_{50} values were determined by Probit analysis and the 95 % confidence interval by Chi-square goodness of fit test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-1: Toxicity of glyphosate K-salt to honey bees (*Apis mellifera* L.) in the contact toxicity test

Dose [µg a.e./bee]	Mean intake of test item [µg a.e./bee]	Mortality [%]			
		1	3	24 h	48 h
Contact toxicity test					
Control	-	0	0	2	4
Farmon Blue control	-	0	0	2	4
100	-	0	0	2	2 (0)

In brackets the Abbot corrected mortality is given

B. OBSERVATIONS

Corrected mortality at 48 h was 0%. No sublethal effects were observed except for one bee one hour after test start, but it recovered by 3 h.
The determined contact 48h LD_{50} for the reference item dimethoate was 0.123 µg/bee for contact toxicity. These results are in line with published values, indicating that the test insects were of suitable sensitivity.

Deviations according to the current guideline OECD 214:

- Relative humidity was slightly above the recommended range
- No mortality assessed at 4 hours.

These deviations are not-expected to have a negative impact on the validity of the study which was valid at the time of conduct.

All validity criteria according to OECD 214 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of glyphosate was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >100 µg glyphosate acid equivalent/bee in the contact toxicity test.

The study is considered valid so LD_{50} >100 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.2/002
Report author	
Report year	2000
Report title	Acute Contact Toxicity of GLIFOSATO IPA TECNICO NUFARM to Honey Bee (*Apis mellifera* L.)
Report No	RF-D4.017/00
Document No	-
Guidelines followed in study	OECD Draft Proposal for a New Guideline: Honey bees, Acute Contact Toxicity Test (1996).
Deviations from current test guideline	Deviations from guideline OECD 214 (1998): Minor: - Mortality observation was not assessed at 4 hours - A water control and an undosed control were reported in chapter 5.7.4 (Experimental test), however results of only one (negative) control group were reported. - The temperature in test cages was higher than the expected range: 27-31 °C instead of 25±2 °C. - Humidity was lower than the expected range: 40-67% instead of 50-70 % - 24-hour LD_{50} with dimethoate is slightly above the requested range of 0.10-0.30 µg a.s./bee..
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In an acute laboratory study the contact toxicity of isopropylamine (IPA) salt of glyphosate to the honey bee, *Apis mellifera* L. was tested. Following a range finding test, adult worker bees were exposed to nominal dose rates of 10.0, 12.5, 24.0, 62.5 and 100.0 µg glyphosate IPA salt/bee. In addition, an untreated control was tested. Technical dimethoate was used as a reference item.

In the test, three replicate cages, each containing 10 bees, were used for the test item treatment, control and reference treatment. Mortality and sublethal effects were recorded at 24 and 48 hours after the treatment.

No significant mortality of bees was observed during the 48 hours observation period. In addition, no sublethal effects were observed. The validity criteria according to guideline OECD 214 are fulfilled.

In conclusion, under the conditions of the present test, the 48 hours contact LD_{50} of was determined to be >100 µg glyphosate IPA salt/bee, equivalent to >61.3 µg a.e./bee.

This study is considered valid in spite of slightly higher LD_{50} for the reference toxicant so o LD_{50} >61.3 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate isopropylamine salt (technical)
Description:	Not stated
Lot/Batch #:	MJRT 02 S 201 04
Purity:	612.7 g/kg salt equivalent (analysed)
Density:	Not stated
Vehicle and/or positive control:	Vehicle: water + acetone Positive control: technical dimethoate

Test organisms:

Species:	Honey bee (*Apis mellifera*)
Age:	Adult worker bees from healthy colonies
Source:	Apiario Silva Unit, Piracicaba, Brasil
Diet/Food:	Sucrose solution *ad libitum*
Acclimatisation:	At 25 ± 2 °C and 65 ± 5% relative humidity between collection of worker bees and test initiation (time span not stated)

Environmental conditions:

Temperature:	27 – 31°C
Relative humidity:	40 – 67%
Photoperiod:	24 hours darkness
Experimental dates	05 June – 14 June 2000

B. STUDY DESIGN

Experimental treatments

Based on the results of a range-finder test, bees in the main test were exposed to the nominal dose rates of 10.0, 12.5, 24.0, 62.5 and 100.0 µg glyphosate IPA salt/bee. The glyphosate concentration was analysed in each of the dosing solutions. In addition, an undosed control was tested. Technical dimethoate was used as a reference item. The test was conducted with 3 replicates chambers (inverted petri dish (50 mm depth x 100 mm diameter) per test concentration/control and 10 bees per cage. Bees were anaesthetised with carbon dioxide and counted onto filters papers inside each petri dish in groups of 5 until all chambers contained 10 bees. Bees were exposed to either the test material, the reference toxicant, water or acetone, by administering 1.0 µL of the appropriate substance to the ventral side of the thorax, using a micro syringe. After dosing the cages, a smaller inverted petri-dish containing sucrose solution was placed inside each chamber, and the chambers were covered with a 100-gauge mesh tissue 'lid' to prevent bee escape. All chambers were kept in darkness for 48 hours. Sucrose solution was available *ad libitum* throughout the whole test period.

Observations

Mortality and sublethal effects were recorded at 24 and 48 hours after treatment.

Validity criteria

For a test to be valid the following conditions apply:

- the average mortality for the total number of controls must not exceed 10% at the end of the test;
- the LD_{50} of the toxic standard meets the specified range.

Statistical calculations
Descriptive statistics for the test item. Data on mortality for dimethoate were analysed using Trimmed Spearman-Karber Method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The measured test concentrations ranged between 90.35 and 103.5% of the nominal values.

Table 0-2: Analytical results

Nominal concentration (g glyphosate IPA salt/L)	Measured concentration (g/L)	Concentration expressed as % of nominal (%)	% of deviation from the nominal
Control	-	-	-
10	10.350	103.50	3.50
12.5	12.778	102.22	2.22
24	23.722	98.84	1.16
62.5	60.369	96.59	3.41
100	90.350	90.35	9.65

Analytical data: Analytical determination of the test concentrations showed that the deviation from the nominal concentrations was below 20 %. Therefore, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

A summary of the mortality is provided below.

Table 0-3: Toxicity of glyphosate IPA salt to honey bees (*Apis mellifera*) in a contact toxicity test

Dose [µg glyphosate IPA salt/bee]	Mortality (mean of 3 replicates) [%]	
	24 h	48 h
Control (undosed)	0.0	0.0
10.0	0.0	0.0
12.5	0.0	0.0
24.0	0.0	0.0
62.5	0.0	0.0
100.0	3.33	3.33

Reference test: The determined 24 h LD_{50} for the reference item was 0.34 µg dimethoate/bee and 48 h LD_{50} for the reference item was 0.12 µg dimethoate/bee. These results show a toxicity level just above the ranges reported by the OECD guidelines.

B. OBSERVATIONS

No sub-lethal effects were observed up to a dose of 100 µg glyphosate IPA salt/bee, equivalent to 61.3 µg a.e./bee. The highest dose that showed no lethal effect was 62.5 µg glyphosate IPA salt/bee.

The test is considered to be valid because the negative control mortality did not exceed 10 % (actual value: 0 %) and the 24-hour LD_{50} of the toxic standard was slightly above the range of 0.10-0.30 µg a.s./bee specified in the guideline 214 (actual value: 0.34 µg dimethoate/bee).

The following points are deviated from the current guideline but are not expected to have any negative on the study validity:

- Mortality observation was not assessed at 4 hours
- A water control and an undosed control were reported in chapter 5.7.4 (Experimental test), however results of only one (negative) control group were reported.
- The temperature in test cages was higher than the expected range: 27-31 °C instead of 25 ± 2 °C.
- Humidity was lower than the expected range: 40 – 67 % instead of 50 – 70 %.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate IPA salt was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >100 µg glyphosate IPA salt/bee, equivalent to >61.3 µg a.e./bee.

This study is considered valid in spite of slightly higher LD_{50} for the reference toxicant so o LD_{50} >61.3 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.3.1.1.2/003
Report author	
Report year	1998
Report title	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*)
Report No	FN9700
Document No	-
Guidelines followed in study	EPPO guidelines (1992) OPPTS 850.3020 Draft OECD 213 (1997) and Draft OECD 214 (1997)
Deviations from current test guideline	Deviation from guideline OECD 214 (1998): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In an acute laboratory study the contact toxicity of glyphosate acid to the honey bee, *Apis mellifera* L., was tested. Following a range finding test, a definitive test was conducted exposing female worker bees to nominal doses of 0.0984, 0.984, 9.84 and 103 µg glyphosate acid/bee.
Three replicate cages, each containing 10 bees, were used for the test item treatments, controls and reference treatments. Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation for contact toxicity.
No mortality of bees or sub-lethal effects were observed after 48 hours of exposure in the test item and the control groups during the 48 hours test period. All validity criteria according to OECD 214 were fulfilled as mortality in the control group did not exceed 10% (actual 0%) and the LD_{50} of the toxic standard met the specified range.
In conclusion, the toxicity of glyphosate acid was tested in an acute contact and an oral toxicity test on honey bees.
The study is considered valid so LD_{50} >103 µg a.s./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Technical Glyphosate acid
Description:	White powder
Lot/Batch #:	TSC 0521/05148
Purity:	97.6 %

Vehicle and/or positive control:
Vehicle for test item: Agral 90
Vehicle for positive control: Triton X100
Positive control: Dimethoate (BASF 40 lot 083.10/96)

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Own colony
Diet/Food:	Not stated

Environmental conditions:

Temperature:	25 ± 1 °C
Humidity:	65 ± 5 %
Photoperiod:	24 hours darkness (except during observation)

Experimental dates: 24 August - 04 September 1998

B. STUDY DESIGN

The definitive test was conducted with 0.0984, 0.984, 9.84 and 103 µg glyphosate acid/bee prepared in an appropriate carrier (deionised water containing 500 mg/L of the wetting agent Agral 90) and administered as a 1.0 µL droplet per bee (dorsal thorax) to each of ten bees in each of three cages per treatment.. A control with 500 mg Agral 90/L and a toxic reference solution containing 1g Triton X100/L were run in parallel. During the observation method a 50 % w/v aqueous sucrose solution was provided.

Observations
Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation for contact toxicity.

Statistical calculations
Doses and LD_{50} calculations were based on the analysed content of glyphosate acid. The mortality results were analysed using a probit programme (toxic reference treatment).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-4: Toxicity of glyphosate acid to honey bees (*Apis mellifera*) in the contact toxicity test

Dose [µg test item/bee]	Mean intake of glyphosate acid [µg a.s./bee]	Mortality [%]		
		24 h	48 h	72 h
Contact toxicity test				
Control	-	0	0	0
0.0984	-	0	0	0
0.984	-	0	0	0
9.84	-	0	0	0
103	-	0	0	0

B. OBSERVATIONS
No mortality of bees was observed in the 48 hours test period. No sub-lethal effects were observed in the test item group and the control group during the 48 hours test period.

All validity criteria according to OECD 214 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of glyphosate acid was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >103 µg glyphosate acid/bee.
The study is considered valid so LD_{50} >103 µg a.s./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.2/004
Report author	
Report year	1996
Report title	Glyphosate: Acute contact and oral toxicity to honeybees.
Report No	1413/3-1018
Document No	-
Guidelines followed in study	EPPO Guideline No. 170: Test methods for evaluating the side-effects of plant protection products on honeybee (1992)
Deviations from current test guideline	Deviations from guideline OECD 214 (1998): Minor: - Mortality observation was not assessed at 4 hours - The relative humidity exceeded the recommended values
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

In an acute laboratory study the contact toxicity of glyphosate to honeybee, *Apis mellifera* was tested. After a preliminary dose range-finding test, adult worker bees were treated with 0, 0.625, 1.25, 2.5, 5.0, 10 and 20 µg glyphosate/bee in the contact test. Three replicate cages, containing 10 bees each, were used. Mortalities and sub-lethal effects were made 1, 4, 24 and 48 h after treatment. No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period. The validity criteria according to current OECD guideline 214 are fulfilled.

In conclusion the 24 and 48-hour oral LD_{50} values for glyphosate were >20 µg a.s./bee for contact exposure (nominal).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: Glyphosate
Description: White powder
Lot/Batch #: H95 D161A
Purity: 95.3 %

Vehicle and/or positive control:

Vehicle: Headland Enhance LF + reverse-osmosis water
Positive control: formulated Dimethoate (BASF Dimethoate 40 EC)

Test organisms:

Species: Honey bee (*Apis mellifera*)
Age: Adult worker bees
Source: The Bee Farm, Wetherby, West Yorkshire, UK
Diet/Food: 50 % sucrose solution *ad libitum*
Acclimatisation: Not stated

Environmental conditions:

Temperature:	24.5 - 25.8°C
Relative humidity:	49.1 - 86.0%
Photoperiod:	darkness
Experimental dates:	27 June – 06 July 1996

B. STUDY DESIGN

Experimental treatments
To determine the test concentrations for the definitive study a range-finding test was performed. The nominal doses of glyphosate used for the range-finding test were 0, 0.1, 1, 10 and 20 µg a.s./bee for contact dosing.
Bees were anaesthetised with carbon dioxide. Contact doses were applied as a 1.0 µL droplet of the test solution was placed on the dorsal thorax of each bee. The nominal doses of glyphosate used for the definitive test contact were 0, 0.625, 1.25, 2.5, 5.0, 10 and 20 µg a.s./bee. The nominal dose of 20 µg a.s./bee was given as a double droplet application (2 × 1 µL). Three replicate cages, containing 10 bees each, were used.

Observations
Assessments of mortality and sub-lethal effects were conducted 1, 4, 24 and 48 h after treatment.

Statistical calculations
Descriptive Statistics; the LD_{50} values of the toxic standard, dimethoate, were calculated by Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS
No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period. The 48 h LD_{50}-value for dimethoate was calculated to be 0.452 µg a.s./bee (95 % confidence limits: 0.374 to 0.557) for contact exposure.

Deviations according to the current guideline OECD 214:

- Mortality observation was not assessed at 4 hours
- The relative humidity exceeded the recommended values

These deviations are not expected to have a negative impact on the validity of the study which was valid at the time of conduct.
The test is considered to be valid according to OECD guideline 214 as mortality in the negative control did not exceed 10 % after 48 hours. In addition, the LD_{50} for the reference item met the specified range.

II. CONCLUSIONS

Assessment and conclusion by applicant: The toxicity of glyphosate was tested in an acute contact toxicity test on honey bees. The contact LD_{50} (24 h/48 h) values for glyphosate were >20 µg a.s./bee for contact exposure (nominal). The study is considered valid so LD_{50} >20 µg a.e./bee can be for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.2/005
Report author	
Report year	1995
Report title	Testing Toxicity to Honeybee - *Apis mellifera* L. (laboratory) according to EPPO Guideline No 170. Glyphosate (tec.)
Report No	95 10 48 065
Document No	-
Guidelines followed in study	EPPO Guideline No. 170
Deviations from current test guideline	Deviations from guideline OECD 214 (1998): Minor: - Mortality observation was not assessed at 4 hours.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a laboratory study, the acute contact toxicity of technical glyphosate to the honey bee, *Apis mellifera* L. was tested. Adult worker bees were exposed to two nominal test doses of 100 and 200 µg a.s/bee.
In the test, three replicate cages, each containing 10 bees were used for the test item treatment, control and reference treatment. Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after treatment initiation.
In the contact exposure test, there was no bee mortality recorded during the 48 hours test period at both test rates. In addition, no behavioural abnormalities were observed in test item groups and control groups during the whole test period. All validity criteria according to the OECD guideline 214 was fulfilled.
The study is considered valid so LD_{50} >200 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	Not stated
Lot/Batch #:	01/07/95
Purity:	98.2 % a.s.
Vehicle and/or positive control:	Positive control: Dimethoate EC 400, containing 411.14 g a.s./L Vehicle: Extravon (surfactant)

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Purchase from the bee-keeper Mr Weimann/Gottscheina
Diet/Food:	50 % aqueous sucrose solution *ad libitum* (except for 1 – 2 hours prior to oral test initiation)

Environmental conditions:

Temperature:	25 – 26 °C
Humidity:	53 – 70 %
Photoperiod:	8 hours diffuse light/16 hours darkness
Experimental dates:	21 August – 01 September 1995

B. STUDY DESIGN

Experimental treatments

The contact toxicity test was performed at two nominal test doses of 100 and 200 µg a.s./bee, with the test substance dissolved into a 1 % watery solution/surfactant Extravon. A negative control group where bees were exposed to 0.1 % Extravon only was also included. Dimethoate was used a toxic reference, at test doses ranging from 0.0313 to 1.0 µg/bee. The contact toxicity test was conducted in triplicate using 10 bees per replicate (30 bees). For contact toxicity test, test solutions containing appropriate concentrations of technical glyphosate were dosed to bees by thorax injection. After administration of the test substance, the bees were provided with 50% sucrose solution.

Observations

Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after test start.

Statistical calculations

Descriptive statistics

II. RESULTS AND DISCUSSION

A. FINDINGS

The LD_{50} value is given below based on nominal concentrations.

Table 0-5: Toxicity of technical glyphosate to honey bees in a contact toxicity tests

Endpoints (48 h)	Technical glyphosate [µg a.s./bee]
Contact LD_{50}	>200

B. OBSERVATIONS

No biologically relevant mortality of bees was observed during the 48-hour test period for test concentrations of up to 200 µg a.s./bee, which was the highest concentration tested. In addition, no behavioural abnormalities were observed at any test item concentration and in the control groups.
For the toxic reference dimethoate, the highest test doses caused 97 % mortalities for contact test.

Table 0-6: Mortality of honey bees in a contact toxicity tests

Test	Time [h]	Mortality [%]			
		Control	Technical glyphosate [µg a.s./bee]		Toxic reference [µg a.s./bee]
		-	100	200	Highest test dose
Contact	24	0	3	3	97
	48	0	0	0	97

Deviations according to the current guideline OECD 214:

- Mortality observation was not assessed at 4 hours;

This deviation is not expected to have a negative impact on the validity of the study.

The validity criteria according to the OECD guideline 214 was fulfilled as the mortality in the control was <10 % at test termination.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of technical glyphosate was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >200 µg a.s/bee.
The study is considered valid so LD_{50} >200 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.3.1.1.2/006
Report author	
Report year	1995
Report title	Honey Bees (*Apis mellifera* L.), contact toxicity study in the laboratory with Glyphosate
Report No	142335
Document No	-
Guidelines followed in study	EPPO guidelines 22, 203 – 215 (1992)
Deviations from current test guideline	Deviations from guideline OECD 214 (1998): Minor: - Mortality observation was not assessed at 4 hours - Humidity was lower than the expected range: 34-40 % instead of 50-70 % - Test extended to 72h with no rising of mortality of 10 %. Additional assessment in regards to guideline requirement. - Water control was not setup.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In an acute laboratory study the contact toxicity of glyphosate technical material (96 % purity) to the honey bee, *Apis mellifera* L., was tested. Following a range finding test, adult worker bees were exposed to a single nominal dose of 100 µg a.s./bee.
In the test, three replicate cages, each containing 10 bees, were used for the test item treatment, control and reference treatment. Mortality and paralysis effects were recorded at least at the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment.
No mortality of bees was observed after 72 hours of exposure. In addition, no paralysis was observed in the test item and the control groups during the 72 hours test period. The validity criteria according to guideline OECD 214 are fulfilled.
In a contact toxicity test, no effects of glyphosate on the mortality and the paralysis of honey bees were observed at concentrations up to and including 100 µg a.s./bee.
The study is considered valid so LD_{50} >100 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: GLYFOSAAT (Spelling for report: GLYPHOSATE)
Description: White powder
Lot/Batch #: 22021
Purity: 96 %

Vehicle and/or positive control:

Vehicle: Tap water
Positive control: Parathion 25 % liquid

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Naïve worker bees
Source:	Research Centre for Insect Pollination and Beekeeping, "Ambrosiushoeve"
Diet/Food:	50 % aqueous sucrose solution *ad libitum* (except during treatment)

Environmental conditions:

Temperature:	24 – 25 °C
Humidity:	34 – 40 %
Photoperiod:	24 hours darkness (except during observation)

Experimental dates: 20 March - 25 March 1995

B. STUDY DESIGN

Experimental treatments
Prior to the main test, a range-finding test was performed exposing adult bees to nominal concentrations of 1.0, 10, 50 and 99 µg a.s./1 µL acetone. The definitive test was conducted as a limit test with a single nominal concentration of 100 µg a.s./1 µL acetone. All test solutions were prepared in an acetone solution. In addition, a control constituted of acetone and the reference substance (Parathion 25 % liquid) were tested. For the definite test, adult worker bees were exposed in triplicates (10 bees/test cage) to the test item, control and reference item. After the test substance was applied on the ventral part of the thorax of the bees with a micropipette (1mm^3/bee), then the bees were provided with sucrose solution 50 %.

Observations
Mortality, paralysis and any other abnormalities were recorded at least the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment start.

Validity criteria
For a test to be valid the following conditions apply:

- the average mortality for the total number of controls must not exceed 10% at the end of the test;
- the LD_{50} of the toxic standard meets the specified range.

Statistical calculations
Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS
The test solution containing a concentration of 100 µg a.s./bee was administered on the ventral part of the thorax of the bees. A summary of the mortality is provided below.

Table 0-7: Toxicity of glyphosate to honey bees (*Apis mellifera* L.) in a contact toxicity test

Dose [µg a.s./bee]	Mortality [%]		
	24 h	48 h	72 h
Control (Acetone)	0.00	0.00	0.00
100	0	0	0

B. OBSERVATIONS

No mortality of bees was observed during the 72 hours test period for the test concentration of 100 µg a.s./bee. In addition, no paralysis was observed in the test item group and the control group during the 72 hours test period.

Deviations according to the current guideline OECD 214 (1998):

- Mortality observation was not assessed at 4 hours
- Humidity was lower than the expected range: 34-40 % instead of 50-70 %
- Test extended to 7 2h with no rising of mortality of 10 %. Additional assessment in regards to guideline requirement.
- Water control was not setup.

These deviations are not expected to have a negative impact on the validity of the study.

All validity criteria according to OECD 214 were fulfilled, since the average mortality in the control group did not exceed 10 % (actual value: 0 %) and the 24-hour LD_{50} of the toxic standard meets the standard of less than 1.0 µg a.s./bee based on historical data (actual value: 0.4 µg a.s./bee)..

In an contact toxicity test, glyphosate had no effects on mortality of honey bees at concentrations of up to and including 100 µg a.s./bee.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate was tested in an acute contact toxicity test on honey bees. The LD_{50} (72 h) was >100 µg a.s/bee.

The study is considered valid so LD_{50} >100 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.2/007
Report author	
Report year	1972
Report title	The acute contact and oral toxicities of CP67573 and MON2139 to worker honey bees
Report No	HU85X094
Document No	-
Guidelines followed in study	Working Document 13 produced by the UK Pesticide Safety Precautions Scheme
Deviations from current test guideline	Deviations from guideline OECD 214 (1998): Major: - Mortality in the control was >10 % at test termination Minor: - Only 2 replicates (10 replicates only for the highest concentration tested) per treatment group - No additional solvent control was tested - Duration of starvation was not reported - Mortality observation was not assessed at 4 hours.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed.
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

* Two test materials were assessed in this study; namely CP67573 and MON2139 (a 36% w/v formulation). MON2139 contains a surfactant that is not present in the representative formulation for the Annex I renewal. This summary therefore only contains information on CP67573 (glyphosate technical).

2. Full summary

Executive summary

The contact toxicity of CP67573 (glyphosate technical) to young adult worker bees (*Apis mellifera* L.) was determined in a limit tests performed at a nominal dose of 100 µg CP67573/bee. The test comprised 10 replicate mesh cages, each containing 10 bees. In a parallel test, honey bees were exposed to a reference item in a dose response test using dimethoate at concentrations ranging from 0.048 to 0.117 µg dimethoate/bee. In both tests, the test substance was applied as 1.0 µL drops onto the ventral thorax of CO_2 anaesthetised bees, dissolved in 50% acetone. Control groups consisting of 2 cages of 10 bees were included alongside each of the tests. Assessments of mortality were conducted after 24 and 48 hours. The validity criteria according to OECD guideline 214 were not fulfilled as mortality in the control was > 10% at test termination.

In the 100 µg CP67573/bee treatment group, at 24 and 48 hours, there was 8 % and 38 % mortality, with corresponding mortality in the control group of 5 % and 15 % respectively.

This resulted in overall control corrected mortality levels of 3 and 27 % achieving a 48 hour LD_{50} of >100 µg CP67573/bee.

The study is considered invalid so endpoints cannot be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: CP67573 (technical active ingredient)

Description: Not stated

Lot/Batch #:	No batch details presented in report
Purity:	Not stated
Density:	Not stated
Vehicle and/or positive control:	Vehicle: 50 % acetone Positive control: Dimethoate
Test organisms:	
Species:	Honey bee (*Apis mellifera*)
Age:	Young adult worker bees
Source:	Experienced apiarist in Huntingdonshire, U.K
Diet/Food:	Bees were fed with 20 % sucrose
Acclimatisation:	Not reported
Environmental conditions:	
Temperature:	26 – 27 °C
Relative humidity:	Not reported
Photoperiod:	Not reported
Experimental dates:	Not reported

B. STUDY DESIGN

Experimental treatments
Honey bees were exposed topically to CP67573 in a limit test conducted at 100 µ test item/bee, in nylon coated 2 mm wire mesh tubes, with 11.5 cm high and 3.5 cm in diameter, closed by corks at both ends. In the contact toxicity test, CP67573 was dissolved in 50% acetone and was applied as 1.0 µL droplets (containing 100 g test item/L) to the ventral thorax of CO_2-anesthetised bees using a micro-applicator. There were 10 cages per test item treatment, with two control cages containing 10 worker bees each. A reference item dose-response test (dimethoate) was conducted in parallel, at five test rates between 0.13 and 0.29 µg test item/bee, with two cages of ten bees per treatment and control group.

Mortality in the test or reference item treatment groups, were corrected for control mortalities using Abbot's correction, to give overall control corrected levels of mortality, on which the endpoint LD_{50} values were based.

Observations
Mortality was recorded 24 and 72 hours after test initiation.

Statistical calculations
Descriptive statistics; LD_{50} for dimethoate were obtained by graphical interpolation on probability/log paper, confidence limits were calculated according to Litchfield & Wilson (1949).

II. RESULTS AND DISCUSSION

A. FINDINGS
A summary of the mortality results is provided below.

Table 0-8: Toxicity of glyphosate to honey bees (*Apis mellifera* L.) in a contact toxicity test

Endpoints (48 h)	CP67573 [µg a.s./bee]
LD_{50} contact	>100

Table 0-9: Contact toxicity of CP67573 to honey bees (*Apis mellifera* L.)

Exposure	Mortality [%]		Corrected mortality * [%]
	Control	100 µg a.s./bee	
contact (24 h)	5	8	3
contact (48 h)	15	38	27

B. OBSERVATIONS

In the test with CP67573, the corrected bee mortality did not reach or exceed 50 % (max mortality was 27 %), resulting in overall control corrected mortality levels of 3 and 27 % at 24 and 48 hour respectively, achieving a 48 hour LD_{50} of >100 µg CP67573/bee.

In the reference item test with dimethoate, a 48 hour contact exposure LD_{50} value of 0.16 µg dimethoate/bee (95 % C.I. of 0.14 - 0.19 µg dimethoate/bee) was observed.

Deviations according to the current guideline OECD 214:

- Only 2 replicates (10 replicates only for the highest concentration tested) per treatment group
- No additional solvent control was tested
- Duration of starvation was not reported
- Mortality observation was not assessed at 4 hours.

These deviations are not expected to have a negative impact on the validity of the study.

- Mortality in the control was >10% at test termination.

This deviation has a negative impact on the validity of the study.

The validity criteria according to the OECD guideline 214 were not fulfilled as mortality in the control was >10% at test termination.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate technical (CP67573) was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >100 µg a.s./bee. The contact LD_{50} for honey bees exposed to MON2139 were determined to be >100 µg a.s./bee.

The study is considered invalid so endpoints cannot be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.3.1.1.2/008
Report author	
Report year	2017
Report title	MON 0139: Acute Oral and Contact Toxicity to the Bumble Bee, *Bombus terrestris* L. under Laboratory Conditions.
Report No	S16-06634
Document No	-
Guidelines followed in study	Based on the proposal for new OECD Guidelines: Bumblebee, acute oral toxicity test (2016) and Bumblebee, acute contact toxicity test (2016)
Deviations from current test guideline	Deviations from guideline OECD 246 (2017): Minor: - analytical verification of dose is missing, however this was not a requirement at the time of study conduct.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
Bumblebees (*Bombus terrestris*) were exposed to MON 0139 via contact administration, i.e. cuticular absorption following the application of a droplet to the dorsal body surface of a solution in deionised water. Adult bees were treated with 62.5, 125, 250, 500 and 1000 µg test item/bumble bee. Mortality was recorded 4 and 24 hours after application and thereafter at 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. No mortality was recorded at the end of the test in the 62.5, 250, 500 and 1000 µg test item/bumble bee treatment groups, however, 3.3% mortality (one dead bee) was observed in the 125 µg test item/bumble bee treatment. The 48 hours contact LD_{50} (Lethal Dose causing 50% mortality) for MON 0139 was determined to be > 1000 µg test item/bumble bee (equivalent to >461 µg a.e./bumble bee). The NOED for mortality after 48 hours was determined to be ≥1000 µg test item/bumble bee (equivalent to ≥461 µg a.e./bumble bee). The study was considered valid as there was no mortality in the control group and in the toxic reference group (dimethoate at 13 µg a.s./bumble bee) 100% mortality was observed.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Test item:	MON 0139
Lot/Batch #:	GLP-1503-23921-T
Actual content of active ingredients:	Glyphosate: 46.1% (a.e.); 574.4 g/ml
Description:	liquid/slightly yellow
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	February 13, 2018
Density:	1.2460 g/cm^3

Treatments

Test rates:	Target doses: 62.5, 125, 250, 500 and 1000 µg test item/bumble bee, equivalent to 28.8, 57.6, 115, 231 and 461 µg a.e./bumble bee
Control:	Deionised water
Toxic standard:	BAS 152 11 I (dimethoate, analysed 405.2 g a.s./L) 13 µg a.s./bumble bee

Test organisms

Species:	*Bombus terrestris* L. (Hymenoptera: Apidae)
Source:	From healthy colony owned and maintained by Biobest Belgium, Ilse Velden 18, 2260 Westerlo, Belgium.
Food:	50% w/v aqueous sucrose solution

Test design

Test cage description:	Nicot cages
Replication:	30
No. of bees/arena:	1
Duration of test:	48 hours

Environmental conditions

Temperature:	24.8 – 25.3°C
Humidity:	50.9 ± 60.4 %
Photoperiod:	Darkness (except during application and observations)

Experimental dates: 10 April - 13 April 2017

B. STUDY DESIGN

Experimental treatment

Adult worker bumblebees (*Bombus terrestris*) were exposed to MON 0139 via two routes of administration: (1) contact, i.e. cuticular absorption following the application of a droplet to the dorsal body surface of a solution in deionised water. To immobilise the bees during the course of treatment, they were anaesthetised using short bursts of CO_2.

Bumblebees were treated with one 2 µl drop of the test solution, control or toxic standard applied to the dorsal surface of the thorax using a micro applicator. The bumblebees were returned to the test unit, allowed to recover and kept in the CE room with a continuous supply of 50 % w/v aqueous sucrose solution.

Assessments

Mortality was recorded 4 and 24 hours after application (after application in the contact toxicity test) and thereafter at 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. In the reference item group, behavioural assessments were not conducted as it was assumed that moribund and affected bumble bees of the reference item group would die by the end of the test.

Statistics

For the statistical evaluation the statistics program ToxRat professional, Version 3.2.1 was used. Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment groups in the contact toxicity test and to determine the NOED based on mortality.

The LD_{50} with 95 % confidence limits could not be calculated in the contact toxicity test since the observed mortalities were below 50 % in all test item groups.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-10: Summary of contact acute toxicity of MON 0139 to the bumblebee

MON 0139	Contact toxicity test	
	[µg test item/bumble bee]	[µg a.e./bumble bee]
LD_{50} (24 h)	>1000	>461
LD_{50} (48 h)	>1000	>461
NOED (48 h)	≥1000	≥461

B. OBSERVATIONS

In the control group treated with deionised water, no mortality occurred during the 48 hours test period. In the test item treatment group, no mortality was recorded at the end of the 48 hours test period in the 62.5, 250, 500 and 1000 µg test item/bumble bee treatment groups. 3.3 % mortality was observed in the 125 µg test item/bumble bee treatment groups after 48 hours (corresponding to 1 dead bumble bee). No behavioural abnormalities were recorded during the 48 hours testing period at any target dose.

Deviations according to the current guideline OECD 246 (2017):

- analytical verification of dose is missing, however this was not a requirement at the time of study conduct.

Validity criteria

The study is considered valid since the control and reference item validity criteria were met:
The mean control mortality was ≤10 % at the end of the test (actual 0 % mortality)
The mean reference item mortality was ≥50 % at the end of the test (actual 100 % mortality)

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 hours contact LD_{50} for MON 0139 was determined to be >1000 µg test item/bumble bee, equivalent to >461 µg a.e./bumble bee. The NOED for mortality after 48 hours was determined to be ≥1000 µg test item/bumble bee, equivalent to ≥461 µg a.e./bumble bee.
The study is considered valid so LD_{50} >461 µg a.e./bumble bee and NOED ≥461 µg a.e./bumble bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.3.1.1.2/009
Report author	
Report year	2017
Report title	MON 0139: Acute Contact Toxicity to the Solitary Bee, *Osmia bicornis* under Laboratory Conditions
Report No	S17-00083
Document No	-
Guidelines followed in study	Based on OEPP/EPPO 170 (4) (2010), OECD 214 (1998) and the minutes of the ICPPR Non-Apis bees workshops (2014, 2015, 2016 and 2017)
Deviations from current test guideline	No specific test guideline available.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
Solitary bees (*Osmia bicornis*) were exposed to MON 0139 by topical application to the thorax following an adapted version of OECD 214. A hand operated micro-applicator was used for contact application of the treatment groups. Adult bees were treated with 62.5, 125, 250, 500 and 1000 µg glyphosate/bee. Three replicate cages each containing 10 bees each were used. Mortality was recorded 4 hours after application and thereafter at 24 hours and 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. Mortality in all glyphosate treated groups was low and did not exceed 6.67% 48 hours after treatment. The 48 hours contact LD_{50} for MON 0139 was determined to be >1000 µg test item/bee (equivalent to >461 µg a.e./bee). The NOED for mortality after 48 hours was determined to be ≥1000 µg test item/bee (equivalent to ≥461 µg a.e./bee). The study was considered valid as there was no mortality in the control group and the toxic reference group (dimethoate at 10 µg a.s./bee) 86.7% mortality was observed.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Test item:	MON 0139
Lot/Batch #:	GLP-1503-23921-T
Actual content of active ingredients:	Glyphosate: 46.1% (a.e.); 574.4 g/ml
Description:	liquid / slightly yellow
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	February 13, 2018
Density:	1.2460 g/cm^3

Treatments

Test rates:	62.5, 125, 250, 500 and 1000 µg test item/bee, equivalent to 28.8, 57.6, 115, 231 and 461 µg a.e./ bee

Control:	Deionised water
Toxic standard:	BAS 152 11 I (dimethoate, analysed 405.2 g a.s./L) 10 µg a.i./bee
Administration:	Topical application in the torax of 2 µL droplet of the application solution with a hand operated micro-applicator

Test organisms

Species:	*Osmia bicornis* (Linnaeus) (Hymenoptera: Apidae)
Source:	Commercial supplier (WAB-Mauerbienenzucht, Sonnentauweg 47, 78467 D-Konstanz, Germany)
Food:	50% w/v aqueous sucrose solution containing 0.1% anise oil

Test design

Test cage description:	Plastic boxes 13 x 17 cm, height: 6cm
Replication:	3
No. of bees/arena :	10
Duration of test:	48 hours

Environmental conditions

Temperature:	Target: 19.2 – 20.3 °C Exposure: 19.1 – 20.4 °C
Humidity:	Target: 50 – 70 % Exposure: 64.4 ± 79.4[1] % [1] Deviations ≥2 hours without impact on the outcome of the study
Photoperiod:	16 hours light : 8 hours dark

Experimental dates: 10 May to 12 May 2017

B. STUDY DESIGN

Experimental treatments
Solitary bees were exposed to MON 0139 by topical application to the thorax. A hand operated micro-applicator was used for application of the treatment groups. The application amount was 2 µL/bee. After anaesthetising the bees by cooling for ~ 1 hour in the refrigerator (~ 10°C) the 2 µL droplet of the application solution was applied individually to the dorsal side of the thorax of each bee. After the application, the bees were returned to the test units, allowed to recover and were fed with a continuous supply of 50 % w/v aqueous sucrose solution with anise oil (0.1 %). Anise oil was used to attract the bees to the food source (phagostimulant).

Assessments
Mortality was recorded 4 hours after application and thereafter at 24 hours and 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. In the reference item group, behavioural abnormalities assessments were not conducted as it can be assumed that moribund and affected bees of the reference item group died by the end of the test.

Statistics
Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment group and to determine the NOED based on mortality. The LD_{50} with 95% confidence limits could not be calculated since the observed mortalities were below 50 % in all test item groups. Statistical calculations were made by using the statistical program TOXRAT Professional 3.2.1.

II. RESULTS AND DISCUSSION

A. FINDINGS

In the control group treated with deionised water no mortality occurred during the 48-hour test period. After the 24 hour assessment two bees escaped through a hole in the lid of one cage of the control group. As none of the remaining bees showed any effects, and all the remaining bees in the control group survived the impact was deemed minor and the study objective was still achieved.

Table 0-11: Summary of contact acute toxicity of MON 0139 to solitary bee

MON 0139	**Contact toxicity test**	
	[µg test item/bee]	**[µg a.e./bee]**
LD_{50} (24 h)	>1000	>461
LD_{50} (48 h)	>1000	>461
NOED (48 h)	≥1000	≥461

B. OBSERVATIONS

Mortalities of 0.0, 0.0, 3.3, 6.7 and 6.7 % were recorded at the dose levels of 62.5, 125, 250, 500 and 1000 µg product/bee at the end of the 48-hour test period, respectively. No exceptional behavioural abnormalities were recorded throughout the test (one affected bee at the dose level of 62.5 µg test item/bee 48 hours after start of exposure).

Validity criteria

There was no bee mortality in the control group over the 48-hour duration of the test. In the reference item group of the contact toxicity test (deionised water containing dimethoate), 86.7% mortality was observed at the end of the 48 hours test period. Consequently, validity criteria for both control (average mortality ≤ 20%) and reference item mortality (mean mortality ≥ 50%) were met and the test was considered valid.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 hours contact LD_{50} for Solitary Bee, *Osmia bicornis* exposed to MON 0139 was determined to be >1000 µg test item/bee, equivalent to >461 µg a.e./bee. The NOED for mortality after 48 hours was determined to be ≥1000 µg test item/bee, equivalent to ≥461 µg a.e./bee.

The study is considered valid so LD_{50} >461 µg a.e./bee and NOED ≥461 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

CA 8.3.1.2 Chronic toxicity to bees

Studies considering the effects of glyphosate on the chronic toxicity to bees were assessed for their validity to current and relevant guidelines and are presented in the following table.

Table 0.3.1.2-1: Chronic toxicity studies of glyphosate to pollinators

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.3.1.2/001	, 2017	Chronic adult	*Apis mellifera L.*	Glyphosate IPA-salt	Valid	

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the chronic impact of glyphosate or its relevant metabolites on bees. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. For discussions of literature regarding toxicity to bees, please refer to document M-CP Section 10.3.

Endpoints of studies considered valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.3.1.2-3: Endpoints: Chronic toxicity of glyphosate to pollinators

Reference	Test item	Species	Test design/ GLP	LDD_{50} (µg a.e./bee/d)	NOEDD (µg a.e./bee/d)
, 2017 CA 8.3.1.2/001	**Glyphosate IPA-salt**	***Apis mellifera***	**Chronic, adult 10 days**	**>179.9**	**179.9**

a.e.: acid equivalents
Endpoints in **bold** is used for risk assessment

Study summaries are provided below.

1. Information on the study

Data point	CA 8.3.1.2/001
Report author	
Report year	2017
Report title	MON 0139: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera* L.) in the Laboratory
Report No	118401136
Document No	-
Guidelines followed in study	OECD (2016), Proposal for a New Guideline for the Testing of Chemicals. Honey Bee (*Apis mellifera* L.), Chronic Oral Toxicity Test. 10 Day Feeding Test in the Laboratory, OECD Publishing, Paris, February 2016
Deviations from current test guideline	Deviation from guideline OECD 245 (2017): none

Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive Summary
To evaluate the chronic effects of the test item on honey bees, a 10 days chronic oral feeding test in the laboratory (dose response test) was performed. Young honey bees were provided with 5 concentrations (256, 640, 1600, 4000, 10000 mg a.s./kg) of the test item treated sugar solutions ad libitum over a period of 10 days. An untreated control and a reference item (BAS 152 11 I; 400 g/L dimethoate) were included in this study. For the study 3 replicates per treatment were used, each consisting of 10 bees per test cage. The number of dead bees in each test replicate was assessed daily until test end (Day 0 – Day 10). Behavioural abnormalities were assessed daily until test end (Day 1 to Day 10). Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded. The food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed on the following assessment day. The quantification of the active ingredient glyphosate of the test item MON 0139 in the feeding solutions was performed using HPLC-method with UV-detection indicating actual doses of 5.6, 10.2, 38.6, 98.0 and 179.9 µg a.s./bee/day (corrected for evaporative losses). Ten days following the start of chronic exposure 3.3 and 6.7 % mortality occurred in the 10000 and 640 ppm (179.9 and 10.2 µg a.s./bee/day) treatment groups, respectively. No mortality occurred in the other test item treatments (4000, 1600 and 256 mg a.s./kg feeding solution). There was 6.7 % mortality in the control (50 % w/v sucrose solution). No behavioural abnormalities occurred following treatment with MON 0139 at any time during the trial.
The chronic oral toxicity of MON 0139 was tested over 10 days.
The LC_{50} value (10 days) was >10000 mg a.s./kg feeding solution.
The LDD_{50} value (10 days) was >179.9 µg a.s./bee/day.
The NOEC and NOEDD values (10 days) were 10000 mg a.s./kg feeding solution and 179.9 µg a.s./bee/day, respectively.
The study is considered valid so LDD_{50} >179.9 µg a.s./bee/day and NOEDD of 179.9 µg a.s./bee/day can be used for the risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	MON 0139
Lot/Batch #:	GLP-1503-23921-T
Actual content of active ingredients:	Glyphosate: 46.1 % (w/w) 574.4 g glyphosate IPA salt/L (analytical), according to certificate of analysis
Description:	Slightly yellow liquid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	February 13, 2018
Density:	1.246 g/cm³ (according to Sponsor); 1.24 g/cm³ (according to MSDS)

Treatments

Test rates:	Concentrations: 256, 640, 1600, 4000, 10000 mg a.s./kg feeding solution Nominal target dose per bee/day: 6.4, 16, 40, 100 and 250 µg a.s./bee/day Actual dose per bee/day: 5.6, 10.2, 38.6, 98.0 and 179.9 µg a.s./bee/day

Control:	50 % w/v sucrose solution (500 g sucrose/L deionised water)
Toxic standard:	BAS 152 11 I (nominally 400 g dimethoate/L; analytical 405.2 g/L) 1 ppm dimethoate (1 mg dimethoate/kg feeding solution)
Administration:	The bees in each test unit were fed *ad libitum,* via a single syringe (feeder) attached to each test unit with a 50 % (w/v) sucrose solution containing the treatments or control

Test organisms

Species:	*Apis mellifera* (Hymenoptera: Apidae)
Source:	Honey bee colonies, disease-free and queen-right, bred by ibacon.
Food:	50 % w/v aqueous sucrose solution. On each day of the test, feeder syringe was replaced with a new syringe containing freshly prepared sucrose solution only (control), or containing the test item or reference item as required.

Test design

Test cage description:	Stainless steel chambers
Replication:	3
No. of bees/arena :	10
Duration of test:	10 days

Environmental conditions

Temperature:	32 – 34 °C
Humidity:	59 – 72 %
Photoperiod:	Darkness (except during observations)

Experimental dates: 20 June 2017 – 04 September 2017

B. STUDY DESIGN

Experimental treatments
To evaluate the chronic effects of the test item on honey bees, a 10 days chronic oral feeding test in the laboratory (dose response test) was performed. Young honey bees were provided with 5 concentrations of the test item treated sugar solutions ad libitum over a period of 10 days. An untreated control and a reference item (BAS 152 11 I; 400 g/L dimethoate) were included in this study. For the study 3 replicates per treatment were used, each consisting of 10 bees per test cage.

Observations
The number of dead bees in each test replicate was assessed daily until test end (Day 0 – Day 10). Behavioural abnormalities were assessed daily until test end (Day 1 to Day 10). Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded**.** The food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed on the following assessment day.

Analysis
The quantification of the active ingredient glyphosate of the test item MON 0139 in the feeding solutions was performed using HPLC-method with UV-detection.

Statistics
Levels of bee mortality in the test item groups were compared with mortality levels achieved in the control group. Since mortality in all test item treatment groups was < 50% the LC_{50} / LDD_{50} values could not be calculated and are therefore considered to be greater than the highest tested rate/dose (10000 ppm/179.9 µg a.s./bee/day). The NOEC/NOEDD of the test item was estimated using Fisher's Exact Test (pairwise

comparison, one-sided greater, α = 0.05), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS

Chemical analysis

The analytical recovery rates of the active ingredient glyphosate in the feeding solutions were as follows:

Table 0-2: Analytical recovery rates

	Recovery rate [%] [1]	
Concentration [2]	**Day 0 [3]**	**Day 9 [4]**
10000	96	93
256	92	91

[1] Recovery rate of the a.s. in feeding solution [ppm]
[2] Nominal concentration of the a.s. in the feeding solution [ppm]
[3] Day 0 = freshly prepared feeding solution on day 0
[4] Day 9 = freshly prepared feeding solution on day 9

As the recoveries were within 100 % ± 20 % nominal concentrations were taken when calculating the dose per bee/day (including correction for evaporative loss).

B. OBSERVATIONS

Effects on honey bees

Over the 10 day chronic exposure period, there was 3.3 and 6.7% mortality in the 10000 and 640 ppm (179.9 and 10.2 µg a.s./bee/day) treatment groups, respectively. No mortality occurred in the other test item treatments (4000, 1600 and 256 mg a.s./kg feeding solution). There was 6.7% mortality in the control (50% w/v sucrose solution). Control mortality was not corrected to the mortality values in the test item treatment. The reference item (dimethoate) at a concentration of 1 ppm (1 mg dimethoate/kg feeding solution) corresponding to 0.015 µg a.s./bee/day caused 100% mortality at day 4.

For each treatment group, based on the actual amount of test solutions consumed (corrected for evaporative losses) within each treatment group, the daily mean doses were 179.9, 98.0, 38.6, 10.2 and 5.6 µg a.s./bee/day after 10 days. The maximum nominal dose levels of the test item (250 µg a.s./bee) could not be achieved, because the bees did not ingest the full targeted volume of treated 50% w/v sucrose solution. Food consumption varies among the treatment group. In the highest dose level (250 µg a.s./bee) the food consumption ranges between 103.7 µg a.s./bee (day 7-8) and 229.0 µg a.s./bee (day 9-10). In the other dose levels the pattern of consumption was more consistent. It is known that there is a high variation of food uptake by the bees within this test. Together with the trophallaxis of the bees the mean values at the end of the test (µg a.s./bee/day) should be seen as the relevant reference point.

No behavioural abnormalities occurred following treatment with MON 0139 at any time during the trial.

Table 0-3: Summary of chronic oral toxicity of glyphosate to the honeybee

Test Organism		***Apis mellifera* L.**	
Exposure		**Oral 10 days chronic exposure**	
Treatment Group	**Concentration [mg a.s./kg]**	**Dose Level [1] [µg a.s./bee]**	**Mortality at day 10 [2] [% Mean]**
Water control	0.0	0.0	6.7
MON 0139	256	5.6	0.0 (n.s.)
MON 0139	640	10.2	6.7 (n.s.)
MON 0139	1600	38.6	0.0 (n.s.)
MON 0139	4000	98.0	0.0 (n.s.)
MON 0139	10000	179.9	3.3 (n.s.)
Reference Item	1.0	0.015	100.0
Endpoint at test termination (day 10)			
LC_{50}	**LDD_{50}**	**NOEC**	**NOEDD**
> 10000 mg a.s./kg	> 179.9 µg a.s./bee	10000 mg a.s./kg	179.9 µg a.s./bee

[1] mean dose per bee per day; dose measured based on consumed feeding solution adjusted for evaporation
[2] Mortality at study termination 10 days after start of first feeding
Statistic: Mortality: Fisher`s Exact Test, pairwise comparison, one-sided greater, $\alpha = 0.05$
NOEC/NOEDD: was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$). n.s. = no statistical significant difference compared to the control,

Validity criteria

The study is considered to be valid because it meets the criteria of OECD 245:

- the mean mortality of the control was ≤ 15 % (6.7 % on day 10)
- the reference item mortality was > 50 % (actual: 100.0 % on day 4)

Ten days following the start of chronic exposure 3.3 and 6.7% mortality occurred in the 10000 and 640 ppm (179.9 and 10.2 µg a.s./bee/day) treatment groups, respectively. No mortality occurred in the other test item treatments (4000, 1600 and 256 mg a.s./kg feeding solution). There was 6.7% mortality in the control (50 % w/v sucrose solution). No behavioural abnormalities occurred following treatment with MON 0139 at any time during the trial. The LC_{50} value (10 days) was > 10000 mg a.s./kg feeding solution. The LDD_{50} value (10 days) was > 179.9 µg a.s./bee/day. The NOEC and NOEDD values (10 days) were 10000 mg a.s./kg feeding solution and 179.9 µg a.s./bee/day, respectively.

III. CONCLUSIONS

Assessment and conclusion by applicant:
This chronic oral toxicity study to honey bees (*Apis mellifera* L.) under laboratory conditions provides relevant and reliable endpoints.
The LC_{50} value (10 days) was > 10000 mg a.s./kg feeding solution. The LDD_{50} value (10 days) was >179.9 µg a.s./bee/day. The NOEC and NOEDD values (10 days) were 10000 mg a.s./kg feeding solution and 179.9 µg a.s./bee/day, respectively.
The study is considered valid so LDD_{50} >179.9 µg a.s./bee/day and NOEDD of 179.9 µg a.s./bee/day can be used for the risk assessment purposes.

Assessment and conclusion by RMS:

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Studies considering the effects of glyphosate on honeybee development and life stages were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table.

Table 0.3.1.3-1: Studies on honey bee development and other honey bee life stages toxicity of glyphosate

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.3.1.3/001	, 2020	Chronic larvae	*Apis mellifera L.*	Glyphosate IPA-salt	Valid	-

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on honeybee development and other honeybee life stages. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. For discussions of literature regarding toxicity to polliantors, please refer to document M-CP Section 10.3.

Endpoints of studies considered valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.3.1.3-3: Endpoints: honey bee development and other honey bee life stages toxicity of glyphosate

Reference	Test item	Species	Test design/ GLP	LD_{50} (µg a.e./larva)	NOED (µg a.e./larva)
, 2020 CA 8.3.1.3/001	Glyphosate IPA-salt	*Apis mellifera*	Chronic larvae, 22-day	-	80

a.e.: acid equivalents

Study summaries are provided below.

1. Information on the study

Data point	CA 8.3.1.3/001
Report author	
Report year	2020
Report title	MON 0139 - Repeated exposure of honey bee larvae (*Apis mellifera* L.) under laboratory conditions
Report No	19 48 BLC 0068
Document No	-
Guidelines followed in study	OECD (2016) No. 239 and Adaptations based on SCHMEHL et al. (2016).
Deviations from current test guideline	Deviation from OECD 239 (2016) with adaptation according to SCHMEHL et al., 2016: none
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs	Category 1

2. Full summary

Executive Summary

The chronic effects of MON 0139 (Glyphosate technical in the form of the IPA salt) on honey bee larvae, was evaluated in a repeat dose laboratory dietary exposure test. Honey bee larvae collected from three different colonies, were exposed to MON 0139 administered at a constant concentration dose in the diet, at five doses of 5.1, 12.8, 31.9, 80 and 200 µg a.s./larva (corresponding to 11.0, 27.5, 68.7, 172 and 429 µg product/larva). An untreated control and a reference item (Dimethoate tech.) were also included in the definitive test. Three replicates per treatment, control or reference item group were prepared, each consisting of 12 larvae, using 48 well plates and polystyrene grafting cells. Cumulative mortality of honey bee larvae treated with the test item was assessed daily from Day 4 to Day 8, with cumulative mortality during the pupal phase assessed on day 15. All mortality was compared to the control. The adult emergence rate was assessed on day 22. Sublethal effects were assessed and recorded daily until test end. The level of glyphosate in the diet was measured using a HPLC-method with UV-detection. In the test item groups, larval mortalities on D8 ranged between 0.0 and 8.3 %. Pupal mortalities on D15, ranged between 11.1 and 23.0 % in the test item treatment groups. Total mortalities on D22 ranged between 19.4 and 36.1 %. Mortality in the toxic reference was above 50% across all replicates on D8 (69.4 %). No sublethal effects (e.g. remaining food or small body size) were observed at the end of the feeding phase and no other observations occurred in any of the test item treatments on D22.

The ED_{50} (successful adult emergence up to D22) was >200 µg a.s./larva, equivalent to an EC_{50} of >1262 mg a.s./kg diet.

The ED_{20} was determined to be 195.7 µg a.s./larva, which is equivalent to an EC_{20} of 1235 mg a.s./kg diet.

Values for ED_{10} and EC_{10} were 75.6 µg a.s./larva and 477 mg a.s./kg diet, respectively.

The respective NOED was 80 µg a.s./larva and the corresponding NOEC was 505 mg a.s./kg diet.

The study is considered valid so NOED of 80 µg a.e./larva can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MON 0139

Lot/Batch #:	11494372
Actual content of active ingredients:	MON 0139 is a 62% technical solution comprising Glyphosate at 46.5 % (w/w); 580 g/L, according to certificate of analysis
Description:	Yellow liquid
Reanalysis/Expiry date:	29 March 2021
Density:	1.2482 g/mL

2. Treatments

Test rates:	Concentrations: 32, 81, 202, 505 and 1262 mg a.s./kg diet Actual dose per larva: 5.1, 12.8, 31.9, 80 and 200 µg a.s./larva
Control:	untreated diet B/C (aqueous sugar solution + royal jelly)
Toxic standard:	Dimethoate tech. (analysed purity: 98.8% w/w) treated diet B/C at a concentration of 48 mg a.i./kg food
Administration:	Each larva was fed with 20 µL of artificial diet A on day 1, with 20 µL of artificial diet B on day 3 and with 30µL, 40µL and 50 µL of diet C on day 4, 5 and 6 respectively.

3. Test organisms

Species:	*Apis mellifera* Subspecies: Buckfast (Hymenoptera: Apidae)
Source:	Honey bee colonies, disease-free and queen-right, reared by Biochem agrar.
Food:	Artificial diets composed of royal jelly and sugar solution according to the guideline requirements. On each feeding day of the test, freshly prepared diets only were administered to control, or containing the test item or reference item as required.

4. Test design

Test cage description:	Crystal polystyrene grafting cells were placed in 48 well plates
Replication:	3
No. of larvae/replicate :	12
Duration of test:	22 days

5. Environmental conditions

Temperature:	34.0 – 34.8 °C
Humidity:	D1-D8: 92 - 100%; D8 - D15: 80-82%; D15 - D22: 60-62%
Photoperiod:	Darkness (except during observations)

6. Experimental dates: 16 September 2019 – 20 November 2019

B. STUDY DESIGN AND METHODS

1. Experimental treatments

To evaluate the chronic effects of the test item MON 0139 on honey bee larvae, a laboratory test (dose response test) after repeated exposure was performed. The test item was administered to the larvae at a constant concentration in the diet according to their growth, within a range of five increasing doses spaced by a factor of ≤3 An untreated control and a reference item (Dimethoate tech.) were included in this study. For the study 3 replicates per treatment, control or reference item were used, each consisting of 12 larvae. All test larvae were collected from three different colonies, each representing a replicate.

2. Observations

Number of dead larvae (an immobile larva or one which did not react to contact stimulus was noted as dead), daily on D4 to D8 (larval mortality); number of dead pupae (larvae that had not transformed into pupae) on D15 (pupal mortality). Recording, e.g. of larger amounts of unconsumed food and/or discolourations and/or abnormal behaviour and/or substantially undersized larvae on D8 in order to support in the interpretation of mortality data. The test ended on D22 (final assessment) and the bees which emerged successfully were counted.

3. Analytical doses verification

Each final diet was sampled in duplicate for analysis and retained directly after diet preparation on each day of use. The test item stock solutions were sampled in parallel as a back-up in case of issues with the final diet analysis. The determination of the active ingredient was conducted by an in-house developed method using HPLC with MS/MS-detection. The analytical method was validated according to SANCO/3029/99 rev. 4.

4. Statistics

Descriptive statistics were carried out; Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$) for determination of NOED/NOEC. $ED/EC_{10/20/50}$ values were determined by Logit analysis using linear maximum likelihood regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analytical recovery rates of the active ingredient glyphosate in the final diets ranged between 86.8 and 111 %. As the measured concentrations always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations. Details are presented below:

Table 0-2: Analytical recovery rates

Sampling Day	Nominal concentration [µg a.s./L]	Nominal concentration [mg a.s./kg]	Measured concentration [mg a.s./kg]	Recovery rate [% of the nominal]
3	5.1	32.3	34.7	107
4			35.5	110
5			33.4	103
6			36.0	111
3	12.8	80.7	81.4	101
4			75.3	93
5			83.1	103
6			76.6	94.9
3	31.9	202	200	99.3
4			175	86.8
5			200	99.3
6			197	97.7
3	80	505	467	92.6
4			477	94.6
5			462	91.6
6			453	89.7
3	200	1262	1098	87.0
4			1186	94.0
5			1316	104
6			1196	94.8

No test item was detected in the control specimen.

B. OBSERVATIONS

On D8, a larval mortality of 2.8 % was observed in the control. Pupal mortality (between D8 and D15) was 19.9 % in the control. The control group showed a total mortality of 22.2 % on D22 (larval mortality, pupal mortality, and adults not emerged by D22). In the test item groups, larval mortalities on D8 ranged between 0.0 and 8.3 %. Pupal mortalities ranged between 11.1 and 23.0 % in the test item treatment groups. Total mortalities on D22 ranged between 19.4 and 36.1 %. Mortality in the toxic reference (AR) was above 50 % across all replicates on D8, being 69.4 %.

No sublethal effects, e.g. remaining food or small body size, were observed at the end of the feeding phase and no other observations occurred in any of the test item treatments on D22.

In the final assessment on D22, an adult emergence rate of 77.8 % was determined for the honey bees in the control group. In the test item groups the adult honey bees emerged at rates ranging between 63.9 % and 80.6 % following an application of 200, 80, 31.9, 12.8 and 5.1 µg a.s./larva.

Table 0-3: Toxicity of MON 0139 to larvae of *Apis mellifera* L. after repeated exposure

	Control	Test item					Tox. Ref.
Nominal concentrations [mg a.s./kg]	0	32	81	202	505	1262	48
Nominal doses [µg a.s./Larva]	0	5.1	12.8	31.9	80	200	7.6
Larval mortality D3 to D8 abs. [%]	2.8	2.8	0	2.8	8.3	2.8	69.4
Larval mortality D3 to D8 corr. [%]	-	0	0	0	5.7	0	68.6
Pupal mortality D8 to D15 abs. [%]	19.9	11.9	11.1	20.2	20.4	23.0	24.4
Pupal mortality D8 to D15 corr. [%]	-	0	0	0.3	0.5	3.8	5.6
Total mortality D3 to D22 abs. [%]	22.2	19.4	25.0	25.0	33.3	36.1	88.9
Total mortality D3 to D22 corr. [%]	-	0	3.6	3.6	14.3	17.9	85.7
Adult emergence rate [%]	77.8	80.6	75.0	75.0	66.7	63.9*	11.1

Results are averages based on 3 replicates, containing 12 larvae each;
corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): test and reference item treated groups were corrected by control; negative values were set to "0"; calculations were performed with non-rounded values;
CL: confidence limit; abs.: absolute mortality as counted from the results;
* Statistically significant if compared to the control (Step-down Cochran-Armitage Test Procedure)

Table 0-4: Endpoints

Endpoints	Nominal doses [µg a.s./Larva]	Endpoints	Nominal concentrations [mg a.s./kg]
ED_{50}[2,3]	>200	EC_{50}[2,3]	>1262
ED_{20}[2] (95% CL)	195.7 (83.9 - 456.7)	EC_{20}[2] (95% CL)	1235 (530 - 2881)
ED_{10}[2] (95% CL)	75.6 (38.8 - 147.3)	EC_{10}[2] (95% CL)	477 (245 - 930)
NOED[1]	80	NOEC[1]	505

[1] Step-down Cochran-Armitage Test Procedure; alpha=0.05; one sided greater
[2] Logit analysis using linear max. likelihood regression
[3] Calculated endpoint was beyond the tested range.

Validity criteria

All the validity criteria according to OECD No. 239 were fulfilled as:

- control mortality was ≤ 15 % on D8 (actual value 2.8 %)
- cumulative mortality in the reference item treatment group was ≥50 % on D8 (actual value 68.6 % corrected form control)
- adult emergence in the control was ≥70 % on D22 ,

The study is reliable and can be considered as valid.

III. CONCLUSIONS

Assessment and conclusion by applicant:
This repeated exposure larval toxicity study with MON 0139 on honey bees larvae (*Apis mellifera* L.) under laboratory conditions provides relevant and reliable endpoints.

The ED_{50} (successful adult emergence up to D22) was determined to be >200 µg a.s./larva, which is equivalent to an EC_{50} of >1262 mg a.s./kg diet. The ED_{20} was determined to be 195.7 µg a.s./larva, which is equivalent to an EC_{20} of 1235 mg a.s./kg diet. Values for ED_{10} and EC_{10} were 75.6 µg a.s./larva and 477 mg a.s./kg diet, respectively. The respective NOED was 80 µg a.s./larva and the corresponding NOEC was 505 mg a.s./kg diet.

The study is considered valid so NOED of 80 µg a.e./larva can be used for risk assessment purposes.

Assessment and conclusion by RMS:

CA 8.3.1.4 Sub-lethal effects

Studies considering the sublethal effects of glyphosate on pollinators were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table.

Table 0.3.1.4-1: Studies on sub-lethal toxicity of glyphosate to pollinators

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.3.1.4/001	2012	bee brood feeding	*Apis mellifera L.*	Glyphosate IPA-salt	Valid	-

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the sub-lethal impact of glyphosate or its relevant metabolites on pollinator species. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. For discussions of literature regarding toxicity to pollinators, please refer to document M-CP Section 10.3.

Endpoints of studies considered valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.3.1.4-3: Endpoints: Sub-lethal toxicity of glyphosate to pollinators

Reference	Test item	Species	Test design/GLP	LD_{50} (µg a.e./L)	NOAEL (µg a.e./L)
2012 CA 8.3.1.4/001	Glyphosate IPA-salt	*Apis mellifera*	Bee brood feeding test Field study	-	≥ 301000 (301 mg a.e./L)

a.e.: acid equivalents

Study summaries are provided below.

1. Information on the study

Data point	CA 8.3.1.4/001
Report author	
Report year	2012
Report title	Glyphosate: Evaluating potential effects on honeybee brood (*Apis mellifera*) development
Report No	V7YH1001
Document No	-
Guidelines followed in study	Oomen *et al.*, 1992
Deviations from current test guideline	Deviations from guideline Oomen (1992): Minor: - Some colonies were slightly smaller in terms of the number of brood frames, but this was not considered to have a significant impact on the study. - Feeding period was extended up to 5 days. This extension of the feeding period is not considered to have had an impact on the validity of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A field study was undertaken to determine the potential for toxicity to developing honey bee larvae and pupae to glyphosate (tested as the IPA salt) when fed directly to honey bee colonies. The IPA salt was selected as the test substance because it is representative of the active substance in glyphosate formulations and the appropriate for this terrestrial study. Three groups of four colonies were treated with 75, 150 and 301 mg a.e./L of glyphosate in 1 litre of 50% w/v sucrose. One group of four colonies was fed with 1 litre 50 % w/v sucrose solution only and one group of four colonies was fed with the toxic reference fenoxycarb dispersed in 1 litre of 50 % w/v sucrose. Brood cells were marked in each colony (100 cells containing eggs, 100 cells containing 1-2 day old larvae, and 100 cells containing 3-4 day old larvae) up to 24 hours to dosing using the standard acetate overlay method. On day 7 and just prior to expected emergence, the marked brood cells (eggs, young and old larvae) were assessed for mortality and appearance in each test colony. The content of the dead bee traps attached to the colonies was counted daily during brood assessment period. All colonies were assessed within one week prior to the dosing and within week 1, 2 and 3 after dosing. Samples of each concentration of test item treated sucrose solution were taken on the

day of dosing. Four to five day old larvae were sampled 4 and 7 days following start of dosing. Both dosing solution and larval samples were analysed for glyphosate content.

Measured glyphosate (a.e.) concentrations in the dosing solutions were within 11 % of the nominal doses. Mean measured glyphosate (a.e.) residues in larvae on 4 days were 13, 37 and 53 mg a.e./kg for the nominal dose levels of 75, 150, and 301 mg a.e./L. Mean measured residues after 7 days were reduced with values of 1.7, 3.2 and 4.1 mg a.e./kg for the nominal dose levels of 75, 150, and 301 mg a.e./L. Glyphosate acid was not detected in the control group.

No biologically significant adult mortality was observed in any treatment group. Over a 16 day observation period after dosing, 2.0 dead pupae/colony were observed in the control and 1.3 – 1.8 dead pupae/colony were observed in the glyphosate treated colonies. Overall survival was 85% for marked eggs, 96 % for marked young larvae and 96 % for marked old larvae in controls and 82-87 % for marked eggs, 87-94 % for marked young larvae and 94-95 % for marked old larvae in the glyphosate treated colonies.
The overall NOAEL for brood development of honey bees was the highest dose tested – 301 mg a.e./L (nominal) equivalent to 245 mg a.e./kg nominal when considering the density of the sucrose solution and 266 mg a.e./kg actually measured.

The study is considered valid so NOAEL of 301 mg a.e./L can be used in risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 0139
Active substance:	Glyphosate isopropylamine salt
Active substance content:	62.27% Glyphosate isopropylamine salt 46.14% glyphosate acid equivalent/L (measured)
Description:	Clear pale yellow liquid
Lot/Batch #:	GLP-1104-21370-T

Vehicle and/or positive control: Vehicle: sucrose solution
Positive control: Fenoxycarb (750 mg a.s./L)

Test organism:

Species:	Apis mellifera L.
Age:	Not stated
Source:	UK national Bee Unit
Acclimatisation:	not required

Test system: Twenty standardised field colonies housed in a single chamber wooden Smith hive with British standard frames each and headed by queens of similar age. The honey bee colonies contained 12000 – 22500 adult bees and consisted of 0.5-3 frames of brood, 0.5-2 frames of honey and 0-1 frame of pollen.

Crop cultivated:	Not applicable; the test site with no nearby flowering crops and few flowering weeds, Dunnington, York, U.K.
Replication:	4 colonies/treatment and control

Environmental conditions:

Temperature:	3.4 – 46.3 °C
Relative humidity:	0 – 100 %
Average wind speed:	4.0 – 13.1 mph
Precipitation:	0.0 – 9.71 mm
Experimental dates:	21 June – 23 August 2011

B. STUDY DESIGN

Experimental treatments

Test system: Twenty standardised honey bee colonies, each equipped with a dead bee trap fitted to the front were used in this study. All colonies were placed on varroa floors and sticky inserts were placed on the trays to trap any fallen mites. Colonies were located on a test site at Dunnington, York and allowed to fly freely, there were no nearby flowering crops and few flowering weeds (clover). Colonies were placed in groups according to treatment and placed at least 20 m apart from each other.

Experimental design: Up to 24 hours prior to dosing, 100 brood cells containing eggs, 100 cells containing 1-2 day old larvae and 100 cells containing 3-4 day old larvae were selected in each colony and marked using the Oomen *et al.* (1992)[14] acetate overlay sheet method.

Test doses: Dose setting was based on measured residues achieved in a glasshouse residues study after spray application onto *Phacelia* plants at 2.88 kg glyphosate a.e./ha. Considering that bee colonies used in the brood study may be up to 50% bigger than those used in the residue study, an additional calculation for the expected total daily intake of glyphosate residues was undertaken assuming that such colonies would collect 9 g pollen and 1944 mL nectar (see table below). Furthermore the determined residue content based on application of 2.88 kg a.e./ha was adjusted to reflect the lower application rate of 2.16 kg a.e./ha.

[14] Oomen, P. A., De Ruijter, A., & Van der Steen J. (1992) Method for honeybee brood feeding tests with insect growth-regulating insecticides. Bulletin OEPP/EPPO Bulletin 22, 613-616.

Table 0-2: Exposure assessment of a brood study colony to glyphosate under two scenarios used to establish test doses for use in the brood study

Scenario	Daily intake of glyphosate residues in nectar (1944 g nectar/d) [mg]	Daily intake of glyphosate residues in pollen (9 g pollen/d) [mg]	Total daily intake of glyphosate residues [mg]	Uptake over 3 days [mg]	Adjustment from 2.88 kg a.e./ha to 2.16 kg a.e./ha [mg] [7]
Day 1 maximum mean residues (31.3 µg a.e./g in nectar, 574 µg a.e./g in pollen)	60.8 [1]	5.2 [2]	66.0	198	148.5 [3]
Mean residues over days 1-3 (15.5 µg a.e./g in nectar, 310 µg a.e./g in pollen)	30.3 [4]	2.8 [5]	33.1	99.3	74.5 [6]

[1] Derived from 1.944 kg nectar consumed/day × 31.3 mg/kg = 60.8 mg glyphosate a.e.
[2] Derived from 0.009 kg pollen consumed/day × 574 mg/kg = 5.2 mg glyphosate a.e.
[3] Value of 148.5 mg was rounded to 150 mg to achieve the nominal mid-dose concentration in brood study
[4] Derived from 1.944 kg nectar consumed/day × 15.5 mg/kg = 30.3 mg glyphosate a.e.
[5] Derived from 0.009 kg pollen consumed/day × 310.1 mg/kg = 2.8 mg glyphosate a.e.
[6] Value of 74.5 was rounded to 75 mg to achieve the nominal low-dose concentration in brood study
[7] The determined residue content based on application of 2.88 kg a.e./ha was adjusted to reflect the lower application rate of 2.16 kg a.e./ha.

Test item application: Three groups of colonies (i.e. four colonies per group) were treated with glyphosate isopropylamine salt added to 1 litre of 50% sucrose solution to achieve doses of 75, 150, and 301 mg a.e./L and one group was an untreated control, i.e. fed 1 litre 50% sucrose solution, only. In addition, one group was treated with the toxic reference fenoxycarb, dispersed in 1 L of 50% sucrose (750 mg a.s./L). Doses were administered by removing frames of stores from the colonies and placing a 1 litre glass container containing the treatment solution within the brood chamber.

Observations

The content of dead bee traps was counted daily during the brood assessment period. All colonies were assessed within one week prior to dosing and within weeks 1, 2 and 3 after dosing, including counts of the number of combs of adults, brood, stores and pollen as well as behavioural or physical abnormalities. The uptake of each sucrose solution was checked daily and the container removed when empty or after 5 days. On day 7 the marked brood cells (eggs, young and old larvae) were assessed for mortality and appearance. On day 13 brood cells marked as containing old larvae, on day 15 cells previously containing young larvae and on day 16 cells previously containing eggs, were assessed. Cells were uncapped; the bee removed carefully with forceps and the age of bee was assessed, weighed and observed for deformities. The temperature and humidity were recorded continuously using a data logger; local (within 10 km) weather data was also collected.

Residues analysis

Analysis of glyphosate acid in larvae samples was conducted following extraction with acetonitrile:water (1:4, v/v), clean up by solid phase extraction on C18 and derivatisation as FMOC-glyphosate and a second clean up (solid phase extraction on Oasis HLB, methanolic elution) by HPLC-MS/MS. Analysis of glyphosate acid in treated sugar solution samples was conducted following extraction with acetonitrile:water (1:4, v/v), solid phase extraction on Oasis HLB, methanolic elution and derivatisation as FMOC-glyphosate by HPLC-MS/MS. Limit of quantification (LOQ) and limit of detection (LOD) were 1.0 and 0.3 mg/kg, respectively. Freshly prepared test treated sucrose solution samples were retained for analysis. On day 4 and 7, samples of ten 4-5 day old larvae were collected from each colony for residue analysis.

Data analysis
Brood mortality was analysed using a generalised linear model (Logit distribution) and an ANOVA for pupae weight data to determine NOAEL statistically.

II. RESULTS AND DISCUSSION

A. FINDINGS
Analytical data: Residues in samples of sucrose treatment solutions were within 11 % of nominal doses. The nominal dose of 75 mg glyphosate a.e./L (corresponding to 61 mg glyphosate a.e./kg) was confirmed to be 65.7 mg glyphosate a.e./kg. The nominal dose of 150 mg glyphosate a.e./L (corresponding to 122 mg glyphosate a.e./kg) was confirmed to be 135 mg glyphosate a.e./kg. The nominal dose of 301 mg glyphosate a.e./L (corresponding to 245 mg glyphosate a.e./kg) was confirmed to be 266 mg glyphosate a.e./kg. (Conversion from nominal dose rate in mg a.e./L to nominal dose rate in mg/kg was based on a density of 50 % w/v sucrose solution of 1.23 kg/L.)
Residues in larvae sampled from the hive on day 4 and day 7 ranged from 7.9 to 18.4 and below LOQ to 3 mg glyphosate a.e./kg, respectively on the dose 75 mg a.e./L, from 26.3 to 53.2 and 1.9 to 4.9 mg glyphosate a.e./kg, respectively on the dose 150 mg a.e./L and from 33.1 to 82.1 and 3.2 to 6.3 mg glyphosate a.e./kg, respectively on the dose 301 mg a.e./L, confirming that larvae were exposed to the test item provided in the sugar solution and consumed it.

B. OBSERVATIONS
Consumption of treated sucrose solution: The control colonies consumed between 0.625 and 1.0 L of untreated sucrose. In the glyphosate treated colonies at least 3 of 4 colonies consumed the total volume of treated sucrose.

Bee brood assessments:

Table 0-3: Survival of marked brood exposed to glyphosate isopropylamine salt

Dose rate [mg/L]	**Control**	**75**	**150**	**301**
Mean dose consumed [mg]		**73 ± 2**	**138 ± 12**	**255 ± 46**
7-d old cells marked as eggs [%]	87.3 ± 1.9	84.8 ± 4.0	87.5 ± 2.7	86.2 ± 3.3
16-d old cells marked as eggs [%]	85.0 ± 2.0	82.3 ± 3.3	86.8 ± 2.7	84.2 ± 3.9
7-d old cells marked as young larvae [%]	96.4 ± 3.0	93.5 ± 1.8	91.5 ± 4.3	95.0 ± 1.8
16-d old cells marked as young larvae [%]	95.9 ± 3.1	93.5 ± 1.8	86.5 ± 4.3	90.0 ± 5.4
7-d old cells marked as old larvae [%]	97.0 ± 0.4	96.8 ± 0.5	96.8 ± 1.7	95.3 ± 2.9
16-d old cells marked as old larvae [%]	95.8 ± 1.3	94.8 ± 1.1	94.3 ± 1.0	95.3 ± 2.9

No significant statistical difference in brood development (eggs, young larvae, old larvae) was observed for all glyphosate treatment groups compared to control ($p<0.05$).

Table 0-4: Pupae weight at final assessment

Dose rate [mg/L]	Control	75	150	301
Mean dose consumed [mg]		**73 ± 2**	**138 ± 12**	**255 ± 46**
Pupae marked as eggs [mg]	127.5 ± 0.7	124.7 ± 0.8	126.7 ± 0.6	135.7 ± 0.6
Pupae marked as young larvae [mg]	128.4 ± 0.6	128.3 ± 1.0	124.4 ± 0.8	125.4 ± 0.6
Pupae marked as old larvae [mg]	128.9 ± 0.4	121.2 ± 0.5	122.6 ± 0.5	125.6 ± 0.4

There were no significant effects of the treatment on the mean weight of the exposed pupae. No biologically significant adult mortality was observed in any treatment group. No adverse effects on colonies were observed in any treatment group apart from an apparent decline in the number of bees and brood in the fenoxycarb treated colonies in the later stages of the study.
In the fenoxycarb toxic reference treated colonies, the overall survival of marked cells was 20 % for marked eggs, 0 % for marked young larvae and 12 % for marked old larvae, meeting the validity criterion for the toxic reference (>40 % effect on all stages).

Deviations according to the guideline Oomen (1992):

- Some colonies used in the study were slightly smaller in terms of the number of brood frames, but this was not considered to have a significant impact on the study as all were viable colonies at the start of the study and a sufficient number of brood cells was available for detailed observations.
- Feeding period was extended up to 5 days (commonly consumed within 24 hours). This extension of the feeding period is not considered to have had an impact on the validity of the study.

III. CONCLUSION

Assessment and conclusion by applicant:
A colony feeding study was undertaken to determine the potential for toxicity to developing honey bee larvae and pupae to glyphosate (tested as the IPA salt) when fed directly to honey bee colonies.
The overall NOAEL for brood development of honey bees was the highest dose tested – 301 mg a.e./L (nominal) equivalent to 245 mg a.e./kg nominal when considering the density of the sucrose solution and 266 mg a.e./kg actually measured.

The study is considered valid so NOAEL of 301 mg a.e./L can be used in risk assessment purposes.

Assessment and conclusion by RMS:

CA 8.3.2 Effects on non-target arthropods other than bees

Non-target arthropods studies were conducted with the representative formulated product MON-52276 rather than the active substance, as permitted in Commission Regulation (EU) No 283/2013. Data for the

effects of the formulated product MON-52276 on non-target arthropods are summarised in document M-CP Section 10.3.2.1 and Section 10.3.2.2.

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

Standard and extended toxicity studies have been submitted with the formulated product on *Aphidius rhopalosiphi* and can be found in document M-CP, Section 10.3.2.1 and Section 10.3.2.2.

CA 8.3.2.2 Effects on *Typhlodromus pyri*

Standard and extended toxicity studies have been submitted with the formulated product on *Typhlodromus pyri* and can be found in document M-CP, Section 10.3.2.1 and Section 10.3.2.2.

CA 8.4 Effects on Non-Target Soil Meso- and Macrofauna

Studies on effects of the active substance glyphosate and its relevant metabolites on soil organisms; earthworms, collembolans and soil mites to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

CA 8.4.1 Earthworms – sub-lethal effects

Earthworm studies have been summarised to evaluate long-term toxicity of glyphosate salts and the glyphosate metabolite AMPA. The results of these studies demonstrate that glyphosate, glyphosate salts and AMPA are of low toxicity to earthworms.

Studies considering the chronic toxicity of glyphosate to earthworms were assessed for their validity to current and relevant guidelines and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 8.3.2.2.4.1-1: Studies on sub-lethal toxicity of glyphosate and metabolites to earthworms

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.4.1/001	, 2009	56 d chronic	*Eisenia andrei*	Glyphosate IPA salt	Valid	-
CA 8.4.1/002	2000	56 days chronic	*Eisenia fetida*	Glyphosate IPA salt and AMPA	Valid	-
CA 8.4.1/003	, 2003	56 days chronic	*Eisenia fetida*	AMPA	Valid	-
CA 8.4.1/004	, 2002	56 days chronic	*Eisenia fetida*	AMPA	Invalid	-

Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate on soil organisms are summarised in the table below. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to soil organisms, please refer to document M-CP Section 10.4.

Table 8.3.2.2.4.1-2: Literature on sub-lethal toxicity of glyphosate and metabolites to earthworms

Annex point	Study	Study type	Substance(s)	Status	Remark

CA 8.4.1/005	Von Mérey *et al.*, 2016	OECD 222; 56 days chronic	Glyphosate IPA salt and AMPA	Relevant and reliable	Evaluates potential effects on earthworm, soil mites, springtails and soil micro-organisms.

Endpoints of studies considered valid for glyphosate are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.4.1-3: Endpoints: Sub-lethal toxicity of glyphosate to earthworms

Reference (Data owner)	Test item	Species	Test design/ GLP	EC_{50} (mg a.e./kg dry soil)	NOEC (mg a.e./kg dry soil)
, 2009 CA 8.4.1/001	Glyphosate IPA salt	*Eisenia andrei*	56 d chronic	> 473	**≥ 473**
, 2000 CA 8.4.1/002	Glyphosate IPA salt	*Eisenia fetida*	56 d chronic	-	≥ 21.31

a.e.: acid equivalent
Endpoints in **bold** is used for risk assessment

Endpoints of studies considered valid for AMPA are shown in the table below.

Table 8.4.1-4: Endpoints: Sub-lethal toxicity of AMPA to earthworms

Reference (Data owner)	Test item	Species	Test design/ GLP	EC_{50} (mg/kg dry soil)	NOEC (mg/kg dry soil)
2000 CA 8.4.1/002	AMPA	*Eisenia fetida*	56 d chronic	-	≥ 28.12
, 2003 CA 8.4.1/003	AMPA	*Eisenia fetida*	56 d chronic	567.2	Recalculated in RAR 2015: **131.90**

Endpoints in **bold** is used for risk assessment

Study summaries are provided below.

1. Information on the study

Data point	CA 8.4.1/001
Report author	
Report year	2009
Report title	MON0139 - Sublethal toxicity to the earthworm *Eisenia fetida*
Report No	09 10 48 056 S
Document No	-
Guidelines followed in study	OECD 222 (2004)
Deviations from current test guideline	Deviation from the guideline OECD 222 (2016): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of MON0139 (glyphosate isopropylamine salt) on *Eisenia fetida* were tested in a 56 days sublethal laboratory test with regard to the parameters mortality, behavioural and pathological symptoms, body weight change and reproduction in OECD soil containing 10 % sphagnum peat. The test was conducted with five nominal test concentrations of 30, 50, 100, 500 and 1000 mg test item/kg dry soil, equivalent to an analysed content of 19.1, 31.9, 63.8, 319.1, and 638.1 mg glyphosate isopropylamine salt/kg dry soil, respectively (*i.e.* 14.2, 23.6, 47.28, 236.4, 472.8 mg glyphosate acid equivalent/kg dry soil, respectively). In addition, a control group was exposed to soil mixed with deionised water only.
After 56 days, the test item caused no mortality at the tested concentrations of 30, 500 and 1000 mg MON0139/kg dry soil. 2.5 % mortality was observed at 50 and 100 mg MON0139/kg dry soil. No mortality occurred in the control group. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass when compared to the control group. All validity criteria according to the OECD guideline 222 were fulfilled. The study is valid so EC_{50} > 473 mg a.e./kg dry soil and NOEC ≥ 473 mg a.e./kg dry soil will be used in the regulatory risk assessment for earthworms exposed to glyphosate technical.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON0139 (glyphosate isopropylamine salt)
Description:	Pale yellow liquid
Lot/Batch #:	A8B60170S0
Purity:	63.81 % w/w glyphosate isopropylamine salt (analysed) 62 % w/w glyphosate isopropylamine salt (nominal) 47.28 % w/w glyphosate acid equivalent (analysed)

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: Nutdazim 50 FLOW (carbendazim, SC 500), tested in a separate study

3. Test organism:

Species:	Earthworm (*Eisenia fetida andrei*)
Age:	Adults, approx. 3 months old with clitellum
Weight:	304 – 472 mg
Source:	In-house rearing (originally from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Food:	Air-dried and finely ground horse manure
Acclimation period:	Approx. 24 hours in the artificial substrate

4. Environmental conditions:

Temperature:	18.6 – 21.8 ºC
Photoperiod:	16 h light (600 Lux)/ 8 h dark
Soil pH:	6.1 – 6.2 (test start); 6.0 – 6.1 (test termination)
Soil moisture content:	35.1 – 35.2 % (test start); 34.6 – 34.8 % (test termination)

B. STUDY DESIGN AND METHODS

1. Experimental treatments: A sublethal test was conducted with five nominal test concentrations of 30, 50, 100, 500 and 1000 mg test item/kg dry soil, equivalent to an analysed content of 19.1, 31.9, 63.8, 319.1, and 638.1 mg glyphosate isopropylamine salt/kg dry soil, respectively. In addition, a control group was exposed to soil mixed with deionised water only. The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water (stock solutions) and thereafter diluted to obtain different test concentrations, which were thoroughly mixed with the artificial soil, achieving desired test concentrations with a final nominal water content of 40 – 60 % of WHC. The artificial soil substrate was composed of 10 % sphagnum peat, 20 % kaolin clay, 69.5 % industrial quartz sand and 0.5 % calcium carbonate. Four replicate test containers (test item) and 8 replicate test containers (control) with 810 g soil (wet weight) and 5 cm soil depth were prepared for each treatment group. 10 adult earthworms were exposed per replicate for 56 days.
As a toxic reference, earthworms were exposed in a separate study to Nutdazim 50 FLOW (carbendazim, SC 500). The results are in line with the OECD requirements (65 % and 92 % of reduction in the number of juveniles at concentrations of 5 and 10 mg product/ kg dry soil respectively).

2. Observations: At test initiation, individual fresh weight and behavioural responses of earthworms were recorded. Behavioural and pathological symptoms including feeding activity were observed on a weekly basis. Four weeks after test initiation, number of surviving adult earthworms and fresh weight of surviving adult earthworms per replicate were recorded. At test termination (8 weeks after test initiation), number of surviving juveniles per replicate, were observed.
The behavioural and pathological symptoms, including morphological alterations were observed 4 and 8 weeks after test initiation. Water content and pH measurements were performed at test initiation and at test termination. The temperature was continuously recoded throughout the test.

3. Statistical calculations: Fisher's Exact Binomial Test and Dunnett's t-test were used for mean comparison. For statistical evaluation of the biomass change, mean fresh weight of surviving worms was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.4.1-2: Sublethal effects of MON0139 (glyphosate isopropylamine salt) on earthworm

MON0139 [mg test item/kg soil d.w.]		Control	30	50	100	500	1000
Mortality of adult worms after 4 weeks (%)		0	0	2.5	2.5	0	0
Mean biomass change (%)		+40.7	+46.7	+39.8	+41.8	+37.5	+36.3
Mean number of juveniles after 8 weeks		79.0	78.5	83.8	71.8	80.3	74.3
CV %		18.7	19.1	15.0	34.1	28.7	22.1
Change of reproduction compared to control (%)		-	0.6	-6.0	9.2	-1.6	6.0
EC_{50}	Test item (MON0139)	> 1000 mg/kg dry soil					
	glyphosate isopropylamine salt	> 638.1/kg dry soil					
NOEC	Test item (MON0139)	1000 mg/kg dry soil					
	glyphosate isopropylamine salt	638.1/kg dry soil					

B. OBSERVATIONS

The test item MON0139 caused no mortality at concentrations of 30, 500 and 1000 mg MON0139/kg dry soil. 2.5 % mortality was observed at concentrations of 500 and 1000 mg MON0139/kg dry soil. No mortality (0%) occurred in the control group. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) when compared to the control.
The validity criteria according to guideline OECD 222 are fulfilled as each replicate (containing 10 adults) has produced ≥ 30 juveniles by the end of the test in the control and the coefficient of variation of reproduction was ≤ 30 % in the control. Also, the adult mortality over the initial 4 weeks of the test was ≤ 10 % in the control.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of glyphosate on mortality and reproduction of earthworms were assessed following application of MON0139 under laboratory conditions.

The EC_{50} of MON0139 for earthworm reproduction was determined to be > 1000 mg test item/kg dry soil, corresponding to > 638.1 mg glyphosate isopropylamine salt/kg dry soil. The overall NOEC was determined to be ≥ 1000 mg/kg dry soil, corresponding to 638.1 mg glyphosate isopropylamine salt/kg dry soil, corresponding to ≥ 473 mg a.e./kg dry soil.

The study is valid so EC_{50} > 473 mg a.e./kg dry soil and NOEC ≥ 473 mg a.e./kg dry soil can be used in risk assessment for earthworms exposed to glyphosate IPA salt.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.4.1/002
Report author	
Report year	2000
Report title	A laboratory investigation of the effects of glyphosate and its breakdown product AMPA on reproduction in the earthworm *Eisenia fetida*
Report No	CEMR-1173
Document No	Not available
Guidelines followed in study	ISO 11268-2 (1998)
Deviations from current test guideline	Deviations from guideline OECD 222 (2016): Minor: - Test design for NOEC required at least 5 concentrations (only 2 of each in this study) and 8 replicates for the negative control (only 4 in this study).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of the isopropylamine (IPA) salt of glyphosate and the metabolite aminomethylphosphonic acid (AMPA) on the earthworm *Eisenia fetida* were tested in a 56-day chronic laboratory test with regard to the parameters mortality, development of body weight and reproduction. The test was conducted with two test concentrations of glyphosate IPA salt (5.76 and 28.79 mg/kg dry soil (equivalent to 4.27 and 21.31 mg glyphosate acid equivalent/kg dry soil) and two test concentrations of AMPA (5.62 and 28.12 mg/kg dry soil) in OECD soil containing 10 % peat. Furthermore, a negative and three concentrations of a positive control (Benlate®) were tested.

Only one adult worm died during the test at the lowest concentration of glyphosate IPA salt (5.76 mg/kg dry soil) tested and thus was not considered to be dose-related. Furthermore, no significant difference in body weight change compared to the untreated controls was noted for adult worms exposed to the glyphosate IPA salt or AMPA at any of the concentrations tested in this study.

No significant differences were observed between the mean juvenile production for the untreated control worms and specimens exposed to glyphosate IPA or AMPA at any concentration tested. Similarly, no significant differences were observed between the numbers of unhatched cocoons present at day 56 in the untreated controls and those in both concentrations of glyphosate IPA salt or AMPA. All validity criteria according to the OECD guideline 222 were fulfilled. The study is valid so NOEC ≥ 21.31 mg a.e./kg dry soil will be used in the regulatory risk assessment for earthworms exposed to glyphosate technical and NOEC ≥ 28.12 mg/kg dry soil will be used in the regulatory risk assessment for earthworms exposed to AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item 1:	MON 0139
Description:	Clear liquid
Lot/Batch #:	A9C 281
Purity:	62 % Isopropylamine (IPA) salt of glyphosate (45.9 % glyphosate acid equivalent)
Test item 2:	AMPA (aminomethylphosphonic acid)
Description:	White crystalline powder
Lot/Batch #:	PIT-8912-1385-A
Purity:	99.1 %
	Vehicle: deionised water

2. Vehicle and/or positive control: Positive controls: Benlate® (50 % w/w benomyl)
Reference item (in a separate study): 2-chloroacetamide

3. Test organism:

Species:	Earthworm (*Eisenia fetida fetida*)
Age:	Adults, 7-10 months old
Weight:	386 - 477 mg (test initiation)
Source:	In-house culture based on a stock of worms obtained from Blades Biological, UK
Food:	Cattle manure
Acclimation period:	Earthworms were acclimatised to the artificial soil for a period of 29 days at 16 – 22.5 °C.

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 h light: 8 h dark
Soil pH:	6.38 – 6.96
Soil temperature:	18.4 – 19.6 °C
Soil moisture content:	37.9 % (60 % of the water holding capacity) (test initiation); 29.6 – 31.1 % (test termination)

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The test was conducted with two test concentrations of glyphosate IPA salt (5.76 and 28.79 mg/kg dry soil, equivalent to 4.27 and 21.31 mg glyphosate acid equivalent/kg dry soil) and two test concentrations of AMPA (5.62 and 28.12 mg/kg dry soil). The test item was dissolved in deionised water and the solution was mixed with the water used for adjusting the soil moisture to 60% of the water holding capacity. Afterwards, the solution was mixed into the artificial soil substrate (10% peat; 20% clay, 70% silica sand and calcium carbonate to obtain a pH of 5.5-6.5). 1 g cow manure/100 g dry soil was added as feed. Four replicate test containers with 600 g dry soil were prepared for each treatment group. 10 adult earthworms were exposed for 56 days per replicate. Earthworms were fed with manure on day 1, 14, 21 and 28. Soil moisture was adjusted once a week by adding deionised water. A negative control was treated with deionised water only. As positive control, earthworms were exposed to three concentrations of Benlate® (2.66, 5.93 and 13.28 mg/kg dry soil). Temperature and light intensity were recorded daily during

the test period. pH and soil temperature were determined at the beginning and the end of the test in one of the replicate vessels at each concentration. Soil moisture content was determined at day 0, 1, 7, 14, 21, 28, 35, 42 and 56. Furthermore, toxicity of 2-chloroacetamide to *Eisenia fetida* was tested in a separate 14 day reference study.

2. Observations:
Mortality and reproduction: The replicates were examined for live and dead adult worms after 28 days at which time all adult worms were removed and the soil was replaced in the vessels. After a further 28 days, the contents of the beakers were examined for juvenile worms and cocoons.
Mean body weights: All surviving earthworms per replicate were weighed as a group and average individual weights were calculated prior to test initiation and at day 28 after application.

3. Statistical calculations: Mean percent changes in weights of live worms at 28 days and mean juvenile production per surviving adult worm at day 56 were tested for significant ($\alpha = 0.05$) inhibition compared to the controls using the Dunnett's Test (one tailed comparison) in the computer program TOXSTAT Release 3.0. The same test, but with a two-tailed comparison, was employed to test for significant differences between mean numbers of un-hatched cocoons because the test substances may have inhibited cocoon production or/and cocoon viability (cocoons may have been produced but unable to hatch). Each set of data was tested for normality before carrying out the parametric multiple comparison procedure using the Chi-square test and the Shapiro Wilks test, the data were also tested for homogeneity of variance using both the Hartley and the Bartletts tests provided in the program TOXSTAT Release 3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.4.1-3: Summary of the effects of glyphosate IPA salt, AMPA and the positive control Benlate® on *Eisenia fetida*

Treatment [mg/kg dry soil]		Adult worms		Juvenile production (at day 56)		Mean number of unhatched cocoons per surviving worm
		Percentage mortality of adult worms (at day 28)	Mean percent weight change (at day 28)	Mean number of juveniles per surviving worm	Coefficient of variation	
Control		0	+ 22	31.0	10	0.1
Benlate®	2.66	0	+ 23	26.0 *	15	0.1
	5.93	0	+ 12 *	7.8 *	23	2.2 *
	13.28	0	- 24 *	0.0 *	0	0.7
Glyphosate(1) (as IPA salt)	5.76	2.5	+ 14	26.2	25	0.3(N)
	28.79	0	+ 20	28.5	12	0.3(N)
AMPA	5.62	0	+ 24	26.0	3	0.3(N)
	28.12	0	+ 24	29.4	16	0.4(N)

* = statistically (P = 0.05) different from controls.
(1) = glyphosate was tested as the IPA salt,
N = The numbers of unhatched cocoons present at the end of the test in the glyphosate and AMPA treatments were slightly higher than the controls but statistical analysis proved that this was probably due to random chance alone and was probably not due to the presence of glyphosate or AMPA.

B. OBSERVATIONS

Mortality: Only one adult worm died during the test at the lowest concentration of glyphosate IPA salt (5.76 mg/kg dry soil). This was not considered to be dose-related since no mortalities were observed at higher concentrations.

Mean body weight: No significant difference in body weight change compared to negative control was noted for adult worms at any concentration or test item treatment.

Behaviour: No abnormal behaviour when compared to untreated controls was observed for adult worms at any concentration or test item treatment.

Reproduction: No significant differences were observed between mean juvenile production for untreated control worms and worms exposed to glyphosate IPA salt, at any concentration tested. Similarly, for worms exposed to AMPA no significant difference from the negative control was seen in terms of juvenile production. No significant differences were observed between number of unhatched cocoons present at day 56 in negative control and both concentrations of glyphosate IPA salt. Similarly, for AMPA, no significant difference from the control was observed in terms of numbers of unhatched cocoons.

Positive control: The adult worms exposed to 5.93 and 13.28 mg Benlate® /kg dry soil showed a significantly reduced growth when compared to negative control at day 28. A significant reduction in juvenile production compared to negative control was seen for 2.66, 5.93 and 13.28 mg Benlate®/kg dry soil. At 5.93 mg Benlate®/kg dry soil a significantly increased number of unhatched cocoons was observed when compared to the negative control.

Reference study with 2-chloroacetamide: The 14 day LC_{50} was determined at 39.4 mg/kg dry soil (95 % confidence limits; 36.0 - 43.1 mg/kg dry soil).

The resulting endpoint values are given below.

Table 8.4.1-4: Toxicity of Glyphosate IPA salt and AMPA to *Eisenia fetida*

Endpoints		Test item [mg/kg dry soil]
LC_{50}	Glyphosate (as IPA salt)	> 28.79
	AMPA	> 28.12
EC_{50}	Glyphosate (as IPA salt)	> 28.79
	AMPA	> 28.12
NOEC	Glyphosate (as IPA salt)	≥ 28.79 (21.31 mg glyphosate a.e./kg dry soil)
	AMPA	≥ 28.12

The following point deviated from the current OECD guideline:

- Test design for NOEC required at least 5 concentrations (only 2 of each in this study) and 8 replicates for the negative control (only 4 in this study).

This deviation is not expected to have any impact on the study validity in that case.

The validity criteria according to guideline OECD 222 are fulfilled as each replicate (containing 10 adults) have produced ≥ 30 juveniles by the end of the test in the control and the coefficient of variation of reproduction was ≤ 30 % in the control. Also, the adult mortality over the initial 4 weeks of the test was ≤ 10 % in the control.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of glyphosate and the metabolite AMPA on mortality and reproduction of *Eisenia fetida* after 56 days of exposure were assessed under laboratory conditions.

Glyphosate, tested as glyphosate IPA salt, and the metabolite aminomethylphosphonic acid (AMPA) had no significant effect on growth or reproduction of *Eisenia fetida* after 56 days of exposure at concentrations up to 28.79 mg glyphosate IPA salt/kg dry soil (21.31 mg glyphosate acid equivalent/kg dry soil) and 28.12 mg AMPA/kg dry soil. Therefore, the NOEC was determined to be ≥ 28.79 mg glyphosate IPA salt/kg dry soil (≥ 21.31 mg glyphosate acid equivalent/kg dry soil) and ≥ 28.12 mg AMPA/kg dry soil.

The study is valid so NOEC ≥ 21.31 mg a.e./kg dry soil can be used in risk assessment for earthworms exposed to glyphosate IPA salt and NOEC ≥ 28.12 mg/kg dry soil can be used in risk assessment for earthworms exposed to AMPA.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.4.1/003
Report author	
Report year	2003
Report title	Laboratory determination of the side-effects of aminomethyl phosphonic acid (AMPA) on the reproductive performance of earthworms (*Eisenia fetida)* using artificial substrate
Report No	01-64-077-ES
Document No	
Guidelines followed in study	OECD draft document (January 2000): Earthworm Reproduction Test – Proposal for a new guideline
Deviations from current test guideline	Deviations from guideline 222 (2016): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The aim of the study was to determine the effects of AMPA (aminomethyl phosphonic acid) on the reproduction of earthworms (*Eisenia fetida)* maintained under laboratory conditions on artificial substrate containing 10 % sphagnum peat for 56 days. The test was conducted with eight nominal test concentrations, encompassing 58.6, 87.8, 131.9, 198.1, 297.1, 445.5, 668.5 and 1002.5 mg test item/kg dry soil thoroughly

mixed into the soil substrate. The water content was adjusted to about 50 % of maximum water holding capacity (WHC). Negative control soil was treated with untreated water only. As a toxic reference, earthworms were exposed to carbendazim at concentrations of 1.0, 2.2 and 5.0 mg/kg dry soil. The test comprised four replicates for each test concentration and toxic reference concentration and eight replicates for the control. The adults were exposed to the test item in the artificial soil substrate for four weeks. Thereafter mortality and mean weight of the survivals were observed. The adults were discarded and after additional four weeks of the test units in the climatic chamber the number of juveniles were assessed.
No test item related mortality was observed up to 1000 mg AMPA/kg dry soil.
The NOEC based on biomass deviation was determined to be 297.1 mg AMPA/kg dry soil and the NOEC based on reproduction was determined to be 198.1 mg AMPA/kg dry soil. The EC_{50} was 562.7 mg AMPA/kg soil. A NOEC of 131.90 mg test item/kg dry soil was suggested for the parameter biomass and number of juveniles. The study is considered valid so EC_{50} of 562.7 mg/kg dry soil and NOEC of 131.9 mg/kg dry soil will be used for risk assessment of earthworms exposed to AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethyl phosphonic acid)
Description:	White powder
Lot/Batch #:	A0164351
Purity:	99.7 % (analysed)

2. Vehicle and/or positive control:

Vehicle: water
Positive control: Carbendazim (99.6%)

3. Test organism:

Species:	Earthworm (*Eisenia fetida*)
Age:	Synchronized adults, > 2 months
Weight:	300 – 600 mg
Source:	In-house rearing (Phytosafe S.A.R.L, 2, rue Marx Dormory, 64000 Pau, France)
Food:	5 g ground cow manure moisten with 6 mL water once per week (days 1, 7, 14, 21 and 28)
Acclimation period:	Not reported

4. Environmental conditions:

Temperature:	19.0 – 21.5 ºC
Photoperiod:	12 h light (416 - 595 Lux)/ 12 h dark
Soil pH:	Control 6.0 (test start), 6.9 (test termination)
	Test item: 5.7 – 6.0 (test start), 6.3 – 6.8 (test termination)
	Reference item: 6.0 (test start), 6.9 – 7.0 (test termination)
Soil moisture content:	Control 43.9 % WHC (water holding capacity, at test termination)
	Test item: 44.3 – 46.2 % WHC (at test termination)
	Reference item: 44.6 – 45.9 WHC (at test termination)

5. Experimental work dates: November 12th, 2002 to January 08th, 2003

B. STUDY DESIGN AND METHODS

Experimental treatments

A sublethal test was conducted with eight nominal test concentrations and one untreated water control. The test substance was prepared by dispersing 10.0249 g of the test item in 500 mL water. Thereafter eight samples containing 1.46, 2.19, 3.29, 4.94, 7.41, 11.11, 16.67 and 25.0 mL test solution were thoroughly mixed into the artificial soil, achieving desired test concentrations of 58.6, 87.8, 131.9, 198.1, 297.1, 445.5, 668.5 and 1002.5 mg test item/kg dry soil, with a final nominal water content of 50% of WHC.
Test units contained 500 g of the oven dried weight artificial soil substrate incorporated into 1.5 to 2 L glass containers, composed of 10% sphagnum peat; 20% kaolinite clay and 70% fine sand, each. Four replicate test containers (test item and reference groups) and 8 replicate test containers (control group) were prepared for each treatment group. 10 adult earthworms were exposed per replicate for 56 days.
As a toxic reference, earthworms were exposed to carbendazim at concentrations of 1.0, 2.2 and 5.0 mg test item/kg dry soil, respectively.

Observations

Four weeks after test initiation, percent mortality and mean weight of the surviving adult earthworms were recorded. At test termination (8 weeks after test initiation), the number of surviving juveniles were determined.
Measurements of pH values were performed at test initiation and at test termination. The soil moisture was recorded at test end. Corresponding percent water holding capacity was calculated. The temperature in the climatic chamber was reported without any detailed information on the respective measurements.

Statistical calculations

For statistical evaluation of the biomass deviation and production of juveniles, F-variance analysis was considered ($\alpha = 0.01$). EC_{50} values including 95% confidence intervals were calculated using Excel calculations. EC_{50} calculations were based on untransformed data due to low confidence of log values.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.4.1-5: Observed effects of AMPA to *Eisenia fetida*

Concentrations [mg test item/kg dry soil]	Observations		
	Mean mortality [%]	Mean biomass deviation [%]	Number of juveniles [Mean ± SD]
Control			
0.0	0.0	- 9.5	120.6 ± 12.4
AMPA			
58.6	0.0	-11.0	114.8 ± 12.1
87.8	0.0	-10.0	112.5 ± 9.8
131.9	0.0	-11.5	110.0 ± 14.8
198.1	0.0	-16.8	109.0 ± 11.2
297.1	0.0	-11.8	93.8 ± 10.2
445.5	0.0	-22.3	66.8 ± 3.4
668.5	2.5	-32.4	41.0 ± 3.2
1002.5	0.0	-34.2	16.3 ± 6.3
Carbendazim			
1.0	0.0	-9.3	56.3 ± 14.9[a]
2.2	2.5	-12.6	9.5 ± 5.8
5.0	2.5	-33.3	0.3 ± 0.5

SD: standard deviation
[a] Percent reduction of the production of juveniles was slightly higher than 50% initially postulated as a maximum.

The EC_{50}, NOEC and LOEC value are given below based on nominal concentrations.

Table 8.4.1-6: Toxicity to *Eisenia fetida* exposed to AMPA

Parameter	AMPA [mg/kg dry soil]
Biomass deviation	
NOEC	297.1
LOEC	445.5
Reproduction	
EC_{50} (95 % CI)	562.7 (381.2 – 744.1)
NOEC	198.1
LOEC	297.1

CI= confidence interval

B. OBSERVATIONS

There was no mortality in the control and a single mortality in the 668.5 mg test item/kg concentration of the test item treated group and in the 2.0 and the 5.0 mg test item/kg dry soil concentration of the reference item group.
Mean percent of biomass deviation was -9.5 % in the control group. In the test item treatment groups, the loss of biomass was similar to the control, ranging from -10.0 to -11.8% in the concentrations between

58.6 and 297.1 mg test item/kg dry soil, with an exception for the 198.1 mg test item/kg dry soil test item with a higher loss in biomass. The loss of biomass was significantly higher for the treatment concentrations of 445.5, 668.5 and 1002.5 mg test item/kg dry soil compared with the control.

Table 8.4.1-7: Percent biomass deviation after 28 days of exposure of adult earthworms to AMPA

Concentrations [mg test item/kg dry soil]	Replicates								Biomass deviation [% ± SD]
	1	2	3	4	5	6	7	8	
Control [%]									
0.0	-9.5	-9.1	-6.0	-10.8	-12.8	-12.9	-4.0	-11.0	-9.5 ± 3.1
AMPA[%]									
58.6	-12.5	-9.5	-8.3	-13.8					-11.0 ± 2.6
87.8	-11.1	-9.8	-9.1	-9.9					-10.0 ± 0.8
131.9	-16.5	-11.4	-12.7	-5.5					-11.5 ± 4.6
198.1	-13.9	-16.3	-21.1	-16.0					-16.8 ± 3.0
297.1	-8.1	-14.7	-17.4	-7.1					-11.8 ± 5.0
445.5	-21.7	-24.8	-19.5	-23.2					-22.3 ± 2.3*
668.5	-35.6	-27.9	-29.2	-36.6					-32.4 ± 4.4*
1002.5	-32.2	-34.4	-36.6	-33.4					-34.2 ± 1.9*
Carbendazim[%]									
1.0	-11.2	-10.4	-6.2	-9.3					-9.3 ± 2.2
2.2	-16.1	-9.7	-19.3	-5.3					-12.6 ± 6.3
5.0	-40.5	-35.0	-29.1	-28.6					-33.3 ± 5.6

SD: standard deviation,
*= statistically significant different from the control according to F-variance analysis.

Mean number of juveniles was 120.6 in the control group, the coefficient of variations was 10.3 %. The production of juveniles was significantly reduced for treatment concentrations ranging between 297.1 and 1002.5 mg AMPA/ kg dry soil.

Table 8.4.1-8: Number of juveniles after 56 days of exposure to AMPA

Concentrations [mg test item/kg dry soil]	Replicates								Number of juveniles [Mean ± SD]	CV in %
	1	2	3	4	5	6	7	8		
Control										
0.0	127	105	125	112	136	134	104	122	120.6 ± 12.4	10.3
AMPA										
58.6	104	122	128	105					114.8 ± 12.1	10.5
87.8	104	121	121	104					112.5 ± 9.8	8.7
131.9	124	106	119	91					110.0 ± 14.8	13.4
198.1	119	94	107	116					109.0 ± 11.2	10.3
297.1	88	109	90	88					93.8 ± 10.2*	10.9
445.5	64	71	64	68					66.8 ± 3.4*	5.1
668.5	45	39	38	42					41.0 ± 3.2*	7.7
1002.5	18	9	24	14					16.3 ± 6.3*	39.0
Carbendazim										

Table 8.4.1-8: Number of juveniles after 56 days of exposure to AMPA

Concentrations [mg test item/kg dry soil]	Replicates								Number of juveniles [Mean ± SD]	CV in %
	1	2	3	4	5	6	7	8		
1.0	49	74	62	40					56.3 ± 14.9[a]	26.5
2.2	8	18	7	5					9.5 ± 5.8	61.1
5.0	0	1	0	0					0.3 ± 0.5	200

SD: standard deviation; CV= Coefficient of variation
*= statistically significant different from the control according to F-variance analysis
[a] Percent reduction of the production of juveniles was slightly higher than 50% initially postulated as a maximum.

Validity of the test according to the current OECD guideline:

- Control mortality < 10% (achieved: 0.0%)
- Production of juveniles in the control > 30 per unit (actual values ranging from 104 to 136)
- Coefficient of variation of reproduction in the control ≤ 30% (achieved: 10.3%)

Therefore, all validity criteria according to guideline OECD 222 are fulfilled.
Moisture content was not monitored throughout the test as requested by the test guideline. However, moisture was in an acceptable range at the end of the test and control criteria passed. Therefore, this is only a minor deviation and has not affected the integrity of the study.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The NOEC based on biomass was determined to be 297.1 mg AMPA/kg dry soil and the NOEC based on reproduction was determined to be 198.1 mg AMPA/kg dry soil. The EC_{50} was 562.7 mg AMPA/kg soil.

Statistical re-evaluation was performed by the RMS (ToXRatPro, Version 2.10) in the RAR 2015. Percent biomass deviation at the end of the exposure period of the adults were re-analysed. Treatments were compared by the t-test procedure after Williams. Significance was α = 0.05.

Table B.9.6-9: Biomass change (%) after 28d of exposure of adult earthworms to AMPA

	AMPA (mg/kg dry soil)								
No.	control	58.6	87.8	131.9	198.1	297.1	445.5	668.5	1002.5
1	90.5	87.5	88.9	83.4	86.1	91.9	78.3	64.4	67.2
2	90.9	90.5	90.2	88.6	83.7	85.3	75.2	72.1	65.6
3	94	91.7	90.9	87.3	78.9	82.6	80.5	70.8	63.4
4	89.2	86.2	90.1	94.5	84	92.9	76.7	63.4	66.6
Replicates	4	4	4	4	4	4	4	4	4
Mean	91.2	89.0	90.0	88.5	83.2*	88.2*	77.7*	67.7*	65.7*
Std.Dev	2.0	2.6	0.8	4.6	3.0	5.0	2.3	4.4	1.7
CV%	2.2	2.9	0.9	5.2	3.7	5.7	2.9	6.5	2.5

*statistically significant different from the control

Table B.9.6-10: Number of earthworm juvenils after 56 days exposure to AMPA

	AMPA (mg/kg dry soil)								
No.	control	58.6	87.8	131.9	198.1	297.1	445.5	668.5	1002.5
1	116	104	104	124	119	88	64	45	18
2	119	122	121	106	94	109	71	39	9
3	135	128	121	119	107	99	64	38	24
4	113	105	104	91	116	88	68	42	14
Replicates	8	4	4	4	4	4	4	4	4
Mean	120.6	114.8	112.5	110.0	109.0*	93.8*	66.8*	41.0*	16.3*
Std.Dev	12.4	12.1	9.8	14.8	11.2	10.2	3.4	3.2	6.3
CV%	10.z3	10.5	8.7	13.4	10.3	10.9	5.1	7.7	39.0

RMS changed from 8 to 4 replicate values in the control (taking into account a mean of two values) and reported the biomass deviation as a mean percentage.

A NOEC of 131.90 mg test item/kg dry soil was suggested for the parameter biomass and number of juveniles. The study is considered valid so EC_{50} of 562.7 mg/kg dry soil and NOEC of 131.9 mg/kg dry soil, can be used for risk assessment of earthworms exposed to AMPA.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.4.1/004
Report author	
Report year	2002
Report title	AMPA - Earthworm (*Eisenia fetida*), effects on reproduction
Report No	RRR84121
Document No	-
Guidelines followed in study	DIN ISO 11268-2: 1998: Soil quality – effects of pollutants on earthworms – Part 2: Determination of effects on reproduction
Deviations from current test guideline	Deviations from the guideline OECD 222 (2016): Major: - Coefficient of variation in the reproduction rate for control was 38 % instead of <30 % required. Minor: - 3 test item concentrations were tested instead of at least 5 - 4 replicates for the negative control used instead of 8 - Food was added just before application instead of 1 day after application
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

In a laboratory study, adult earthworms (*Eisenia fetida*) were exposed for 56 days to three test concentrations of AMPA in artificial soil containing 10% sphagnum peat and observed for mortality, growth, and reproduction. A negative control group was maintained concurrently. Four replicate test chambers were maintained in each treatment with 10 worms in each test chamber. Nominal test concentrations were 0.79, 3.94 and 19.7 mg AMPA/kg dry soil. After 28 days, number and weight of surviving adult worms was determined. After a further 28 days the reproduction rate was determined by counting the numbers of juvenile earthworms and cocoons in each test vessel.

No mortality was observed in any treatment group. The body weight of the earthworms exposed to AMPA were not statistically different when compared to the control up to and including the highest test concentration of 19.7 mg AMPA/kg dry soil. There were no statistically significant effects on reproduction were observed up to and including the highest test concentration of 19.7 mg/kg dry soil. No behavioural abnormalities were observed in any of the treatment groups.

The coefficient of variation for reproduction in control groups was higher than 30 % at the end of the test. The validity criteria according to guideline OECD 222 are therefore not considered fulfilled. The no-observed-effect-concentration (NOEC) of AMPA for mortality, growth and reproduction of the earthworm *Eisenia fetida* was found to be 19.7 mg test item/kg dry soil, which was the highest concentration tested.

However, due to the guideline deviations, the study is considered invalid and not acceptable for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (aminomethyl phosphonic acid)
Description:	White powder
Lot/Batch #:	FA005563
Purity:	99 %

2. Vehicle and/or positive control: Vehicle: demineralised water
Positive control: Derosal flüssig (31.5% carbendazim)

3. Test organism:

Species:	Earthworm (*Eisenia fetida*)
Age:	synchronized adults with clitellum, 4 months
Weight:	300 – 600 mg
Source:	Biologische Bundesanstalt (BBA), Braunschweig, Germany
Food:	Dried litter of stinging nettle and porridge oats
Acclimation period:	2 days in artificial soil under test conditions

4. Environmental conditions:

Temperature:	20 ± 2 °C
Relative humidity	70 – 80 %
Photoperiod:	16 h light / 8 hours dark (400 - 800 lux)
pH:	5.45 – 5.57 (test start), 6.03 – 6.30 (test termination)
Water content:	46.11-51.53%

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (OECD 207, 10% Sphagnum-peat, air dried, finely ground; 20 % kaolin clay, 69 % industrial quartz sand and 0.43 % calcium carbonate). Four replicate test chambers were maintained in each treatment, with 10 worms in each test chamber. Nominal test concentrations of 0.97, 3.94 and 19.7 mg AMPA/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted to about 50 % of maximum water holding capacity (WHC) using demineralised water. Negative control soil was treated with demineralised water only.
As a toxic reference, earthworms were exposed in a separate study to Derosal flüssig (31.5 % carbendazim). The adult earthworms were exposed to the test item for 4 weeks; the adult worms were counted, removed and weighed per replicate. The remaining soil was returned to the reproductive test for additional 4 weeks. Thereafter, juveniles were counted. Temperature and relative humidity were monitored continuously. Water content and pH were determined at the beginning and the end of the test.
2. Observations: The adult earthworms were exposed to the test item for 4 weeks, after which the artificial soil was emptied onto a tray and the adult worms were counted, removed and weighed per replicate after they were washed under tap water and dried on filter paper. Missing worms and the earthworms, which failed to respond to gentle stimulation, were considered to be dead.
The number of damaged earthworms (e.g. lack of movement, rigidity, etc.) was assessed at day 28 after application.
Individual weight of the earthworms was recorded at day 28 after application.
Reproduction was recorded 8 weeks after the test initiation as mean number of juveniles per test container and replicate.

3. Statistical analysis: As data for body weight changes and the reproduction were normally distributed and homogeneous, the Dunnett's test was used (multiple comparison, two-sided for weight and one sided smaller for reproduction, $\alpha = 0.05$). NOEC and EC-values for reproduction were determined by regression analysis in an appropriate dose-response function.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.4.1-9: Effects of AMPA on survival, growth and reproduction of *Eisenia fetida*

Test parameter	Control	AMPA [mg test item/kg dry soil]		
		0.79	3.94	19.7
Mortality (day 28) [%]	0	0	0	0
Weight change (day 28) [%][1)]	-	+10.71	+1.79	+7.14
No. of juveniles (day 56)	60 ± 23	64 ± 23	61 ± 5	68 ± 10
CV [%]	38	36	9	14
Reproduction [%] of control (56 days)[1)]	-	+7	+2	+13

[1)] negative values indicate a decrease, positive values an increase when compared to the control

The LC_{50} and NOEC values are given below based on nominal concentrations.

Endpoints	AMPA [mg test item/kg dry soil]	Reference item [mg/kg]
LC_{50} (28 d)	>19.7	>5.04
$NOEC_{mortality}$ (28 d)	19.7	5.04
$EC_{50,\ biomass}$ (28 d)	>19.7	n.d.
$NOEC_{biomass}$ (28 d)	19.7	1.26
$EC_{50,\ repro}$ (56 d)	>19.7	2.9 (2.60 - 3.23)
$NOEC_{repro}$ (56 d)	19.7	1.26

B. OBSERVATIONS

No pathological symptoms or chances in behaviour of the adult earthworms were notes in any of the test item treatments and the control. During test period, body weights of earthworms in treated and control groups slightly increased or remained at starting level. No mortality was observed in any of the treatment groups and in the control. Different test item concentrations had no effects on the number of offspring. There was no statistically significant difference between the treated groups and the control.

The LC_{50}-value of the reference test item was determined to be 2.9 mg/kg dry substrate.
Each control replicate containing 10 adults produced ≥ 30 juveniles and adult mortality in the control treatments after four weeks did not exceed 10%. The coefficient of variation for reproduction in control groups was higher than 30% at the end of the test. The validity criteria according to guideline OECD 222 are therefore not considered fulfilled.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The no-observed-effect-concentration (NOEC) of AMPA for mortality, growth and reproduction of the earthworm *Eisenia fetida* was found to be 19.7 mg test item/kg dry soil, which was the highest concentration tested.
However, due to the following deviations, the study is considered invalid and not acceptable for risk assessment:

- 3 test item concentrations were tested instead of at least 5
- 4 replicates for the negative control used instead of 8
- Food was added just before application instead of 1 day after application
- Coefficient of variation in the reproduction rate for control was 38% instead of <30% required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.4.1/005
Report author	von Mérey, G. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota
Document No	DOI: 10.1002/etc.3438 E-ISSN: 1552-8618
Guidelines followed in study	OECD 222; OECD 226; OECD 232; OECD 216
Deviations from current test guideline	Earthworm cocoons were not counted, in accordance with OECD 222.
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

The exposure risk from glyphosate and the primary soil metabolite aminomethylphosphonic acid (AMPA) on representative species of earthworms, springtails, and predatory soil mites and the effects on nitrogen-transformation processes by soil microorganisms were assessed under laboratory conditions based on internationally recognized guidelines. For earthworms, the reproductive no-observed-effect concentration (NOEC) was 472.8 mg glyphosate acid equivalent (a.e.)/kg dry soil, which was the highest concentration tested, and 198.1 mg/kg dry soil for AMPA. For predatory mites, the reproductive NOEC was 472.8 mg a.e./kg dry soil for glyphosate and 320 mg/kg dry soil for AMPA, the highest concentrations tested. For springtails, the reproductive NOEC was 472.8 mg a.e./kg dry soil for glyphosate and 315 mg/kg dry soil for AMPA, the highest concentrations tested. Soil nitrogen-transformation processes were unaffected by glyphosate and AMPA at 33.1 mg a.e./kg dry soil and 160 mg/kg dry soil, respectively. Comparison of these endpoints with worst-case soil concentrations expected for glyphosate (6.62 mg a.e./kg dry soil) and AMPA (6.18 mg/kg dry soil) for annual applications at the highest annual rate of 4.32 kg a.e./ha indicate very low likelihood of adverse effects on soil biota.

Materials and methods

Test substances

Glyphosate (N-phosphonomethylglycine) is an acidic substance, which is manufactured and formulated as a salt to increase the solubility in water and compatibility with other formulation components. In water, AMPA is highly soluble (56 g/L at 20 °C), whereas neither glyphosate nor AMPA is significantly soluble in common organic solvents. Therefore, no cosolvent was required, and both stock solutions of glyphosate and AMPA test items were prepared in deionized water (5 - 20 Mohm at 25 °C). Two batches of AMPA analytical reference standards with purity of 98.7% (synthesized by Chemir) and 99.7 % (Acros Organics BVBA) were dissolved in deionized water. For soil nitrogen-transformation tests, stock solutions of glyphosate acid technical grade (96.59 % purity; Monsanto Europe) were prepared by direct addition of test item to deionized water. For all other tests, glyphosate isopropylamine salt (nominal purity 62 % w/w, measured purity 63.81 ± 0.29 % w/w; MON 0139), corresponding to 45.9% w/w glyphosate a.e. (measured 47.28 ± 0.21 % w/w; Monsanto Europe), were prepared in deionized water.

Earthworm reproduction tests

The earthworm reproduction test with glyphosate was conducted according to OECD guideline 222. For AMPA, an earthworm reproduction test was conducted according to the OECD 222. Both testing guidelines are equivalent in terms of the procedures employed during the tests (soil pH, temperature, lighting regime, soil composition and humidity, rearing, feeding quantities, test design, endpoints, number of replicates, growth stage of worms at test initiation, and so on). Therefore, to avoid repetition, the procedures used in the glyphosate study only are described.

Glyphosate - Earthworm reproduction test. In the earthworm reproduction glyphosate study *Eisenia fetida* (Haplotaxida: Lumbricidae, Savigny, 1826) were used as the test species. Mature adult *E. fetida* (~3 mo old with clitellum), weighing between 300 mg and 600 mg, were obtained from an age-synchronized stock culture from the test facility and reared under ambient laboratory conditions in the test facility. The original breeding animals were purchased from W. Neudorff. A detailed description of earthworm culturing is provided in Annex 4 of OECD 222. The *E. fetida* were reared in the laboratory on standard breeding medium (1:1:1 mixture of straw, horse manure, and peat; straw and horse manure were purchased from farmers, and peat was purchased from Torfwerk Moorkultur Ramsloh); no exposure to the test item was allowed prior to use in testing. Testing was conducted in artificial soil, equivalent to the soil in which the worms were originally cultured. The test aims to evaluate effects on adult body weight and survival percentage (according to treatment) during an initial 4-wk adult exposure period. Effects on juvenile production were then assessed at the end of a 4-wk period that followed directly after adult removal from the test. Behavior (including feeding activity) and pathological symptoms (e.g., lethargy, morphological alterations) of adults and juveniles were also assessed.

On the day before the test start, earthworms (from aged-synchronized batches, to ensure that similar-sized earthworms were used) were acclimated to test conditions in a separate batch of artificial soil supplemented with pasteurized horse manure, purchased from farmers and collected from horses not treated with growth promoters, nematicides, or other veterinary products - also used as the food source during testing. On test start day, volumes of the test solution (prepared by direct addition of glyphosate isopropylamine to deionized water) were mixed into bulk samples of artificial soil, to achieve nominal glyphosate soil concentrations of 14.18 mg a.e./kg dry soil, 23.64 mg a.e./kg dry soil, 47.28 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil. Glyphosate test concentrations were selected to cover the range and exceed field exposure concentrations. A toxic reference test was also performed in a separate test with carbendazim (Nutdazim 50 Flow, SC 500) at concentrations of 5 mg/kg dry soil and 10 mg/kg dry soil.

Test vessels were filled with the appropriate treated soil (810 g wet wt corresponding to 600 g dry wt). Groups of 10 individually weighed earthworms were randomly assigned to replicates within each treatment group, with a total of 40 earthworms used per treatment group divided equally between 4 replicates. For the control group (water only), 80 worms were used, divided equally between 8 replicates. Groups of 10 earthworms were placed onto the assigned replicate soil surface and closed with perforated transparent lids (following a brief burrowing period) to reduce evaporative water loss, allow gaseous exchange, and prevent worms from escaping the replicate vessels. Test vessels were then randomly positioned in an environmental test chamber under continuous light (to maintain worms in the soil). On day 1 and weekly thereafter for the

4-wk adult exposure period, 5 g of air-dried finely ground horse manure was scattered on the soil surface of each test vessel and wetted with 5mL of deionized water. The amount of manure applied each week (up to 5 g) was dictated by feeding activity.

After 4 wk, adult earthworms were removed from the vessels by emptying the contents of each replicate vessel onto a tray and removing the adult worms. Care was taken not to remove any cocoons from the soil. Cocoons were not counted, in accordance with OECD 222. It can be reasonably assumed that effects on cocoon numbers would lead to effects on numbers of juveniles; hence, the endpoint number of juveniles accounts for effects at earlier life stages of earthworm progeny. All worms were rinsed with deionized water and dried on filter paper before recording body weights (by replicate and by treatment). Behavioral (including feeding activity) and pathological symptoms were also recorded during the exposure period and at the time of adult removal. The adult worms were then discarded. The soil in each replicate vessel was then mixed carefully with 5 g of manure, and the mixture was returned to the vessels. The test continued for a further 4 wk. At test termination (8 wk after adult addition) the number of surviving juveniles in each test vessel was recorded on manual inspection of the substrate. Soil was emptied on the lower edge of a white tray (30 cm × 40 cm). Subportions of the soil were spread in the middle of the tray, resulting in a thin layer of soil of approximately 10 cm × 10 cm. The subportion was examined thoroughly for juvenile worms, after which it was moved to the upper edge of the tray. This procedure was repeated until the entire soil from a vessel was examined. The entire procedure was repeated until there were no additional juvenile counts in 2 consecutive counting procedures, resulting in an average of 5 counting procedures per vessel. The counting tray and soil samples were illuminated using a fiber-optic light source connected with a double gooseneck light guide. The water content and pH of the artificial soil were determined. Adult body weights and the effects on reproduction (juvenile numbers) were analyzed using a lower-tailed Dunnett's multiple comparisons test ($\alpha = 0.05$). The Kolmogorov-Smirnov test and Cochran's test procedure were used, respectively, to test the biomass data for normality and homogeneity of variance. Survival was analyzed with a 1-sided Fisher's exact binomial test with Bonferroni correction ($\alpha = 0.05$).

AMPA - Earthworm reproduction tests. The procedures used during the AMPA earthworm study are considered equivalent to those employed in the glyphosate earthworm reproduction study described above in Glyphosate—Earthworm reproduction test. Mature adult *E. fetida* (~3mo old with clitellum), weighing between 300 mg and 600 mg, were obtained from an age-synchronized stock culture from the test facility and reared under ambient laboratory conditions in the test facility. A detailed description of earthworm culturing is provided in Annex 4 of OECD 222.

In the AMPA earthworm reproduction study, mature (clitellated) adult *E. fetida* were exposed to AMPA (99.7 % purity; Acros Organics BVBA) mixed into artificial soil at nominal soil concentrations of 58.6 mg AMPA/kg dry soil, 87.8 mg AMPA/kg dry soil, 131.9 mg AMPA/kg dry soil, 198.1 mg AMPA/kg dry soil, 297.1 mg AMPA/kg dry soil, 445.5 mg AMPA/kg dry soil, 668.5 mg AMPA/kg dry soil, and 1002.5 mg AMPA/kg dry soil. A control group was prepared using deionized water only. A toxic reference test was also performed in parallel using earthworms from the same batch, exposed to carbendazim at concentrations of 1.0 mg active substance (a.s.)/kg dry soil, 2.2 mg a.s./kg dry soil, and 5.0 mg a.s./kg dry soil. For effects on biomass and production of juveniles, homogeneity was tested with the Brown-Forsythe and Bartlett tests. Dunnett's multiple comparison test was conducted using GraphPad Prism, Ver 6.03, because a continuous response could not be observed for all the test concentrations, as recommended by the OECD 222 test guideline and the OECD statistical guidance. The 50% effect rate on reproduction was calculated using GraphPad Prism.

Soil predatory mite reproduction test

The soil predatory mite reproduction tests for glyphosate and AMPA were both conducted according to OECD guideline 226 predatory mite (*Hypoaspis* [Geolaelaps] *aculeifer*) reproduction test in soil. The procedures used in the 2 studies were identical. Full details of the procedures are presented for glyphosate only. The *Hypoaspis* used in these studies were originally purchased from Katz Biotech and reared in the test facility under ambient conditions since June 2005.

Glyphosate - Soil predatory mite reproduction test. The glyphosate soil predatory mite reproduction test was conducted using glyphosate isopropylamine salt (MON 0139). Survival of mites (*H. aculeifer*) and their reproductive performance were evaluated at 4 nominal concentrations, equivalent to 50 mg MON 0139/kg dry soil, 100 mg MON 0139/kg dry soil, 500 mg MON 0139/kg dry soil, and 1000 mg

MON 0139/kg dry soil (= 23.64 mg a.e./kg dry soil, 47.28 mg a.e./kg dry soil, 236.40 mg a.e./kg dry soil, and 472.80 mg a.e./kg dry soil, respectively). A negative control with deionized water only was also included. A toxic reference test was performed in parallel using dimethoate EC400 (422.4 g/L; Perfekthion) at concentrations of 4.1 mg active ingredient (a.i.)/kg dry soil, 5.12 mg a.i./kg dry soil, 6.4 mg a.i./kg dry soil, 8.0 mg a.i./kg dry soil, and 10 mg a.i./kg dry soil. Mites were reared in the laboratory under ambient conditions on a mixture of plaster of paris, activated charcoal, and deionized water (8:1:9). Adults with no more than a 3-d age difference were used at the start of the test. No exposure of the mites to glyphosate was allowed prior to the test. Each treatment group contained 40 mites divided equally between 4 replicate vessels, with the control group comprising 8 replicates, each containing 10 mites. In addition, 2 test vessels without mites were included with each test concentration and in the control group for soil pH measurements. Glass bottles (100mL nominal volume) with screw tops were filled with 20 g (dry wt) artificial soil at the required test concentrations. Cheese mites were added as a food source to the surface of the soil, and vessels were then covered to prevent mites from escaping. Bottles were opened every second day during the 14-d test for the addition of food and to allow aeration. At the end of the test (day 14), the parental mites and juveniles were counted, after extraction using a MacFayden high-gradient extractor (heat/light extraction method). This was achieved by adding the soil substrate from each test vessel into a canister placed inverted onto the extraction system. Soil substrate was retained within the canister using a plastic net (2mm mesh size) on the bottom. Beneath the canister was a funnel attached to a collecting flask with 25mL of a fixing liquid. A temperature gradient was created between the upper and the lower parts of the system, by circulating heated air in the canister area and cooled air in the collection area. Over the 48-h extraction time, the following regime was applied: 25 °C for 12 h, 35 °C for 12 h, and 45 °C for 24 h. During this time, adults and juveniles moved down through the soil away from the heat source and fell through the funnel into the fixing liquid. Extraction efficiency was determined to be 95% in a separate extraction using vessels containing a known number of juvenile and adult mites in untreated substrate. Water content and pH were determined at test start and end. Statistical analysis was performed with the software ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni-Holm correction for mortality and a 1-sided Dunnett multiple comparisons test for reproduction ($\alpha = 0.05$) were used to compare the control with independent test item groups. Abbott's formula was used to correct for control mortality.

AMPA - Soil predatory mite reproduction test. The soil predatory mite reproduction test with AMPA was conducted at 5 nominal application rates, equivalent to 40 mg test item/kg dry soil, 80 mg test item/kg dry soil, 160 mg test item/kg dry soil, 240 mg test item/kg dry soil, and 320 mg test item/kg dry soil. A negative control (deionized water only) group was also included. All procedures and observations in the test with AMPA were as described for the mite (OECD 226) test with glyphosate in Glyphosate—Soil predatory mite reproduction test. A reference test was performed with dimethoate EC400 (414.8 g/L) at test concentrations of 0 mg a.i./kg dry soil, 4.1 mg a.i./kg dry soil, 5.12 mg a.i./kg dry soil, 6.4 mg a.i./kg dry soil, 8.0 mg a.i./kg dry soil, and 10 mg a.i./kg dry soil.

Springtail reproduction tests

The springtail reproduction tests for glyphosate and AMPA were both conducted according to OECD guideline 232. The procedures used in the 2 studies were identical. Full details of the procedures are presented for glyphosate only. Springtails used in these studies were originally purchased from Biologische Bundesanstalt in May 2000 and reared in the laboratory of the test facility under ambient laboratory conditions.

Glyphosate - Springtail reproduction test. The springtail reproduction test conducted for glyphosate was conducted using glyphosate isopropylamine salt. Survival of springtails (*Folsomia candida*) and their reproductive performance were evaluated at 5 nominal application rates of 32 µL MON 0139/kg dry soil, 50 µL MON 0139/kg dry soil, 100 µL MON 0139/kg dry soil, 500 µL MON 0139/kg dry soil, and 1000 µL MON 0139/kg dry soil (= 15.1 mg a.e./kg dry soil, 23.6 mg a.e./kg dry soil, 47.3 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil, respectively). A negative control with deionized water only was also included. In a reference toxicity test with Betosip (15.7 % phenmedipham), concentrations of 50 mg/kg dry soil, 100 mg/kg dry soil, 200 mg/kg dry soil, and 400 mg/kg dry soil were tested. Each treatment group, including the control group, comprised 50 mites divided equally between 5 replicate vessels. For each treatment group and for the control group, 2 test vessels without springtails were provided for pH measurement purposes. Glass containers (150 mL nominal volume) were filled with

30 g (wet wt) of the required treated or control soil. Springtails were reared in the laboratory under ambient conditions on a mixture of plaster for stucco, activated charcoal, and water (8:1:9). No exposure to the test item was allowed prior to testing. Juvenile springtails, 10 d to 12 d old and from a synchronized cohort, were added to each test vessel and then covered with a glass lid for 28 d, following which the surviving adults and juveniles were counted. Water content and pH were determined at test start and end. Adult and juvenile springtails were counted at test end. Statistical analysis was performed with the software ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni correction ($\alpha = 0.05$) and Welch's t test ($\alpha = 0.05$), because of non-heterogeneity of variance, were used to compare the control with the independent test item groups for significance of parental mortality and reproductive reduction, respectively. Abbott's formula was used to correct for control mortality.

AMPA = Springtail reproduction test. The springtail reproductive test for AMPA was conducted with AMPA (98.7 % purity) mixed into artificial soil at 5 nominal application rates, equivalent to 30 mg/kg dry soil, 54 mg/kg dry soil, 97.2 mg/kg dry soil, 175 mg/kg dry soil, and 315 mg/kg dry soil. The negative control used deionized water only. In a separate toxic reference test with 100% crystalline boric acid (BDH Prolabo) mixed with the soil, also included in the test design, the sensitivity of the population was determined with test concentrations of 0 mg/kg dry soil, 44 mg/kg dry soil, 67 mg/kg dry soil, 97.2 mg/kg dry soil, 150 mg/kg dry soil, and 225 mg/kg dry soil. The procedures used during the Springtail reproduction study were essentially equivalent to those used in the springtail test with glyphosate (described in Glyphosate - Springtail reproduction test) with the following exceptions. Each treatment group comprised 40 springtails (10 per test vessel), whereas the control group comprised 8 replicates. Statistical evaluation was performed with ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni correction and a 1-sided Dunnett test were used to compare the control with independent test item groups. Mortality of adult springtails

Soil nitrogen-transformation tests

Soil nitrogen-transformation tests were conducted with glyphosate and AMPA according to OECD guideline 216 and performed according to good laboratory practice. The procedures used in the 2 tests were identical, although tested rates differed. Full details of procedures used are presented for glyphosate only.

Glyphosate - Soil nitrogen-transformation test. The soil nitrogen-transformation test for glyphosate was conducted using glyphosate acid (96.59 % purity; Monsanto Europe) applied at 2 soil concentrations, 6.62 mg a.e./kg dry soil and 33.1 mg a.e./kg dry soil. The tested rates were equivalent to 1 and 5 times the maximum predicted environmental concentration in soil following a worst-case application of glyphosate to bare soil in the EU. Each treatment group and the control comprised 3 replicate test vessels. The control was treated with water only. Field-collected soil was used (LUFA standard soil, type 2.3). On collection, the soil was manually cleared of large objects, such as stones and parts of plants, and then moist-sieved to a particle size ≤ 2 mm. The soil was stored under aerobic conditions in the dark at 4 ± 2 °C until required for use.

Glyphosate was prepared in deionized water and then mixed into a bulk sample of soil at the start of the test. The soil moisture content was 40 % (± 5 %) of the maximum water holding capacity. During the test, the weight of a moisture control vessel maintained under the same test conditions was used as a guide to correct for test vessel water loss. Control and treated bulk samples of soil were amended with ground lucerne meal (0.5 %) as a nitrogen source with a C to N ratio of 16:4:1. Bulk samples were then subsampled (~500 g) into replicate vessels and incubated at 20 ± 2 °C for 28 d. All containers were covered with a perforated lid to avoid evaporative water loss and stored in the dark. Soil (10 g) was taken from 1 replicate from each treatment for pH (water) determination at the start and end of the Glyphosate - Soil nitrogen-transformation study. An additional soil sample was taken from 1 replicate per treatment for moisture and dry matter content determination at the end of the study. As soon as possible after dosing (day 0) and after 7 d, 14 d, and 28 d, a 50-g soil sample (based on dry wt) was removed from each replicate to determine NH_4^+, NO_2^-, and NO^3. Soil extracts were prepared by adding 250mL of 2 M KCl, then shaking for 2 h and centrifuging for 15 min. The supernatant was analyzed using a Bran+Luebbe Autoanalyzer AA3 system. Effects below 25% deviation from control were not considered to be biologically significant.

AMPA - Soil nitrogen-transformation test. In the soil nitrogen-transformation test conducted for AMPA, the bulk samples of field-sampled soil were prepared at AMPA (98.7% purity) soil concentrations of 40 mg/kg dry soil, 80 mg/kg dry soil, 160 mg/kg dry soil, 320 mg/kg dry soil, and 640 mg/kg dry soil. In

addition, a negative control (nontreated soil) was tested. Although conducting reference tests and use of positive controls are not guideline requirements, in a separate reference test with dinoterb (2-tert-butyl-4,6-dinitrophenol, 99.9 % purity; Sigma-Aldrich Chemie), test concentrations of 6.8 mg/kg dry soil, 16 mg/kg dry soil, and 27 mg/kg dry soil were applied, in addition to the control (0 mg/kg dry soil), with 3 replicates per treatment group.

Results

Glyphosate - Earthworm reproduction test

There was 0 % mortality of adult *E. fetida* at glyphosate concentrations of 14.18 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil. Mortality (2.5%) was observed at 23.64 mg a.e./kg dry soil and 47.28 mg a.e./kg dry soil, which is considered incidental background mortality given the 10 % validity criterion for adult mortality in the control (p > 0.3). No statistically significant differences were detected for adult biomass (p > 0.05; Figure A below) and for the numbers of juveniles produced at each of the treatment groups when compared to the control (p > 0.05; Figure A below). Adult and juvenile feeding behavior was also not adversely affected over the duration of the test (56 d). The resulting no-observed-effect concentration (NOEC) for effects on reproduction was determined therefore to be the maximum test concentration of 472.8 mg a.e./kg dry soil.

In the reference test with carbendazim, juveniles were reduced by 65 % and 92 % at 5 mg reference item/kg dry soil and 10 mg reference item/kg dry soil, respectively. The control treatment had a mean number of 143 juveniles, whereas 5 mg/kg dry soil and 10 mg/kg dry soil treatments with carbendazim had a mean number of 51 juveniles and 11 juveniles, respectively. These reference test values show that the test system was appropriate to detect toxic effects on earthworm reproduction. The validity criteria, namely adult mortality < 20 % and number of juveniles per replicate ≥ 30 in the control treatment, and coefficient of variance between control replicates < 30 % were all met. The guideline requirements for water content, temperature, and pH were all met.

AMPA - Earthworm reproduction test

In the earthworm reproduction study with AMPA, there were no significant effects on *E. fetida* adult mortality across concentrations compared to the control (p > 0.22). In all treatment groups, all 10 adults survived the treatments, except for 1 mortality in a single replicate of the 668.5 mg/kg dry soil treatment (Figure B below). Adult earthworm biomass was significantly lower compared to the control at the 445.5 mg AMPA/kg dry soil, 668.5 mg AMPA/kg dry soil, and 1002.5 mg AMPA/kg dry soil test concentrations (p < 0.0001; Figure B below). Adult biomass at 198.1 mg AMPA/kg dry soil was also significantly lower than the control (p = 0.007), but at 297.1 mg AMPA/kg dry soil there was no significant difference (p > 0.802) because the biomass (in percentage of control) was 88.5 % and 88.2 % in the 131.9 mg AMPA/kg dry soil and the 297.1 mg AMPA/kg dry soil treatment groups, respectively. The effect at 198.1 mg AMPA/kg dry soil is therefore considered to not be treatment-related. Juvenile production was not significantly affected at concentrations up to 198.1 mg/kg dry soil (p > 0.342). At 297.1 mg AMPA/kg dry soil and higher concentrations juvenile *E. fetida* numbers decreased significantly compared to the control (p = 0.0013). The resulting NOEC for effects on reproduction therefore was concluded to be 198.1 mg/kg dry soil, with a reproductive lowest-observed effect concentration (LOEC) at 297.1 mg AMPA/kg dry soil. The calculated 50% effective concentration (EC50) value for AMPA on earthworm survival was > 1000 mg/kg dry soil. The reproduction EC50 value was calculated at 654.7 mg AMPA/kg dry soil (95 % confidence interval 610.9 - 705.5 mg/kg dry soil). The resulting regression equation was y = - 0.1108 (± 0.005) AMPA mg/kg + 122.6 (± 2.271), with an R^2 of 0.92. The reference test item carbendazim resulted in decreased biomass of 33.3 % at 5.0 mg/kg dry soil and no reproduction, showing that the test system was sensitive to pesticide application. The validity criteria and guideline requirements were all met.

Glyphosate - Soil predatory mite reproduction test

No significant effects were observed on soil mite survival (p > 0.3) or reproduction (p > 0.05) up to and including the highest test concentration (472.8 mg a.e./kg dry soil; Figure C below) after 14 d of continuous exposure. All validity criteria and guideline recommendations were met. In the reference test with dimethoate the EC50 on reproduction was determined to be 4.9 mg a.i./kg dry soil. The reference test

demonstrated the sensitivity of the test system to detect reproductive toxicity in soil mites. The NOEC was therefore set at the highest test concentration.

AMPA - Soil predatory mite reproduction test
No significant effects were observed on soil mite survival ($p > 0.1$) or reproduction ($p > 0.05$) up to and including the highest test concentration (320 mg AMPA/kg dry soil; Figure D below). All validity criteria and guideline recommendations were met. The reference test with dimethoate showed that the test was sensitive at detecting reproductive toxicity in soil mites. The NOEC for AMPA was therefore concluded to be at the highest test concentration, 320 mg/kg dry soil.

Glyphosate - Springtail reproduction test
No significant effects were observed on springtail survival ($p > 0.5$) or reproduction ($p > 0.05$) up to and including the highest test concentration (472.8 mg a.e./kg dry soil; Figure E below). The validity criteria and guideline recommendations were all met. In the reference test with phenmedipham, the EC50 on reproduction was determined to be 28.4 mg phenmedipham/kg dry soil, which demonstrates that the test system was sensitive for reproductive toxicity. The NOEC for glyphosate was therefore concluded to be the highest test concentration.

AMPA - Springtail reproduction test
No significant effects were observed on springtail survival ($p > 0.5$) or reproduction ($p > 0.06$, $\alpha = 0.01$) up to and including the highest test concentration (315 mg AMPA/kg dry soil; Figure F below). The validity criteria and the guideline recommendations were all met. In the reference test with boric acid, the EC50 for reproduction was determined to be 108.6 mg/kg dry soil, demonstrating sensitivity to reproductive toxicity of the test system. The NOEC for AMPA was therefore concluded to be the highest test concentration.

Glyphosate - Soil nitrogen transformation test
Nitrogen-transformation rates in the soil treated at glyphosate rates equivalent to 6.62 mg a.e./kg dry soil and 33.1 mg a.e./kg dry soil were - 0.13% and 2.13% different compared to the control between day 14 and day 28, respectively (Figure G below). The validity criterion of < 15% variation between control treatments was met in the test. As the rates of nitrate formation between the control and the treated groups were < 25 % on day 28, glyphosate can be evaluated as having no long-term influence on nitrogen transformation in LUFA soils at concentrations ≤ 33.1 mg a.e./kg dry soil. No reference test was conducted, in line with the OECD guideline.

AMPA - Soil nitrogen-transformation test
Stimulation of nitrogen-transformation rates was observed across all treatments on day 7 and day 14, which was possibly linked to the high levels of nitrogen and phosphorus released from the degradation of AMPA in the biologically active soil. Only in the 2 highest test concentrations did the increase exceed 25 % compared to the control at 28 d. The test was therefore prolonged from 28 d to 56 d for the 2 highest test concentrations, 320 mg/kg dry soil and 640 mg/kg dry soil (Figure H below). At 56 d, the deviation from the control was 26.7 % at 320 mg/kg dry soil and 43.1 % at 640 mg/kg dry soil. The reference test results with dinoterb showed increases of 37.6 % at 6.8 mg/kg dry soil, 51.4 % at 16.00 mg/kg dry soil, and 27.1 % at 27 mg/kg dry soil. The validity criterion of < 15% variation between controls was met at all sampling intervals.

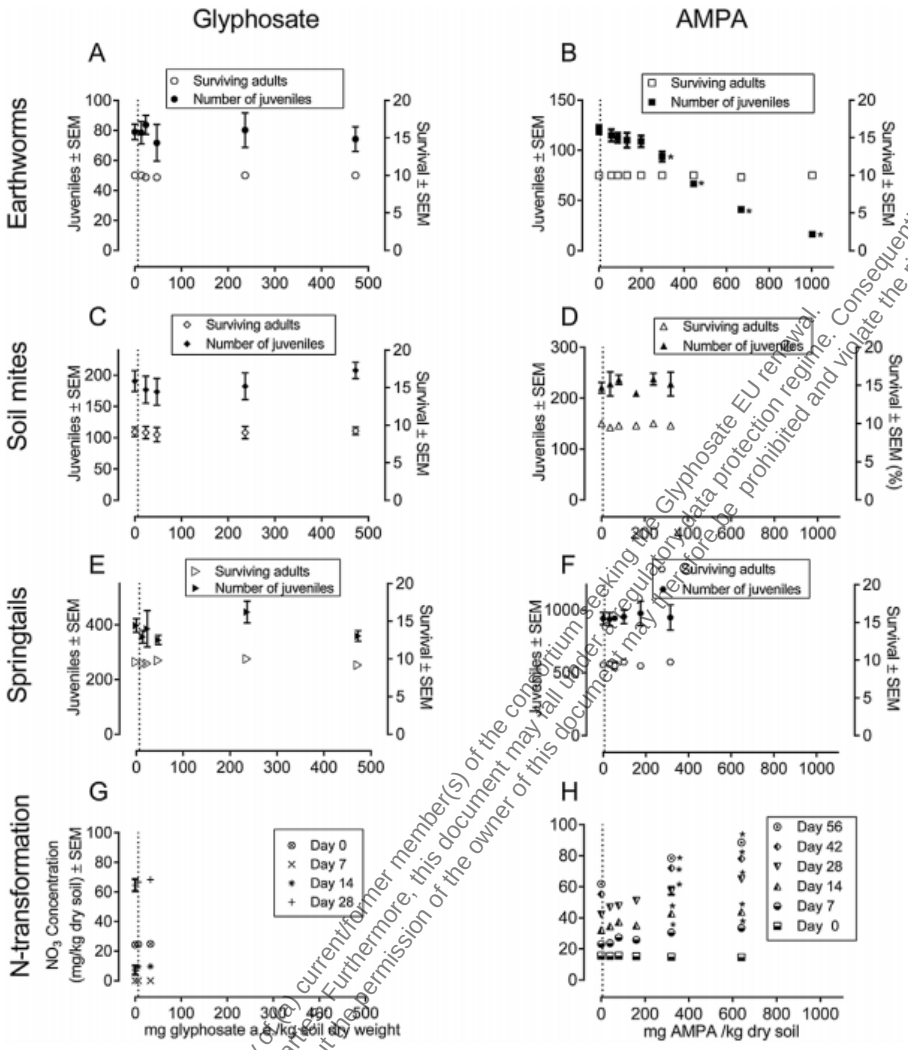


Figure 8.4.1-1: Chronic risk assessment for glyphosate (left) and aminomethylphosphonic acid (AMPA; right) in soil. Number of surviving adults (28 d) and number of juveniles (56 d) in earthworms exposed to glyphosate (A) and AMPA (B), (* statistically significant effect [$p \leq 0.05$] compared with control treatment), in soil predatory mites (*Hypoaspis aculeifer*) exposed to glyphosate (C) and AMPA (D) for 14 d, and in springtails (*Folsomia candida*) exposed to glyphosate (E) and AMPA (F) for 28 d. Effects on nitrogen transformation in soil treated with glyphosate (G) and AMPA (H) for 0 d, 7 d, 14 d,28 d, 42 d, and 56 d (* > 25 % effect compared with control treatment). Vertical dotted line in each graph indicates the worst-case predicted environmental concentration of glyphosate/AMPA. Vertical bars indicate standard error of the mean (SEM).

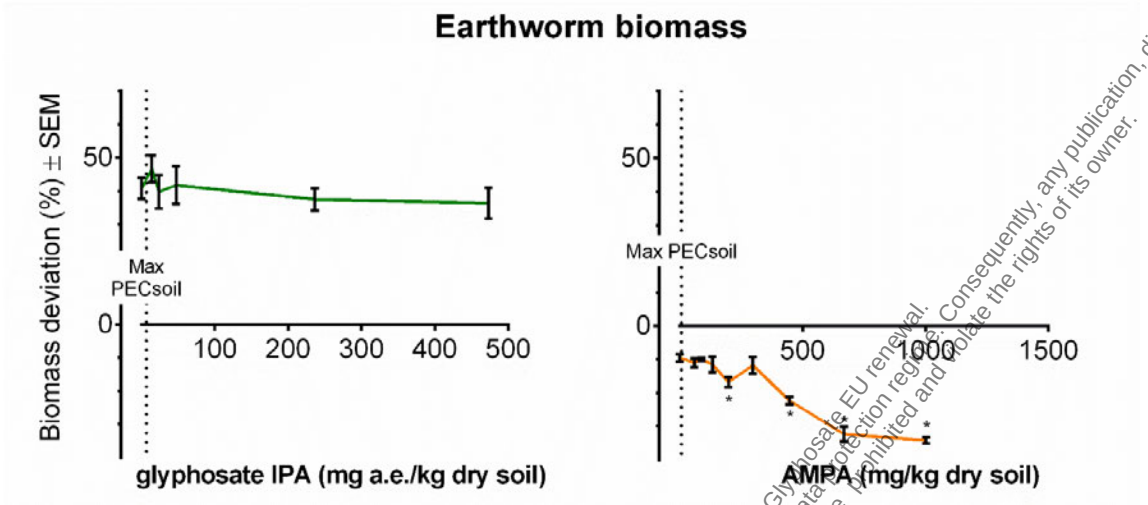


Figure 8.4.1-2: Effects of glyphosate (A) and AMPA (B) on earthworm biomass after 28 d of exposure. The vertical dotted line indicates the predicted environmental concentration for AMPA and glyphosate. An asterisk next to a data point indicates a significant difference ($P < 0.05$) when compared against the control treatment.

Risk assessment

The chronic effects of exposure to glyphosate and the major soil metabolite AMPA to representative taxonomic groups of soil macroorganisms and nitrogen transformation were assessed following standard practices outlined under Annex VI Uniform Principles of the EU's Plant Protection Products Regulation (EC) No 1107/2009. At soil concentrations relevant to recommended glyphosate field application rates, no significant adverse effects were observed in any of the test species or systems exposed to glyphosate or AMPA. The risk assessment for soil macroorganisms in the EU compares the lowest NOEC achieved for each of the taxonomic groups with worst-case initial predicted soil concentrations (soil $PEC_{initial}$) achieved directly following a bare soil application and the potential for accumulation in soil following applications over multiple years to the same field (soil PEC_{accu}) The ratio of the endpoint to the predicted soil concentration is determined (toxicity exposure ratio = NOEC - $PEC_{initial}$) and compared against trigger values in accordance with Annex VI Uniform Principles of the EU's Plant Protection Products Regulation 1107/2009. Where trigger values are exceeded, a low exposure risk may be concluded. The long-term trigger value of 5 using NOECs derived from laboratory tests accounts for uncertainty related to interspecies sensitivity, predicted exposure estimates, and extrapolation from laboratory to field exposure.

For glyphosate and AMPA, the initial soil concentration ($PEC_{initial}$) at a soil depth of 5 cm has been determined based on a bare soil application (without foliar/crop interception), at the maximum cumulative annual application rate of 4.32 kg glyphosate a.e./ha for the EU. The risk of glyphosate and AMPA residues accumulating in soil over multiple years is considered by deriving the PEC_{accu} value. This is the sum of the $PEC_{initial}$ and plateau concentrations in soil, achieved in the top 5 cm (tillage depth for permanent crops) soil layer, following applications to bare soil at the maximum cumulative annual application rate (4.32 kg a.e./ha) each year for 10 yr.

It is important to mention that a single application rate of 4.32 kg glyphosate a.e./ha is not supported in the representative use rate but rather represents the recommended maximum cumulative (total) annual application rate for all uses and, therefore, a very conservative worst-case approach.

For exposure of soil mites, springtails, and earthworms to glyphosate in soil, the achieved chronic endpoints exceed the worst-case predicted glyphosate $PEC_{initial}$ and PEC_{accu} soil concentration by factors of 82 and 71, respectively.

For exposure of soil mites, springtails, and earthworms to AMPA in soil, the achieved chronic endpoints exceed worst-case AMPA $PEC_{initial}$ soil concentrations by factors of between 97 and 491, whereas the chronic endpoints exceed the PEC_{accu} soil concentrations by factors of between 32 and 162.

For soil nitrogen transformation, the endpoints achieved for glyphosate and AMPA (33.1 mg a.e./kg dry soil [glyphosate] and 160 mg a.e./kg dry soil [AMPA]) both achieved a < 25 % effect on nitrogen-

transformation rates following a 28-d soil exposure to either glyphosate or AMPA. These soil exposure rates exceed the worst-case predicted $PEC_{initial}$ soil concentrations by factors of 6 (glyphosate) and 78 (AMPA). The achieved endpoints also exceed the PEC_{accu} soil concentrations, by factors of 5 for glyphosate and 26 for AMPA.

For the soil mite, springtail, and earthworm reproduction chronic endpoints, the toxicity exposure ratio values exceed the EU Regulation No 546/2011 Annex VI trigger (5), indicating that for the ecotoxicologically relevant endpoints achieved for survival and reproduction, the use of glyphosate according to label recommendations is unlikely to result in adverse effects inside the treated area for soil biota - from exposure to both glyphosate and AMPA.

For the soil microbial community, relative to expected field application rates for exposure to glyphosate there is at least a 5-fold safety margin. For exposure to AMPA, a 26-fold safety margin applies. The observed increases in nitrate concentrations at the higher test concentrations are expected to be related to the large quantity of nitrogen and phosphate provided to the microbes via degradation of AMPA in the biologically active soil.

Table 8.4.1-1. Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil organisms[a]

Test species	Test item	Test duration (d)	Endpoint type	NOEC (mg a.e. or AMPA/kg soil)	$PEC_{initial}$ (mg a.e./kg soil)	PEC_{accu} (mg a.e./kg soil)	$TER_{initial}$	TER_{accu}
Earthworm	Glyphosate IPA salt	56	Adult mortality	472.8	5.76	6.62	82	71
			Biomass	472.8			82	71
			Reproduction	472.8			82	71
	AMPA	56	Adult mortality	1002.5	2.04	6.18	491	162
			Biomass	297.1			146	48
			Reproduction	198.1			97	32
Soil mite	Glyphosate IPA salt	14	Adult mortality	472.8	5.76	6.62	82	71
			Reproduction	472.8			82	71
	AMPA	14	Adult mortality	320	2.04	6.18	157	52
			Reproduction	320			157	52
Springtail	Glyphosate IPA salt	28	Adult mortality	472.8	5.76	6.62	82	71
			Biomass	472.8			82	71
	AMPA	28	Adult mortality	315	2.04	6.18	154	51
			Biomass	315			154	51
N-transformation	Glyphosate acid	28	Effect <25%	33.1	5.76	6.62	6	5
	AMPA	28	Effect <25%	160	2.04	6.18	78	26

a.e. = acid equivalent; AMPA = aminomethylphosphonic acid; IPA = isopropylamine; NOEC = no-observed-effect concentration; PEC_{accu} = accumulative predicted environmental concentration, cumulative worst-case application of 4.32 kg a.e./ha of glyphosate for 10 yr; $PEC_{initial}$ = initial predicted environmental concentration, assuming single worst-case application of 4.32 kg a.e./ha of glyphosate; TER_{accu} = toxicity to exposure ratio (= NOEC - PEC_{accu}); $TER_{initial}$ = toxicity to exposure ratio (= NOEC - $PEC_{initial}$).

Conclusion

The risks from exposure to glyphosate and the primary soil metabolite AMPA at levels that exceed commercial application rates were evaluated against a battery of representative soil macroorganisms and microorganisms under controlled laboratory conditions. Results from the present studies demonstrate that the potential impact to beneficial soil macro-organisms and nutrient cycling soil microorganisms under environmentally relevant exposure scenarios is low.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The aim of the paper was to evaluate potential effects of Glyphosate, Glyphosate salt and AMPA on earthworm, soil mites, springtails and soil micro-organisms.

The studies have been conducted according to recognised guidelines and validity criteria were presented. Test substance information, test organism origin, study designs and toxicity effects were adequately described. The study is considered reliable.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Studies considering the toxicity of glyphosate to soil organisms (other than earthworms) were assessed for their validity to current and relevant guidelines for glyphosate, glyphosate salts and the metabolite AMPA and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

CA 8.4.2.1 Species level testing

Regulatory studies have been conducted with *Folsomia candida* and *Hypoaspis aculeifer* to evaluate the toxicity of glyphosate, glyphosate salts and the glyphosate metabolite AMPA on soil macrofauna. The results of these studies demonstrate that glyphosate, glyphosate salts and AMPA are of low toxicity to soil macrofauna.

Table 0.4.2.1-1: Studies on toxicity of glyphosate and metabolites to soil organisms other than earthworms

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.4.2.1/001	, 2010	28 d	*Folsomia candida*	Glyphosate IPA salt	Valid	-
CA 8.4.2.1/002	2009	14 d	*Hypoaspis aculeifer*	Glyphosate IPA salt	Valid	-
CA 8.4.2.1/003	, 2010	28 d	*Folsomia candida*	AMPA	Valid	-
CA 8.4.2.1/004	2010	14 d	*Hypoaspis aculeifer*	AMPA	Valid	-

Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate on soil organisms (other than earthworms) are summarised in the table below. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to soil organisms other than otherworms, please refer to document M-CP Section 10.4.

Table 0.4.2.1-2: Literature on toxicity of glyphosate and metabolites to soil organisms other than earthworms

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.4.2.1/005	Von Mérey *et al*., 2016	OECD 226: *Hypoaspis aculeifer* and OECD 232: *Folsomia candida*	Glyphosate IPA salt and AMPA	Relevant and reliable	Evaluates potential effects on earthworm, soil mites, springtails and soil micro-organisms.

Endpoints of studies considered valid for glyphosate are shown in the table below.

Table 8.4.2.1-3: Toxicity of glyphosate to non-target soil meso- and macrofauna (other than earthworms)

Reference (Data owner)	Test item	Species	Test design/ GLP	EC_{50} (mg a.e./kg dry soil)	NOEC (mg a.e./kg dry soil)
, 2010 CA 8.4.1/001	Glyphosate IPA salt	*Folsomia candida*	Chronic, 28-day	> 587	**≥ 587**
2009 CA 8.4.1/002	Glyphosate IPA salt	*Hypoaspis aculeifer*	Chronic, 14-day	> 473	**≥ 473**

a.e.: acid equivalents
Endpoints in **bold** is used for risk assessment

Endpoints of studies considered valid for AMPA are shown in the table below.

Table 8.4.2.1-4: Toxicity of AMPA to non-target soil meso- and macrofauna (other than earthworms)

Reference (Data owner)	Test item	Species	Test design/ GLP	EC_{50} (mg/kg dry soil)	NOEC (mg/kg dry soil)
2010 CA 8.4.2.1/003	AMPA	*Folsomia candida*	Chronic, 28-day	>315	**≥ 315**
2010 CA 8.4.2.1/004	AMPA	*Hypoaspis aculeifer*	Chronic, 14-day	>320	**≥ 320**

Endpoints in **bold** is used for risk assessment

Study summaries are provided below.

1. Information on the study

Data point	CA 8.4.2.1/001
Report author	
Report year	2010
Report title	MON0139 – Effects on the reproduction of the collembolans *Folsomia candida*
Report No	09 10 48 057 S
Document No	-
Guidelines followed in study	ISO 11267 (1999)
Deviations from current test guideline	Deviations from guideline OECD 232 (2016): Minor: - 5 replicates were used for the test item treatment groups and the control, instead of 4 in the test item group and 8 in the control - 10 % sphagnum peat was used instead of 5 %

	- 30 g wet weight per test vessel was used instead of 30 g dry weight.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a laboratory study the toxicity and reproductive inhibition of Glyphosate isopropylamine salt to *Folsomia candida* was tested. Juvenile springtails, approximately 10 - 12 days old, were exposed to 35, 50, 100, 500 and 1000 µL glyphosate isopropylamine salt/kg dry soil (equivalent to 19, 29, 59, 294 and 587 mg glyphosate acid equivalent/kg dry soil) and to a control with deionised water. A toxic reference (Betosip) was tested in a separate study.

50 springtails (10/ test vessel) per test concentration and control were put in a glass container on artificial soil with incorporated test item and adults and juveniles counted after 28 days. All validity criteria according to OECD 232 were fulfilled. The study is considered valid and the NOEC ≥ 587 mg a.e./kg dry soil will be used in the regulatory risk assessment for *Folsomia* exposed to glyphosate technical.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Glyphosate isopropylamine salt

Description: Pale yellow liquid

Lot/Batch #: A8B60170S0

Purity: Nominal: 62% w/w glyphosate isopropylamine salt (corresponding to 45.9% w/w glyphosate acid equivalent)
Analysed: 63.81 ± 0.29% w/w glyphosate isopropylamine salt (corresponding to 47.28 ± 0.21% w/w glyphosate acid equivalent)

2. Vehicle and/or positive control:

Vehicle: deionised water

Positive control: Betosip (Phenmedipham EC 157 g/L)

3. Test organisms:

Species: *Folsomia candida* (Willem)

Age: Juvenile springtails (10 – 12 d old)

Source: In-house culture originally obtained from Biologische Bundesanstalt (BBA), Berlin, Germany

Diet/Food: Approximately 2 mg granulated dry yeast at test start and after 14 days

4. Environmental conditions:

Temperature: 20.4 – 21.1 °C

Composition of artificial soil	10% sphagnum peat 20% kaolin clay 0.5% calcium carbonate 69.5% quartz sand Deionised water
Soil water content:	Test start: 34.9 – 35.2% (54.4 – 54.9% of WHC) Test end: 34.5 – 34.7% (53.8 – 54.1% of WHC)
pH:	Test start: 6.01 – 6.08 Test end: 5.79 – 5.91
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	580 lux

B. STUDY DESIGN AND METHODS

1. Experimental treatments: MON0139 was evaluated for mortality and reproductive reduction in a test with *Folsomia candida* at five application rates of 35, 50, 100, 500 and 1000 µL MON0139/kg dry soil (19, 29, 59, 294 and 587 mg glyphosate acid equivalent/kg dry soil). In addition, a blank control with deionised water and a toxic reference (Betosip) were conducted. Each test item concentration and the control were tested with 50 springtails (10/ test vessel). For each test item concentration and for the control group 2 test vessels without springtails were provided for measurement purposes. The springtails were put in a glass container (~ 150 mL) containing 30 g (wet weight) artificial soil with the requested test item concentrations and covered with a glass lid for 28 days. Four weeks after introducing the test organisms the parental and juvenile collembolans were counted.
2. Observations: Water content and pH were determined at test start and end. Adults and juvenile springtails were counted at test end.
3. Statistical calculations: Fisher's Exact Binomial test with Bonferroni Correction for significance of parental mortality. Welch-t-test ($p \leq 0.05$) for significance of reproductive reduction. Statistical program: ToxRat Professional 2.10 (2009).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-2: Mortality and reproductive reduction of *Folsomia candida* after application of MON0139 in a 28 days laboratory study

Test rate [µL MON0139/kg dry soil]	Test concentration [mg glyphosate a.e./kg dry soil]	Mortality of parental collembolans after 4 weeks [%]	Corrected mortality [1)] [%]	Mean number of juveniles after 4 weeks [%]	Reduction of reproduction compared to control [%]	Coefficient of variation [%]
Control	Control	4	-	397.2	-	14.2
32	19	6	2	355.6	10	14.3
50	29	6	2	384.6	3	38.4
100	59	2	-2	344.4	13	10.8
500	294	0	-4	446.4	-12	20.0
1000	587	8	4	358.8	10	12.1

[1)] calculated with Abbott 1925

Reference test:
After treatment with the reference item Betosip (Phenmedipham EC 157 g/L) at concentrations of 50, 100, 200 and 400 mg test item/ kg dry soil an EC_{50} of 181.0 mg Betosip/kg dry soil was determined.

B. OBSERVATIONS

No statistically significant effects on parental mortality (Fishers's Exact Binomial Test, $p > 0.05$) or the number of offspring (Welch-t-test, $p > 0.05$) compared to the control was found.

The LC_{50} and EC_{50} values as well as the NOEC are given below based on nominal concentrations.

Endpoints	MON0139 [µL test item/kg dry soil]	Glyphosate acid equivalent [mg a.e./kg dry soil]
NOEC (mortality)	1000	587
NOEC (reproduction)	1000	587
EC_{50} (28 d)	> 1000	> 587

Reference test:
The EC_{50} reproduction with the reference item Betosip (Phenmedipham EC 157 g/L) demonstrated the sensitivity of the test system.

All validity criteria according to OECD 232 were fulfilled, since the mean adult mortality did not exceed 20 %, the mean number of juveniles per vessel was ≥ 100 and the coefficient of variation of juveniles was less than 30 %.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of glyphosate on mortality and reproduction of *Folsomia candida* were assessed following application of MON0139 under laboratory conditions.

The 28-day EC_{50} was > 1000 µL MON0139/kg dry soil (>587 mg glyphosate acid equivalent/kg dry soil). The NOEC was ≥ 1000 µL MON0139/kg dry soil (≥ 587 mg glyphosate acid equivalent/kg dry soil), the highest tested concentrations, since MON0139 had no negative effect on the test organisms. There were some deviations to the current guideline. However, these deviations did not affect the scientific validity of the study.

The study is considered valid and NOEC ≥ 587 mg a.e./kg dry soil can be used in risk assessment for *Folsomia* exposed to glyphosate IPA salt.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.4.2.1/002
Report author	
Report year	2009
Report title	MON0139 – Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Report No	09 10 48 058 S
Document No	-
Guidelines followed in study	OECD 226 (2008)
Deviations from current test guideline	Deviations from guideline OECD 226 (2016): Minor: - Four concentrations of the test item were tested instead of at least five for a NOEC test design.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
In the laboratory study the toxicity and reproductive inhibition of MON0139 to *Hypoaspis aculeifer* was tested. Adult mites were exposed to 50, 100, 500 and 1000 mg MON0139/kg dry soil (equivalent to 23.64, 47.28, 236.40 and 472.80 mg glyphosate acid equivalent/kg dry soil) and to a control with deionised water. A toxic reference (Perfekthion) was tested in a separate study.
40 mites (10/test vessel) per test concentration and 80 mites per control (10/test vessel) were put in a glass bottle on artificial soil with incorporated test item and adults and juveniles counted after 14 days. The test item MON0139 caused no statistically significant mortality of adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Also, no significant decrease in reproduction was observed. All validity criteria according to OECD 226 were fulfilled. The study is considered valid so $EC_{50} > 473$ mg a.e./kg dry soil and NOEC ≥ 473 mg a.e./kg dry soil will be used in the regulatory risk assessment for *Hypoaspis* exposed to glyphosate technical.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON0139 (glyphosate isopropylamine salt)
Description:	Pale yellow liquid
Lot/Batch #:	A8B60170S0
Purity:	Nominal: 62 % w/w glyphosate isopropylamine salt (corresponding to 45.9 % w/w glyphosate acid equivalent) Analysed: 63.81 ± 0.29 % w/w glyphosate isopropylamine salt (corresponding to 47.28 ± 0.21 % w/w glyphosate acid equivalent)

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: Perfekthion (Dimethoate, EC 400, 422.4 g/L analysed)

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini)
Age:	Adult mites
Source:	In-house culture originally obtained from Katz Biotech AG, 15837 Baruth, Germany
Diet/Food:	*Tyrophagus putrescentiae* (Schrank) were fed every 2 days, before and during the test

4. Environmental conditions:

Temperature:	19.7 – 21.9 °C
Composition of artificial soil	5 % sphagnum peat 20 % kaolin clay 0.3 % calcium carbonate 74.7 % quartz sand Deionised water
Soil water content:	Test start: 18.79 – 20.21 % (47.52 – 51.11 % of WHC) Test end: 18.65 – 20.11 % (47.17 – 50.87 % of WHC)
pH	Test start: 5.9 – 6.2 Test end: 5.3 – 5.4
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	588 lux

B. STUDY DESIGN AND METHODS

1. Experimental treatments: MON0139 was evaluated for mortality and reproductive reduction in a test with *Hypoaspis aculeifer* at four application rates of 50, 100, 500 and 1000 mg MON0139/kg dry soil (equivalent to 23.64, 47.28, 236.40 and 472.80 mg glyphosate acid equivalent/kg dry soil). In addition, a control with deionised water and a toxic reference (Perfekthion, 422.4 g/L dimethoate) were tested.
Each test item concentration was tested with 40 mites (10/test vessel), while the control group consisted of 80 mites (10/test vessel). For each test item concentration and for the control group 2 test vessels without mites were provided for measurement purposes.
The mites were put in glass bottles with screw tops of 100 mL containing 20 g (dry weight) artificial soil with the requested test item concentrations and closed. Test vessels were opened every two days for food supply and aeration. Two weeks after introducing the test organisms the parental and juvenile mites were counted.
2. Observations: Water content and pH were determined at test start and end. Temperature was recorded continuously. Adult and juvenile mites were counted at test end.
3. Statistical calculations: Fisher's Exact Binomial test with Bonferroni Correction for significance of parental mortality. Dunnett-t-test ($p \leq 0.05$) for significance of reproductive reduction. Statistical program: ToxRat Professional 2.10 (2009).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-3: Mortality and reproductive reduction of *Hypoaspis aculeifer* after application of MON0139 in a 14 day laboratory study

Test rate [mg MON0139/ kg dry soil]	Test rate [mg a.e./ kg dry soil]	Mortality of adults after 14days [%]	Corrected mortality [1] [%]	Mean number of juveniles after 14 days [%]	Reduction of reproduction compared to control [%]	Coefficient of variation [%]
Control	Control	8.8	-	190.5	-	8.9
50	23.64	10	1.4	176.8	7.2	12.3
100	47.28	12.5	4.1	173.5	8.9	12.4
500	236.40	10.0	1.4	182.3	4.3	11.7
1000	472.80	7.5	-1.4	207.8	-9.1	6.3

[1] calculated with Abbott 1925

Reference test:
After treatment with the reference item Perfekthion (Dimethoate, EC 400, 422.4 g/L analysed) at concentrations of 4.1, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil an EC_{50} (reproduction) of 4.9 mg test item/kg dry soil was concluded.

B. OBSERVATIONS

The test item MON0139 caused no statistically significant mortality (Fishers's Exact Binomial Test, $p > 0.05$) of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Also, no significant decrease in reproduction was observed (Dunnett-t-test, $p > 0.05$).

The EC_{50} value and the NOEC are given below.

Endpoints	MON0139 [mg/kg dry soil]	Glyphosate acid equivalent [mg/kg dry soil]
NOEC	1000	472.80
EC_{50} (14 d)	> 1000	>472.80

Reference test:
The EC_{50} (reproduction) with the reference item Dimethoate EC 400 was in line with the range defined in the guideline to demonstrate the sensitivity of the test system.

All validity criteria according to OECD 226 were fulfilled, as adult mortality in the control treatments did not exceed 20 %, the mean number of juveniles per replicates was > 50 at test end and the coefficient of variation of the number of juveniles per replicate was not higher than 30 % at test end.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of MON0139 on mortality and reproduction of *Hypoaspis aculeifer* were assessed for 14 days under laboratory conditions.

The 14-day EC_{50} was >1000 mg MON0139/kg dry soil (473 mg glyphosate acid equivalent/kg dry soil). The NOEC was ≥ 1000 mg test item/kg dry soil (≥ 473 mg glyphosate acid equivalent/kg dry soil), the highest tested concentration, since MON0139 had no negative effect on the test organisms.

The study is considered valid so EC_{50} > 473 mg a.e./kg dry soil and NOEC ≥ 473 mg a.e./kg dry soil can be used in risk assessment for *Hypoaspis* exposed to glyphosate IPA salt.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.4.2.1/003
Report author	
Report year	2010
Report title	AMPA – Effects on the Reproduction of the collembolans *Folsomia candida*
Report No	10 10 48 054 S
Document No	Not available
Guidelines followed in study	OECD 232 (2009) ISO 11267 (1999)
Deviations from current test guideline	Deviations from guideline OECD 232 (2016)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
In the laboratory study the toxicity and reproductive inhibition of AMPA to *Folsomia candida* was tested. Juvenile springtails, 9-12 days old, were exposed to 30, 54, 97.2, 175 and 315 mg test item/kg dry soil and to a control with deionised water. A toxic reference (100% boric acid) was tested in a separate study. 40 springtails (10/ test vessel) per test concentration and 80 springtails per control (10/ test vessel) were put in a glass container on artificial soil with incorporated test item and adults and juveniles counted after 28 days.

No statistically significant effects on parental mortality and number of offspring were observed. All validity criteria according to OECD 232 were fulfilled. The study is considered valid so EC_{50} > 315 mg/kg dry soil and NOEC ≥ 315 mg/kg dry soil will be used in the regulatory risk assessment for *Folsomia* exposed to

AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	White crystalline solid
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7 %

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: Reference item: Boric acid (100%)

3. Test organisms:

Species:	*Folsomia candida* (Willem)
Age:	Juvenile springtails (9 – 12 d old)
Source:	In-house culture originally obtained from Biologische Bundesanstalt (BBA), Berlin, Germany
Diet/Food:	Approximately 2 mg granulated dry yeast at test start and after 14 days

4. Environmental conditions:

Temperature:	20.4 – 22.0 °C
Composition of artificial soil	5 % sphagnum peat 20 % kaolin clay 0.3 % calcium carbonate 74.7 % quartz sand Deionised water
Soil water content:	Test start: 24.9 – 25.1 % (57.8 – 58.2 % of WHC) Test end: 24.3 – 25.0 % (56.4 – 58.0 % of WHC)
Soil pH:	Test start: 5.78 – 5.98 (test start) Test end: 5.60 – 5.78
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	750 lux

B. STUDY DESIGN AND METHODS

1. Experimental treatments: AMPA at five concentrations, 30, 54, 97.2, 175 and 315 mg test item/kg dry soil, was evaluated for mortality and reproductive reduction in a test with *Folsomia candida*. In addition, a control with deionised water and a toxic reference (100 % boric acid) were conducted. Each test item concentration was tested with 40 springtails (10/ test vessel), while the control group consisted of 8 replicates. For each test item concentration and for the control group 2 test vessels without springtails were provided for measurement purposes. The springtails were held in a glass container (~ 150 mL), containing 30 g (wet weight) artificial soil including the requested test item concentrations and covered with a glass lid for 28 days. Four weeks after introducing the test organisms the parental and juvenile collembolans were counted.

2. Observations: Water content and pH values were determined at test start and end. Adults and juvenile springtails were counted at test end as well as physiological or pathological symptoms.

3. Statistical calculations: Fisher's Exact Binomial test with Bonferroni Correction for significance of parental mortality Dunnett-t-test ($p \leq 0.05$) for significance of reproductive reduction Statistical program:

ToxRat Professional 2.10 (2009).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-4: Mortality and reproductive reduction of *Folsomia candida* after application of AMPA in a 28-day laboratory study

AMPA [mg test item/kg dry soil]	Mortality of parental collembolans after 4 weeks [%]	Corrected mortality [1)] [%]	Mean number of juveniles after 4 weeks [%]	Reduction of reproduction compared to control [%]	Coefficient of variation [%]
Control	6.3	-	931	-	15.1
30	5.0	-1	925	1	11.6
54	7.5	1	934	0	5.2
97.2	2.5	-4	946	-2	11.8
175	7.5	1	973	-4	20.1
315	2.5	-4	939	-1	21.3

[1)] calculated with Abbott 1925

Reference test:
After treatment with the reference item boric acid at concentrations of 44, 67, 97.2, 150 and 225 mg test item/ kg dry soil an EC_{50} of 108.6 mg test item/ kg dry soil.

B. OBSERVATIONS

No statistically significant effects on parental mortality (Fishers's Exact Binomial Test, $p > 0.05$) or the number of offspring (Dunnett-t-test, $p > 0.05$) compared to the control was found.

The LC_{50} and EC_{50} values as well as the NOEC are given below based on nominal concentrations.

Endpoints	AMPA [mg/kg dry soil]
NOEC (mortality)	315
NOEC (reproduction)	315
LC_{50} (28 d)	> 315
EC_{50} (28 d)	> 315

Reference test:
The EC_{50} reproduction with the reference item boric acid was in line the expected result defined in the guideline to demonstrate the sensitivity of the test system (about 100 mg test item/kg dry soil).

All validity criteria according to OECD 232 were fulfilled, since the mean adult mortality did not exceed 20 %, the mean number of juveniles per vessel was ≥ 100 and the coefficient of variation of juveniles was less than 30 %.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of AMPA on mortality and reproduction of *Folsomia candida* were assessed for 28 days under laboratory conditions.

The 28-day LC_{50} and EC_{50} were > 315 mg test item/kg dry soil. The NOEC was ≥ 315 mg AMPA/kg dry soil, the highest tested concentration, since AMPA had no negative effects on the test organisms.

The study is considered valid so EC_{50} > 315 mg/kg dry soil and NOEC ≥ 315 mg/kg dry soil can be used in the regulatory risk assessment for *Folsomia* exposed to AMPA.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.4.2.1/004
Report author	
Report year	2010
Report title	AMPA – Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*
Report No	10 10 48 053 S
Document No	-
Guidelines followed in study	OECD 226 (2008)
Deviations from current test guideline	Deviations from guideline OECD 226 (2016): Minor: - A combined approach design (determination of NOEC and EC_{50}) was conducted with only 5 test item concentrations and a spacing factor of 2 (8 concentrations and spacing factor not exceeding 1.8 are required).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

In the laboratory study the toxicity and reproductive inhibition of AMPA to *Hypoaspis aculeifer* was tested. Adult mites were exposed to 40, 80, 160, 240 and 320 mg test item/kg dry soil and to deionised water only as control. A toxic reference (Dimethoate EC 400) was tested in a separate study.

40 mites (10/test vessel) per test concentration and 80 mites per control (10/test vessel) were put in a glass bottle on artificial soil with incorporated test item and adults and juveniles counted after 14 days. The test item AMPA caused no statistically significant mortality of adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Also, no significant decrease in reproduction was observed. All validity criteria according to OECD 226 were fulfilled. The study is considered valid so EC_{50} > 320 mg/kg dry soil and NOEC ≥ 320 mg/kg dry soil will be used in the regulatory risk assessment for *Hypoaspis* exposed to AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	White crystalline solid
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7 %
	Vehicle: deionised water

2. Vehicle and/or positive control: Positive control: Reference item: Dimethoate EC 400 (414.8 g/L analysed)

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini)
Age:	Adult mites
Source:	In-house culture originally obtained from Katz Biotech AG, 15837 Baruth, Germany
Diet/Food:	*Tyrophagus putrescentiae* (Schrank) were fed every 2 days, before and during the test

4. Environmental conditions:

Temperature:	19.7 – 21.8 °C
Composition of artificial soil	5 % sphagnum peat 20 % kaolin clay 0.3 % calcium carbonate 74.7 % quartz sand Deionised water
Soil water content:	Test start: 17.40- 18.07 % (47.81 – 49.64 % of WHC) Test end: 17.10 – 17.55 % (46.98 – 48.22 % of WHC)
pH	Test start: 5.8 – 6.1 Test end: 5.4 – 6.3
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	472 lux

B. STUDY DESIGN AND METHODS

1. Experimental treatments: AMPA was evaluated for mortality and reproductive reduction in a test with *Hypoaspis aculeifer* at five test item concentrations of 40, 80, 160, 240 and 320 mg test item/kg dry soil. In addition, a control with deionised water and a toxic reference (Dimethoate EC 400) were conducted. Each test item concentration was tested with 40 mites (10/test vessel), while the control group consisted of 80 mites (10/test vessel). For each test item concentration and for the control group 2 test vessels without mites were provided for measurement purposes. The mites were put in glass bottles with screw tops of 100 mL, each containing 20 g (dry weight) artificial soil with the requested test item concentrations and closed. Every two days test vessels were opened for food supply and aeration. Two weeks after introducing the test organisms the parental and juvenile mites were counted.

2. Observations: Water content and pH were determined at test start and end. Adults and juvenile mites were counted at test end. The temperature was continuously measured and recorded.

3. Statistical calculations: Fisher's Exact Binomial test with Bonferroni Correction for significance of parental mortality. Dunnett-t-test ($p \leq 0.05$) for significance of reproductive reduction. Statistical programs: ToxRat Professional 2.10 (2009).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 0-5: Mortality and reproductive reduction of *Hypoaspis aculeifer* after application of AMPA in a 14 day laboratory study

Test concentration [mg test item/kg dry soil]	Mortality of adults after 14 days [%]	Corrected mortality [1] [%]	Mean number of juveniles after 14 days [%]	Reduction of reproduction compared to control [%]	Coefficient of variation [%]
Control	0.0	-	220.6	-	13.3
40	5.0	5.0	228.0	-3.3	20.7
80	2.5	2.5	236.3	-7.1	7.7
160	2.5	2.5	209.3	5.2	6.0
240	0.0	0.0	237.3	-7.5	9.9
320	2.5	2.5	227.5	-3.1	20.7

[1] calculated with Abbott 1925

Reference test:
After treatment with the reference item Dimethoate EC 400 at concentrations of 4.1, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil and EC_{50} (reproduction) of 6.6 mg test item/kg dry soil was concluded.

B. OBSERVATIONS

The test item AMPA caused no statistically significant mortality (Fishers's Exact Binomial Test, $p > 0.05$) of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Also, no significant decrease in reproduction was observed (Dunnett-t-test, $p > 0.05$).

The EC_{50} value and the NOEC are given below based on nominal concentrations.

Endpoints	AMPA [mg/kg dry soil]
NOEC	320
EC_{50} (14 d)	> 320

Reference test:
The EC_{50} (reproduction) with the reference item Dimethoate EC 400 was in line with the range defined in the guideline to demonstrate the sensitivity of the test system.

Following point deviated from the guideline OECD 226 (2016):
A combined approach design (determination of NOEC and EC_{50}) was conducted with only 5 test item concentrations and a spacing factor of 2 (8 concentrations and spacing factor not exceeding 1.8 are required). Since an EC_{50} could not be calculated and would be greater than the highest test concentration, the design is in line with the requirement for determination of NOEC only.

All validity criteria according to OECD 226 were fulfilled, as adult mortality did not exceed 20 %, the mean number of juveniles per replicate was > 50 at test end and the coefficient of variation of the number of juveniles per replicate was not higher than 30 % at test end.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of AMPA on mortality and reproduction of *Hypoaspis aculeifer* were assessed for 14 days under laboratory conditions.

The 14-day EC_{50} was > 320 mg test item/kg dry soil. The NOEC was ≥ 320 mg AMPA/kg dry soil, the highest tested concentration, since AMPA had no negative effect on the test organisms.

The study is considered valid so EC_{50} > 320 mg/kg dry soil and NOEC ≥ 320 mg/kg dry soil can be used in risk assessment for *Hypoaspis* exposed to AMPA.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.4.2.1/005
Report author	von Mérey G. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota
Document No	DOI: 10.1002/etc.3438 E-ISSN: 1552-8618
Guidelines followed in study	OECD 222; OECD 226; OECD 232; OECD 216
Deviations from current test guideline	Earthworm cocoons were not counted, in accordance with OECD 222.
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

The exposure risk from glyphosate and the primary soil metabolite aminomethylphosphonic acid (AMPA) on representative species of earthworms, springtails, and predatory soil mites and the effects on nitrogen-transformation processes by soil microorganisms were assessed under laboratory conditions based on internationally recognized guidelines. For earthworms, the reproductive no-observed-effect concentration (NOEC) was 472.8 mg glyphosate acid equivalent (a.e.)/kg dry soil, which was the highest concentration tested, and 198.1 mg/kg dry soil for AMPA. For predatory mites, the reproductive NOEC was 472.8 mg a.e./kg dry soil for glyphosate and 320 mg/kg dry soil for AMPA, the highest concentrations tested. For springtails, the reproductive NOEC was 472.8 mg a.e./kg dry soil for glyphosate and 315 mg/kg dry soil for AMPA, the highest concentrations tested. Soil nitrogen-transformation processes were unaffected by glyphosate and AMPA at 33.1 mg a.e./kg dry soil and 160 mg/kg dry soil, respectively. Comparison of these endpoints with worst-case soil concentrations expected for glyphosate (6.62 mg a.e./kg dry soil) and AMPA (6.18 mg/kg dry soil) for annual applications at the highest annual rate of 4.32 kg a.e./ha indicate very low likelihood of adverse effects on soil biota.

Materials and methods

Test substances

Glyphosate (N-phosphonomethylglycine) is an acidic substance, which is manufactured and formulated as a salt to increase the solubility in water and compatibility with other formulation components. In water, AMPA is highly soluble (56 g/L at 20 °C), whereas neither glyphosate nor AMPA is significantly soluble in common organic solvents. Therefore, no cosolvent was required, and both stock solutions of glyphosate and AMPA test items were prepared in deionized water (5 - 20 Mohm at 25 °C). Two batches of AMPA analytical reference standards with purity of 98.7 % (synthesized by Chemir) and 99.7% (Acros Organics BVBA) were dissolved in deionized water. For soil nitrogen-transformation tests, stock solutions of glyphosate acid technical grade (96.59 % purity; Monsanto Europe) were prepared by direct addition of test item to deionized water. For all other tests, glyphosate isopropylamine salt (nominal purity 62 % w/w, measured purity 63.81 ± 0.29 % w/w; MON 0139), corresponding to 45.9 % w/w glyphosate a.e. (measured 47.28 ± 0.21 % w/w; Monsanto Europe), were prepared in deionized water.

Earthworm reproduction tests

The earthworm reproduction test with glyphosate was conducted according to OECD guideline 222. For AMPA, an earthworm reproduction test was conducted according to the OECD 222. Both testing guidelines are equivalent in terms of the procedures employed during the tests (soil pH, temperature, lighting regime, soil composition and humidity, rearing, feeding quantities, test design, endpoints, number of replicates, growth stage of worms at test initiation, and so on). Therefore, to avoid repetition, the procedures used in the glyphosate study only are described.

Glyphosate - Earthworm reproduction test. In the earthworm reproduction glyphosate study *Eisenia fetida* (Haplotaxida: Lumbricidae, Savigny, 1826) were used as the test species. Mature adult *E. fetida* (~3 mo old with clitellum), weighing between 300 mg and 600 mg, were obtained from an age-synchronized stock culture from the test facility and reared under ambient laboratory conditions in the test facility. The original breeding animals were purchased from W. Neudorff. A detailed description of earthworm culturing is provided in Annex 4 of OECD 222. The *E. fetida* were reared in the laboratory on standard breeding medium (1:1:1 mixture of straw, horse manure, and peat; straw and horse manure were purchased from farmers, and peat was purchased from Torfwerk Moorkultur Ramsloh); no exposure to the test item was allowed prior to use in testing. Testing was conducted in artificial soil, equivalent to the soil in which the worms were originally cultured. The test aims to evaluate effects on adult body weight and survival percentage (according to treatment) during an initial 4-wk adult exposure period. Effects on juvenile production were then assessed at the end of a 4-wk period that followed directly after adult removal from the test. Behavior (including feeding activity) and pathological symptoms (e.g., lethargy, morphological alterations) of adults and juveniles were also assessed.

On the day before the test start, earthworms (from aged-synchronized batches, to ensure that similar-sized earthworms were used) were acclimated to test conditions in a separate batch of artificial soil supplemented with pasteurized horse manure, purchased from farmers and collected from horses not treated with growth promoters, nematicides, or other veterinary products - also used as the food source during testing. On test start day, volumes of the test solution (prepared by direct addition of glyphosate isopropylamine to deionized water) were mixed into bulk samples of artificial soil, to achieve nominal glyphosate soil concentrations of 14.18 mg a.e./kg dry soil, 23.64 mg a.e./kg dry soil, 47.28 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil. Glyphosate test concentrations were selected to cover the range and exceed field exposure concentrations. A toxic reference test was also performed in a separate test with carbendazim (Nutdazim 50 Flow, SC 500) at concentrations of 5 mg/kg dry soil and 10 mg/kg dry soil.

Test vessels were filled with the appropriate treated soil (810 g wet wt corresponding to 600 g dry wt). Groups of 10 individually weighed earthworms were randomly assigned to replicates within each treatment group, with a total of 40 earthworms used per treatment group divided equally between 4 replicates. For the control group (water only), 80 worms were used, divided equally between 8 replicates. Groups of 10 earthworms were placed onto the assigned replicate soil surface and closed with perforated transparent lids (following a brief burrowing period) to reduce evaporative water loss, allow gaseous exchange, and prevent worms from escaping the replicate vessels. Test vessels were then randomly positioned in an environmental test chamber under continuous light (to maintain worms in the soil). On day 1 and weekly

thereafter for the 4-wk adult exposure period, 5 g of air-dried finely ground horse manure was scattered on the soil surface of each test vessel and wetted with 5mL of deionized water. The amount of manure applied each week (up to 5 g) was dictated by feeding activity.

After 4 wk, adult earthworms were removed from the vessels by emptying the contents of each replicate vessel onto a tray and removing the adult worms. Care was taken not to remove any cocoons from the soil. Cocoons were not counted, in accordance with OECD 222. It can be reasonably assumed that effects on cocoon numbers would lead to effects on numbers of juveniles; hence, the endpoint number of juveniles accounts for effects at earlier life stages of earthworm progeny. All worms were rinsed with deionized water and dried on filter paper before recording body weights (by replicate and by treatment). Behavioral (including feeding activity) and pathological symptoms were also recorded during the exposure period and at the time of adult removal. The adult worms were then discarded. The soil in each replicate vessel was then mixed carefully with 5 g of manure, and the mixture was returned to the vessels. The test continued for a further 4 wk. At test termination (8 wk after adult addition) the number of surviving juveniles in each test vessel was recorded on manual inspection of the substrate. Soil was emptied on the lower edge of a white tray (30 cm × 40 cm). Subportions of the soil were spread in the middle of the tray, resulting in a thin layer of soil of approximately 10 cm × 10 cm. The subportion was examined thoroughly for juvenile worms, after which it was moved to the upper edge of the tray. This procedure was repeated until the entire soil from a vessel was examined. The entire procedure was repeated until there were no additional juvenile counts in 2 consecutive counting procedures, resulting in an average of 5 counting procedures per vessel. The counting tray and soil samples were illuminated using a fiber-optic light source connected with a double gooseneck light guide. The water content and pH of the artificial soil were determined. Adult body weights and the effects on reproduction (juvenile numbers) were analyzed using a lower-tailed Dunnett's multiple comparisons test ($\alpha = 0.05$). The Kolmogorov-Smirnov test and Cochran's test procedure were used, respectively, to test the biomass data for normality and homogeneity of variance. Survival was analyzed with a 1-sided Fisher's exact binomial test with Bonferroni correction ($\alpha = 0.05$).

AMPA - Earthworm reproduction tests. The procedures used during the AMPA earthworm study are considered equivalent to those employed in the glyphosate earthworm reproduction study described above in Glyphosate—Earthworm reproduction test. Mature adult *E. fetida* (~3mo old with clitellum), weighing between 300 mg and 600 mg, were obtained from an age-synchronized stock culture from the test facility and reared under ambient laboratory conditions in the test facility. A detailed description of earthworm culturing is provided in Annex 4 of OECD 222.

In the AMPA earthworm reproduction study, mature (clitellated) adult *E. fetida* were exposed to AMPA (99.7% purity; Acros Organics BVBA) mixed into artificial soil at nominal soil concentrations of 58.6 mg AMPA/kg dry soil, 87.8 mg AMPA/kg dry soil, 131.9 mg AMPA/kg dry soil, 198.1 mg AMPA/kg dry soil, 297.1 mg AMPA/kg dry soil, 445.5 mg AMPA/kg dry soil, 668.5 mg AMPA/kg dry soil, and 1002.5 mg AMPA/kg dry soil. A control group was prepared using deionized water only. A toxic reference test was also performed in parallel using earthworms from the same batch, exposed to carbendazim at concentrations of 1.0 mg active substance (a.s.)/kg dry soil, 2.2 mg a.s./kg dry soil, and 5.0 mg a.s./kg dry soil. For effects on biomass and production of juveniles, homogeneity was tested with the Brown-Forsythe and Bartlett tests. Dunnett's multiple comparison test was conducted using GraphPad Prism, Ver 6.03, because a continuous response could not be observed for all the test concentrations, as recommended by the OECD 222 test guideline and the OECD statistical guidance. The 50 % effect rate on reproduction was calculated using GraphPad Prism.

Soil predatory mite reproduction test

The soil predatory mite reproduction tests for glyphosate and AMPA were both conducted according to OECD guideline 226 predatory mite (*Hypoaspis* [Geolaelaps] *aculeifer*) reproduction test in soil. The procedures used in the 2 studies were identical. Full details of the procedures are presented for glyphosate only. The *Hypoaspis* used in these studies were originally purchased from Katz Biotech and reared in the test facility under ambient conditions since June 2005.

Glyphosate - Soil predatory mite reproduction test. The glyphosate soil predatory mite reproduction test was conducted using glyphosate isopropylamine salt (MON 0139). Survival of mites (*H. aculeifer*) and their reproductive performance were evaluated at 4 nominal concentrations, equivalent to 50 mg MON 0139/kg dry soil, 100 mg MON 0139/kg dry soil, 500 mg MON 0139/kg dry soil, and 1000 mg

MON 0139/kg dry soil (= 23.64 mg a.e./kg dry soil, 47.28 mg a.e./kg dry soil, 236.40 mg a.e./kg dry soil, and 472.80 mg a.e./kg dry soil, respectively). A negative control with deionized water only was also included. A toxic reference test was performed in parallel using dimethoate EC400 (422.4 g/L; Perfekthion) at concentrations of 4.1 mg active ingredient (a.i.)/kg dry soil, 5.12 mg a.i./kg dry soil, 6.4 mg a.i./kg dry soil, 8.0 mg a.i./kg dry soil, and 10 mg a.i./kg dry soil. Mites were reared in the laboratory under ambient conditions on a mixture of plaster of paris, activated charcoal, and deionized water (8:1:9). Adults with no more than a 3-d age difference were used at the start of the test. No exposure of the mites to glyphosate was allowed prior to the test. Each treatment group contained 40 mites divided equally between 4 replicate vessels, with the control group comprising 8 replicates, each containing 10 mites. In addition, 2 test vessels without mites were included with each test concentration and in the control group for soil pH measurements. Glass bottles (100mL nominal volume) with screw tops were filled with 20 g (dry wt) artificial soil at the required test concentrations. Cheese mites were added as a food source to the surface of the soil, and vessels were then covered to prevent mites from escaping. Bottles were opened every second day during the 14-d test for the addition of food and to allow aeration. At the end of the test (day 14), the parental mites and juveniles were counted, after extraction using a MacFayden high-gradient extractor (heat/light extraction method). This was achieved by adding the soil substrate from each test vessel into a canister placed inverted onto the extraction system. Soil substrate was retained within the canister using a plastic net (2 mm mesh size) on the bottom. Beneath the canister was a funnel attached to a collecting flask with 25 mL of a fixing liquid. A temperature gradient was created between the upper and the lower parts of the system, by circulating heated air in the canister area and cooled air in the collection area. Over the 48-h extraction time, the following regime was applied: 25 °C for 12 h, 35 °C for 12 h, and 45 °C for 24 h. During this time, adults and juveniles moved down through the soil away from the heat source and fell through the funnel into the fixing liquid. Extraction efficiency was determined to be 95% in a separate extraction using vessels containing a known number of juvenile and adult mites in untreated substrate. Water content and pH were determined at test start and end. Statistical analysis was performed with the software ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni-Holm correction for mortality and a 1-sided Dunnett multiple comparisons test for reproduction ($\alpha = 0.05$) were used to compare the control with independent test item groups. Abbott's formula was used to correct for control mortality.

AMPA - Soil predatory mite reproduction test. The soil predatory mite reproduction test with AMPA was conducted at 5 nominal application rates, equivalent to 40 mg test item/kg dry soil, 80 mg test item/kg dry soil, 160 mg test item/kg dry soil, 240 mg test item/kg dry soil, and 320 mg test item/kg dry soil. A negative control (deionized water only) group was also included. All procedures and observations in the test with AMPA were as described for the mite (OECD 226) test with glyphosate in Glyphosate—Soil predatory mite reproduction test. A reference test was performed with dimethoate EC400 (414.8 g/L) at test concentrations of 0 mg a.i./kg dry soil, 4.1 mg a.i./kg dry soil, 5.12 mg a.i./kg dry soil, 6.4 mg a.i./kg dry soil, 8.0 mg a.i./kg dry soil, and 10 mg a.i./kg dry soil.

Springtail reproduction tests

The springtail reproduction tests for glyphosate and AMPA were both conducted according to OECD guideline 232. The procedures used in the 2 studies were identical. Full details of the procedures are presented for glyphosate only. Springtails used in these studies were originally purchased from Biologische Bundesanstalt in May 2000 and reared in the laboratory of the test facility under ambient laboratory conditions.

Glyphosate - Springtail reproduction test. The springtail reproduction test conducted for glyphosate was conducted using glyphosate isopropylamine salt. Survival of springtails (*Folsomia candida*) and their reproductive performance were evaluated at 5 nominal application rates of 32 µL MON 0139/kg dry soil, 50 µL MON 0139/kg dry soil, 100 µL MON 0139/kg dry soil, 500 µL MON 0139/kg dry soil, and 1000 µL MON 0139/kg dry soil (= 15.1 mg a.e./kg dry soil, 23.6 mg a.e./kg dry soil, 47.3 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil, respectively). A negative control with deionized water only was also included. In a reference toxicity test with Betosip (15.7% phenmedipham), concentrations of 50 mg/kg dry soil, 100 mg/kg dry soil, 200 mg/kg dry soil, and 400 mg/kg dry soil were tested. Each treatment group, including the control group, comprised 50 mites divided equally between 5 replicate vessels. For each treatment group and for the control group, 2 test vessels without springtails were provided for pH measurement purposes. Glass containers (150mL nominal volume) were filled with

30 g (wet wt) of the required treated or control soil. Springtails were reared in the laboratory under ambient conditions on a mixture of plaster for stucco, activated charcoal, and water (8:1:9). No exposure to the test item was allowed prior to testing. Juvenile springtails, 10 d to 12 d old and from a synchronized cohort, were added to each test vessel and then covered with a glass lid for 28 d, following which the surviving adults and juveniles were counted. Water content and pH were determined at test start and end. Adult and juvenile springtails were counted at test end. Statistical analysis was performed with the software ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni correction ($\alpha = 0.05$) and Welch's t test ($\alpha = 0.05$), because of non-heterogeneity of variance, were used to compare the control with the independent test item groups for significance of parental mortality and reproductive reduction, respectively. Abbott's formula was used to correct for control mortality.

AMPA = Springtail reproduction test. The springtail reproductive test for AMPA was conducted with AMPA (98.7 % purity) mixed into artificial soil at 5 nominal application rates, equivalent to 30 mg/kg dry soil, 54 mg/kg dry soil, 97.2 mg/kg dry soil, 175 mg/kg dry soil, and 315 mg/kg dry soil. The negative control used deionized water only. In a separate toxic reference test with 100 % crystalline boric acid (BDH Prolabo) mixed with the soil, also included in the test design, the sensitivity of the population was determined with test concentrations of 0 mg/kg dry soil, 44 mg/kg dry soil, 67 mg/kg dry soil, 97.2 mg/kg dry soil, 150 mg/kg dry soil, and 225 mg/kg dry soil. The procedures used during the Springtail reproduction study were essentially equivalent to those used in the springtail test with glyphosate (described in Glyphosate - Springtail reproduction test) with the following exceptions. Each treatment group comprised 40 springtails (10 per test vessel), whereas the control group comprised 8 replicates. Statistical evaluation was performed with ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni correction and a 1-sided Dunnett test were used to compare the control with independent test item groups. Mortality of adult springtails

Soil nitrogen-transformation tests

Soil nitrogen-transformation tests were conducted with glyphosate and AMPA according to OECD guideline 216 and performed according to good laboratory practice. The procedures used in the 2 tests were identical, although tested rates differed. Full details of procedures used are presented for glyphosate only.

Glyphosate - Soil nitrogen-transformation test. The soil nitrogen-transformation test for glyphosate was conducted using glyphosate acid (96.59 % purity; Monsanto Europe) applied at 2 soil concentrations, 6.62 mg a.e./kg dry soil and 33.1 mg a.e./kg dry soil. The tested rates were equivalent to 1 and 5 times the maximum predicted environmental concentration in soil following a worst-case application of glyphosate to bare soil in the EU. Each treatment group and the control comprised 3 replicate test vessels. The control was treated with water only. Field-collected soil was used (LUFA standard soil, type 2.3). On collection, the soil was manually cleared of large objects, such as stones and parts of plants, and then moist-sieved to a particle size ≤ 2 mm. The soil was stored under aerobic conditions in the dark at 4 ± 2 °C until required for use.

Glyphosate was prepared in deionized water and then mixed into a bulk sample of soil at the start of the test. The soil moisture content was 40% (± 5%) of the maximum water holding capacity. During the test, the weight of a moisture control vessel maintained under the same test conditions was used as a guide to correct for test vessel water loss. Control and treated bulk samples of soil were amended with ground lucerne meal (0.5%) as a nitrogen source with a C to N ratio of 16:4:1. Bulk samples were then subsampled (~500 g) into replicate vessels and incubated at 20 ± 2 °C for 28 d. All containers were covered with a perforated lid to avoid evaporative water loss and stored in the dark. Soil (10 g) was taken from 1 replicate from each treatment for pH (water) determination at the start and end of the Glyphosate - Soil nitrogen-transformation study. An additional soil sample was taken from 1 replicate per treatment for moisture and dry matter content determination at the end of the study. As soon as possible after dosing (day 0) and after 7 d, 14 d, and 28 d, a 50-g soil sample (based on dry wt) was removed from each replicate to determine NH_4^+, NO_2^-, and NO^3. Soil extracts were prepared by adding 250mL of 2 M KCl, then shaking for 2 h and centrifuging for 15 min. The supernatant was analyzed using a Bran+Luebbe Autoanalyzer AA3 system. Effects below 25 % deviation from control were not considered to be biologically significant.

AMPA - Soil nitrogen-transformation test. In the soil nitrogen-transformation test conducted for AMPA, the bulk samples of field-sampled soil were prepared at AMPA (98.7% purity) soil concentrations of 40 mg/kg dry soil, 80 mg/kg dry soil, 160 mg/kg dry soil, 320 mg/kg dry soil, and 640 mg/kg dry soil. In

addition, a negative control (nontreated soil) was tested. Although conducting reference tests and use of positive controls are not guideline requirements, in a separate reference test with dinoterb (2-tert-butyl-4,6-dinitrophenol, 99.9 % purity; Sigma-Aldrich Chemie), test concentrations of 6.8 mg/kg dry soil, 16 mg/kg dry soil, and 27 mg/kg dry soil were applied, in addition to the control (0 mg/kg dry soil), with 3 replicates per treatment group.

Results

Glyphosate - Earthworm reproduction test

There was 0 % mortality of adult *E. fetida* at glyphosate concentrations of 14.18 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil. Mortality (2.5%) was observed at 23.64 mg a.e./kg dry soil and 47.28 mg a.e./kg dry soil, which is considered incidental background mortality given the 10 % validity criterion for adult mortality in the control ($p > 0.3$). No statistically significant differences were detected for adult biomass ($p > 0.05$; Figure A below) and for the numbers of juveniles produced at each of the treatment groups when compared to the control ($p > 0.05$; Figure A below). Adult and juvenile feeding behavior was also not adversely affected over the duration of the test (56 d). The resulting no-observed-effect concentration (NOEC) for effects on reproduction was determined therefore to be the maximum test concentration of 472.8 mg a.e./kg dry soil.

In the reference test with carbendazim, juveniles were reduced by 65 % and 92 % at 5 mg reference item/kg dry soil and 10 mg reference item/kg dry soil, respectively. The control treatment had a mean number of 143 juveniles, whereas 5 mg/kg dry soil and 10 mg/kg dry soil treatments with carbendazim had a mean number of 51 juveniles and 11 juveniles, respectively. These reference test values show that the test system was appropriate to detect toxic effects on earthworm reproduction. The validity criteria, namely adult mortality < 20 % and number of juveniles per replicate > 30 in the control treatment, and coefficient of variance between control replicates < 30 % were all met. The guideline requirements for water content, temperature, and pH were all met.

AMPA - Earthworm reproduction test

In the earthworm reproduction study with AMPA, there were no significant effects on *E. fetida* adult mortality across concentrations compared to the control ($p > 0.22$). In all treatment groups, all 10 adults survived the treatments, except for 1 mortality in a single replicate of the 668.5 mg/kg dry soil treatment (Figure B below). Adult earthworm biomass was significantly lower compared to the control at the 445.5 mg AMPA/kg dry soil, 668.5 mg AMPA/kg dry soil, and 1002.5 mg AMPA/kg dry soil test concentrations ($p < 0.0001$; Figure B below). Adult biomass at 198.1 mg AMPA/kg dry soil was also significantly lower than the control ($p = 0.007$), but at 297.1 mg AMPA/kg dry soil there was no significant difference ($p > 0.802$) because the biomass (in percentage of control) was 88.5% and 88.2% in the 131.9 mg AMPA/kg dry soil and the 297.1 mg AMPA/kg dry soil treatment groups, respectively. The effect at 198.1 mg AMPA/kg dry soil is therefore considered to not be treatment-related. Juvenile production was not significantly affected at concentrations up to 198.1 mg/kg dry soil ($p > 0.342$). At 297.1 mg AMPA/kg dry soil and higher concentrations juvenile *E. fetida* numbers decreased significantly compared to the control ($p = 0.0013$). The resulting NOEC for effects on reproduction therefore was concluded to be 198.1 mg/kg dry soil, with a reproductive lowest-observed effect concentration (LOEC) at 297.1 mg AMPA/kg dry soil. The calculated 50% effective concentration (EC50) value for AMPA on earthworm survival was > 1000 mg/kg dry soil. The reproduction EC50 value was calculated at 654.7 mg AMPA/kg dry soil (95 % confidence interval 610.9 - 705.5 mg/kg dry soil). The resulting regression equation was $y = - 0.1108 (\pm 0.005)$ AMPA mg/kg + 122.6 ($\pm$ 2.271), with an R^2 of 0.92. The reference test item carbendazim resulted in decreased biomass of 33.3% at 5.0 mg/kg dry soil and no reproduction, showing that the test system was sensitive to pesticide application. The validity criteria and guideline requirements were all met.

Glyphosate - Soil predatory mite reproduction test

No significant effects were observed on soil mite survival ($p > 0.3$) or reproduction ($p > 0.05$) up to and including the highest test concentration (472.8 mg a.e./kg dry soil; Figure C below) after 14 d of continuous exposure. All validity criteria and guideline recommendations were met. In the reference test with dimethoate the EC50 on reproduction was determined to be 4.9 mg a.i./kg dry soil. The reference test

demonstrated the sensitivity of the test system to detect reproductive toxicity in soil mites. The NOEC was therefore set at the highest test concentration.

AMPA - Soil predatory mite reproduction test
No significant effects were observed on soil mite survival ($p > 0.1$) or reproduction ($p > 0.05$) up to and including the highest test concentration (320 mg AMPA/kg dry soil; Figure D below). All validity criteria and guideline recommendations were met. The reference test with dimethoate showed that the test was sensitive at detecting reproductive toxicity in soil mites. The NOEC for AMPA was therefore concluded to be at the highest test concentration, 320 mg/kg dry soil.

Glyphosate - Springtail reproduction test
No significant effects were observed on springtail survival ($p > 0.5$) or reproduction ($p > 0.05$) up to and including the highest test concentration (472.8 mg a.e./kg dry soil; Figure E below). The validity criteria and guideline recommendations were all met. In the reference test with phenmedipham, the EC50 on reproduction was determined to be 28.4 mg phenmedipham/kg dry soil, which demonstrates that the test system was sensitive for reproductive toxicity. The NOEC for glyphosate was therefore concluded to be the highest test concentration.

AMPA - Springtail reproduction test
No significant effects were observed on springtail survival ($p > 0.5$) or reproduction ($p > 0.06$, $\alpha = 0.01$) up to and including the highest test concentration (315 mg AMPA/kg dry soil; Figure F below). The validity criteria and the guideline recommendations were all met. In the reference test with boric acid, the EC50 for reproduction was determined to be 108.6 mg/kg dry soil, demonstrating sensitivity to reproductive toxicity of the test system. The NOEC for AMPA was therefore concluded to be the highest test concentration.

Glyphosate - Soil nitrogen transformation test
Nitrogen-transformation rates in the soil treated at glyphosate rates equivalent to 6.62 mg a.e./kg dry soil and 33.1 mg a.e./kg dry soil were - 0.13% and 2.13% different compared to the control between day 14 and day 28, respectively (Figure G below). The validity criterion of < 15% variation between control treatments was met in the test. As the rates of nitrate formation between the control and the treated groups were < 25 % on day 28, glyphosate can be evaluated as having no long-term influence on nitrogen transformation in LUFA soils at concentrations ≤ 33.1 mg a.e./kg dry soil. No reference test was conducted, in line with the OECD guideline.

AMPA - Soil nitrogen-transformation test
Stimulation of nitrogen-transformation rates was observed across all treatments on day 7 and day 14, which was possibly linked to the high levels of nitrogen and phosphorus released from the degradation of AMPA in the biologically active soil. Only in the 2 highest test concentrations did the increase exceed 25 % compared to the control at 28 d. The test was therefore prolonged from 28 d to 56 d for the 2 highest test concentrations, 320 mg/kg dry soil and 640 mg/kg dry soil (Figure H below). At 56 d, the deviation from the control was 26.7 % at 320 mg/kg dry soil and 43.1 % at 640 mg/kg dry soil. The reference test results with dinoterb showed increases of 37.6 % at 6.8 mg/kg dry soil, 51.4 % at 16.00 mg/kg dry soil, and 27.1 % at 27 mg/kg dry soil. The validity criterion of < 15% variation between controls was met at all sampling intervals.

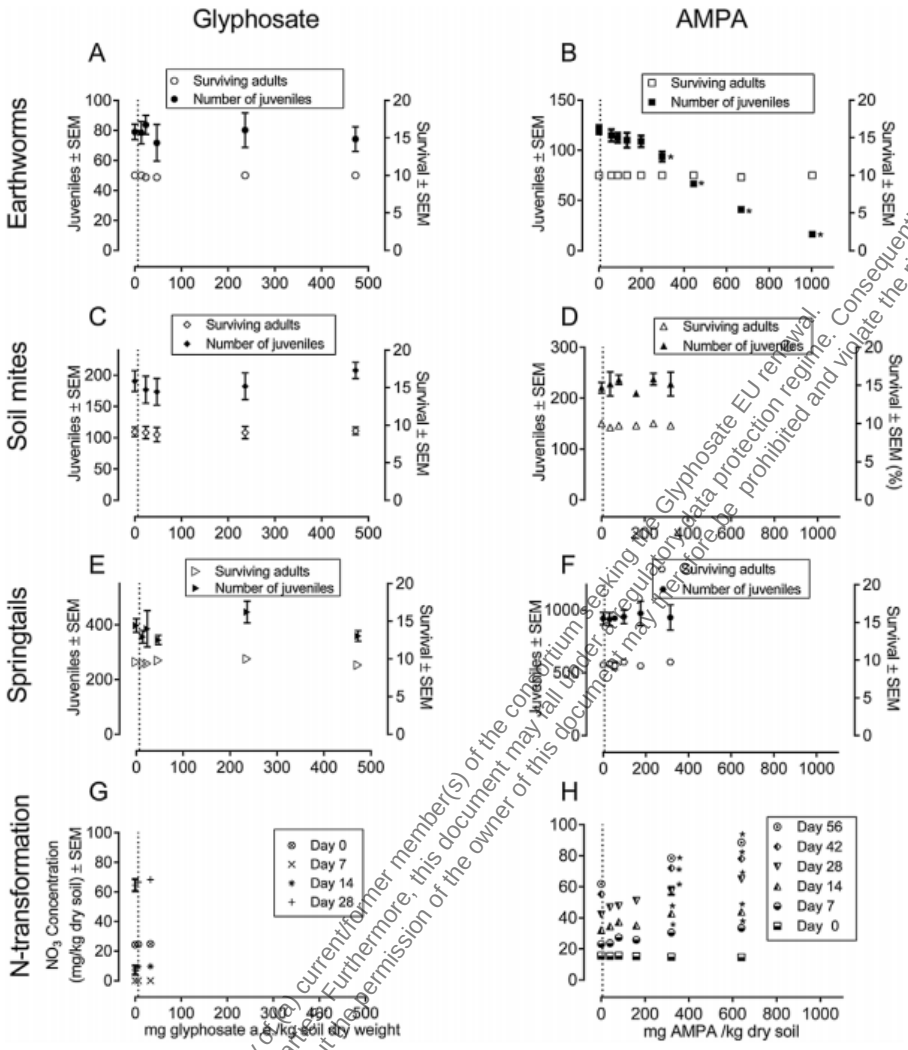


Figure 8.4.2.1-1. Chronic risk assessment for glyphosate (left) and aminomethylphosphonic acid (AMPA; right) in soil. Number of surviving adults (28 d) and number of juveniles (56 d) in earthworms exposed to glyphosate (A) and AMPA (B), (* statistically significant effect [$p \leq 0.05$] compared with control treatment), in soil predatory mites (*Hypoaspis aculeifer*) exposed to glyphosate (C) and AMPA (D) for 14 d, and in springtails (*Folsomia candida*) exposed to glyphosate (E) and AMPA (F) for 28 d. Effects on nitrogen transformation in soil treated with glyphosate (G) and AMPA (H) for 0 d, 7 d, 14 d,28 d, 42 d, and 56 d(* > 25 % effect compared with control treatment). Vertical dotted line in each graph indicates the worst-case predicted environmental concentration of glyphosate/AMPA. Vertical bars indicate standard error of the mean (SEM).

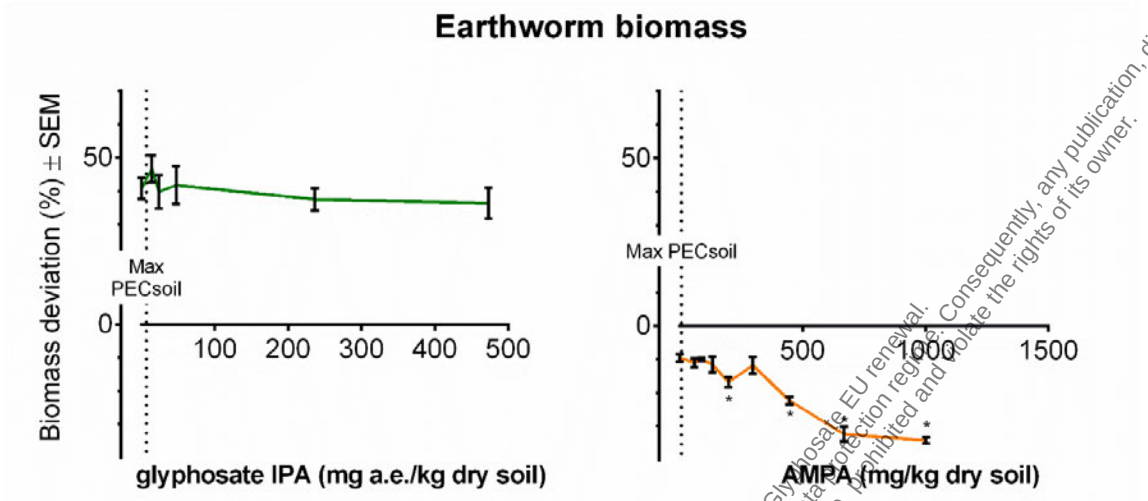


Figure 8.4.2.1-2. Effects of glyphosate (A) and AMPA (B) on earthworm biomass after 28 d of exposure. The vertical dotted line indicates the predicted environmental concentration for AMPA and glyphosate. An asterisk next to a data point indicates a significant difference ($P < 0.05$) when compared against the control treatment.

Risk assessment

The chronic effects of exposure to glyphosate and the major soil metabolite AMPA to representative taxonomic groups of soil macroorganisms and nitrogen transformation were assessed following standard practices outlined under Annex VI Uniform Principles of the EU's Plant Protection Products Regulation (EC) No 1107/2009. At soil concentrations relevant to recommended glyphosate field application rates, no significant adverse effects were observed in any of the test species or systems exposed to glyphosate or AMPA. The risk assessment for soil macroorganisms in the EU compares the lowest NOEC achieved for each of the taxonomic groups with worst-case initial predicted soil concentrations (soil $PEC_{initial}$) achieved directly following a bare soil application and the potential for accumulation in soil following applications over multiple years to the same field (soil PEC_{accu}) The ratio of the endpoint to the predicted soil concentration is determined (toxicity exposure ratio = NOEC - $PEC_{initial}$) and compared against trigger values in accordance with Annex VI Uniform Principles of the EU's Plant Protection Products Regulation 1107/2009. Where trigger values are exceeded, a low exposure risk may be concluded. The long-term trigger value of 5 using NOECs derived from laboratory tests accounts for uncertainty related to interspecies sensitivity, predicted exposure estimates, and extrapolation from laboratory to field exposure.

For glyphosate and AMPA, the initial soil concentration ($PEC_{initial}$) at a soil depth of 5 cm has been determined based on a bare soil application (without foliar/crop interception), at the maximum cumulative annual application rate of 4.32 kg glyphosate a.e./ha for the EU. The risk of glyphosate and AMPA residues accumulating in soil over multiple years is considered by deriving the PEC_{accu} value. This is the sum of the $PEC_{initial}$ and plateau concentrations in soil, achieved in the top 5 cm (tillage depth for permanent crops) soil layer, following applications to bare soil at the maximum cumulative annual application rate (4.32 kg a.e./ha) each year for 10 yr.

It is important to mention that a single application rate of 4.32 kg glyphosate a.e./ha is not supported in the representative use rate but rather represents the recommended maximum cumulative (total) annual application rate for all uses and, therefore, a very conservative worst-case approach.

For exposure of soil mites, springtails, and earthworms to glyphosate in soil, the achieved chronic endpoints exceed the worst-case predicted glyphosate $PEC_{initial}$ and PEC_{accu} soil concentration by factors of 82 and 71, respectively.

For exposure of soil mites, springtails, and earthworms to AMPA in soil, the achieved chronic endpoints exceed worst-case AMPA $PEC_{initial}$ soil concentrations by factors of between 97 and 491, whereas the chronic endpoints exceed the PEC_{accu} soil concentrations by factors of between 32 and 162.

For soil nitrogen transformation, the endpoints achieved for glyphosate and AMPA (33.1 mg a.e./kg dry soil [glyphosate] and 160 mg a.e./kg dry soil [AMPA]) both achieved a < 25 % effect on nitrogen-

transformation rates following a 28-d soil exposure to either glyphosate or AMPA. These soil exposure rates exceed the worst-case predicted $PEC_{initial}$ soil concentrations by factors of 6 (glyphosate) and 78 (AMPA). The achieved endpoints also exceed the PEC_{accu} soil concentrations, by factors of 5 for glyphosate and 26 for AMPA.
For the soil mite, springtail, and earthworm reproduction chronic endpoints, the toxicity exposure ratio values exceed the EU Regulation No 546/2011 Annex VI trigger (5), indicating that, for the ecotoxicologically relevant endpoints achieved for survival and reproduction, the use of glyphosate according to label recommendations is unlikely to result in adverse effects inside the treated area for soil biota - from exposure to both glyphosate and AMPA.
For the soil microbial community, relative to expected field application rates for exposure to glyphosate there is at least a 5-fold safety margin. For exposure to AMPA, a 26-fold safety margin applies. The observed increases in nitrate concentrations at the higher test concentrations are expected to be related to the large quantity of nitrogen and phosphate provided to the microbes via degradation of AMPA in the biologically active soil.

Table 8.4.2.1-1. Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil organisms[a]

Test species	Test item	Test duration (d)	Endpoint type	NOEC (mg a.e. or AMPA/kg soil)	$PEC_{initial}$ (mg a.e./kg soil)	PEC_{accu} (mg a.e./kg soil)	$TER_{initial}$	TER_{accu}
Earthworm	Glyphosate IPA salt	56	Adult mortality	472.8	5.76	6.62	82	71
			Biomass	472.8			82	71
			Reproduction	472.8			82	71
	AMPA	56	Adult mortality	1002.5	2.04	6.18	491	162
			Biomass	297.1			146	48
			Reproduction	198.1			97	32
Soil mite	Glyphosate IPA salt	14	Adult mortality	472.8	5.76	6.62	82	71
			Reproduction	472.8			82	71
	AMPA	14	Adult mortality	320	2.04	6.18	157	52
			Reproduction	320			157	52
Springtail	Glyphosate IPA salt	28	Adult mortality	472.8	5.76	6.62	82	71
			Biomass	472.8			82	71
	AMPA	28	Adult mortality	315	2.04	6.18	154	51
			Biomass	315			154	51
N-transformation	Glyphosate acid	28	Effect <25%	33.1	5.76	6.62	6	5
	AMPA	28	Effect <25%	160	2.04	6.18	78	26

a.e. = acid equivalent; AMPA = aminomethylphosphonic acid; IPA = isopropylamine; NOEC = no-observed-effect concentration; PEC_{accu} = accumulative predicted environmental concentration, cumulative worst-case application of 4.32 kg a.e./ha of glyphosate for 10 yr; $PEC_{initial}$ = initial predicted environmental concentration, assuming single worst-case application of 4.32 kg a.e./ha of glyphosate; TER_{accu} = toxicity to exposure ratio (= NOEC - PEC_{accu}); $TER_{initial}$ = toxicity to exposure ratio (= NOEC - $PEC_{initial}$).

Conclusion

The risks from exposure to glyphosate and the primary soil metabolite AMPA at levels that exceed commercial application rates were evaluated against a battery of representative soil macroorganisms and microorganisms under controlled laboratory conditions. Results from the present studies demonstrate that the potential impact to beneficial soil macro-organisms and nutrient cycling soil microorganisms under environmentally relevant exposure scenarios is low.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The aim of the paper was to evaluate potential effects of Glyphosate, Glyphosate salt and AMPA on earthworm, soil mites, springtails and soil micro-organisms.

The studies have been conducted according to recognised guidelines and validity criteria were presented. Test substance information, test organism origin, study designs and toxicity effects were adequately described. The study is considered reliable.

CA 8.5 Effects on Nitrogen Transformation

A regulatory database on toxicity to soil nitrogen transformation has been summarised to evaluate toxicity of glyphosate and AMPA. The results of these studies demonstrate that glyphosate and AMPA are of low toxicity to soil microflora.

Studies considering the effects of glyphosate on soil microflora were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 0.5-1: Studies on toxicity of glyphosate to soil nitrogen transformation

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.5/001	2014	Nitrogen-mineralisation	Soil microorganisms	Glyphosate acid	Valid	-
CA 8.5/002	, 2000	Nitrogen cycle Carbon cycle	Soil microorganisms	Glyphosate technical	Invalid	Validity criteria for variation in control replicates not met
CA 8.5/003	1995	Nitrogen cycle Carbon cycle	Soil microorganisms	Glyphosate	Invalid	-
CA 8.5/004	2010	Nitrogen and Carbon mineralisation	Soil microorganisms	AMPA	Valid	Not sufficient information provided to check for validity criteria

Literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate on soil microflora are summarised in the table below. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. Each literature article summary is presented below according to the respective annex point. For discussions of literature regarding toxicity to soil microflora, please refer to document M-CP Section 10.5.

Table 0.5-2: Literature on toxicity of glyphosate to soil nitrogen transformation

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 8.5/005	Von Mérey *et al*., 2016	OECD 222; 56 days chronic	Glyphosate IPA salt and AMPA	Relevant and reliable	Evaluates potential effects on earthworm, soil mites, springtails and soil micro-organisms.

Endpoints of studies considered valid for glyphosate are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.5-3: Endpoints: Toxicity of glyphosate to soil nitrogen transformation

Reference (Data owner)	Test item	Test design/ GLP	Endpoint (mg a.e./kg dry soil)	Endpoint (kg a.e./ha)
, 2014 CA 8.5/001	Glyphosate technical (MON 77973)	Nitrogen mineralisation, 28-days	NOEC ≥ 33.1	**NOEC ≥ 24.83**

a.e.: acid equivalents
Endpoint in **bold** used for risk assessment

Endpoints of studies considered valid for AMPA are shown in the table below.

Table 8.5-4: Endpoints: Toxicity of AMPA to soil nitrogen transformation

Reference (Data owner)	Test item	Test design/ GLP	Endpoint (mg/kg dry soil)	Endpoint (kg/ha)
,, 2010 CA 8.5/004	AMPA	Nitrogen and Carbon mineralisation, 56-days	NOEC = 160	**NOEC = 120**

Endpoint in **bold** used for risk assessment

Study summaries are provided below.

1. Information on the study

Data point	CA 8.5/001
Report author	
Report year	2014
Report title	Glyphosate technical (MON77973): Effect on Soil Microbial Nitrogen Transformations
Report No	CEMR-6237
Document No	-
Guidelines followed in study	OECD Guideline 216 (2000)
Deviations from current test guideline	Deviation from the guideline OECD 2016 (2000): None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of Glyphosate technical (MON 77973) on the nitrogen transformation pathways were assessed in a LUFA standard soil type 2.3. The transformation rates were determined in replicate soil samples treated with MON 77973 at rates of 6.62 and 33.1 mg acid equivalent/kg dry soil (equivalent to 1 and 5 × the initial Predicted Environmental Concentration in soil) and compared to a control (deionised water). The products of the process of nitrification (nitrate, ammonium and nitrite) were extracted from the soil on Day 0, 7, 14 and 28 after treatment.
As the average rate of production of nitrate (mg/kg/day) from Day 14 to Day 28 between the treatment rates of MON 77973 (6.62 and 33.1 mg a.e./kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long-term influence on nitrogen transformation in soils.
The study is considered valid and NOEC ≥ 33.1 mg a.e./kg dry soil (corresponding to 24.8 kg a.e./ha) can be used in risk assessment for micro-organisms exposed to glyphosate technical.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 77973
Description:	White Powder
Lot/Batch #:	GLP-0807-19475-T
Purity:	96.59 % Glyphosate Acid
Vehicle and/or positive control:	Vehicle: deionised water
	Positive control: none

Test system:

Soil:	Sandy loam soil "LUFA standard soil 2.3" (Batch number Sp2.33113)
Source:	LUFA-Speyer, Obere Langgasse 40, 67346 Speyer, Germany
Water holding capacity:	36.2 % (g water/100 g dry soil)
Water content:	35 ± 5 %
pH:	6.5
Org. Carbon:	0.67%
Microbial biomass:	4.35% to C_{org}
Clay (< 0.002 mm):	5.9 ± 2.5 %
Silt (0.002 - 0.050 mm):	33.9 ± 0.0 %
Sand (0.050 – 2.0 mm):	60.3 ± 2.5 %
Acclimation:	35 % (± 5 %) of MWHC at 20 ± 2 °C for 5 days

Environmental conditions:

Temperature:	20 ± 2 °C
pH:	6.0 - 6.6 (range between Day 0 and Day 28)
Water content:	42 % of MWHC
Photoperiod:	24 hours darkness
Experimental Dates:	20 September - 24 October 2013

B. STUDY DESIGN AND METHODS

Experimental treatments

Soil samples were bulk dosed with MON 77973 at nominal rates equivalent to 1 and 5 × $PEC_{plateau}$ (6.62 and 33.1 mg a.e./kg dry soil, respectively).

Five days before the start of the exposure phase, the soil moisture content was nominally adjusted to 35 % (± 5 %) of the MWHC. The soil was placed in the test cabinet in the dark at 20 ± 2 ºC. On the day of dosing, the moisture of the soil was adjusted to 40 % (± 5%) of the MWHC with deionised water with the appropriate dose of test item. Three replicates (each of them contained 500 g dry weight equivalent of soil) were prepared for the control treatment (deionised water) and the test item treatments. Each replicate of soil was transferred to plastic test vessels (2 L). The test soil was amended with lucerne (2.5 g of lucerne/500 g of soil) to the control and treatment groups on Day 0. Additionally, 500 g (dry weight equivalent) of soil was prepared which had no lucerne amendment to serve as the unamended control sample. The moisture content of soil samples was maintained during the test at 40 % of the maximum water holding capacity of the soil with a range of ± 5%.

Inorganic ammonium, nitrate and nitrite were extracted from each sub-sample of soil with 2 M potassium chloride solution (250 mL) and shaking for 2 hours. The extract was separated from the soil by centrifugation (15 minutes, 2500 rpm). Approximately 20 mL of the supernatant was stored refrigerated prior to analysis. Each extract was analysed for nitrate, ammonium and nitrite using the Bran + Luebbe Autoanalyser AA3 system.

Observations

As soon as possible after treatment, a sub-sample of soil was taken from each replicate for the determination of nitrate, nitrite and ammonium concentration. Further sub-samples were taken after 7, 14 and 28 days. All samples were analysed for nitrate, ammonium and nitrite on Day 28. Concentrations of nitrate (as TON) and ammonium were measured (mg/kg dry soil) from Day 0 to Day 28. The nitrite values were not reported as no nitrite-N was detected, and therefore considered not to have nitrite present in any of the extracted soil solutions. Changes in concentration of nitrate and nitrate transformation rates (mg/kg/day) over the duration of the study were measured. The changes in nitrate production from 0 - 7, 7 - 14 and 14 - 28 days were also determined.

Statistical calculations

Shapiro-Wilks and Bartlett's Test followed by Dunnett's two-tailed test (α= 0.05).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.5-2: Effects of MON 77973 on soil nitrogen transformation

		Nitrogen concentration [mg/kg soil]		% deviation from control	
Concentration in MON 77973	**Control**	**6.62 mg/kg dws**	**33.1 mg/kg dws**	**6.62 mg/kg dws**	**33.1 mg/kg dws**
Nitrate transformation rates					
Day 0-7	-3.47	-3.51	-3.56	+1.26	+2.52
Day 7-14	+1.04	+1.34	+1.39	+29.47	+33.68
Day 14-28	+4.10	4.09	+4.18	-0.13	+2.13
Nitrate (NO_3^-)					
Day 0	24.3	24.6	24.9	+1.23	+2.47

Table 8.5-2: Effects of MON 77973 on soil nitrogen transformation

		Nitrogen concentration [mg/kg soil]		% deviation from control	
Concentration in MON 77973	**Control**	**6.62 mg/kg dws**	**33.1 mg/kg dws**	**6.62 mg/kg dws**	**33.1 mg/kg dws**
Day 7	0	0	0	-	-
Day 14	7.3	9.4	9.7	+28.77	+32.88
Day 28	64.6	66.7	68.3	+3.25	+5.73
Ammonium (NH_4^+)					
Day 0	7.0	7.0	6.6	0	-5.71
Day 7	2.4	2.4	2.4	0	0
Day 14	1.8	1.7	1.7	-5.56	-5.56
Day 28	0.8	0.8	0.8	0	0

dws: dry weight soil
- = inhibition, + = stimulation

B. OBSERVATIONS

Statistical analysis showed there was no significant difference ($p<0.05$) between the treatment rates of 6.62 and 33.1 mg a.e./kg dry soil and the control treatment for nitrate production from Day 14 to 28.

As the difference in nitrate production between the treatment rates of MON 77973 (6.62 and 33.1 mg a.e./kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long-term influence on nitrogen transformation in soils at concentrations ≤33.1 mg a.e./kg dry soil.

The variation within the control treatment ranged from -4.2 to 2.6 % at Day 0; from -0.9 to 1.8 % at Day 7; from -49.5 to 26.3 % at Day 14 and from -7.1 to 5.4 % at Day 28.

The changes in nitrate production were determined between each time point and not on the whole test from 0-28 days.

Validity criteria

The validity criterion according to OECD 216 guideline was met at study termination, as the variation between replicate control treatments did not vary by more than ± 15 % at Day 28 for nitrogen transformation (actual values from -7.1 to 5.4 %).

III. CONCLUSIONS

Assessment and conclusion by applicant:

The study provides relevant and reliable endpoints to be used in the regulatory risk assessment for Glyphosate. At soil concentrations of 6.62 and 33.1 mg glyphosate acid equivalent/kg dry soil, there were <25% effect at Day 28 in nitrogen transformation, so MON 77973 is expected to have no long-term influence on the nitrogen transformation pathways in soils up to and including a test concentration ≤33.1 mg glyphosate acid equivalent/kg dry soil.

The study is considered valid and NOEC ≥ 33.1 mg a.e./kg dry soil (corresponding to 24.8 kg a.e./ha) can be used in risk assessment for micro-organisms exposed to glyphosate technical.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.5/002
Report author	.
Report year	2000
Report title	Side-Effects of Glifosate Técnico Nufarm on soil microflora: Carbon and Nitrogen Cycles
Report No	RF-D1.113/99
Document No	-
Guidelines followed in study	Instituto Brasileiro do Meio ambiente e dos Recursos naturais Renováveis_Ibama, portaria Normativa no 84 of October, 15 1996
Deviations from current test guideline	Deviations from guidelines OECD 216 (2000) and OECD 217 (2000): Major: - Nitrogen cycle evaluation should have been prolonged until deviation from control dropped under ±25 %. Minor: - Detail of soil storage and pre-incubation period are not reported. - The alfalfa amendment was also added in samples used for carbon cycle. - Carbon cycle was assessed for one hour instead of 12 consecutive hours. - The assessments after 7 days were missing for both nitrogen and carbon cycles.
Previous evaluation	Not accepted in RAR (2015) for nitrogen Yes, accepted in RAR (2015) for carbon
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of glyphosate technical on soil carbon cycle and nitrogen cycle were investigated in two soil types, a “Typic Hapludox” and a “Rhodic Hapludox” under laboratory conditions. The test substance was applied at two concentration rates of 2.4 and 4.8 kg test item/ha in three replicates. In addition, negative controls (without test item) with or without organic matter amendment were tested. 150 g soil samples were amended with organic matter at a rate of 0.5 % dry soil equivalent for all treatments, except for control without organic matter amendment. Soils were incubated at a temperature range of 19 to 22 °C in the dark in covered glass flasks. Soil samples were removed from the jars 0, 14 and 28 days after treatment and analysed for soil dry mass, pH, nitrite, nitrate, ammoniacal nitrogen and short term respiration.
The results showed no adverse effects of glyphosate technical on soil carbon cycle for both concentrations tested after 28 days. In addition, all validity criteria according to OECD 217 were fulfilled.
For the soil nitrogen cycle test however, the validity criteria according to OECD 216 were not fulfilled, as

the variation between replicate control samples was more than ± 15 %. Therefore, no consistent conclusions could be drawn from the study. The study is therefore considered invalid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	037-919-113
Purity:	95 % a.s. (nominal), 95.49 % a.s. (measured)

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: none

3. Test system:

Soil	LE (Typic Hapludox) and LR (Rhodic Hapludox)
Source:	Not stated
Water content of soil:	Not stated
Water holding capacity	Not stated
pH:	5.5 (LR), 7.0 (LE)
Organic matter:	31 g/md^3 (LR) and 20 g/dm^3
Microbial biomass:	2.63 mg C/g soil (LR), 2.24 mg C/g soil (LE)
Clay (< 0.002 mm):	39 % (LR), 24 % (LE)
Silt (0.002 mm - 0.063 mm):	10 % (LR), 9 % (LE)
Sand (0.063 – 2.00 mm):	51 % (LR), 67 % (LE)

4. Environmental conditions:

Temperature:	19 – 22 °C
pH:	5.53 – 6.27 (LR); 6.34 – 6.84 (LE)
Water content:	40- 60 % of WHC
Photoperiod:	24 hours dark

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The test substance was applied at two concentration rates of 2.4 and 4.8 kg test item/ha using three replicates per concentration. In addition, negative controls (without test item) with or without organic matter amendment were tested. 150 g soil samples were amended with organic matter at a rate of 0.5 % dry soil equivalent for all treatments, except for control without organic matter amendment. Soils were incubated at a temperature range of 19 to 22 °C in the dark in covered glass flasks. Soil samples were removed from the jars, 0, 14 and 28 days after treatment and analysed for soil dry mass, pH, nitrite, nitrate, ammoniacal nitrogen and short term respiration test.

2. Observations:
Nitrogen cycle: For the preparation of soil extract for ammonium-N analysis, 10 g of soil was placed in 250 mL wide-mouth bottle, to which 100 mL of 2M KCl was added. 1 mL of the filtered aliquot containing between 0.5 and 12 µg of NH_4+-N was placed into 25 mL volumetric flasks. 1 mL EDTA, 2 mL phenol nitroprussid and 4 mL hypochlorite buffer were successively added. The concentration of NH_4+-N was thereafter determined using a photometric method at 636 nm. For nitrate-N and nitrite-N analysis, 10 g of soil was placed in a 500 mL Erlenmeyer flask, then 0.5 g of $CaSO_4$ and 250 mL distilled water were added.

For the analysis of nitrate-N, an aliquot of 25 mL of the extract was pipetted into 10 mL round bottom flask and 0.05 g of $CaCO_3$ was added. Subsequently, 2 mL of phenoldisulfonic acid (25 g phenol in 150 mL of concentrated H_2SO_4) was added. After 10 min, 20 mL of distilled water was added. The nitrate-N concentration was determined using a Hach Model DR 2010 absorbance spectrophotometer at 410 nm. For the analysis of nitrite-N, an aliquot of 25 mL of the extract was pipetted into a 25 mL cell. The visual absorbance of each sample was determined at 507 nm using a Hach Model DR 2010 absorbance spectrophotometer.
Carbon cycle: 2 g of soil samples were placed in 50 mL Erlenmeyer flasks, adding 0.5 mL of 2 µmol/mL of glucose- ^{14}C. In order to absorb CO_2 evolved from glucose degradation by soil microorganisms, a small glass flask (1 mL) was hung from the rubber cap, containing 0.2 mL of NaOH. After one hour of incubation in dark conditions, the glucose degradation was then stopped. The NaOH and filter paper strips were transferred into scintillation vials. The radioactivity was assessed in a Liquid Scintillation Analyzer Packard model Tri-carb 1900, during 5 min/sample.

3. Statistical calculations: Results were evaluated using Duncan's Multiple range Test at $\alpha = 0.01$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.5-3: Effects of glyphosate technical on soil nitrogen cycle

		Glyphosate technical [kg test item./ha]				
		Control	2.4		4.8	
		[mg N/kg dry soil]	[mg N/kg dry soil]	Dev. [a]	[mg N/kg dry soil]	Dev. [a]
Soil: LR (Rhodic Hapludox)						
Day 0	Ammonium	22.66	21.61	-4.6	24.31	+7.3
	Nitrite	0.30	0.29	-3.3	0.40	+33.3*
	Nitrate	22.51	22.54	+0.1	23.11	+2.7
Day 14	Ammonium	27.34	34.92	+27.7*	37.50	+37.2*
	Nitrite	0.29	0.21	-27.6*	0.23	-20.7
	Nitrate	30.02	36.47	+21.5*	44.10	+46.9*
Day 28	Ammonium	13.13	11.32	-13.8	9.38	-28.6*
	Nitrite	0.26	0.24	-7.7	0.24	-7.7
	Nitrate	18.39	24.16	+31.4*	34.61	+88.2*
Soil: LE (Typic Hapludox)						
Day 0	Ammonium	30.01	27.87	-7.1	34.72	+15.7*
	Nitrite	0.32	0.27	-15.6	0.27	-15.6*
	Nitrate	22.58	22.74	+0.7	23.34	+3.4
Day 14	Ammonium	26.19	22.60	-13.7	24.50	-6.5
	Nitrite	0.26	0.29	+11.5	0.27	+3.8
	Nitrate	21.78	39.26	+80.3*	41.01	+88.3*
Day 28	Ammonium	16.82	18.71	+11.2	18.72	+11.3
	Nitrite	0.40	0.24	-40.0*	0.26	-35.0*
	Nitrate	18.39	31.67	+72.2*	25.77	+40.1*

[a] - = Deviation from control
* = Significant deviation from control according to OECD Guideline 216
- = inhibition, + = stimulation

Table 8.5-4: Effects of glyphosate technical on soil carbon cycle

	Glyphosate technical [kg test item/ha]				
	Control	2.4		4.8	
	Soil respiration [b]	Soil respiration [b]	Dev. [a]	Soil respiration [b]	Dev. [a]
Soil: LR (Rhodic Hapludox)					
Day 0	9.00	8.33	-7.4	9.06	+0.7
Day 14	16.06	16.19	+0.8	16.76	+4.4
Day 28	15.13	14.63	-3.3	16.53	+9.3
Soil: LE (Typic Hapludox)					
Day 0	12.80	13.00	+1.6	11.56	-9.7
Day 14	16.69	20.16	+20.8	17.56	+5.2
Day 28	16.43	18.06	+9.9	17.26	+5.1

[a] - = Deviation from the control
[b] = Activity of soil microorganism in mmoles metabolized glucose/g soil/h
- = inhibition, + = stimulation

B. OBSERVATIONS

No adverse effects of glyphosate technical on soil carbon cycle were observed for both concentrations 28 days after application. In addition, all validity criteria according to OECD 217 were fulfilled. For the soil nitrogen cycle test validity criteria according to OECD 216 were not fulfilled, as the variation between replicate control samples was more than ± 15 %.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The test item glyphosate technical caused no significant adverse effects on soil carbon cycle at test concentrations of 2.4 and 4.8 kg test item/ha, 28 days after treatment.
All validity criteria according to OECD 217 were fulfilled. For the soil nitrogen cycle test however, the validity criteria according to OECD 216 were not fulfilled, as the variation between replicate control samples was more than ± 15%. Therefore, no consistent conclusions could be drawn from the study.
The study is therefore considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.5/003
Report author	
Report year	1995
Report title	The Effects of Glyfosaat on Soil Respiration and Nitrification
Report No	141885
Document No	-
Guidelines followed in study	BBA-Guideline: Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln Teil VI 1-1 (2. Auflage). "Auswirkungen auf die Aktivität der Bodenmikroflora", (März, 1990)
Deviations from current test guideline	Deviations from guidelines OECD 216 (2000) and OECD 217 (2000): Major: - No indication on the variation between replicate control samples Minor: - Biomass carbon content was not mentioned (should be at least 1% of total organic carbon) - Day 7 assessment is missing - Nitrogen transformation rate was not calculated (mg nitrate/kg dry soil/day) - Respiration rate was not calculated (mg carbon dioxide/kg dry soil/h or mg oxygen/dry soil/h) - The Westmaas soil did not reach the sand percentage of at least 50 %
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

The effect of glyphosate on soil respiration and soil nitrification was investigated in two different agricultural soil types, a loamy sand soil and a loamy soil. The test substance was applied at concentration rates of 2.16 kg glyphosate/ha equivalent to 2.88 mg a.s./kg dry soil and 10.8 kg glyphosate/ha equivalent to 14.4 mg a.s./kg dry soil representing the maximum recommended application rate and five-fold the maximum recommended application rate. 40 g triplicate samples of each treatment were removed for analysis on day 0, 14, 29, 56 and 91 after treatment for loamy sand soil (Speyer soil 2.3) and on day 0, 14, 29 and 56 for loamy soil (Westmaas soil).
In both the loamy sand soil and the loamy soil, no treatment related effects on soil microbial respiration and nitrogen transformation were observed over the duration of the test.
It is not possible to conclude on the study validity according to current OECD guideline requirements. The study is therefore considered invalid.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	22021
Purity:	96 %
Vehicle and/or positive control:	Vehicle: water
	Positive control: Dinosebacetate

Test system:

Soil	Speyer Soil 2.3 (loamy sand soil) and Westmaas soil (loamy soil)
Source:	Speyer Soil 2.3 was originated from Offenbach in Rheinland-Pfalz, "Im Bildgärten", Nr. 510 and 510/2, Germany.
	Westmaas soil was originated from ROC Westmaas, the Netherlands.
Water content of soil:	5.5 – 8.6 % (Speyer Soil 2.3)
pH:	6.4 (Speyer Soil 2.3), 7.4 (Westmaas soil)
Total Org. C:	1.22 % (Speyer Soil 2.3); 1.23 % (Westmaas soil)
Clay (< 0.002 mm):	9.5 % (Speyer Soil 2.3); 17.5 % (Westmaas soil)
Silt (0.063 mm > 0.002 mm):	29.6 % (Speyer Soil 2.3); 50.6 % (Westmaas soil)
Sand (≥ 0.063 – 2.00 mm):	60.9 % (Speyer Soil 2.3); 31.9 % (Westmaas soil)

Environmental conditions:

Temperature:	20 ± 2 °C
pH:	6.8 – 9.0 (Speyer Soil 2.3), 7.1 – 7.9 (Westmaas soil)
Water content:	50 % WHC
Photoperiod:	Not specified
Incubation period:	5 days
Experimental Dates:	Not stated

B. STUDY DESIGN

Experimental treatments

Glyphosate was tested at two treatment concentrations, the maximum field rate of 2.16 kg glyphosate/ha (equivalent to 2.88 mg glyphosate/kg dry soil) and at 5 × the maximum field rate, 10.8 kg glyphosate/ha (equivalent to 14.4 mg glyphosate/kg dry soil) using 3 replicates. In addition, a negative control and a toxic reference were tested. To determine soil respiration and soil nitrification, treated and untreated soils were incubated at a water content of 50 % WHC in 100 mL Erlenmeyer flasks at 20 ± 2 °C. The flasks were covered with cotton wool. For soil nitrification, each soil group was amended with 0.5 % lucerne meal (14.4 C/N ratio) as a nitrogen source at the time of preparation.

Observations

Soil microflora respiration: 40 g samples (based on the dry weight) of sieved soil samples were incubated per treatment. After 0 - 3 hours, 14, 29, 56 and 91 days, each soil sample was amended by 500 mg glucose. The amount of glucose to be added was determined during the microbial biomass determination. CO_2 was collected in $Ba(OH)_2$ traps during 24 hours (T = 0, 14, 29 and 56 days) or 16 hours (T = 91 days). The

amount of CO_2 formed was determined by titration of the contents of the traps.
Soil nitrification: 40 g samples (based on the dry weight) of sieved soil were amended with lucerne meal (41.7 % C; 2.9 % N) and incubated. Soil samples were taken after 0 - 3 hours, 14, 29, 56 and 91 days. NH_4-N, NO_2-N and NO_3-N were measured according to NEN norms 6472, 6652 and 6777. The pH (KCl) was determined at the day of application, after 29 days and at each sampling day after 29 days.
The soil water content was checked at weekly intervals. If necessary, the water content was adjusted with Milli-Q water.
The microbial biomass was measured, but there were no indications whether its carbon content was at least 1% of the total soil organic carbon.

Statistical calculations
The results were statistically evaluated at $\alpha = 0.05$ using Dunnett's test. For data with outliers the Bonferroni t-test was used at $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS
The control samples showed that the respiration and nitrification of both soils were sufficient at the start of the test.

Table 8.5-5: Effects of glyphosate on soil nitrification in Speyer Soil 2.3 (loamy sand soil)

	Nitrogen concentration [mg N/kg soil]:			% deviation from control[1)]	
	Control	2.88 mg /kg dws	14.4 mg /kg dws	2.88 mg/kg dws	14.4 mg/kg dws
Nitrate					
Day 0	2.33	1.41	1.48	-40*	-36*
Day 14	8.30	5.23	6.12	-37	-26
Day 29	5.14	4.55	4.94	-11	-4
Day 56	5.58	5.41	7.20	-3	+29*
Day 91	12.78	7.45	6.30	-42	-51
Nitrite					
Day 0	0.42	0.47	0.41	+11	-2
Day 14	0.022	0.021	0.021	-6	-6
Day 29	0.032	0.044	0.039	+38*	+23
Day 56	0.048	0.058	0.061	+19	+26
Day 91	0.019	0.018	0.017	-4	-9
Ammonium					
Day 0	1.81	1.96	2.11	+8	+16
Day 14	0.882	0.883	1.003	0	+14
Day 29	0.624	0.776	0.666	+24*	+7
Day 56	1.59	2.29	2.04	+44*	+28
Day 91	0.352	0.340	0.492	-3	+40*

dws = dry weight soil
0 - = inhibition; + = stimulation
* = Significantly different from control (at $\alpha = 0.05$)

Table 8.5-6: Effects of glyphosate on soil nitrification in Westmaas soil (loamy soil)

	Nitrogen concentration [mg N/kg soil]:			% deviation from control[1)]	
	Control	2.88 mg/kg dws	14.4 mg/kg dws	2.88 mg./kg dws	14.4 mg/kg dws
Nitrate					
Day 0	41.0	35.9	34.2	-13	-17
Day 14	40.1	35.3	35.9	-12	-10
Day 29	32.5	29.4	24.8	-10	-24*
Day 56	29.9	33.6	29.3	+13	-2
Nitrite					
Day 0	2.34	2.38	1.86	+2	-20
Day 14	0.026	0.022	0.019	-15	-26
Day 29	0.040	0.038	0.039	-4	-2
Day 56	0.040	0.042	0.033	+7	-16
Ammonium					
Day 0	10.9	10.4	9.41	-4	-13*
Day 14	0.688	0.685	0.640	0	-7
Day 29	0.624	0.776	0.666	+24*	+7
Day 56	0.372	0.369	0.363	-1	-2

dws = dry weight soil
1) - = inhibition; + = stimulation
* = Significantly different from control (at α = 0.05)

Table 8.5-7: Effects of glyphosate on soil respiration

	Biomass concentration [mg/100g soil]			% deviation from control[1)]	
	Control	2.88 mg/kg dws	14.4 mg/kg dws	2.88 mg/kg dws	14.4 mg/kg dws
Speyer Soil 2.3 (loamy sand soil)					
Day 0	22	36	28	+68	+28
Day 14	54.9	58.0	71.4	+6	+30*
Day 29	67.9	73.2	69.6	+8	+2
Day 56	94.6	89.5	88.9	-5	-6
Day 91	30.1	30.7	33.6	+2	+11
Westmaas soil (loamy soil)					
Day 0	107	103	94	-4	-12
Day 14	106	87.0	102	-18*	-3
Day 29	102	109	105	+7	+3

dws = dry weight soil
1) - = inhibition; + = stimulation
* = Significantly different from control (at α = 0.05)

B. OBSERVATIONS

Soil microflora respiration: No significant effect on the microbial biomass could be determined in either loamy sand soil or loamy soil.

The biomass was evaluated but the respiration rates were not calculated (mg carbon dioxide/kg dry soil/h or mg oxygen/dry soil/h). The percent deviation from the control is based on the respiration rates per hour in the current guideline, so the respiration rate cannot be evaluated according to the current guideline requirements in this study.

Soil nitrification: In the control treatments of the loamy sand soil, the amount of nitrate increased from 2.33 mg N/kg soil at the beginning of the exposure to 12.78 mg N/kg dry soil after 91 days, whereas the amount of ammonia decreased from 1.81 mg N/kg dry soil to 0.352 mg N/kg dry soil. This increase is also reflected in the two treatment concentrations. No treatment related effects on nitrogen transformation were observed during the exposure. Differences observed between treated and untreated soils fall within natural soil variability. In contrast to that, total amount of mineralised nitrogen slightly decreased in the loamy soil treatments and control. In the control treatments, ammonia decreased from 10.9 mg N/kg dry soil to 0.372 mg N/kg dry soil. This decrease of mineralised nitrogen may be attributed to anaerobic nitrogen denitrification caused by insufficient homogenisation of soil samples, due to an extremely sticky soil texture. No treatment related effects on nitrogen transformation were observed during the experiments.

The nitrogen content was evaluated but the nitrogen transformation rate was not calculated (mg nitrate/kg dry soil/day). The percentage deviation from the control is based on the nitrogen transformation rate per day in the current guideline, so the nitrate formation rate cannot be evaluated according to the current guideline requirements in this study.

The toxic standard had significant effects on soil nitrification in both loamy sand soil and loamy soil and also on soil respiration in both types.

The validity criteria according to guideline OECD 216 and OECD 217 require a variation of less than ± 15 % between replicate control samples for nitrogen transformation and soil respiration. In the study report, only mean values are provided; therefore, it is not possible to give any indication about validity.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Glyphosate had no significant long term detrimental effect on microbial biomass and nitrogen content in soil at concentrations of 2.88 and 14.4 mg/kg dry soil. It is not possible to conclude on the study validity according to current OECD guideline requirements. The study is therefore considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 8.5/004
Report author	
Report year	2010
Report title	AMPA - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests)
Report No	10 10 48 010 C/N
Document No	-
Guidelines followed in study	OECD 216 (2000) OECD 217 (2000)
Deviations from current test guideline	Deviations from guidelines OECD 216 (2000) and 217 (2000): Minor: - Deviation from nitrate formation rate is missing.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The effects of AMPA on soil nitrogen transformation and soil carbon transformation were investigated in a loamy sand soil. The test substance was applied at concentration rates of 40, 80, 160, 320 and 640 mg test item/kg dry soil using three replicates per treatment. In addition, a negative control (untreated soil) was tested. A reference item was tested in a separated study.
The results showed no adverse effects of the test item 28 days after application on nitrogen and carbon transformation in soil up to and including a test concentration of 160 mg test item/kg dry soil. Due to measured deviations of > 25% observed in the treatment groups treated with 320 and 640 mg test item/kg dry soil, 28 days after application, the test was prolonged to 56 days for both treatment levels. After the test prolongation, the measured variations of nitrogen and carbon transformations of >25% could be observed until the end of the study (56 days). All validity criteria according to OECD 216 and 217 were fulfilled. The study is considered valid so NOEC of 160 mg/kg of dry soil (corresponding to 120 kg/ha) can be used in risk assessment for micro-organisms exposed to AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: AMPA (Aminomethylphosphonic acid)
Description: White crystalline solid
Lot/Batch #: GLP-0908-19984-A
Purity: 98.7 %

Vehicle and/or positive control: Vehicle: deionised water
Positive control: Dinoterb

Test system:

Soil	Loamy sand soil "Wassergut Canitz" (agricultural soil)
Source:	Field "Schag 34/3" in the municipality of Canitz, Saxony, Germany.
Water content of soil:	11.30 % (g water/100 g dry soil)
Water holding capacity	36.56 % (g water/100 g dry soil)
pH:	6.3
Total Org. C:	1.43 %
Microbial biomass:	2.37 % to C_{org}.
Clay (< 0.002 mm):	9.1 %
Silt (≥0.002 mm - 0.063 mm):	40.2 %
Sand (≥ 0.063 – 2.00 mm):	50.7 %

Environmental conditions:

Temperature:	19.7 – 21.8 °C
pH:	5.9 – 6.3
Water content:	41.46 – 44.71 % of WHC (nitrogen transformation test) 41.84 – 45.09 % of WHC (carbon transformation test)
Photoperiod:	24 hours darkness
Experimental work dates:	20 May to 15 July 2010

B. STUDY DESIGN

Experimental treatments

The test substance was applied at concentration rates encompassing 40, 80, 160, 320 and 640 mg test item/kg dry soil. In addition, a negative control (untreated soil) was tested. Three replicate soil samples were prepared for each treatment rate and the control for the carbon transformation and nitrogen transformation tests.

Soil carbon transformation: For each replicate a sub-sample of 1000 g dry soil was mixed with deionised water. Water was added to the soil to achieve a water content of approximately 45% WHC. Water content was adjusted weekly to the required range of 40-50% of WHC. The prepared soil was transferred to steel test vessels (4 L) and incubation was carried out at 19.7 – 21.8°C in a climatic room.

Soil nitrogen transformation: Sub-samples of 200 g dry soil were weighed into each test vessel (500 mL wide mouth glass flask). Lucerne meal (5 g/kg dry soil) was then added to provide 1.0 g Lucerne meal per 200 g dry soil. One additional soil sample (without Lucerne meal) was used for determination of initial NH_4-N- and NO_3-N-content. The initial NH_4-N and NO_3-N content was 0.01 mg and 1.48 mg/100 g dry soil, respectively. Incubation of the prepared soil was carried out in wide-mouth glass flasks (500 mL) at 19.7 – 21.8°C in a climatic room.

Observations

Soil carbon transformation: Carbon transformation was determined for a measurement period of 12 hours on sampling days 0 (3 hours after application), 7, 14, 28, 42 and 56 days after application. On each sampling occasion, 100 g samples of soil (dry soil) were taken, mixed with glucose using a hand-stirrer and placed into glass reaction flasks (500 mL). Then, glass vessels containing 18 mL of 1 M NaOH solution were placed in the reaction flasks and connected with a respirometer (BSB digi SELUTEC). Cumulative oxygen production (corresponding to the O_2 consumption by micro-organisms) was determined over a 12-hour measurement period.

Soil nitrogen transformation: Soil samples (10 g dry soil per replicate) were sampled at intervals of 3 hours, 7, 14, 28, 42 and 56 days after application and NH_4-N, NO_3-N and NO_2-N contents were determined. Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g dry soil. Quantitative

determination of mineralized nitrogen was performed using an Autoanalyzer II.

Statistical calculations

Two-sided Students t-test for homogenous variances at $\alpha = 0.05$. For carbon transformation, a two-sided Welch t-test for inhomogeneous variance was additionally performed.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

28 days after application, no adverse effects on nitrogen content and carbon transformation were observed up to and including a test concentration of 160 mg test item/kg dry soil. After the prolongation of the test to 56 days for the test concentrations 320 and 640 mg test item/kg dry soil, the measured variations of nitrogen content and carbon transformations of >25% could be observed till the end of the study (56 days). This can be most likely attributed to the high phosphorus/nutrient content in AMPA.

Table 8.5-8: Effects of AMPA on soil nitrogen transformation

	AMPA [mg test item/kg dry soil]										
	Control	40		80		160		320		640	
	NO_3-N	NO_3-N	Dev. [a]	NO_3-N	Dev. [a]	NO_3-N	Dev. [a]	NO_3-N	Dev. [a]	NO_3-N	Dev. [a]
Day 0	15.7	15.5	-1.1	15.7	0.2	15.4	-1.9	14.9*	-4.9	14.6*	-6.6
Day 7	23.1	23.6	2.5	27.3*	18.5	25.8	11.7	30.5*	32.2	33.5*	45.2
Day 14	32.2	34.6	7.5	37.4*	16.3	35.1*	9.2	42.9*	33.3	43.9*	36.5
Day 28	42.2	46.8*	10.7	47.7*	13	51.0*	20.8	57.4*	35.8	65.0*	53.8
Day 42	55.4	-	-	-	-	-	-	72.1*	30.2	78.1*	41.1
Day 56	61.9	-	-	-	-	-	-	78.4*	26.7	88.6*	43.1

[a] - = Deviation from the control based on NO_3-nitrogen content
* = Significantly different from control (two-sided Student- t test for homogenous variances at $\alpha = 0.05$)
- = inhibition, + = stimulation

Table 8.5-9: Effects of AMPA on soil carbon transformation

	AMPA [mg test item/kg dry soil]										
	Control	40		80		160		320		640	
	O_2 [a]	O_2 [a]	Dev. [b]	O_2 [a]	Dev. [b]	O_2 [a]	Dev. [b]	O_2 [a]	Dev. [b]	O_2 [a]	Dev. [b]
Day 0	12.0	11.9	-0.8	11.4*	-5.3	11.1*	-8.0	10.8*	-10.4	10.1*	-16.2
Day 7	11.9	11.0*	-7.1	10.3*	-13.2	9.9*	-16.9	9.5*	-20.2	8.4*	-29.7
Day 14	11.7	10.9*	-7.0	10.6*	-9.1	9.9*	-15.4	9.1*	-22.6	8.0*	-31.3
Day 28	10.9	10.0*	-7.9	9.5*	-12.9	8.9*	-18.5	8.1*	-25.7	7.0*	-35.3
Day 42	10.7	-	-	-	-	-	-	7.9*	-26.6	6.8*	-37.0
Day 56	10.1	-	-	-	-	-	-	7.4*	-26.1	6.2*	-38.8

[a] = Oxygen consumption
[b] - = Deviation from the control
* = Significantly different from control (two-sided Student- t test or two-sided Welch-t-test, for homogenous or inhomogeneous variances at $\alpha = 0.05$, respectively)
- = inhibition, + = stimulation

In a different test, 28 days after application the toxic standard dinoterb caused effects of +37.6 %, +51.4 % and +27.1 % on nitrogen content and -30.5 %, -34.5 % and -28.8 % on carbon transformation at concentrations of 6.80, 16.0 and 27.0 mg dinoterb/kg dry soil respectively, and thus demonstrates the sensitivity of the test system.

All validity criteria according to OECD 216 and 217 were fulfilled, as the variation between replicate control samples was less than ± 15 %.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The test item AMPA caused no adverse effects on soil nitrogen content and on soil carbon transformation up to and including a test concentration of 160 mg test item/kg dry soil at the end of the 28-day incubation period.
The study is considered valid so NOEC of 160 mg/kg of dry soil (corresponding to 120 kg/ha) can be used in risk assessment for micro-organisms exposed to AMPA.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.5/005
Report author	von Mérey, G. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota
Document No	DOI: 10.1002/etc.3438 E-ISSN: 1552-8618
Guidelines followed in study	OECD 222; OECD 226; OECD 232; OECD 216
Deviations from current test guideline	Earthworm cocoons were not counted, in accordance with OECD 222.
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

The exposure risk from glyphosate and the primary soil metabolite aminomethylphosphonic acid (AMPA) on representative species of earthworms, springtails, and predatory soil mites and the effects on nitrogen-transformation processes by soil microorganisms were assessed under laboratory conditions based on internationally recognized guidelines. For earthworms, the reproductive no-observed-effect concentration (NOEC) was 472.8 mg glyphosate acid equivalent (a.e.)/kg dry soil, which was the highest concentration tested, and 198.1 mg/kg dry soil for AMPA. For predatory mites, the reproductive NOEC was 472.8 mg a.e./kg dry soil for glyphosate and 320 mg/kg dry soil for AMPA, the highest concentrations tested. For springtails, the reproductive NOEC was 472.8 mg a.e./kg dry soil for glyphosate and 315 mg/kg dry soil for AMPA, the highest concentrations tested. Soil nitrogen-transformation processes were unaffected by glyphosate and AMPA at 33.1 mg a.e./kg dry soil and 160 mg/kg dry soil, respectively. Comparison of these endpoints with worst-case soil concentrations expected for glyphosate (6.62 mg a.e./kg dry soil) and AMPA (6.18 mg/kg dry soil) for annual applications at the highest annual rate of 4.32 kg a.e./ha indicate very low likelihood of adverse effects on soil biota.

Materials and methods

Test substances

Glyphosate (N-phosphonomethylglycine) is an acidic substance, which is manufactured and formulated as a salt to increase the solubility in water and compatibility with other formulation components. In water, AMPA is highly soluble (56 g/L at 20 °C), whereas neither glyphosate nor AMPA is significantly soluble in common organic solvents. Therefore, no cosolvent was required, and both stock solutions of glyphosate and AMPA test items were prepared in deionized water (5 - 20 Mohm at 25 °C). Two batches of AMPA analytical reference standards with purity of 98.7% (synthesized by Chemir) and 99.7 % (Acros Organics BVBA) were dissolved in deionized water. For soil nitrogen-transformation tests, stock solutions of glyphosate acid technical grade (96.59% purity; Monsanto Europe) were prepared by direct addition of test item to deionized water. For all other tests, glyphosate isopropylamine salt (nominal purity 62 % w/w, measured purity 63.81 ± 0.29 % w/w; MON 0139), corresponding to 45.9 % w/w glyphosate a.e. (measured 47.28 ± 0.21 % w/w; Monsanto Europe), were prepared in deionized water.

Earthworm reproduction tests

The earthworm reproduction test with glyphosate was conducted according to OECD guideline 222. For AMPA, an earthworm reproduction test was conducted according to the OECD 222. Both testing guidelines are equivalent in terms of the procedures employed during the tests (soil pH, temperature, lighting regime, soil composition and humidity, rearing, feeding quantities, test design, endpoints, number of replicates, growth stage of worms at test initiation, and so on). Therefore, to avoid repetition, the procedures used in the glyphosate study only are described.

Glyphosate - Earthworm reproduction test. In the earthworm reproduction glyphosate study *Eisenia fetida* (Haplotaxida: Lumbricidae, Savigny, 1826) were used as the test species. Mature adult *E. fetida* (~3 mo old with clitellum), weighing between 300 mg and 600 mg, were obtained from an age-synchronized stock culture from the test facility and reared under ambient laboratory conditions in the test facility. The original breeding animals were purchased from W. Neudorff. A detailed description of earthworm culturing is provided in Annex 4 of OECD 222. The *E. fetida* were reared in the laboratory on standard breeding medium (1:1:1 mixture of straw, horse manure, and peat; straw and horse manure were purchased from farmers, and peat was purchased from Torfwerk Moorkultur Ramsloh); no exposure to the test item was allowed prior to use in testing. Testing was conducted in artificial soil, equivalent to the soil in which the worms were originally cultured. The test aims to evaluate effects on adult body weight and survival percentage (according to treatment) during an initial 4-wk adult exposure period. Effects on juvenile production were then assessed at the end of a 4-wk period that followed directly after adult removal from the test. Behavior (including feeding activity) and pathological symptoms (e.g., lethargy, morphological alterations) of adults and juveniles were also assessed.

On the day before the test start, earthworms (from aged-synchronized batches, to ensure that similar-sized earthworms were used) were acclimated to test conditions in a separate batch of artificial soil supplemented with pasteurized horse manure, purchased from farmers and collected from horses not treated with growth promoters, nematicides, or other veterinary products - also used as the food source during testing. On test start day, volumes of the test solution (prepared by direct addition of glyphosate isopropylamine to deionized water) were mixed into bulk samples of artificial soil, to achieve nominal glyphosate soil concentrations of 14.18 mg a.e./kg dry soil, 23.64 mg a.e./kg dry soil, 47.28 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil. Glyphosate test concentrations were selected to cover the range and exceed field exposure concentrations. A toxic reference test was also performed in a separate test with carbendazim (Nutdazim 50 Flow, SC 500) at concentrations of 5 mg/kg dry soil and 10 mg/kg dry soil.

Test vessels were filled with the appropriate treated soil (810 g wet wt corresponding to 600 g dry wt). Groups of 10 individually weighed earthworms were randomly assigned to replicates within each treatment group, with a total of 40 earthworms used per treatment group divided equally between 4 replicates. For the control group (water only), 80 worms were used, divided equally between 8 replicates. Groups of 10 earthworms were placed onto the assigned replicate soil surface and closed with perforated transparent lids (following a brief burrowing period) to reduce evaporative water loss, allow gaseous exchange, and prevent worms from escaping the replicate vessels. Test vessels were then randomly positioned in an environmental test chamber under continuous light (to maintain worms in the soil). On day 1 and weekly

thereafter for the 4-wk adult exposure period, 5 g of air-dried finely ground horse manure was scattered on the soil surface of each test vessel and wetted with 5mL of deionized water. The amount of manure applied each week (up to 5 g) was dictated by feeding activity.

After 4 wk, adult earthworms were removed from the vessels by emptying the contents of each replicate vessel onto a tray and removing the adult worms. Care was taken not to remove any cocoons from the soil. Cocoons were not counted, in accordance with OECD 222. It can be reasonably assumed that effects on cocoon numbers would lead to effects on numbers of juveniles; hence, the endpoint number of juveniles accounts for effects at earlier life stages of earthworm progeny. All worms were rinsed with deionized water and dried on filter paper before recording body weights (by replicate and by treatment). Behavioral (including feeding activity) and pathological symptoms were also recorded during the exposure period and at the time of adult removal. The adult worms were then discarded. The soil in each replicate vessel was then mixed carefully with 5 g of manure, and the mixture was returned to the vessels. The test continued for a further 4 wk. At test termination (8 wk after adult addition) the number of surviving juveniles in each test vessel was recorded on manual inspection of the substrate. Soil was emptied on the lower edge of a white tray (30 cm × 40 cm). Subportions of the soil were spread in the middle of the tray, resulting in a thin layer of soil of approximately 10 cm × 10 cm. The subportion was examined thoroughly for juvenile worms, after which it was moved to the upper edge of the tray. This procedure was repeated until the entire soil from a vessel was examined. The entire procedure was repeated until there were no additional juvenile counts in 2 consecutive counting procedures, resulting in an average of 5 counting procedures per vessel. The counting tray and soil samples were illuminated using a fiber-optic light source connected with a double gooseneck light guide. The water content and pH of the artificial soil were determined. Adult body weights and the effects on reproduction (juvenile numbers) were analyzed using a lower-tailed Dunnett's multiple comparisons test ($\alpha = 0.05$). The Kolmogorov-Smirnov test and Cochran's test procedure were used, respectively, to test the biomass data for normality and homogeneity of variance. Survival was analyzed with a 1-sided Fisher's exact binomial test with Bonferroni correction ($\alpha = 0.05$).

AMPA - Earthworm reproduction tests. The procedures used during the AMPA earthworm study are considered equivalent to those employed in the glyphosate earthworm reproduction study described above in Glyphosate—Earthworm reproduction test. Mature adult *E. fetida* (~3mo old with clitellum), weighing between 300 mg and 600 mg, were obtained from an age-synchronized stock culture from the test facility and reared under ambient laboratory conditions in the test facility. A detailed description of earthworm culturing is provided in Annex 4 of OECD 222.

In the AMPA earthworm reproduction study, mature (clitellated) adult *E. fetida* were exposed to AMPA (99.7% purity; Acros Organics BVBA) mixed into artificial soil at nominal soil concentrations of 58.6 mg AMPA/kg dry soil, 87.8 mg AMPA/kg dry soil, 131.9 mg AMPA/kg dry soil, 198.1 mg AMPA/kg dry soil, 297.1 mg AMPA/kg dry soil, 445.5 mg AMPA/kg dry soil, 668.5 mg AMPA/kg dry soil, and 1002.5 mg AMPA/kg dry soil. A control group was prepared using deionized water only. A toxic reference test was also performed in parallel using earthworms from the same batch, exposed to carbendazim at concentrations of 1.0 mg active substance (a.s.)/kg dry soil, 2.2 mg a.s./kg dry soil, and 5.0 mg a.s./kg dry soil. For effects on biomass and production of juveniles, homogeneity was tested with the Brown-Forsythe and Bartlett tests. Dunnett's multiple comparison test was conducted using GraphPad Prism, Ver 6.03, because a continuous response could not be observed for all the test concentrations, as recommended by the OECD 222 test guideline and the OECD statistical guidance. The 50% effect rate on reproduction was calculated using GraphPad Prism.

Soil predatory mite reproduction test

The soil predatory mite reproduction tests for glyphosate and AMPA were both conducted according to OECD guideline 226 predatory mite (*Hypoaspis* [Geolaelaps] *aculeifer*) reproduction test in soil. The procedures used in the 2 studies were identical. Full details of the procedures are presented for glyphosate only. The *Hypoaspis* used in these studies were originally purchased from Katz Biotech and reared in the test facility under ambient conditions since June 2005.

Glyphosate - Soil predatory mite reproduction test. The glyphosate soil predatory mite reproduction test was conducted using glyphosate isopropylamine salt (MON 0139). Survival of mites (*H. aculeifer*) and their reproductive performance were evaluated at 4 nominal concentrations, equivalent to 50 mg MON 0139/kg dry soil, 100 mg MON 0139/kg dry soil, 500 mg MON 0139/kg dry soil, and 1000 mg

MON 0139/kg dry soil (= 23.64 mg a.e./kg dry soil, 47.28 mg a.e./kg dry soil, 236.40 mg a.e./kg dry soil, and 472.80 mg a.e./kg dry soil, respectively). A negative control with deionized water only was also included. A toxic reference test was performed in parallel using dimethoate EC400 (422.4 g/L; Perfekthion) at concentrations of 4.1 mg active ingredient (a.i.)/kg dry soil, 5.12 mg a.i./kg dry soil, 6.4 mg a.i./kg dry soil, 8.0 mg a.i./kg dry soil, and 10 mg a.i./kg dry soil. Mites were reared in the laboratory under ambient conditions on a mixture of plaster of paris, activated charcoal, and deionized water (8:1:9). Adults with no more than a 3-d age difference were used at the start of the test. No exposure of the mites to glyphosate was allowed prior to the test. Each treatment group contained 40 mites divided equally between 4 replicate vessels, with the control group comprising 8 replicates, each containing 10 mites. In addition, 2 test vessels without mites were included with each test concentration and in the control group for soil pH measurements. Glass bottles (100mL nominal volume) with screw tops were filled with 20 g (dry wt) artificial soil at the required test concentrations. Cheese mites were added as a food source to the surface of the soil, and vessels were then covered to prevent mites from escaping. Bottles were opened every second day during the 14-d test for the addition of food and to allow aeration. At the end of the test (day 14), the parental mites and juveniles were counted, after extraction using a MacFayden high-gradient extractor (heat/light extraction method). This was achieved by adding the soil substrate from each test vessel into a canister placed inverted onto the extraction system. Soil substrate was retained within the canister using a plastic net (2mm mesh size) on the bottom. Beneath the canister was a funnel attached to a collecting flask with 25mL of a fixing liquid. A temperature gradient was created between the upper and the lower parts of the system, by circulating heated air in the canister area and cooled air in the collection area. Over the 48-h extraction time, the following regime was applied: 25 °C for 12 h, 35 °C for 12 h, and 45 °C for 24 h. During this time, adults and juveniles moved down through the soil away from the heat source and fell through the funnel into the fixing liquid. Extraction efficiency was determined to be 95% in a separate extraction using vessels containing a known number of juvenile and adult mites in untreated substrate. Water content and pH were determined at test start and end. Statistical analysis was performed with the software ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni-Holm correction for mortality and a 1-sided Dunnett multiple comparisons test for reproduction ($\alpha = 0.05$) were used to compare the control with independent test item groups. Abbott's formula was used to correct for control mortality.

AMPA - Soil predatory mite reproduction test. The soil predatory mite reproduction test with AMPA was conducted at 5 nominal application rates, equivalent to 40 mg test item/kg dry soil, 80 mg test item/kg dry soil, 160 mg test item/kg dry soil, 240 mg test item/kg dry soil, and 320 mg test item/kg dry soil. A negative control (deionized water only) group was also included. All procedures and observations in the test with AMPA were as described for the mite (OECD 226) test with glyphosate in Glyphosate—Soil predatory mite reproduction test. A reference test was performed with dimethoate EC400 (414.8 g/L) at test concentrations of 0 mg a.i./kg dry soil, 4.1 mg a.i./kg dry soil, 5.12 mg a.i./kg dry soil, 6.4 mg a.i./kg dry soil, 8.0 mg a.i./kg dry soil, and 10 mg a.i./kg dry soil.

Springtail reproduction tests

The springtail reproduction tests for glyphosate and AMPA were both conducted according to OECD guideline 232. The procedures used in the 2 studies were identical. Full details of the procedures are presented for glyphosate only. Springtails used in these studies were originally purchased from Biologische Bundesanstalt in May 2000 and reared in the laboratory of the test facility under ambient laboratory conditions.

Glyphosate - Springtail reproduction test. The springtail reproduction test conducted for glyphosate was conducted using glyphosate isopropylamine salt. Survival of springtails (*Folsomia candida*) and their reproductive performance were evaluated at 5 nominal application rates of 32 µL MON 0139/kg dry soil, 50 µL MON 0139/kg dry soil, 100 µL MON 0139/kg dry soil, 500 µL MON 0139/kg dry soil, and 1000 µL MON 0139/kg dry soil (= 15.1 mg a.e./kg dry soil, 23.6 mg a.e./kg dry soil, 47.3 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil, respectively). A negative control with deionized water only was also included. In a reference toxicity test with Betosip (15.7% phenmedipham), concentrations of 50 mg/kg dry soil, 100 mg/kg dry soil, 200 mg/kg dry soil, and 400 mg/kg dry soil were tested. Each treatment group, including the control group, comprised 50 mites divided equally between 5 replicate vessels. For each treatment group and for the control group, 2 test vessels without springtails were provided for pH measurement purposes. Glass containers (150mL nominal volume) were filled with

30 g (wet wt) of the required treated or control soil. Springtails were reared in the laboratory under ambient conditions on a mixture of plaster for stucco, activated charcoal, and water (8:1:9). No exposure to the test item was allowed prior to testing. Juvenile springtails, 10 d to 12 d old and from a synchronized cohort, were added to each test vessel and then covered with a glass lid for 28 d, following which the surviving adults and juveniles were counted. Water content and pH were determined at test start and end. Adult and juvenile springtails were counted at test end. Statistical analysis was performed with the software ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni correction ($\alpha = 0.05$) and Welch's t test ($\alpha = 0.05$), because of non-heterogeneity of variance, were used to compare the control with the independent test item groups for significance of parental mortality and reproductive reduction, respectively. Abbott's formula was used to correct for control mortality.

AMPA = Springtail reproduction test. The springtail reproductive test for AMPA was conducted with AMPA (98.7 % purity) mixed into artificial soil at 5 nominal application rates, equivalent to 30 mg/kg dry soil, 54 mg/kg dry soil, 97.2 mg/kg dry soil, 175 mg/kg dry soil, and 315 mg/kg dry soil. The negative control used deionized water only. In a separate toxic reference test with 100% crystalline boric acid (BDH Prolabo) mixed with the soil, also included in the test design, the sensitivity of the population was determined with test concentrations of 0 mg/kg dry soil, 44 mg/kg dry soil, 67 mg/kg dry soil, 97.2 mg/kg dry soil, 150 mg/kg dry soil, and 225 mg/kg dry soil. The procedures used during the Springtail reproduction study were essentially equivalent to those used in the springtail test with glyphosate (described in Glyphosate - Springtail reproduction test) with the following exceptions. Each treatment group comprised 40 springtails (10 per test vessel), whereas the control group comprised 8 replicates. Statistical evaluation was performed with ToxRat Professional 2.10. A 1-sided Fisher exact binomial test with Bonferroni correction and a 1-sided Dunnett test were used to compare the control with independent test item groups. Mortality of adult springtails

Soil nitrogen-transformation tests

Soil nitrogen-transformation tests were conducted with glyphosate and AMPA according to OECD guideline 216 and performed according to good laboratory practice. The procedures used in the 2 tests were identical, although tested rates differed. Full details of procedures used are presented for glyphosate only.

Glyphosate - Soil nitrogen-transformation test. The soil nitrogen-transformation test for glyphosate was conducted using glyphosate acid (96.59% purity; Monsanto Europe) applied at 2 soil concentrations, 6.62 mg a.e./kg dry soil and 33.1 mg a.e./kg dry soil. The tested rates were equivalent to 1 and 5 times the maximum predicted environmental concentration in soil following a worst-case application of glyphosate to bare soil in the EU. Each treatment group and the control comprised 3 replicate test vessels. The control was treated with water only. Field-collected soil was used (LUFA standard soil, type 2.3). On collection, the soil was manually cleared of large objects, such as stones and parts of plants, and then moist-sieved to a particle size ≤ 2 mm. The soil was stored under aerobic conditions in the dark at 4 ± 2 °C until required for use.

Glyphosate was prepared in deionized water and then mixed into a bulk sample of soil at the start of the test. The soil moisture content was 40% (± 5%) of the maximum water holding capacity. During the test, the weight of a moisture control vessel maintained under the same test conditions was used as a guide to correct for test vessel water loss. Control and treated bulk samples of soil were amended with ground lucerne meal (0.5%) as a nitrogen source with a C to N ratio of 16:4:1. Bulk samples were then subsampled (~500 g) into replicate vessels and incubated at 20 ± 2 °C for 28 d. All containers were covered with a perforated lid to avoid evaporative water loss and stored in the dark. Soil (10 g) was taken from 1 replicate from each treatment for pH (water) determination at the start and end of the Glyphosate - Soil nitrogen-transformation study. An additional soil sample was taken from 1 replicate per treatment for moisture and dry matter content determination at the end of the study. As soon as possible after dosing (day 0) and after 7 d, 14 d, and 28 d, a 50-g soil sample (based on dry wt) was removed from each replicate to determine NH_4^+, NO_2^-, and NO^3. Soil extracts were prepared by adding 250mL of 2 M KCl, then shaking for 2 h and centrifuging for 15 min. The supernatant was analyzed using a Bran+Luebbe Autoanalyzer AA3 system. Effects below 25% deviation from control were not considered to be biologically significant.

AMPA - Soil nitrogen-transformation test. In the soil nitrogen-transformation test conducted for AMPA, the bulk samples of field-sampled soil were prepared at AMPA (98.7% purity) soil concentrations of 40 mg/kg dry soil, 80 mg/kg dry soil, 160 mg/kg dry soil, 320 mg/kg dry soil, and 640 mg/kg dry soil. In

addition, a negative control (nontreated soil) was tested. Although conducting reference tests and use of positive controls are not guideline requirements, in a separate reference test with dinoterb (2-tert-butyl-4,6-dinitrophenol, 99.9% purity; Sigma-Aldrich Chemie), test concentrations of 6.8 mg/kg dry soil, 16 mg/kg dry soil, and 27 mg/kg dry soil were applied, in addition to the control (0 mg/kg dry soil), with 3 replicates per treatment group.

Results

Glyphosate - Earthworm reproduction test

There was 0 % mortality of adult *E. fetida* at glyphosate concentrations of 14.18 mg a.e./kg dry soil, 236.4 mg a.e./kg dry soil, and 472.8 mg a.e./kg dry soil. Mortality (2.5%) was observed at 23.64 mg a.e./kg dry soil and 47.28 mg a.e./kg dry soil, which is considered incidental background mortality given the 10 % validity criterion for adult mortality in the control ($p > 0.3$). No statistically significant differences were detected for adult biomass ($p > 0.05$; Figure A below) and for the numbers of juveniles produced at each of the treatment groups when compared to the control ($p > 0.05$; Figure A below). Adult and juvenile feeding behavior was also not adversely affected over the duration of the test (56 d). The resulting no-observed-effect concentration (NOEC) for effects on reproduction was determined therefore to be the maximum test concentration of 472.8 mg a.e./kg dry soil.

In the reference test with carbendazim, juveniles were reduced by 65 % and 92 % at 5 mg reference item/kg dry soil and 10 mg reference item/kg dry soil, respectively. The control treatment had a mean number of 143 juveniles, whereas 5 mg/kg dry soil and 10 mg/kg dry soil treatments with carbendazim had a mean number of 51 juveniles and 11 juveniles, respectively. These reference test values show that the test system was appropriate to detect toxic effects on earthworm reproduction. The validity criteria, namely adult mortality < 20 % and number of juveniles per replicate > 30 in the control treatment, and coefficient of variance between control replicates < 30 % were all met. The guideline requirements for water content, temperature, and pH were all met.

AMPA - Earthworm reproduction test

In the earthworm reproduction study with AMPA, there were no significant effects on *E. fetida* adult mortality across concentrations compared to the control ($p > 0.22$). In all treatment groups, all 10 adults survived the treatments, except for 1 mortality in a single replicate of the 668.5 mg/kg dry soil treatment (Figure B below). Adult earthworm biomass was significantly lower compared to the control at the 445.5 mg AMPA/kg dry soil, 668.5 mg AMPA/kg dry soil, and 1002.5 mg AMPA/kg dry soil test concentrations ($p < 0.0001$; Figure B below). Adult biomass at 198.1 mg AMPA/kg dry soil was also significantly lower than the control ($p = 0.007$), but at 297.1 mg AMPA/kg dry soil there was no significant difference ($p > 0.802$) because the biomass (in percentage of control) was 88.5% and 88.2% in the 131.9 mg AMPA/kg dry soil and the 297.1 mg AMPA/kg dry soil treatment groups, respectively. The effect at 198.1 mg AMPA/kg dry soil is therefore considered to not be treatment-related. Juvenile production was not significantly affected at concentrations up to 198.1 mg/kg dry soil ($p > 0.342$). At 297.1 mg AMPA/kg dry soil and higher concentrations juvenile *E. fetida* numbers decreased significantly compared to the control ($p = 0.0013$). The resulting NOEC for effects on reproduction therefore was concluded to be 198.1 mg/kg dry soil, with a reproductive lowest-observed effect concentration (LOEC) at 297.1 mg AMPA/kg dry soil. The calculated 50% effective concentration (EC50) value for AMPA on earthworm survival was >1000 mg/kg dry soil. The reproduction EC50 value was calculated at 654.7 mg AMPA/kg dry soil (95% confidence interval 610.9 - 705.5 mg/kg dry soil). The resulting regression equation was $y = -0.1108 (\pm 0.005)$ AMPA mg/kg + 122.6 ($\pm$ 2.271), with an R^2 of 0.92. The reference test item carbendazim resulted in decreased biomass of 33.3% at 5.0 mg/kg dry soil and no reproduction, showing that the test system was sensitive to pesticide application. The validity criteria and guideline requirements were all met.

Glyphosate - Soil predatory mite reproduction test

No significant effects were observed on soil mite survival ($p > 0.3$) or reproduction ($p > 0.05$) up to and including the highest test concentration (472.8 mg a.e./kg dry soil; Figure C below) after 14 d of continuous exposure. All validity criteria and guideline recommendations were met. In the reference test with dimethoate the EC50 on reproduction was determined to be 4.9 mg a.i./kg dry soil. The reference test

demonstrated the sensitivity of the test system to detect reproductive toxicity in soil mites. The NOEC was therefore set at the highest test concentration.

AMPA - Soil predatory mite reproduction test
No significant effects were observed on soil mite survival ($p > 0.1$) or reproduction ($p > 0.05$) up to and including the highest test concentration (320 mg AMPA/kg dry soil; Figure D below). All validity criteria and guideline recommendations were met. The reference test with dimethoate showed that the test was sensitive at detecting reproductive toxicity in soil mites. The NOEC for AMPA was therefore concluded to be at the highest test concentration, 320 mg/kg dry soil.

Glyphosate - Springtail reproduction test
No significant effects were observed on springtail survival ($p > 0.5$) or reproduction ($p > 0.05$) up to and including the highest test concentration (472.8 mg a.e./kg dry soil; Figure E below). The validity criteria and guideline recommendations were all met. In the reference test with phenmedipham, the EC50 on reproduction was determined to be 28.4 mg phenmedipham/kg dry soil, which demonstrates that the test system was sensitive for reproductive toxicity. The NOEC for glyphosate was therefore concluded to be the highest test concentration.

AMPA - Springtail reproduction test
No significant effects were observed on springtail survival ($p > 0.5$) or reproduction ($p > 0.06$, $\alpha = 0.01$) up to and including the highest test concentration (315 mg AMPA/kg dry soil; Figure F below). The validity criteria and the guideline recommendations were all met. In the reference test with boric acid, the EC50 for reproduction was determined to be 108.6 mg/kg dry soil, demonstrating sensitivity to reproductive toxicity of the test system. The NOEC for AMPA was therefore concluded to be the highest test concentration.

Glyphosate - Soil nitrogen transformation test
Nitrogen-transformation rates in the soil treated at glyphosate rates equivalent to 6.62 mg a.e./kg dry soil and 33.1 mg a.e./kg dry soil were - 0.13% and 2.13% different compared to the control between day 14 and day 28, respectively (Figure G below). The validity criterion of < 15% variation between control treatments was met in the test. As the rates of nitrate formation between the control and the treated groups were < 25 % on day 28, glyphosate can be evaluated as having no long-term influence on nitrogen transformation in LUFA soils at concentrations ≤ 33.1 mg a.e./kg dry soil. No reference test was conducted, in line with the OECD guideline.

AMPA - Soil nitrogen-transformation test
Stimulation of nitrogen-transformation rates was observed across all treatments on day 7 and day 14, which was possibly linked to the high levels of nitrogen and phosphorus released from the degradation of AMPA in the biologically active soil. Only in the 2 highest test concentrations did the increase exceed 25 % compared to the control at 28 d. The test was therefore prolonged from 28 d to 56 d for the 2 highest test concentrations, 320 mg/kg dry soil and 640 mg/kg dry soil (Figure H below). At 56 d, the deviation from the control was 26.7 % at 320 mg/kg dry soil and 43.1 % at 640 mg/kg dry soil. The reference test results with dinoterb showed increases of 37.6 % at 6.8 mg/kg dry soil, 51.4 % at 16.00 mg/kg dry soil, and 27.1 % at 27 mg/kg dry soil. The validity criterion of < 15 % variation between controls was met at all sampling intervals.

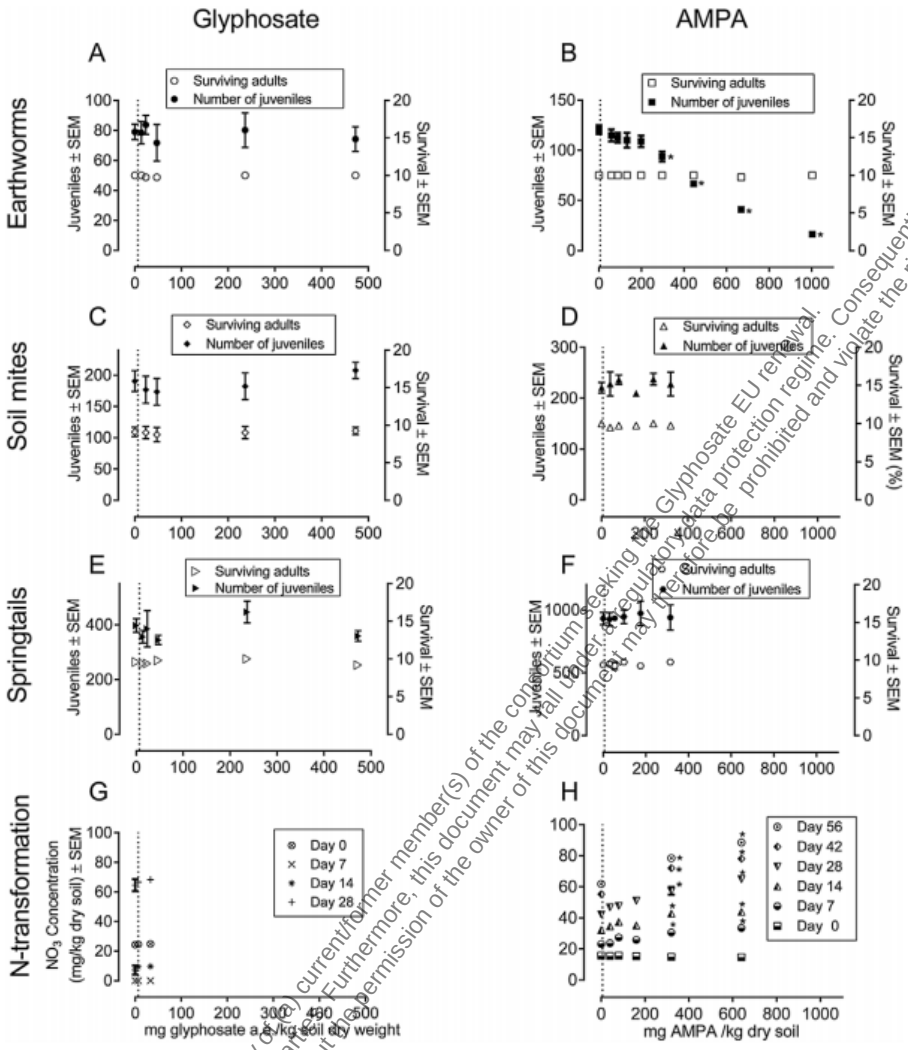


Fig. 1. Chronic risk assessment for glyphosate (left) and aminomethylphosphonic acid (AMPA; right) in soil. Number of surviving adults (28d) and number of juveniles (56 d) in earthworms exposed to glyphosate (A) and AMPA (B), (* statistically significant effect [$p \leq 0.05$] compared with control treatment), in soil predatory mites (*Hypoaspis aculeifer*) exposed to glyphosate (C) and AMPA (D) for 14 d, and in springtails (*Folsomia candida*) exposed to glyphosate (E) and AMPA (F) for 28 d. Effects on nitrogen transformation in soil treated with glyphosate (G) and AMPA (H) for 0 d, 7 d, 14 d,28 d, 42 d, and 56 d(* > 25 % effect compared with control treatment). Vertical dotted line in each graph indicates the worst-case predicted environmental concentration of glyphosate/AMPA. Vertical bars indicate standard error of the mean (SEM).

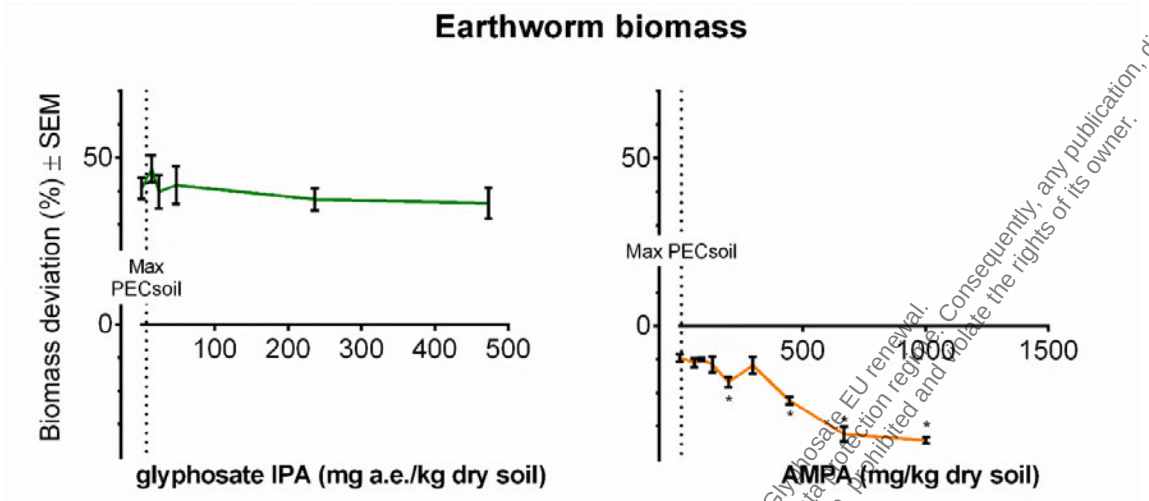


Fig. 2. Effects of glyphosate (A) and AMPA (B) on earthworm biomass after 28 d of exposure. The vertical dotted line indicates the predicted environmental concentration for AMPA and glyphosate. An asterisk next to a data point indicates a significant difference ($P < 0.05$) when compared against the control treatment.

Risk assessment

The chronic effects of exposure to glyphosate and the major soil metabolite AMPA to representative taxonomic groups of soil macroorganisms and nitrogen transformation were assessed following standard practices outlined under Annex VI Uniform Principles of the EU's Plant Protection Products Regulation (EC) No 1107/2009. At soil concentrations relevant to recommended glyphosate field application rates, no significant adverse effects were observed in any of the test species or systems exposed to glyphosate or AMPA. The risk assessment for soil macroorganisms in the EU compares the lowest NOEC achieved for each of the taxonomic groups with worst-case initial predicted soil concentrations (soil $PEC_{initial}$) achieved directly following a bare soil application and the potential for accumulation in soil following applications over multiple years to the same field (soil PEC_{accu}) The ratio of the endpoint to the predicted soil concentration is determined (toxicity exposure ratio = NOEC - $PEC_{initial}$) and compared against trigger values in accordance with Annex VI Uniform Principles of the EU's Plant Protection Products Regulation 1107/2009. Where trigger values are exceeded, a low exposure risk may be concluded. The long-term trigger value of 5 using NOECs derived from laboratory tests accounts for uncertainty related to interspecies sensitivity, predicted exposure estimates, and extrapolation from laboratory to field exposure.

For glyphosate and AMPA, the initial soil concentration ($PEC_{initial}$) at a soil depth of 5 cm has been determined based on a bare soil application (without foliar/crop interception), at the maximum cumulative annual application rate of 4.32 kg glyphosate a.e./ha for the EU. The risk of glyphosate and AMPA residues accumulating in soil over multiple years is considered by deriving the PEC_{accu} value. This is the sum of the $PEC_{initial}$ and plateau concentrations in soil, achieved in the top 5 cm (tillage depth for permanent crops) soil layer, following applications to bare soil at the maximum cumulative annual application rate (4.32 kg a.e./ha) each year for 10 yr.

It is important to mention that a single application rate of 4.32 kg glyphosate a.e./ha is not supported in the representative use rate but rather represents the recommended maximum cumulative (total) annual application rate for all uses and, therefore, a very conservative worst-case approach.

For exposure of soil mites, springtails, and earthworms to glyphosate in soil, the achieved chronic endpoints exceed the worst-case predicted glyphosate $PEC_{initial}$ and PEC_{accu} soil concentration by factors of 82 and 71, respectively.

For exposure of soil mites, springtails, and earthworms to AMPA in soil, the achieved chronic endpoints exceed worst-case AMPA $PEC_{initial}$ soil concentrations by factors of between 97 and 491, whereas the chronic endpoints exceed the PEC_{accu} soil concentrations by factors of between 32 and 162.

For soil nitrogen transformation, the endpoints achieved for glyphosate and AMPA (33.1 mg a.e./kg dry soil [glyphosate] and 160 mg a.e./kg dry soil [AMPA]) both achieved a < 25 % effect on nitrogen-transformation rates following a 28-d soil exposure to either glyphosate or AMPA. These soil exposure

rates exceed the worst-case predicted $PEC_{initial}$ soil concentrations by factors of 6 (glyphosate) and 78 (AMPA). The achieved endpoints also exceed the PEC_{accu} soil concentrations, by factors of 5 for glyphosate and 26 for AMPA.
For the soil mite, springtail, and earthworm reproduction chronic endpoints, the toxicity exposure ratio values exceed the EU Regulation No 546/2011 Annex VI trigger (5), indicating that for the ecotoxicologically relevant endpoints achieved for survival and reproduction, the use of glyphosate according to label recommendations is unlikely to result in adverse effects inside the treated area for soil biota - from exposure to both glyphosate and AMPA.
For the soil microbial community, relative to expected field application rates for exposure to glyphosate there is at least a 5-fold safety margin. For exposure to AMPA, a 26-fold safety margin applies. The observed increases in nitrate concentrations at the higher test concentrations are expected to be related to the large quantity of nitrogen and phosphate provided to the microbes via degradation of AMPA in the biologically active soil.

Table 8.4.2.1-1: Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil organisms[a]

Test species	Test item	Test duration (d)	Endpoint type	NOEC (mg a.e. or AMPA/kg soil)	$PEC_{initial}$ (mg a.e./kg soil)	PEC_{accu} (mg a.e./kg soil)	$TER_{initial}$	TER_{accu}
Earthworm	Glyphosate IPA salt	56	Adult mortality	472.8	5.76	6.62	82	71
			Biomass	472.8			82	71
			Reproduction	472.8			82	71
	AMPA	56	Adult mortality	1002.5	2.04	6.18	491	162
			Biomass	297.1			146	48
			Reproduction	198.1			97	32
Soil mite	Glyphosate IPA salt	14	Adult mortality	472.8	5.76	6.62	82	71
			Reproduction	472.8			82	71
	AMPA	14	Adult mortality	320	2.04	6.18	157	52
			Reproduction	320			157	52
Springtail	Glyphosate IPA salt	28	Adult mortality	472.8	5.76	6.62	82	71
			Biomass	472.8			82	71
	AMPA	28	Adult mortality	315	2.04	6.18	154	51
			Biomass	315			154	51
N-transformation	Glyphosate acid	28	Effect E_{25}%	33.1	5.76	6.62	6	5
	AMPA	28	Effect E_{25}%	160	2.04	6.18	78	26

a.e. = acid equivalent; AMPA = aminomethylphosphonic acid; IPA = isopropylamine; NOEC = no-observed-effect concentration; PEC_{accu} = accumulative predicted environmental concentration, cumulative worst-case application of 4.32 kg a.e./ha of glyphosate for 10 yr; $PEC_{initial}$ = initial predicted environmental concentration, assuming single worst-case application of 4.32 kg a.e./ha of glyphosate; TER_{accu} = toxicity to exposure ratio (= NOEC - PEC_{accu}); $TER_{initial}$ = toxicity to exposure ratio (= NOEC - $PEC_{initial}$).

Conclusion

The risks from exposure to glyphosate and the primary soil metabolite AMPA at levels that exceed commercial application rates were evaluated against a battery of representative soil macroorganisms and microorganisms under controlled laboratory conditions. Results from the present studies demonstrate that the potential impact to beneficial soil macro-organisms and nutrient cycling soil microorganisms under environmentally relevant exposure scenarios is low.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The aim of the paper was to evaluate potential effects of Glyphosate, Glyphosate salt and AMPA on earthworm, soil mites, springtails and soil micro-organisms.

The studies have been conducted according to recognised guidelines and validity criteria were presented. Test substance information, test organism origin, study designs and toxicity effects were adequately described. The study is considered reliable.

CA 8.6 Effects on Terrestrial Non-Target Higher Plants

Studies on the effects of the active substance glyphosate on vegetative vigour and seedling emergence of terrestrial non-target plants are available and are presented.

CA 8.6.1 Summary of screening data

Screening data is not considered to be required, since toxicity of glyphosate and the representative product MON 52276 (see MCP section 10.6.2) to terrestrial non-target plants is adequately addressed within the framework of vegetative vigour and seedling emergence tests with 10 different representative plant species.

CA 8.6.2 Testing on non-target plants

Studies considering the effects of glyphosate on terrestrial non-target plants were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in this section below.

Table 8.6.2-1: Studies on toxicity of glyphosate to terrestrial non-target higher plants

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.6.2/001	, 1994	21 d vegetative vigour	*Solanum lycopersicum* *Glycine max* *Lactuca sativa* *Raphanus sativus* *Cucmis sativus* *Brassica oleracea* *Avena sativa* *Lolium perenne* *Zea mays* *Allium cepa*	Glyphosate technical	Valid	-
CA 8.6.2/002	, 1994	21 d vegetative vigour	*Onion* *Field corn* *Oat* *Wheat* *Soybean* *Radish* *Cucumber* *Sunflower* *Tomato* *Carrot*	Glyphosate technical	Invalid	Emergence rate is not available

There are no literature articles or peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on non-target terrestrial plants. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document. For discussions of literature regarding toxicity to non-target terrestrial plants, please refer to document M-CP Section 10.6.

Endpoints of studies considered valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct

comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.6.2-2: Endpoint: Toxicity of glyphosate to terrestrial non-target higher plants

Reference (Data owner)	Test item	Species	Test design/ GLP	ER_{50} (g a.e./ha)	NOER (g a.e./ha)
1994 CA 8.6.2/001	Glyphosate technical	*Solanum lycopersicum* *Glycine max* *Lactuca sativa* *Raphanus sativus* *Cucmis sativus* *Brassica oleracea* *Avena sativa* *Lolium perenne* *Zea mays* *Allium cepa*	Vegetative vigour, 21-day	146 (tomato)	78.5 (tomato and corn)

a.e.: acid equivalents

Study summaries are provided below.

1. Information on the study

Data point:	CA 8.6.2/001
Report author	
Report year	1994
Report title	Tier 2 Vegetative Vigor Nontarget Phytotoxicity Study Using Glyphosate
Report No	93235
Document No	-
Guidelines followed in study	EPA Guidelines, Subdivision J, Series 123-1 (b)
Deviations from current test guideline	Deviations from test guideline OECD 227 (2006): Minor: - Five plant per 4 inches pot instead of one or two for bigger plants as corn, soybean, tomato, cucumber. - No reference substance or historical data were mentioned in the report. - Temperature rose above 22±10 °C, the light period was less than 16 h per day and the hygrometry dropped under 70±25 %.
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A vegetative vigour study was conducted exposing six dicotyledonous (soybean, lettuce, cabbage, cucumber, radish and tomato) and four monocotyledonous (oat, ryegrass, corn and onion) plant species to seven nominal test concentrations of glyphosate, encompassing 0.0785, 0.1569, 0.3138, 0.6276, 1.2329, 2.5778 and 5.0436 kg a.e./ha. In addition, one negative control group (treated with deionized water) was tested. Each test concentration was applied in four replicates containing five plants each. In addition radish and tomato were tested using five further nominal concentrations of 0.0049, 0.0099, 0.0202, 0.0392 and 0.0785 kg a.e./ha. Plant height was recorded prior to treatment and 21 days after treatment. Phytotoxicity ratings were recorded 7, 14, and 21 days after treatment. 21 days after treatment, plant material was dried at approximately 100 °C for a minimum of 48 hours and dry weight was recorded.
Result showed significant effects of glyphosate treatments on visual phytotoxicity, plant height and plant dry weight in all crops. Except for soybean and onion, glyphosate treatments significantly affected plant survival of all species tested. The validity criteria according to the OECD 227 were fulfilled.
The study is considered valid so EC_{50} of 146 g a.e./ha and a NOEC of 78.5 g a.e./ha can be used in risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	Glyphosate (N-phosphonomethylglycine)
Description:	White powder
Lot/Batch #:	RUD-9302-4778-T (technical) RUD-9203-3961-A (analytical standard)
Purity:	96.6 % (technical) 99.8 % (analytical standard)

2. Vehicle and/or positive control:

Vehicle: deionised water

Positive control: none

3. Test organism:

Species: sources	6 Dicotyledons: - soybean: Azlin Seed Co. - lettuce: Germain's Seed Co. - cabbage, radish and tomato: Burpee Seed Co. - cucumber: Carolina Seed Co. 4 Monocotyledons: - corn and onion: Burpee Seed Co. - cucumber: Carolina Seed Co. - oat: Northrup King - Ryegrass: Omni Seed Co

4. Environmental conditions:

Temperature:	19 °C – 44 °C (base test) 17 °C – 40 °C (test continuation)
Relative humidity:	40% - 90% (base test) 37% - 90% (test continuation)
Photoperiod:	Approx. 14 h light/ 10 h dark at 38212 – 45639 Lux (base test) Approx. 13 h light/ 11 h dark at 24542 – 19052 Lux (test continuation)

Soil pH: 7.9
Soil organic matter content: 1.1 %

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Prior to treatment, seedlings were grown in plastic pots (approx.10 cm x 10 cm x 7.6 cm) completely filled with soil/perlite mixture. Soybean, cucumber, oat and corn were planted at a depth of 2.5 cm while the remaining six crops were planted at a depth of 1.3 cm. Each treatment/crop combination was replicated four times. Prior to treatment, seedlings were grown to 1-3 true leaves and then thinned to five plants of uniform height per pot. The plants were treated with seven nominal concentrations, encompassing 0.0785, 0.1569, 0.3138, 0.6277, 1.2329, 2.5780 and 5.0438 kg a.e./ha. In addition, one negative control group (treated with deionized water) was tested. All applications of glyphosate were performed indoors with a spray booth equipped with a single TeeJet 8001-E nozzle and a compressed air cylinder. After treatment plants were placed in greenhouse. During the first 48 hours after treatment, pots were hand watered to prevent the test item from being washed off. As a no-observable effect concentration level was not reached for radish and tomato, a test continuation was initiated for both species using five nominal concentrations, encompassing 0.0049, 0.0099, 0.0202, 0.0392 and 0.0785 kg a.e./ha and a control.

2. Observations: Plant height was recorded prior to treatment and 21 days after treatment. Phytotoxicity ratings were recorded 7, 14, and 21 days after treatment. 21 days after treatment, surviving plants were cut at soil level and dry weight was recorded. Prior to application, samples (10 mL) of each test solution were collected and analysed immediately by HPLC method to verify the concentrations of the test item in the test solutions.

3. Statistical calculations: Analysis of variance, followed by a one-tailed Dunnett's multiple comparison test were used for data analysis. The EC_x values were determined using regression analysis (TableCurve™ Curve Fitting Software).

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

Visual phytotoxicity, plant height and plant dry weight of all crops were significantly affected by glyphosate treatments.

Table 8.6.2-3: Effects of glyphosate on survival, plant height and plant dry weight at 21DAT (all species, test 1)

	Glyphosate [kg a.e./ha]						
	0.0785	**0.1569**	**0.3138**	**0.6277**	**1.2329**	**2.5780**	**5.0438**
Mean effect on plant survival [% deviation from control]							
Soybean	0	0	0	0	0	0	-15
Lettuce	0	0	0	0	0	-60*	-95*
Radish	0	0	0	-20*	-70*	-100*	-100*
Tomato	0	0	0	-55*	-100*	-100*	-100*
Cucumber	0	0	0	0	0	-20	-75*
Cabbage	0	0	0	0	0	-15*	-60*
Oat	0	0	0	0	-5	-15	-25*
Ryegrass	0	0	0	0	-5	-25*	-50*

Table 8.6.2-3: Effects of glyphosate on survival, plant height and plant dry weight at 21DAT (all species, test 1)

	Glyphosate [kg a.e./ha]						
	0.0785	**0.1569**	**0.3138**	**0.6277**	**1.2329**	**2.5780**	**5.0438**
Corn	0	0	0	0	-25*	-85*	-70*
Onion	0	0	0	0	0	0	0
Mean effect on plant height [% deviation from control]							
Soybean	0	-7	-3	-10	-52*	-69*	-80*
Lettuce	9	-1	-1	-7	-50*	-86*	-99*
Radish	-11	-16*	-41*	-68*	-89*	-100*	-100*
Tomato	-9*	-11*	-32*	-88*	-100*	-100*	-100*
Cucumber	2	4	-12	-38*	-44*	-66*	-91*
Cabbage	-7	-5	-14	-10	-52*	-74*	-91*
Oat	0	-6	-8	-16	-46*	-77*	-82*
Ryegrass	4	1	5	-1	-22*	-68*	-80*
Corn	-2	-4	-7	-14	-79*	-97*	-92*
Onion	-2	0	-8	0	-27*	-40*	-48*
Mean effect on plant dry weight [% deviation from control]							
Soybean	4	-5	-10	-32*	-66*	-82*	-92*
Lettuce	12	7	-4	-35*	-83*	-97*	-100*
Radish	-25*	-24*	-63*	-85*	-96*	-100*	-100*
Tomato	-11*	-37*	-69*	-98*	-100*	-100*	-100*
Cucumber	6	1	-11	-39*	-63*	-85*	-96*
Cabbage	-5	-3	-24*	-43*	-87*	-96*	-98*
Oat	-3	-2	-17*	-29*	-66*	-92*	-94*
Ryegrass	39	50	27	3	-38*	-91*	-97*
Corn	2	5	-14	-23	-91*	-99*	-98*
Onion	4	15	-10	11	-41*	-71*	-83*

* = Significantly different from the control ($p < 0.05$)

Table 8.6.2-4: Effects of glyphosate on survival, plant height and plant dry weight (test 2)

	Glyphosate [kg a.e./ha]				
	0.0049	**0.0099**	**0.0202**	**0.0392**	**0.0785**
Mean effect on plant survival [% deviation from control]					
Radish	0	0	0	0	0
Tomato	0	0	0	0	0
Mean effect on plant height [% deviation from control]					
Radish	-3	0	3	-2	-3
Tomato	5	-2	7	0	2
Mean effect on plant dry weight [% deviation from control]					
Radish	15	13	7	4	-9
Tomato	54	33	33	34	5

Analytical results: The average recovery of glyphosate in test media ranged from 100 % to 107 % and 105 % to 110 % of the nominal test concentrations for the first test and the test continuation, respectively. As the mean measured content of the test item always ranged between 80 and 120% of nominal in both tests, ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Except for soybean and onion, a significant effect on mortality was observed for all species exposed to glyphosate. The resulting EC_{50} and NOEC values are presented in the table below.

Table 8.6.2-5: Toxicity of glyphosate to monocotyledonous and dicotyledonous plants

Crop	Endpoint [kg a.e./ha] (21 days)			
	Phytotoxicity	Percentage survival		
	NOEC	EC_{25}	NOEC	EC_{50}
Ryegrass	0.6277	2.578	1.2329	4.5955
Corn	0.0785	0.8855	0.6277	1.6813
Onion	0.6277	> 5.0438	5.0438	> 5.0438
Soybean	0.3138	> 5.0438	5.0438	> 5.0438
Lettuce	0.3138	1.5692	1.2329	2.8021
Cucumber	0.1569	2.9142	2.5780	4.0351
Cabbage	0.6277	3.2505	1.2329	4.5955
Oat	0.6277	4.9318	2.5780	> 5.0438
Radish	0.1569	0.4932	0.3138	0.9191
Tomato	0.0785	0.2914	0.3138	0.5156

Table 8.6.2-5: Toxicity of glyphosate to monocotyledonous and dicotyledonous plants (continued)

Crop	Endpoint [kg a.e./ha] (21 days)					
	Plant height			Dry weight		
	NOEC	EC_{25}	EC_{50}	NOEC	EC_{25}	EC_{50}
Ryegrass	0.6277	1.0760	2.3538	0.6277	0.8967	1.3450
Corn	0.6277	0.4708	0.9191	0.6277	0.4147	0.7510
Onion	0.6277	1.3450	> 5.0438	0.6277	0.9527	1.7934
Soybean	0.6277	0.6389	1.5692	0.3138	0.4708	0.9751
Lettuce	0.6277	0.7173	1.3450	0.3138	0.4483	0.7622
Cucumber	0.3128	0.5160	1.4571	0.3138	0.4596	0.8967
Cabbage	0.6277	0.7510	1.4571	0.1569	0.3363	0.7398
Oat	0.6277	0.6164	1.3450	0.1569	0.4259	0.8743
Radish	0.0785	0.1569	0.3587	0.0392	0.1569	0.2466
Tomato	0.0392	0.2242	0.3363	0.0392	0.1009	0.1457

The validity criteria according to the OECD 227 were fulfilled. The seedling emergence was at least 70 % (actual values from 80 to 99 %). In the control, the plants did not exhibit visible phytotoxic effects; the mean plant survival is at least 90 % for the duration of the study (actual value 100 %); environmental conditions for a particular species were identical and growing media contain the same amount of soil matrix,

support media, or substrate from the same source.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The lowest (worst case) 21 day EC_{50} values of glyphosate were observed for tomato plants and were calculated to be 0.5156, 0.3363 and 0.1457 kg a.e./ha for survival, plant height and dry weight, respectively. The lowest 21-day NOEC values were determined to be 0.0785 kg a.e./ha (tomato and corn), 0.3138 kg a.e./ha (tomato and radish), 0.0392 kg a.e./ha (tomato and radish), and 0.0392 kg a.e./ha (tomato) respectively for visual phytotoxicity, survival, dry weight and plant height.
The study is considered valid so EC_{50} of 146 g a.e./ha and a NOEC of 78.5 g a.e./ha can be used in risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.6.2/002
Report author	
Report year	1994
Report title	LX1146-02 (Glyphosate techn.) Tier II Non-Target plant hazard evaluation – Terrestrial vegetative vigor
Report No	14625B018
Document No	236 GLY
Guidelines followed in study	EPA Guidelines, Subdivision J, Series 123-1 (b)
Deviations from current test guideline	Deviations from the test guideline OECD 227 (2006): Major: - No data on seedling emergence were reported. - No analytical verification was performed. Minor: - Five plant per 6 inch pot instead of one or two for bigger plants as corn, soybean, tomato, cucumber. - Phytotoxicity and mortality at 21 DAT were missing for initial test. - No reference substance or historical data are mentioned in the report. - Temperature rose above and below 22±10 °C and light period was under 16 h per day.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

A vegetative vigour study was conducted exposing six dicotyledonous (carrot, cucumber, radish, soybean, sunflower, tomato) and four monocotyledonous (field corn, oat, onion, wheat) plant species to five nominal test concentrations of glyphosate, encompassing 0.0056, 0.0112, 0.0235, 0.0471 and 0.0930 kg a.e./ha in

four replicates per treatment. In addition, a negative control group treated with deionized water was tested. The application was performed using a single nozzle hand-held, CO_2 pressurized sprayer. Because of poor rate response in most crops, five additional treatment rates were included, encompassing 0.0930, 0.1861, 0.3721, 0.5582 and 0.7442 kg a.e./ha.
Seedling number and plant height were recorded 7 days before treatment (6 DBT for the continuation test), on the day of treatment, 14 days after treatment (13 DAT for the continuation test) and 28 DAT (21 DAT for the continuation test). For the dry weight measurements, plants within a treated replicate were harvested 21 or 28 DAT and dried for a minimum of 24 h at approximately 100°C. Plant survival observations and phytotoxicity were recorded at 7, 14 and 28 DAT for initial test and 6, 13 and 21 DAT for the continuation test.
Plant height, plant dry weight and survival were significantly affected by glyphosate treatments in all species tested. Among monocotyledonous species, oat was most tolerant to glyphosate, while all other species exhibited approximately the same level of sensitivity to glyphosate. Among dicotyledonous species, sunflower and radish were most sensitive for glyphosate, whilst tomato, carrot and soybean showed a moderate sensitivity to glyphosate. Cucumber was the most tolerant species to glyphosate. For phytotoxicity, monocots and dicots were also affected by glyphosate treatments. The lowest 21-day NOEC value was observed for plant height and visual phytotoxicity and determined to be 0.0930 kg a.e./ha. Not all the validity criteria according to the OECD 227 were fulfilled, no data on seedling emergence in control group were reported.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	Glyphosate technical
Description:	Solid, white
Lot/Batch #:	206-JAK-119-1
Purity:	98.5 % (technical)

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: none

3. Test organism:

Source: species: 6 Dicotyledons:
- Burpee Seed, Warmister, PA: carrot, cucumber, radish, tomato
- Farmers supply, Co., Valdosta, GA: sunflower
- Pineland Plantation, Newton; GA: soybean

4 Monocotyledons:
- Burpee Seed, Warmister, PA: onion, oat
- Farmers supply, Co., Valdosta, GA: field corn, wheat

4. Environmental conditions:

Temperature:	Approx. 11.7°C – 37.8 °C
Relative humidity:	70 % - 94 %
Photoperiod:	10 h light / 14 h dark , 43-336 Wm^{-2} (approx. 3071– 24000 Lux for sunlight)
Soil pH:	5.5 - 5.6
Soil organic matter content:	0.94 - 1.5 %

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Prior to treatment, seedlings were grown in plastic pots (approx.15 cm round) containing approximately 1 kg of pasteurised sandy soil. Small seeds (carrot, onion, radish and tomato) were planted at a depth of 0.5 to 1 cm and large seeds (field corn, wheat, oat, cucumber, sunflower and soybean) were planted at a depth of 1 to 1.5 cm. Soybean seeds were inoculated with commercial *Rhizobium japonicum*. Four replicate pots for each treatment were prepared for each species tested. At least 7 days prior to application, seedlings were grown to 1-3 true leaves and then thinned to five plants per replicate and their height recorded. The plants were treated with 5 nominal concentrations (adjusted to test item purity), encompassing 0.0056, 0.0112, 0.0235, 0.0471 and 0.0930 kg a.e./ha. In addition, one negative control group (treated with deionized water) was tested. Application was performed using a single nozzle hand-held CO_2 pressurized sprayer, starting with the water control. Plants were not watered during the first 24-hour period to avoid wetting the plants foliage and dislodging spray residue. Because of poor rate response in most crops, a test continuation was initiated at five additional concentration rates, encompassing 0.0930, 0.1861, 0.3721, 0.5582 and 0.7442 kg a.e./ha.

2. Observations: Plant height were recorded 6 or 7 days before treatment (DBT), on the day of treatment, 13 or 14 days after treatment (DAT) and 21 or 28 DAT. For dry weight measurements, plants were harvested 21 or 28 DAT and dried for a minimum of 24 h at approximately 100°C. Plant survival observations were recorded 7 DAT (6 DAT for the continuation test), 14 DAT (13 DAT for the continuation test) and 28 DAT (21 DAT for the continuation test). Phytotoxicity was evaluated 7, 14 and 21 DAT for initial test and 6, 13 and 21 DAT for the continuation test.

3. Statistical calculations: Data were analysed using two-way ANOVA and an LSD test was performed as post-hoc. The actual EC_x values were estimated by regression analysis using Lotus 1,2,3 Software.

II. RESULTS AND DISCUSSION

A. FINDINGS and OBSERVATIONS

Plant height, dry weight and survival: Height, dry weight and survival of plants were significantly affected by glyphosate treatments in all species tested. Among the monocotyledonous species, oat was most tolerant to glyphosate while all other species exhibited approximately the same level of sensitivity to glyphosate. Among the dicotyledonous species, sunflower and radish were the most sensitive species, whilst tomato, carrot and soybean exhibited moderate sensitivity to glyphosate. Cucumber was the most tolerant species to glyphosate.

Visual phytotoxicity: Visual phytotoxicity was generally expressed within 13 days after the treatment and did not substantially increase by 21 days. Onion exhibited tip burn (necrosis at the leaf tip and margins) at 0.7442 kg a.e./ha but no visual phytotoxicity at any of the lower rates. Oat exhibited visual phytotoxicity at a rate of 0.3721 kg a.e./ha, whereas wheat and field corn showed signs of visual phytotoxicity at rates as lower as 0.1861 kg a.e./ha. For phytotoxicity, onion was the most tolerant monocot while other monocots tested showed approximately the same level of sensitivity to glyphosate. Glyphosate caused multiple shoots to develop at the soil line; higher application rates caused necrosis at the leaf tips. Despite the levels of visual injury observed on field corn, wheat and oat for all concentration tested, the plant height and dry weight were not significantly affected by glyphosate treatments.

For dicots, visual phytotoxicity occurred within 13 DAT and did no increase significantly by 21 days.

Table 8.6.2-6: Effects of glyphosate on height, dry weight and survival of non-target plants at 21 DAT (test continuation, all species)

Crop	Glyphosate [kg a.e./ha]				
	0.0930	**0.1861**	**0.3721**	**0.5582**	**0.7442**
Mean plant height [% deviation from control]					
Onion	-20.34*	-20.67*	-13.03*	-10.67*	-32.53*
Field corn	2.50*	-15.48*	-15.94*	-28.17*	-44.76*
Oat	6.50	13.93	9.68	1.72	0.27
Wheat	-4.77*	-22.43*	-22.98*	-23.77*	-37.89*
Soybean	5.41	-5.41*	-35.33*	-48.36*	-49.72*
Radish	-14.64*	-33.67*	-23.16*	-100.00*	-100.00*
Cucumber	5.66	-7.03*	-27.96*	-28.53*	-32.86*
Sunflower	25.92*	-47.28*	-62.93*	-100.00*	-100.00*
Tomato	-1.49*	-17.54*	-28.73*	-30.60*	-43.28*
Carrot	0.48	-12.28*	-22.66*	-35.34*	-40.62*
Mean plant dry weight [% deviation from control]					
Onion	-39.06	-50.00	-12.50	3.13	-34.38
Field corn	-5.83*	-24.27*	-33.01*	-45.63*	-53.88*
Oat	5.77	-9.62	-13.46	-20.19	-11.06*
Wheat	-18.33*	-34.58*	-50.00*	-45.28*	-45.14*
Soybean	-8.90	-10.99*	-33.51*	-46.86*	-49.21*
Radish	-29.07*	-54.46*	-57.36*	-100.00*	-100.00*
Cucumber	12.60	13.39	-11.81	20.73	10.43
Sunflower	0.00	-50.22*	-57.24*	-100.00*	-100.00*
Tomato	-18.10	-11.21*	-44.83*	-55.17*	-62.93*
Carrot	13.04	33.70	30.43*	46.74*	50.72*
Mean plant survival [% deviation from control]					
Onion	-5.00	0.00	0.00	-5.00	-5.00
Field corn	0.00	0.00	0.00	0.00	0.00
Oat	0.00	0.00	0.00	-5.00	-5.00
Wheat	0.00	-5.00	0.00	-15.00	-20.00
Soybean	0.00	0.00	0.00	-5.00	0.00
Radish	0.00	-40.00*	-80.00*	-100.00*	-100.00*
Cucumber	0.00	0.00	-10.00*	-40.00*	-20.00*
Sunflower	0.00	-25.00*	-55.00*	-100.00*	-100.00*
Tomato	0.00	0.00	0.00	0.00	0.00
Carrot	5.26	0.00	5.26	-5.26	-5.26

* = significantly different when compared to the control ($\alpha = 0.05$)

Table 8.6.2-7: Effects of glyphosate on plant height and dry weigh and survival 21 DAT (initial test, onion and radish)

Crop	Glyphosate [kg a.e./ha]				
	0.0056	**0.0112**	**0.0235**	**0.0471**	**0.0930**
Mean plant height [% deviation from control]					
Onion	-2.68	-10.92	-15.52	-11.30	-20.31*
Mean plant dry weight [% deviation from control]					
Onion	-19.23	-26.92	-19.23	-13.46	-28.85
Radish	-33.33	-20.99	-23.46	33.33	-4.94*

* = significantly different when compared to the control ($\alpha = 0.05$)

When comparing the 21-day data, carrot was the most tolerant dicot with a NOEC of 0.3721 kg a.e./ha and exhibited no phytotoxicity at rates below 0.5582 kg a.e./ha. The only injury observed from the glyphosate was slight chlorosis and stunting for carrot. With the exception of soybean (NOEC = 0.1861 kg a.e./ha), the NOEC for dicots was 0.0930 kg a.e./ha. For radish and sunflower, mortality was observed at the two highest rates tested and significant treatment effects were also noted in plant height and dry weight.
The resulting EC_{50} and NOEC values are presented in the table below.

Table 8.6.2-8: Toxicity of glyphosate to monocotyledonous and dicotyledonous pants

Crop	Endpoint [kg a.e./ha]		
	Survival		
	NOEC	**EC_{25}**	**EC_{50}**
Onion	0.7442	> 0.7442	> 0.7442
Field corn	0.7442	> 0.7442	> 0.7442
Oat	0.7442	> 0.7442	> 0.7442
Wheat	0.7442	> 0.7442	> 0.7442
Soybean	0.7442	0.7442	> 0.7442
Radish	0.0930	0.1412	**0.2488**
Cucumber	0.3721	0.6277	> 0.7442
Sunflower	0.1861	0.1939	0.3508
Tomato	0.7442	> 0.7442	> 0.7442
Carrot	0.7442	> 0.7442	> 0.7442

n.d. = not determined

Table 8.6.2-9: Toxicity of glyphosate to monocotyledonous and dicotyledonous pants

Crop	Endpoint [kg a.e./ha]					
	Dry weight			Plant height		
	NOEC	EC_{25}	EC_{50}	NOEC	EC_{25}	EC_{50}
Onion	0.0930	n.d.	n.d.	0.0930	0.7442	> 0.7442
Field corn	0.0930	0.297	0.6400	0.0930	0.4607	> 0.7442
Oat	0.7442	> 0.7442	>0.7442	0.7442	> 0.7442	>0.7442
Wheat	0.0930	0.195	0.6478	0.0930	0.4696	> 0.7442
Soybean	0.1861	0.3262	0.6759	0.1861	0.3587	0.6591
Radish	0.0930	0.0942	**0.2623**	0.0930	0.2802	0.6904
Cucumber	0.7442	> 0.7442	> 0.7442	0.1861	0.51	> 0.7442
Sunflower	0.0930	0.1524	0.2959	0.0930	0.1816	**0.2993**
Tomato	0.1861	0.2443	0.5335	0.0930	0.4069	> 0.7442
Carrot	0.3721	0.3284	0.6512	0.1861	0.4349	> 0.7442

n.d. = not determined

The validity criteria according to the OECD 227 were fulfilled, except the fact that no data on seedling emergence in control group were reported.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The lowest (worst case) 21 day EC_{50} values of glyphosate were determined for radish and were calculated to be 0.2488 and 0.2623 kg a.e./ha for survival and dry weight, respectively.
The lowest (worst case) 21 day EC_{50} value of glyphosate was determined for sunflower and was calculated to be 0.2993 kg a.e./ha for plant height.
The lowest 21-day NOEC value was observed for plant height and visual phytotoxicity and determined to be 0.0930 kg a.e./ha. Not all of the validity criteria according to the OECD 227 were fulfilled, because no data on seedling emergence in control group were reported. Due to these limitations, the study is therefore considered invalid for risk assessment purposes.

Assessment and conclusion by RMS:

CA 8.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

As acceptable risk has been shown for all standard test organisms, further testing on additional species is not considered necessary. However, a report has been prepared to further address the impact on biodiversity, namely 'Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment[15]'. The purpose of this report is two-fold: (1) provide a biodiversity assessment that principally informs on indirect effects through trophic interactions and (2) to inform risk assessors and managers on

[15] (2020) Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment (TRR0000305).

risk mitigation options that are protective of aquatic and terrestrial biodiversity. The outcome of the present biodiversity assessment for glyphosate is summarized for the different environmental compartments and taxa where appropriate in the document M-CP Section 10.

CA 8.8 Effects on Biological Methods for Sewage Treatment

Studies on effects of the active substance glyphosate on sewage to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

The results of these studies demonstrate that glyphosate is of low toxicity to biological methods for sewage treatment.

Studies considering the effects of glyphosate on biological methods for sewage treatment were assessed for their validity to current and relevant guidelines for glyphosate and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for these studies are presented in this section below.

Table 0.8-1: Studies on toxicity of glyphosate to biological methods for sewage treatment

Annex point	Study	Study type	Test species	Substance(s)	Status	Remark
CA 8.8/001	2000	Growth inhibition test	*Pseudomonas putida*	Glyphosate	Invalid	Multiplication factor of control inoculum not provided
CA 8.8/002	, 1990	Respiration	Activated sludge bacteria	Glyphosate	Valid	

There are no literature articles and peer-reviewed published data considered to be relevant and reliable or reliable with restrictions with regards to the impact of glyphosate or its relevant metabolites on biological methods for sewage treatment. Full literature evaluation is provided in document M-CA Section 9. A summary of previously evaluated peer reviewed literature from the RAR 2015 is also available in Annex M-CA 8-01 to this document.

Endpoints of studies considered valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 8.8-3: Endpoints: Toxicity of glyphosate on biological methods for sewage treatment

Reference (Data owner)	Test item	Species	Test design/ GLP	EC_{50} (mg a.e./L)	NOEC (mg a.e./L)
, 1990 CA 8.8/002	Glyphosate technical	Activated sludge bacteria	Oxygen consumption of activate sludge over 3 h	> 100	100

a.e.: acid equivalents

Study summaries are provided below.

1. Information on the study

Data point:	CA 8.8/001
Report author	
Report year	2000
Report title	Glyphosate technical: Determination of toxicity to *Pseudomonas putida*
Report No	AH0149/A
Document No	-
Guidelines followed in study	Water quality - *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test) International Standard ISO 10712: 1995.
Deviations from current test guideline	Deviation from the current ISO guideline: Major: - The control inoculum multiplication factor cannot be evaluated (at least 60 is required within the test period) Minor: - Only two replicates were setup for each test item dilution instead of three. - The test solutions were maintained at 27±0.5 °C instead of 23±1 °C.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

Executive Summary

The effects of glyphosate on *Pseudomonas putida* growth inhibition were evaluated in a 16-hour static toxicity test. The test concentrations of 1.0, 3.2, 10, 32, and 100 mg/L in test medium were prepared in duplicate and sterile conditions in conical flasks. Flasks containing 1.0, 3.2, 10, 32, and 100 mg/L (single replicates) of the reference toxic substance (3,5-dichlorophenol) and three control flasks were also prepared. Four mL growth medium, 1 mL inoculum and deionised water were added to obtain a final volume of 50 mL test solution. After shaking at 27.0±0.5°C (in an incubator) for 16±1 hours the optical density of the contents of each flask were measured with a spectrophotometer. The 16-h IC_{50} for *Pseudomonas putida* exposed to glyphosate technical was >100 mg a.e./L based on nominal concentration. The NOEC after 16 h was 100 mg a.e./L, however the study is considered invalid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Aspect:	White solid
Lot/Batch #:	R061837P30
Purity:	97.6 %

2. Vehicle and/or positive control: Vehicle: deionised water
Positive control: 3,5-dichlorophenol (97%)
Growth medium

3. Test organism:

Species:	*Pseudomonas putida*, strain NCIMB9494
Source of organisms:	National Collections of Industrial and Marine Bacteria Ltd., Aberdeen, UK

4. Environmental conditions:

Temperature:	27.0±0.5 °C

5. Experimental dates: May 11, 2000 (first run) and May 17, 2000 to May 18, 2000

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate on *Pseudomonas putida* growth inhibition were evaluated in a 16-hour static toxicity test. The test concentrations of 1.0, 3.2, 10, 32, and 100 mg/L in test medium were prepared in duplicate and sterile conditions in conical flasks. These test solutions were prepared by adding the appropriate amount of a 500 mg/L stock solution (0.125 g glyphosate in 250 mL deionised water) directly into the flasks. Flasks containing 1.0, 3.2, 10, 32, and 100 mg/L (single replicates) of the reference toxic substance (3,5-dichlorophenol) and three control flasks were also prepared. Four mL growth medium, 1 mL inoculum and deionised water were added to obtain a final volume of 50 mL test solution. After shaking at 27.0±0.5°C (in an in incubator) at 150 rpm for 16±1 hours the optical density of the contents of each flask were measured at 600 nm with a Uvikon 930 spectrophotometer.

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of glyphosate on *Pseudomonas putida* are shown below.

Table 8.8-2: Effects of glyphosate on *Pseudomonas putida*

Nominal concentration [mg test item/L]	Mean optical density	Mean % inhibition
Control	0.859	-0
1.0	0.836	3
3.2	0.838	2
10	0.842	2
32	0.868	0
100	0.878	0
3,5-DCP 1.0	0.839	2
3,5-DCP 3.2	0.857	0
3,5-DCP 10	0.851	1

Table 8.8-2: Effects of glyphosate on *Pseudomonas putida*

Nominal concentration [mg test item/L]	Mean optical density	Mean % inhibition
3,5-DCP 32	0.055	94
3,5-DCP 100	0.047	95

B. OBSERVATIONS

Based on the obtained results, the IC_{50} is > 100 mg/L and the highest concentration at which no effect was observed (NOEC) to be 100 mg/L. The reference substance 3,5-dichlorophenol gave an IC_{50} of 18 mg/L.

The following validity criterion was fulfilled according to the guideline:

- The EC_{50} of the reference substance 3,5-dichlorophenol was between 10 mg/L and 30 mg/L (actual value: 18 mg/L)

The following points deviated from the current guideline requirements:

- The inoculum concentration was given as 0.532. Then 1 mL of this inoculum was added to each final 50 mL test solution (including the control solution). The control inoculum concentration was measured as 0.859 at the end of the test but the initial optical density of the control solution was not provided, so it is not possible to conclude on the study validity according to guideline requirements.
- Only two replicates were setup for each test item dilution. The guideline requires three parallel batches for each dilution step.
- The test solutions were maintained at 27±0.5 °C for 16 hours instead of 23±1 °C.

It is not possible to conclude on the study validity, regarding the requested control inoculum multiplication factor so the study is not considered as valid for the risk assessment.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 16-h IC_{50} for *Pseudomonas putida* exposed to glyphosate technical was >100 mg a.e./L based on nominal concentration. The NOEC after 16 h was 100 mg a.e./L.

It is not possible to conclude on the study validity regarding the requested control inoculum multiplication factor so the study is considered invalid for risk assessment purposes. Nevertheless, the results of the study are in line with the additional sludge study (CA 8.8/002)

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 8.8/002
Report author	
Report year	1990
Report title	Assessment of the acute toxicity of glyphosate technical on aerobic waste-water bacteria
Report No	277830
Document No	-
Guidelines followed in study	OECD No.209 (1984)
Deviations from current test guideline	Deviation from the guideline OECD 209 (2010): Minor: - Only one replicate in each treatment concentration - No indication on the dissolved oxygen concentration
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The effects of glyphosate technical on activated sludge were determined in a 3-hour exposure laboratory study. Activated sludge from a domestic waste-water treatment plant was exposed to the test item at concentrations of 3.2, 10, 32, 50, and 100 mg./L, 2 untreated controls and a toxic reference (3,5-dichlorophenol at concentrations of 1.0, 3.2, 10, 32, and 50 mg/L). After 180 minutes of aeration at 22°C, samples were taken from the test flasks for oxygen measurement over a period of up to 10 minutes. The inhibitory effect of the test item is expressed as oxygen consumption per minute. This study is considered valid and the EC_{50} > 100 mg a.e./L and the NOEC of 100 mg a.e./L can be used in risk assessment for micro-organisms exposed to glyphosate technical.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Aspect:	White solid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9 %

2. Vehicle and/or positive control: Vehicle: distilled water
Positive control 3,5-dichlorophenol

3. Test system:

Test system:	Activated sludge
Source:	Domestic waste-water treatment plant (ARA, Sissach, Switzerland
Nutrient solution:	Synthetic sewage feed

Dry sludge concentration: 4 g/L
Test vessel: 500 mL glass flasks

4. Environmental conditions:

Temperature: 20-25°C until use. 22°C during the test.
pH: 7.5-7.7

5. Experimental dates: July 19, 1990 (3 hours duration)

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate technical on activated sludge were determined in a 3-hour exposure laboratory study. Activated sludge from a domestic waste-water treatment plant was exposed to the test item at concentrations of 3.2, 10, 32, 50, and 100 mg/L, 2 replicates of untreated controls and a toxic reference (3,5-dichlorophenol at concentrations of 1.0, 3.2, 10, 32, 50, and 100 mg/L). A stock solution of 500 mg/L was prepared by dissolving glyphosate in distilled water. The sludge was sieved, centrifuged and the solid material resuspended in tap water and again centrifuged. This procedure was repeated a further 2 times. An aliquot of the final sludge suspension was made up with Soerensen buffer to 1 liter. To that mixture, 50 mL OECD recommended synthetic sewage feed were added.
Glass flasks were filled with appropriate aliquots of stock solutions, water and activated sludge up to 500 mL final volume and aerated with an air flow of about 0.2 L/minute.
2. Observations: After 180 minutes of aeration at 22°C, samples were taken from the test flasks for oxygen measurement over a period of up to 10 minutes. The inhibitory effect of the test item is expressed as oxygen consumption per minute. Respiration rate was expressed as percent inhibition relative to the control.
3. Statistical calculations: EC values were calculated using linear regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

The influence of glyphosate on oxygen consumption of activate sludge is presented below.

Table 8.8-3: Influence of glyphosate on oxygen consumption of activate sludge

Nominal concentration [mg test item/L]	Oxygen consumption [mg O_2 per litre per min]	Mean [deviation]	Inhibition [%]
Control	1.02	1.085 (12.7%)	-
Control	1.15		-
3.2	1.16	-	-6.9
10	1.09	-	-0.5
32	1.15	-	-6.0
50	1.09	-	-0.5
100	1.17	-	-7.8
3,5-DCP 1.0	1.11	-	-2.3
3,5-DCP 3.2	1.07	-	1.4
3,5-DCP 10	0.38	-	65.0
3,5-DCP 32	0.07	-	93.5
3,5-DCP 50	0.05	-	95.4

B. OBSERVATIONS

No inhibition of the respiration rate of the sludge was observed (-7.8%) at the highest concentration of glyphosate of 100 mg a.e./L. The EC_{50} for the toxic reference 3,5-DCP was found to be 8.6 mg/L.

The validity criteria were fulfilled according to OECD 209 (2010):

- the coefficient of variance for oxygen uptake in the control replicates was not more than 30 % (actual value: 12.7%)
- the EC_{50} of 3,5-dichlorophenol was in the expected range (actual value: 8.6mg/L)
- Control oxygen uptake rate was more than 20 mg/g of activated sludge (dry weight of suspended solids) in an hour. Based on 4 g/L dry sludge concentration with a dilution ratio of 200 mL in 500 mL final solution and oxygen uptake of 1.085 mgO_2/L.min.

The following points deviated from the current guideline requirements but are not expected to have impact on the study validity:

- Only one replicate in each treatment concentration instead of 5 replicates.
- No indication on the dissolved oxygen concentration. It should be maintained above 60 – 70 % saturation. The air-flow was 0.2 L/min instead of 0.5 to 1 L/min recommended due to foam.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The EC_{50} for waste-water micro-organisms exposed to glyphosate was determined to be >100 mg/L. The NOEC for waste-water micro-organisms exposed to glyphosate was determined to be 100 mg/L.

This study is considered valid and the EC_{50} > 100 mg a.e./L and the NOEC of 100 mg a.e./L can be used in the risk assessment for micro-organisms exposed to glyphosate technical.

Assessment and conclusion by RMS:

CA 8.9 Monitoring Data

Available monitoring data for glyphosate and its metabolites in soil, water, sediment and air are presented and discussed in detail in MCA Section 7.5

Glyphosate

Annex M-CA 8-01: PUBLIC LITERATURE RAR 2015

Annex to the Documen M of the technical section[16]:

ECOTOXICOLOGY

[16] Annex to the Doc ID: 110054-MCA8_GRG_Jun_2020

AIR 5 introduction

During the AIR 2 evaluation process of glyphosate, in the Renewal Assessment Report October 2015 version[17], the RMS Germany included public literature articles as part of the B.6 section. **All articles** included in this version of the RAR Vol 3 October 2015 have been included in this annex for the sake of completeness, with the aim of providing the EU authorities during the AIR 5 EU process with all information available for glyphosate from previous EU evaluations.

All information presented in this Annex 1, is an exact copy of the literature information included in the RAR Vol 3 October 2015 version. When reading the present annex, please note:

- This annex only present articles and not regulatory studies.
- Some references are made to the former Monograph glyphosate 1998.
- If text was strickethrough in the RAR Vol 3 October 2015, then those sentences were not included in the present annex.
- The numbering of tables in the present annex have not been changed and remain as original presented in the RAR Vol 3 Oct 2015 version.
- If text was highlighted in the RAR Vol 3 October 2015, then those sentences are also highlighted in the present annex.
- If text was given in italic style in the RAR Vol 3 October 2015, then those sentences are also given in italic in the present annex.

[17] Renewal Assessment Report, Revised 29 January 2015 Vol 3, annex B.6.4

B.9 Ecotoxicology

B.9.13 Evaluation of peer-reviewed literature regarding ecotoxicity of glyphosate

B.9.13.1 Purpose and matter of subject of the literature survey

Background

Article 8.5 of the regulation 1107/2009/EC stipulates the addition of open, peer-reviewed scientific literature with a submission of a dossier approval of an active substance in the European Union. Literature that was published within the last 10 years before the submission should be included. For this reason, the notifier Glyphosate Task Force (GTF) provided the available peer-reviewed literature from the public domain that refers to effects of glyphosate, the glyphosate-salt, its relevant metabolites and the representative formulation MON52276 to the regulatory bodies. This survey reviews the literature provided under Section 6, Point 8 of the dossier. The relevance of peer-reviewed literature that has been submitted by the notifier for consideration in the environmental risk assessment of the active ingredient glyphosate was assessed. Moreover, also several Non-Governmental Organization (NGO) provided also peer-reviewed literature from the public domain that refers to effects of glyphosate and glyphosate- based products.

Process of retrieving literature by the Notifier-search strategy and data bases

The pre-selection of the studies that were subjected by the notifier to in-deep reliability and relevance analysis after Klimisch et al. (1997) was reviewed. For this purpose, the criteria of Carr and Bleeke (2012) to rank the available literature were adjusted where appropriate. In RMS opinion, the strictly formal criteria employed by the notifier according to Klimisch et al. (1997) would possibly not cover all environmental impacts that have been described in publicly available literature in direct relationship to glyphosate use. In particular the following evaluation-steps were performed:

1. Revision of identified and submitted literature
2. Evaluation of the notifier's selection for detailed description with the cumulative bibliography
3. Supplement of relevant studies
4. Evaluation of the published literature regarding significance of results, the quality of statistical evaluation, plausibility of conclusions after EFSA 2011a (complemented by Klimisch et al. (1997) and Küster et al. (2009, 2010))
5. Assessment and supplementation of the evaluation of the notifier after Klimisch et al. 1997, as found in DocMIIA, section 6, point 8 (Anonymous 2012a, in the following 'DocM')
6. Summary of the results and evidence on outstanding publications regarding the ERA of glyphosate

B.9.13.1 Methodology of the literature search

B.9.13.2 Process of retrieving literature by the notifier

The notifier GTF conducted systematic literature research as stipulated by Article 8.5 of the regulation 1107/2009/EC for the period between the years 2001 and 2011. The notifier did not proceed exactly after EFSA (2011a).

For this purpose, five literature databases, namely 'Web of Science (SM)', 'Biosis Previews®', CAB Abstracts ®', 'Medline ®' and 'Chemical Abstracts Plus' were queried for Glyphosate- or its metabolites related peer-reviewed literature. The exact strategy has been described by Carr and Bleeke (2012) in detail. The results that were obtained by querying the databases for glyphosate-specific keywords were further filtered for the question of focusing on the fate and the characterization of unintended effects of glyphosate. In each of the years 2001 and 2011, between about 200 and 300 papers were identified, summing up to 2.770 citations in total (80% peer-reviewed).

The area of ecotoxicology was covered by 483 peer reviewed publications that came with the submission of the dossier. Additional 180 publications were cited in the text, but t were not submitted (coded 'rel0_nosub_cit', see below), referring e.g. to an Earth Open Source publication on glyphosate effects (Antoniou 2011), mainly older than 10 years and therefore not necessarily included in the submission.

B.9.13.3 Procedures of sighting and classifying the submitted literature by RMS

Steps of the procedure

- DocMIII, pp. 288-651, (glyphapplic_007) was scanned systematically, and all the references to the literature found were marked and labelled unambiguously in the pdf. Bookmarks with chapter headers and identity numbers for easy lookup were added to the pdf.
- Bookmarks for ease of navigation were also added to DocL (glyphapplic_016).
- Unique identity-numbers were assigned to each of the literature entries of DocM, DocL and the pdf-files of the submitted peer-reviewed publications. Notation is as follows:
  - glyphapplic = essential documents submitted by the notifier with the application for renewal of approval as substantial part
  - glyphnosubm = documents that were not submitted with the application but cited in the text of DocM as supplementary data
  - glyphecotox = literature submitted with the application
- The exact sources (journal/issue/pages) necessary to identify a publication, which were provided by DocL (glyphapplic_016) were added to the original reference list of the managing body BVL. This was necessary for all of the publications.
- The information on the relevance and reliability given by the notifier on available literature was added to the references (relevance, reliability, Klimisch rating).
- Citations were added to the references that were not submitted but cited in the text of DocM (glyphnosubm_xxx).
- Non-classified literature by the notifier (rel1_sub_nocit+norev) was assigned in a screening to the best-fit test area (e.g. fish, amphibians, soil microbes, see chapter 'References').
- Comprehensive analysis and classification of the open literature.

Completion and assignment of 'assessment-area' categories

It was desired to follow the notifier's categorisation in general. These are the 13 chapters of the survey at hand (sub-structure of Annex B.9). The literature was categorised as 'birds', 'fish', 'amphibians', 'aquatic invertebrates', 'aquatic plants including algae', 'bees', 'terrestrial non-target arthropods', 'soil microbial community', 'plant disease and plant nutrient status', 'earthworms and soil macro-organisms', 'terrestrial non-target plants', 'POEA' and 'DART/ED'.

However, to characterise the main topics of a study it has been necessary to introduce some new classes of study topics, especially for the studies that were completely out of the scope of the ERA. The assignment has been done for 431 studies that were submitted but neither cited nor reviewed in DocM (290 were rated *rel1_sub_nocit+norev*).

- **Other**: not assignable to a category
- **Soil Organisms**: Collembolans, soil mites, additional to **'Earthworms'** category
- **General**: not assignable to a certain category, e.g. 'general consideration of herbicide use on terrestrial ecosystems'
- Reptiles
- Molluscs
- **Fate:** falsely assigned by the notifier to the 'ecotoxicology' category
- **Soil quality**: includes indicators of soil quality other than organism related

- Aquatic Microbes
- Monitoring
- Vertebrates/Mammals
- **NTP:** studies not assigned by the notifier, were classified 'NTP' while dealing with the effectiveness of herbicides to antagonize weeds. This was because the studies could give indication of the sensitivity range of weed species.
- Modelling
- Genotoxicity
- **Pathogens**: Refers to the section 'plant disease', which was dealt with by the notifier in the fate section of DocM (Anonymous 2012b).

B.9.13.4 Analysis of reliability and relevance of peer-reviewed literature

Notifier

The notifier explained how studies were evaluated introducing an (arbitrary) categorization of the publications (taken from the definitions of Carr and Bleeke 2012). The notifier described the strategy of filtering and classifying the available literature as reproduced below from DocM (Anonymous 2012a).

'The peer-reviewed publications were divided into the four key disciplines within the submission that address exposure and hazard (toxicology, ecotoxicology, residues and environmental fate). Some publications contained information relevant to more than one technical discipline. In some cases, the disciplines originally assigned during the search process were revised to match the disciplines within the submission (for example, publications on effects of glyphosate on soil microorganisms were classified as 'environmental fate' in the original literature search but were reclassified as 'ecotoxicology' for the submission). The peer-reviewed publications identified for inclusion during the literature search were reviewed within each discipline and classified into one of the categories listed below by the notifier.

- Category 0 publications
  These are publications in which glyphosate is only mentioned as an example substance or is discussed/studied in a context that is not relevant or related to any of the regulatory sections or the exposure/hazard assessments within this submission; the publication is therefore outside of the scope of this submission.

- Category 1 publications
  These are publications that discuss glyphosate in a context relevant or related to the regulatory dossier sections and the conclusions fall within the conclusions of the exposure/hazard assessment. The publication is submitted with minimal or no comment or discussion.

- Category 2 publications
  These are publications that discuss glyphosate in a context relevant or related to the regulatory dossier sections and have conclusions that call into question the endpoints/conclusions in the exposure/hazard assessment. Additionally, Category 2 also includes publications with conclusions that support the risk/hazard assessment, and may be included in discussion of other relevant publications. For selected Category 2 publications, an OECD Tier-II type summary is provided in addition to a reliability assessment according to Klimisch et al 1997 5 (Klimisch rating); limited comments and critical remarks are provided, as appropriate.

- Category 3 publications
  These are publications that discuss glyphosate in a context relevant or related to (1) non-regulatory endpoints that need to be addressed as per new Regulation (EC) 1107/2009; (2) sensitive allegations that have emerged or could emerge in the media; or (3) the regulatory dossier sections

and have conclusions that are in disagreement with endpoints/conclusions in the exposure/hazard assessment (although the experimental design seems relevant at first glance). An OECD Tier-II type summary was provided and a Klimisch rating assigned, and supplemented with critical review and discussion.

- Category 'E' publications
  These are peer-reviewed publications that were cited in the Earth Open Source document. This category includes publications that were already captured by the notifier literature search and are addressed within the appropriate discipline, as well as publications that were out of scope of the search (primarily as a result of being published prior to 2001). Publications already captured in the literature search are assigned a Category 1, 2 or 3 rating (as appropriate) in addition to a Category 'E' rating. An OECD Tier-II type summary has been prepared and a Klimisch rating assigned for each of the Category E publications. All Category 'E' publications are reviewed within the appropriate discipline, with most of the reviews provided within the toxicology dossier under Section IIA 5.10.'

For notifier relevance category (2 and) 3 studies, a formal and more or less comprehensive evaluation based on Klimisch et al. (1997) on the reliability of a study was conducted by the notifier. There are 4 categories: 'reliable without restriction', 'reliable with restriction', 'not reliable' and 'not assignable'.
The relevance categories triggering the Klimisch evaluation are indicated by column 'UBA Classification study according to notifier treatment' in the comprehensive reference list (refer to chapter 7 and MS Excel sheet attached).

Rapporteur member state

Based on the general criteria of Klimisch et al. (1997), Küster et al. (2009) aimed to further develop and specify the demands on the reliability of peer-reviewed literature data, in particular for regulatory requirements on the ERA of pharmaceuticals. The categories of Küster et al. (2009) were taken over as follows:

- **Category I**
  Data are reliable without restriction according to the instructions in the EMEA guideline (EMEA 2008) and are therefore usable within the environmental risk assessment. This category includes data from the literature or reports,
  - Which were carried out or generated according to internationally accepted test guidelines (e.g. OECD, ISO).
  - In which the test parameters documented are based on a specific (e.g. national) testing guideline (e.g. DIN).
  - In which all parameters described are closely related/comparable to a guideline method.

- **Category II**
  Data are reliable with restriction and are usable within the environmental risk assessment. This category includes data from the literature or reports
  - In which the test parameters documented do not totally comply with the respective test guideline, but are sufficient to evaluate the data.
  - In which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented, plausible and scientifically acceptable.

- **Category III**
  Data are not reliable and are not usable within the environmental risk assessment. This category includes data from the literature or reports,
  - Where the documentation is not sufficient for an assessment.

- Which were carried out or generated using a non-accepted method or which are not well documented, plausible and scientifically acceptable?

- Which are only listed in short information (e.g. abstracts, summaries, data safety sheets) or non-peer-reviewed literature (e.g. diploma/master thesis).

The evaluation criteria set by Küster et al. (2009) were further refined by international groups of regulators, leading to the latest publicly available initiative on the harmonisation of evaluation criteria by Kase et al. (2012). The reliability and the relevance of literature underlie separate examination. Therefore, even not reliable data can have supporting character. Test data with high reliability and high relevance will have high weight in risk assessment. Test data with either high reliability or high relevance could function as supportive evidence in risk assessments: see Küster et al. (2012).

RMS opted for following evaluation criteria:

Figure B.9.13-1:Assignment of literature data to categories of high and low reliability and relevance for ERA. Modified after Klimisch et al. (1997).

There are criteria to identify 'critical', 'supporting' data and 'low weight data' for the risk assessment. Critical data are data of sensitive species, typically including endpoint for risk assessment (as long as they are reliable and relevant). Supporting data are not described as critical datasince they may suffer from deficiencies. All reliable and relevant data are used. These include studies using less sensitive species/endpoints, studies using non-standard statistical methods. This can help e.g. identifying sensitive taxa, results and conclusions can be combined for risk assessment and for derivation of uncertainty. All available toxicity data, both critical and supporting, are subject to assessment and are reported. Uncertainty levels in risk assessment are estimated by the use of critical and supporting data and extrapolation from all available data.

Finally, the information provided by the experimental studies that were evaluated has been summarized in tables and concluded for its use in ERA. The table below shows a blank table with dummy records that was developed for the particular use in this study.

Table B.9.13-1:Blank general evaluation table with dummy records used as a form to be filled in this survey

litID Assessment area	Author	Year	Title	Source	
Reliability					
Purpose of the study, Description of endpoints					…
Test compound, application procedure, exposure period, protocol					…
Experimental approach, Statistical design, test environment					…
Test organisms					…
Biological effects					…
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints					
Biological Relevance					
1 Is an appropriate test species/ life-stage(s) studied?					…
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?					…
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?					…
Environmental Relevance					
1 Is the substance tested representative and relevant for the substance being assessed?					…
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?					…
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?					…
Concluding weight of evidence/proposed action	…				
Type of information (Critical, supporting, low weight)	…				
Consideration/concluding score	…				

B.9.13.5 Assessment of the open literature submitted

The literature on the ecotoxicological effects of glyphosate, the glyphosate salt, its metabolites and diverse commercial formulations was categorized according to 12 areas. The areas of risk assessment were covered for birds, fish, amphibians, aquatic invertebrates, aquatic plants including algae, terrestrial non-target arthropods, soil microbes, plant diseases and plant nutrient status, earthworms and soil macro-organisms, terrestrial non-target plants, the special case of polyethoxylated tallowamines as surfactive additives and endocrine disruption/reproductive toxicity.

For the whole area of ecotoxicological studies, the notifier submitted many studies that were rated category 1 or 2 and were therefore not considered for further analysis. Some studies were labelled category 3 (DocL) but not processed further in the text. Those studies were not categorised in one of the twelve areas of risk assessment and were for this reason not listed in the overview paragraph of each section. The following table gives an overview on the total dataset and the structure of the classification by the notifier.

Table B.9.13-2: Classification of glyphosate related open, peer-reviewed literature that is used by this evaluation according to its submission, citation and revision status. Frequencies of the submitted peer-reviewed data is given.

Category	Notifier relevance, submittance, citation, review status	No. of publications
Category 0: not submitted, but cited in DocM	rel0_nosub_cit	180
Category 0: not submitted and cited in DocM	rel0_nosub_nocit	2
Category 1: submitted and cited but not reviewed	rel1_sub_cit+norev	6
Category 1: submitted only, not used and	rel1_sub_nocit+norev	290
Category 2: Submitted and revied in DocM	rel2_sub_cit+rev	7
Category 2: submitted only, not used and	rel2_sub_nocit+norev	106
Category 3: not submitted, but used in DocM	rel3_nosub_cit+rev	11
Category 3: Submitted and reviewed in DocM	rel3_sub_cit+rev	59
Category 3: submitted only, not used and	rel3_sub_nocit+norev	14
Official documents provided with the application,	submit_notifier	19
	Total number of studies submitted with	**694**

B.9.13.6 Assessment of the open literature from Non-Governmental Organization (NGO)

In total, over 100 studies on ecological risk assessment were cited and submitted by NGOs. All submitted publications were evaluated by RMS. Out of the submitted publications, over 60 studies were recognized as overlapping with publications submitted by the notifer. Relevant and reliable studies (UBA2) have been described in the above mentioned evaluation table and have been considered for the literature review process.

B.9.13.7 Effects on birds (KIIA 8.16)

All of the four submitted publications regarding the toxicity of glyphosate to birds have been subjected to detailed description and evaluation in the following.

Oliveira et al. (2007)

glyphecotox_001 Birds+DART/ED	Oliveira, AG, Telles LF, Hess RA, Mahecha GAB, Oliveira CA	2007	Effects of the herbicide Roundup on the epididymal region of drakes Anas platyrhynchos.	Reprod Toxicol 23 (2):182-91. DOI: 10.1016/j reprotox.2006.11.004.

Reliability	
Purpose of the study Description of endpoints	Test in vivo the hypothesis that glyphosate affects the steroidogenic acute regulatory protein (StAR) and aromatase activities responsible for androgenic and estrogenic hormones in birds
Test compound, application procedure, exposure period	Weights of testes and epididymal regions; morphometric, histochemical analyses (enzymatic activities), immunohistochemical analyses of ductules and ducts (androgen receptor expression) and hormone level analyses (testosterone, estradiol) were performed
Experimental approach, Statistics, test environment	Glyphosate as Roundup with 360 g glyphosate /L; 480 g/L isopropylamine salt,
Test organisms	(Presumably) daily application of 2 water diluted solutions of 5 and 100 mg/kg bw of Roundup via gavage for 15 days
Biological effects	Non-GLP
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The mallard duck as a standard model species is considered appropriate for the respective research question.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Since the daily doses tested were below the relevant NOELs in standard tests (201 mg ai/kg bw/day) even though statistically significant, effects on the endocrine system and the reproductive tract of male individuals are considered relevant for the population indeed.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The endpoints reflect a broad range of possible deformations of the male reproductive system.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate was tested in a Roundup formulation that was not further specified (e.g. surfactants used). The effects could not be assigned to the active ingredient glyphosate alone.
2 Do the tested concentrations relate to predicted environmental concentrations?	PEC_{SW} FOCUS step 1 = 0.101 mg ai/L
3 Have parameters influencing the endpoints been considered adequately?	Environmental parameters and the conditions of animal housing during the experimental period were not mentioned, but the study was conducted under the ethical principles of the researching University.
Concluding weight of evidence/proposed action	The unusual way of exposure for reproductive studies via gavage and not via dietary uptake is criticized by the notifier. For the mallard duck as an aquatic dabbling bird it is assumed acceptable as a worst case exposure scenario.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Eason, T.H., Scanlon, P.F. (2007)

glyphecotox_358	**Eason, T.H., Scanlon, P.F.**	***2002***	**Effects of Atrazine and Glyphosate ingestion on body weight and nutritional well-being of Coturnix quail**	**Zeitschrift Fur Jagdwissenschaft 48:281-285**

Reliability		
Purpose of the study Description of endpoints	The aim of this project was to test the effects on a quail species (Coturnix coturnix japonica) of ingesting foods treated with Glyphosate. Atrazine was tested in the same experimental design. Body weight, liver weight, body fat content	
Test compound, application procedure, exposure period	The procedure of calculating the exposure concentrations is very fiddly and has not been resulted in definite body weight related values. In the summary, concentrations in the food items? Of 347, 1388, and 3470 ppm have been mentioned. The calculations were based on recommended field application rates, according to the supplier's labels, but have never been specified by the authors. The common name of the test compound 'glyphosate' was misspelled as 'glycophosphate' in the German and English summaries.	
Experimental approach	12 control quails, 10 male quails used for each of the three treatments.	
Test organisms	Adult male, Japanese quail, (Coturnix japonica)	
Biological effects	No effects of glyphosate reported.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		-/-
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?		-/-
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		-/-
2 Do the tested concentrations relate to predicted environmental concentrations?		-/-
3 Have parameters influencing the endpoints been considered adequately?		-/-
Concluding weight of evidence/proposed action	The poor quality of the paper suggests that the experimenters had no or minor experience with animal toxicological testing and should have never given the permission to kill in sum 72 vertebrate organisms. The study was not further considered relevant after checking the experimental design.	
Type of information (Critical, supporting, low weight)	**Low weight**	
Consideration/concluding score	**UBA3**	

Stoleson et al. (2011)

glyphecotox_614	**Stoleson, S.H., Ristau, T.E., deCalesta, D.S., Horsley, S.B.**	**2011**	**Ten-year response of bird communities to an operational herbicide-shelterwood treatment in a northern hardwood forest**	**Forest Ecology and Management 262 (7):1205-1214. DOI: 10.1016/j foreco.2011.06.017.**

Reliability	
Purpose of the study Description of endpoints	Long-term monitoring study of bird occurrence in a 'Shelterwood system' after seed cut and herbicide application (a silvicultural system in which overstory trees are removed in a series of cuts designed to achieve a new, even-aged stand under the shelter of remaining trees, http://en.wikipedia.org/wiki/Shelterwood_cutting). Fixed-radius point counts of birds at two points per plot: overall abundance, abundance of migratory guilds, nesting guilds, vegetation cover, avian community similarity

Test compound, application procedure, exposure period	Herbicide-treated plots were sprayed with a tank mix containing 364 ml glyphosate (Accord®) and 24 ml sulfometuron methyl (Oust ®) in 38 l water per ha
Experimental approach, Statistics, test environment	Repeated measures randomized split-plot experimental design, half of each of 10 plots was treated once with herbicide in August of 1994, remaining 5 plots as controls Time-series between 1992-1994 (pre-treatment period) and 1994-2004 (post-treatment period) **Statistics:** Generalized linear mixed models to model the effects of year, site, herbicide treatment and cutting sequence on vegetation and avian target variables. Site as a random effect, and year, herbicide treatment, and cutting sequence as fixed effects. Shannon Evenness scores were modelled using a Beta distribution and a logit link function, other diversity indices with a Gaussian distribution and identity link, vegetation cover modelled using a lognormal distribution and identity link, whereas bird abundances were modelled using a Poisson distribution and a log link function (Littell et al., 2006). maximum-likelihood (REML) method and the Kenward–Roger procedure to adjust the denominator degrees of freedom Post-hoc tests to identify years with significant differences between control and experimental treatments were conducted using Tukey–Kramer tests Multiple regression analyses to determine the effects of understory vegetation variables on the abundance of ground and shrub birds, and the effects of overall bird abundance and time since treatment on the similarity of avian communities pre- and post-treatment. We used analysis of similarities to test the null hypothesis that avian community structure did not differ significantly between herbicide and control plots.
Test organisms	Naturally occurring North American bird species, vegetation
Biological effects	Long-term monitoring study of bird occurrence in a 'Shelterwood system' after seed cut and herbicide application (a silvicultural system in which overstory trees are removed in a series of cuts designed to achieve a new, even-aged stand under the shelter of remaining trees, http://en.wikipedia.org/wiki/Shelterwood_cutting). Fixed-radius point counts of birds at two points per plot: overall abundance, abundance of migratory guilds, nesting guilds, vegetation cover, avian community similarity
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The 'natural' composition of plant and bird species at all life stages has been analysed. This level of complexity is highly appropriate for ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Endpoints refer to population and ecosystem level effects
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Since a tank mix has been tested consisting of a mixture of glyphosate and sulfometuron methyl, the effects could not be assigned to a single substance. The toxicity of the mixture used can be estimated assuming a 'concentration addition model', probably leading to glyphosate as the determining factor (*analysis not conducted within the scope of this survey*).
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes, the tank mixture was applied at recommended application rates
3 Have parameters influencing the endpoints been considered adequately?	The statistical design (GLM) included time, herbicide treatment, site, cutting sequence as explanatory variables in a model; so ecologically potential influencing factors in this design have been adequately considered.

Concluding weight of evidence/proposed action	**The silvicultural practice of shelterwood systems and thus the experimental approach is quite specific for North American practices and not transferable to European agricultural practices. Nevertheless, the authors describe an impressive example of indirect effects of herbicides on 'ecosystem level' and the complexity of an assessment that is not covered by 'standard ERA' by far. The transferability is further restricted by the use of a tank mixture of two herbicides. The study is recommended to be considered as an example of indirect effects and profound statistical analysis of monitoring data.**
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Sullivan, T.P., Sullivan, D.S. (2003)

glyphecotox_615	**Sullivan, T.P., Sullivan, D.S.**	**2003**	**Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems**	**Environ. Rev. 11 (1):37-59. DOI: 10.1139/a03-005.**

Reliability	
Purpose of the study Description of endpoints	Comprehensive review on effects of Glyphosate on the biodiversity of NTP, NTA, birds and mammals under the general assumption of a relatively non-harmful environmental impact of the substance within vegetation management practices
	Species abundance, numbers (richness) and diversity indices (Shannon)
Test compound, application procedure, exposure period	Not possible to describe in detail, since the study is a literature compilation, not an original experimental work Application rates: Forest ecosystems: between 1.1 and 3.3 kg Glyphosate/ha once a year Agriculture/Wetland: variable dose-rates, nor further specified by the authors
Experimental approach, Statistics, test environment	Considered only: Measures within 'vegetation control' or 'vegetation management' programs for enhancing crop production (not the same as weed control in European countries) Peer-reviewed journal publications describing studies on vascular plants, small mammals, large mammalian herbivores, terrestrial invertebrates in forests or agricultural landscapes. Considered here: **findings on birds** Studies must provide numbers and composition of species (for richness estimation) in terrestrial ecosystems For birds, 7 studies have been analysed, the total number of replicate situations was 10 for statistical comparisons. Effects were given mainly in relative changes compared to the pre-treatment period and between control and treatment
Test organisms	Birds (songbirds and waterfowl)
Biological effects	Short-term (mainly first year after application) effects on species numbers (decline) and abundance (increase), dominance of most common species (increase) Over the whole study periods, most effects disappeared In total: Very small differences between controls and treatments over several years of the studies

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Communities of naturally occurring bird species in field monitoring studies have been assessed over 2-4 years, which could be ecologically highly relevant
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Since the methodology was not described in detail for each of the studies the statistical significance could not be judged. The studies were conducted on population level and could therefore considered relevant on this particular level of organisation
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Population changes over time is amongst the highest possible levels of manifestation
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The test substances were not uniform and not described in more detail than the mere mentioning of 'glyphosate' as the test substance.
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes, because recommended field rates have been tested
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	**The paper deals with the impact of the Anglo-Saxon practice of managing the vegetation for purposes of enhancing forest and other crop yields. This includes especially the control of roadside vegetation and intends the maintenance of ecological processes in terrestrial ecosystems.** **However, the review shows the transiency and indirectness of effects of Glyphosate treatments on the biodiversity of birds, most probably mediated by ephemeral changes of the (shrub) vegetation.**
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

- **B.9.13 7.1 Summary of the relevant literature on birds**

There was no critical data that must imperatively be included in an environmental risk assessment for the active substance glyphosate. It was not possible to distinguish between the effect of the technical glyphosate and the surface-active substances added to the commercial formulations by the experimental designs used. Two publications gave clues on indirect effects of glyphosate use on the biodiversity of birds on a regional scale in a long-term by subtle alterations of the vegetation structure.

- **B.9.13.8 Effects on other terrestrial vertebrates**

- **B.9.13 8.1 Summary of the relevant literature on terrestrial vertebrates**

Please refer to sections regarding amphibians and to the summary of the relevant literature on surface active substances in glyphosate-based formulations (Vol. 3; chapters B.9.9.2 and B.9.11).

- **B.9.13.9 Effects on aquatic organisms**

- **B.9.13 9.1 Fish (KIIA 8.16)**

Filizadeh, Y., Islami, H.R. (2011)

glyphecotox_007	**Filizadeh, Y., Islami, H.R.**	**2011**	**Toxicity determination of three sturgeon species exposed to glyphosate**	**Iranian Journal of Fisheries Sciences 10 (3):383-392**

Reliability	
Purpose of the study Description of endpoints	Determination of the acute toxicity (lethal concentration LC_{50}) of the Glyphosate formulation roundup towards three juvenile Sturgeon species Mortality, swimming behaviour (not analysed)
Test compound, application procedure, exposure period	Roundup formulation (not further specified) with 41 weight % Glyphosate, content of POEA not specified Non-GLP, protocol resembles acute fish toxicity testing after guideline OECD 203
Experimental approach, Statistics, test environment	Dose-response study, 10 doses between 10 and 100 mg a.i./L, irregular spacing, non-geometric series; three treatment replicates with 8 fishes each; mortality was recorded after 6, 12, 24, 48, 96, 168 hours; static exposure in 100L replicate tanks Finney's Probit regression and 95% confidence limits for derivation of LC_{50}; comparison between species by One-way ANOVA and Tukey's post-hoc test, data tested for normality by Kolmogorov-Smirnov-test Fish were fed daily
Test organisms	*Huso huso*, *Acipenser stellatus*, *Acipenser persicus*
Biological effects	LC_{50} between 70-74 mg glyphosate/L after 6h and 8-137 after 168 h of exposure; reference to acute studies is the value after 96h: between 20 and 26 mg a.i./L; differences in the sensitivities of the three species 96 and 168h after exposure (ANOVA); no mortality in control groups
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The test species are considered of temperate to sub-tropical origin and generally as suitable for an indication of intrinsic sensitivity as the standard species in ERA. The juveniles could be considered most sensitive stages.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Since mortality was assessed by the study, the biological level of assessment is appropriate for population level effects.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	This could not be judged because a detailed description of the test item, i.e. proportion of surfactant in the respective formulation is unknown.
2 Do the tested concentrations relate to predicted environmental concentrations?	The predicted environmental concentration of the application applied by the notifier was about 0.1 mg a.i./L and thus far below the concentrations tested here.
3 Have parameters influencing the endpoints been considered adequately?	The deviations in oxygen, ammonia and nitrite were rather high, but it was not indicated how the SD was calculated (all systems, only controls?). If differences in water parameters occurred treatment related, this could cause effects beyond the toxic effects of Glyphosate.
Concluding weight of evidence/proposed action	**The description of the study is deficient; however, the LC_{50}'s 96 hours after exposure (20-26 mg a i./L) are below the acute studies provided by the notifier and are located rather near the chronic toxicity of a full life cycle test (25.7 mg/L). Because the content of POEA that is usually grossly determining the toxicity of Roundup formulations was not stated by the authors, the study could not be taken into account as additional or critical information on the ERA of the active substance glyphosate.**

Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Guilherme et al. (2010)

glyphecotox_008	**Guilherme, S., Gaivão, I., Santos, M.A., Pacheco, M.**	**2010**	**European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® a glyphosate-based herbicide.**	**Archives of Environmental Contamination and Toxicology 62 (1):107-117. DOI: 10.1007/s00244-011-9686-7.**

Reliability	
Purpose of the study Description of endpoints	Description of genotoxicity and oxidative stress indicating endpoints in fish at environmentally relevant concentrations after short term exposure of 1 and 3 days Genotoxicity: Comet assay: strand breaks, alkali labile sites expressed in a Genetic Damage Index (GDI) ; ENA - erythrocytic nuclear abnormalities: irreparable lesions Oxidative stress by: catalase activity, glutathione-S-transferase, glutathione peroxidase and glutathione reductase) and non-enzymatic (total glutathione content) antioxidants, lipid peroxidation (LPO)
Test compound, application procedure, exposure period	Glyphosate as Roundup with isopropylammonium-salt 485 g/L (360 g/L = 30.8 % a.i.) and 16 % POEA as surfactant. Exposure for 1 and 3 days. Application procedure not described in detail. Non-GLP, but procedures were well described and referenced to other peer-reviewed protocols, sounds reliable.
Experimental approach, Statistics, test environment	Static exposure for 1 and 3 days of 6 fishes in each of six 20L aquaria; divided into 2 treatment replicates for control, 58 µg Roundup/L (equals 18 µg glyphosate/L) and 116 µg Roundup/L (36 µg glyphosate/L).
Test organisms	European eel *Anguilla anguilla*, average length 25 cm, average weight 32 g
Biological effects	Increasing DNA damage with increasing exposure time and glyphosate concentration was measured in the Comet assay; for ENA more pronounced effects after 3-days exposure; no oxidative stress was recorded.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The European eel is species that can be considered both to water and sediment phase of cold and warm temperate environments, and is thus most suitable to cover worst-case exposure scenarios
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The true replication of the experiment was poor in fact. There were 6 (pseudo-)replicate fish that should be taken as averages for further statistical evaluation. It was not mentioned clearly but suspected that 1 aquarium equalled the true treatment replicates and the 6 replicate fish per treatment have been used for statistics as pseudo-replicates. The replication was not considered independent. Hence, it was not possible to judge the reliability of the data analyses.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The endpoints measured can be taken as early warning indicators of genotoxic and oxidative stress at the individual level but could not be used for the risk assessment for populations of eels and other temperate fishes in a real environment.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation containing POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	According to the paper at hand (max. PEC surface water 677 µg/L) and the RA of the notifier of 101 µg/L in a FOCUS step 1 scenario, the concentrations are quite realistic.
3 Have parameters influencing the endpoints been considered adequately?	No, environmental parameters were not measured explicitly during the experimental period.

Concluding weight of evidence/proposed action	**The study is well conducted, except for the statistical evaluation. Regardless, the results can be taken as an indicator of genotoxic but not of oxidative stress effects of realistically low water concentrations in fish. Study describes physiological parameters, no mortality endpoints are stated.**
Type of information (Critical, supporting, low weight)	**supporting information**
Consideration/concluding score	**UBA2**

Hued, A.C. et al. (2012)

glyphecotox_009 Fish	**Hued, A.C., Oberhofer, S., de los Ángeles Bistoni, M.**	**2012**	**Exposure to a Commercial Glyphosate Formulation (Roundup) Alters Normal Gill and Liver Histology and Affects Male Sexual Activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes)**	**Archives of Environmental Contamination and Toxicology 62 (1):107-117. DOI: 10.1007/s00244-011-9686-7.**

Reliability	
Purpose of the study Description of endpoints	Histological lesions of the neotropical, South-American fish species *Jenynsia multidentata* after acute and subchronic exposure to sublethal concentrations of Glyphosate LC_{50} after 96h, Male sexual activity after 7d and 28d of exposure, gill and liver histopathological analyses after termination of toxicity experiments; scores from 0-6 alterations to describe the degree of histological findings
Test compound, application procedure, exposure period	Roundup Max granular (Monsanto, Argentina), containing 74.7 % Glyphosate and 25.3 % surfactants (presumably POEA). Application procedure is not described in detail. Static exposure for 96h, 7d and 28d Non-GLP, but procedures used are well documented and referenced in the literature cited
Experimental approach, Statistics, test environment	Short-term testing: static test, nominal 5, 10, 20, 35, 60, and 100 mg Roundup/L for 96 h, duplicates of control and treatment groups, 4 male, 4 female fishes per duplicate Subchronic testing: 0.5 mg Roundup/L of two groups of 9 individuals (5 male, 4 female) for 7 and 28 days No clear indication if the duplicates of the treatments have been the replicates for statistics, or if the replicate fishes have been taken for the testing procedures and for the calculation of the importance indices.
Test organisms	*Jenynsia multidentata* (Onesided livebearer) Male and female fish standard lengths (means ± SDs) were 36.34 ± 4.16 and 43.71 ± 7.46 mm, respectively. The mean weight was 0.58 ± 0.21 g for male fish and 1.12 ± 0.5 g for female fish.
Biological effects	LC_{50} (96h)= 19.02 mg Roundup/L = 14.2 mg a.i./L; subchronic exposure caused significantly lower numbers of copulations per male, similarly for 7d and 28d exposure. Several dose-dependent pathological alterations of gill and liver histology in the acute tests, for the subchronic testing the effects were more pronounced in the 28d-exposure group; since the single histological endpoints did not show unambiguous results, the total histopathogical index showed significant dependent effects at 0.5 mg Roundup/L (equals 0.37 mg a.i./L)

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The species has a special mode of reproduction, as being sexually dimorphic (male differentiate a gonopodium if needed) and livebearers. It could be seen as a well suited model for sexual behaviour of males as claimed by the authors, but the species is very unlikely a taxonomically and behaviourally representative of temperate European fish species. It is therefore considered as not relevant for ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The histological endpoints 'gill' and 'liver' measured could serve as indicators of general individual vitality (stress level) of an organism and its reproduction fitness. It has been shown that the sexual system of males was affected. This could have severe effects on the stability of a fish population on the long term.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Although not specified precisely, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Most of the concentrations tested in the acute testing exceeded environmentally realistic concentrations by far. The concentration of 500 µg/L tested in the subchronic test resemble real measured concentrations of surface waters..
3 Have parameters influencing the endpoints been considered adequately?	The environmental parameters have been holding constant, the light cycle was 12:12 hours light/dark. There was no measurement of e.g. water quality parameters that could cause additional stress concealing toxicant effects.
Concluding weight of evidence/proposed action	**There are some obscurities in the description of the statistics and the test substance. The study could be taken as a further source of information that realistic concentrations of Glyphosate in surface waters could have a pronounced long-term effect on the populations of fishes. It is not distinguishable if the effect on the endpoints was due to the active ingredient glyphosate or (more likely) to the surfactant that was contained at 15.3 % of the formulation.**
Type of information (Critical, supporting, low weight)	**supporting information**
Consideration/concluding score	**UBA2**

Kelly et al. (2010)

glyphecotox_010 Fish	**Kelly, D.W., Poulin, R., Tompkins, D.M., Townsend, C.R.**	**2010**	**Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival**	**Journal of Applied Ecology Volume: 47 Issue: 2 Pages: 498-504 Url: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2010.01791.x/pdf DOI: 10.1111/j.1365-2664.2010.01791.x ISSN: 1365-2664 (online)**

Reliability	
Purpose of the study Description of endpoints	Synergistic effects of multiple stressors, i.e. the combined effect of glyphosate and trematode parasites *Telogaster opisthorchis* on juvenile *Galaxias anomalus* freshwater fish (only the 1st of two independent experiments is evaluated here) Survival and spinal deformation of juvenile fish
Test compound, application procedure, exposure period	Glyphosate 360 (commercial formulation, Ravensdown, New Zealand), 360 mg a.i/L, 10-20 % POEA surfactant Infective trematodes *Telogaster opisthorchis* were provided via the intermediate host *Potamopyrgus antipodarum* snails Non-GLP
Experimental approach, Statistics, test environment	The toxic potential of the herbicide glyphosate is assumed to enhance the disastrous effect of parasite infections: Experiment combined 4 treatments, 8-fold replicated in 32 aquaria, each equipped with 4 fish: 1) Controls: No parasite, no glyphosate, 2) parasite, no glyphosate, 3) No parasite, 0.36 mg glyphosate/L, 4) Parasite, 0.36 mg glyphosate/L Tested on significance by first log10 or square root transforming the data and then applying ANOVA procedure followed by 'Fisher's protected least significant difference (FPLSD)'
Test organisms	juvenile *Galaxias anomalus* freshwater fish, *Telogaster opisthorchis* trematode infection mediated by infected host snails *Potamopyrgus antipodarum*
Biological effects	No difference in survival for herbicide and parasite treatments alone, but for the combination treatment 4); spinal deformations were more frequent in parasitized fish treatment 2) and in the combination 4)
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, or rather there is no indication that juvenile stage sof New Zealand freshwater fish should be less suitable for ERA than others are
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The effects described in this study regarding the synergistic effect were clear
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Survival and spinal deformation are relevant endpoints
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	Yes, 0.36 mg a.i./L it could be seen as the ‚upper edge' of the distribution of possible concentration in real surface waters
3 Have parameters influencing the endpoints been considered adequately?	No, parameters of water quality that could cause further stress have not been regarded by the authors
Concluding weight of evidence/proposed action	**The experiment shows a general mechanism of the ecotoxicological theory: many multiple stressors act additively on the endpoints observed; It is of limited value for ERA because the factors are not considered separately and safety factors should cover uncertainties caused by synergisms or enhanced toxicity.**
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Salbego et al. (2010)

glyphecotox_020 Fish	**Salbego, J., Pretto, A., Gioda, C.R., de Menezes, C.C., Lazzari, R., Radunz Neto, J., Baldisserotto, B., Loro, V.L.**	**2010**	**Herbicide formulation with glyphosate affects growth, acetylcholinesterase activity, and metabolic and hematological parameters in piava (Leporinus obtusidens)**	**Arch Environ Contam Toxicol 58 (3):740-5. DOI: 10.1007/s00244-009-9464-y.**

Reliability	
Purpose of the study Description of endpoints	Effects of long-term glyphosate exposure on growth, Acetylcholinesterase activity and various metabolic and hematological endpoints in the omnivorous fish Leporinus obtusidens (Piva) Weight gain, condition factor (weight * length-3), daily food consumption Hematocrit, haemoglobin, total erythrocyte counts, total leukocyte counts and blood protein from blood samples Liver and muscle glycogen, tissue protein
	Acetylcholinesterase activity from homogenates of brain and muscles
Test compound, application procedure, exposure period	Glyphosate as the isopropylamine salt in the commercial formulation ‚Roundup' that contained 48% of the acid equivalent of the salt; content of POEA in the formulation not stated. Exposure time 90 days water renewal conditions: every 4 days water exchange, water conc. were followed for 8 days by chemical analysis of glyphosate and the main metabolite AMPA: remained nearly constant over the test period of 8 days to check for appropriate exposure, it is questionable if the measurements were realistic because of identical parent and metabolite concentrations
Experimental approach, Statistics, test environment	2 replicate 250 L tanks for the control, 1 mg Roundup/L, 5 mg Roundup/L treatments; 30 fish per tank, 180 fish in sum Weight and length measurements of 15 individuals per tank at days 30 and 60, 90 days after start of the experiment measurement of the remaining individuals Blood samples of 8 ind. at day 90 No indication of how many ind. were sampled for brain and liver tissues Multiple Comparison by ANOVA followed by Tukey's post-hoc test, prior test for homogeneity of variances
Test organisms	Omnivorous fish Leporinus obtusidens (Piva)
Biological effects	No mortality, no effects on condition factor and daily food consumption but significantly reduced, dose- and time dependent weight and length gains over the whole experimental period recorded. Significant effects have been observed at the lower concentration of 1 mg Roundup/L (equals 0.48 mg a.e. of the salt/L). Most metabolic and hematologic endpoints showed significantly reduced or enhanced parameters for both of the concentrations tested.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	This subtropical fish species is indigenous for few rivers in Brazil and thus could not be seen as a good representative for general ecotoxicological effect assessment.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Most endpoints showed significant effects around the NOEC of glyphosate for Zebrafish (43.2 mg a.i./L)

3 Is the ecotoxicological manifestation level appropriate for the assessment?	The authors propose to take enzymatic activities and the hematological parameters as indicators for exposure to glyphosate rather than as assessment endpoints. It remained unclear whether the endpoints measured could be taken as good indicators of the individual fitness of a fish, which would affect the population integrity in the end.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	There was some confusion and obscurities regarding the indication of the test substance as glyphosate or Roundup in the text.
2 Do the tested concentrations relate to predicted environmental concentrations?	Glyphosate concentrations exceed expectable surface water conc. by far (at least Factor 10) as reported by the paper.
3 Have parameters influencing the endpoints been considered adequately?	No
Concluding weight of evidence/proposed action	**Physiological endpoints measured do not contribute to an ERA and the species is not representative for common protection goals. There was no indication of the percentage content of tallow amine surfactants within the Roundup formulation tested.**
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Tierney et al. (2006)

glyphecotox_022 Fish	**Tierney, K.B., Ross, P.S., Jarrard, H.E., Delaney, K.R., Kennedy, C.J.**	**2006**	**Changes in juvenile coho salmon electro-olfactogram during and after short-term exposure to current-use pesticides**	**Environ Toxicol Chem 25 (10):2809-17**

Reliability	
Purpose of the study Description of endpoints	Effects of glyphosate on the olfactory sense of the coho salmon by recording the electro-olfactogram after exposure to an odorant Inhibition of the field potential of olfactory sensory neurons as the EOG peak amplitude size (in mV), determination of a median inhibitory concentration (IC50) The study tested six pesticides in a joint approach, thus the controls of each 3 compounds have been pooled for strengthen the statistical analysis
Test compound, application procedure, exposure period	Glyphosate technical (purity 99 %) was directly added to the test aquaria, concentrations of 0.1, 1, 10, 100 mg a.i./L were chosen well below the LC50 of 22 mg a.i./L for the Coho salmon because the authors assume an enhanced effect of the usually added surfactants (e.g. POEA) Exposure for 30 minutes, post exposure 60 minutes
Experimental approach, Statistics, test environment	Fish were fixed in a water flow-through system and exposed to the test compound and olfactorily stimulated by 2 second-pulses of L-serine as the odorant Measurements 2, 5, 10, 15, 20, 25, and 30 min during exposure; at 2, 5, 10, 15, 20, 30, 40, 50, and 60 min post-exposure. Pooled controls of 3 pesticides, N = 18; N = 6 for each of the treatments Differences between treatments: Two-way (time and treatment), repeated-measures analysis of variance followed by a Holm-Sidak post hoc test EOG response curves (% pre-exposure potential) were fitted to a three- parameter exponential decay model
Test organisms	Coho salmons (Canada), Oncorhynchus kisutch
Biological effects	Significant drops of the EOG occurred at 1 mg a.i./L that is far below the LC50 for the Coho salmon. The IC50 was 10.9 mg/L (95% CI, 6.72–16.8 mg/L) 2 min after the exposure.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The study was very thoroughly designed and conducted. Despite the fact that no standardised guideline was followed, the results appear to be reliable. There was a relatively high number of replicates and the findings are emphasised by analysing time series, which allows for an assessment of the persistence of an effect.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The authors state the main importance of the olfactory sense for the salmons survival. It is not discussed if the extent and duration of a reduction of the EOG may have ecological consequences to natural populations of salmons. The experimental assembly was very artificial and could cause stress whilst preventing behavioural responses of the fish (e.g. avoidance of exposure). The transferability into realistic scenarios seems to be difficult.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphosate was tested as the technical substance, bearing in mind the enhanced toxicity of formulations containing surfactants such as POEA. The results are considered relevant for ERA
2 Do the tested concentrations relate to predicted environmental concentrations?	It relates more to the standard toxicity endpoints than to environmental concentrations.
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	**Very interesting and well conducted study with a comprehensive description of the experimental design and statistics. The endpoints are considered ecologically relevant, but the validity and relevance are lowered by the artificial design.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Soso et al. (2007)

glyphecotox_021 Fish	**Soso, A.B., Barcellos, L.J.G., Ranzani-Paiva, M.J., Kreutz, L.C., Quevedo, R.M., Anziliero, D., Lima, M., da Silva, L.B., Ritter. F., Bedin, A.C., Finco, J.A.**	**2007**	**Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundia (Rhamdia quelen)**	**Environmental Toxicology and Pharmacology 23 (3):308-313. DOI 10.1016/j.etap.2006.11.008.**

Reliability	
Purpose of the study Description of endpoints	Description of the effect of a glyphosate based herbicide on hormones of female Rhamdia quelen Gonado-somatic index (GSI) and liver-somatic index (LSI); Cortisol (F), 17 –beta-estradiol (E2) and testosterone (T) concentrations; swim-up fry production; liver enzymes AST (aspartate aminotransferase), ALT (alanine aminotransferase)
Test compound, application procedure, exposure period	Roundup WG, 640 g a.i./kg powder weight, reapplied every 9th day of the experimental period, Renewal experiment, Water concentration: 3.6 mg a.i./L

Experimental approach, Statistics, test environment	Eight females per treatment and sampling date were sampled prior to glyphosate inoculation and at 1, 10, 20, 30 and 40 days following exposure Student's t-test or ANOVA followed by Tukey's multiple range test	
Test organisms	Adult Jundia, Rhamdia quelen, a South American catfish, 400-600 g body weight	
Biological effects	No significant differences between control and treatment groups for both GSI and LSI at any of the sampling dates; slightly higher concentrations of cortisol at day 20 and 40, lower conc. of estradiol at day 40, no differences in testosterone levels, fertility parameters were only significantly lowered in the treatment group for the endpoint 'transferred swim-up fry'	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?	As reproduction parameters have been tested.	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The cortisol levels of the treatment group have been slightly higher from the start of the experiment. The authors argue that fish have been generally stressed by the experimental environment. This could lead to non-representative responses to additional stress events (such as toxic, chemical stressors). The very indiscernible or inconsistent effects might be of minor ecological meaning.	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Since not only biochemical endpoints as indicators have been measured but also the realised reproduction rate as the number of viable fry has been measured, a comparison of different endpoints and thus an appropriate assessment is possible.	
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Except of the indication that the glyphosate formulation consists of ‚water-dispersible granules' (WG), no further specification of the test substance was made
2 Do the tested concentrations relate to		The single concentration used is far above expectable concentrations
predicted environmental concentrations?		in the environment (roughly around 500 µg a.i./L).
3 Have parameters influencing the endpoints been considered adequately?		No
Concluding weight of evidence/proposed action		**The design of the study as a limit-test makes the interpretation of the tendencies and results difficult. The study has shown slight effects at elevated concentrations. An extrapolation to lower doses would most probably not reveal significant effects, in the given statistical design. The POEA content of the formulation was not stated.**
Type of information (Critical, supporting, low weight)		**supporting**
Consideration/concluding score		**UBA2**

De Menezes et al. (2011)

glyphecotox_338 Fish	De Menezes, C.C., da Fonseca, M.B., Loro, V.L., Santi, A., Cattaneo, R., Clasen, B., Pretto, A., Morsch, V.M.	2011	Roundup Effects on Oxidative Stress Parameters and Recovery Pattern of *Rhamdia quelen*	Archives of Environmental Contamination and Toxicology 60 (4):665-671. DOI: 10.1007/s00244-010-9574-6.

Reliability	
Purpose of the study Description of endpoints	The impact of the herbicide Glyphosate in the commercial formulation Roundup was tested on enzyme biomarkers in tissues of the juvenile silver catfish (Rhamdia quelen) Lipid peroxidation (LPO, thiobarbituric acid reactive species assay), Protein carbonylation both in liver, brain and muscle tissues Oxidative stress by Catalase enzymatic activity, by Superoxide dismutase activity, by Glutathion S-transferase levels, by nonenzymatic antioxidants (ascorbic acid, nonprotein thiols)
Test compound, application procedure, exposure period	Glyphosate as 'Roundup 48%' (control, 0.45 mg a.i./L, 0.95 mg a.i/L), containing POEA as surfactant After 4 days, 50% water renewal an new application of a.i. to maintain exposure levels (amount not specified, but measured concentrations prove the sufficient exposure)
Experimental approach, Statistics, test environment	Exposure for 8 days, recovery period in clean water for further 8 days 3 treatments, 2 replicate 250 L tanks, 8 fish per tank Two-way ANOVA, followed by Tukey´s post-hoc test, N = 8 was taken as the replication in statistical tests
Test organisms	Juvenile *Rhamdia quelen* fish (mean 20 g weight, 11 cm length) from aquaculture
Biological effects	Oxidative stress markers, as Lipid peroxidation and protein carbonyl levels, were significantly affected in most tissues after the exposure period, but returned to the control level after the recovery period Data for 5 antioxidant endpoints showed only very few deviations from control (GST, which decreased during exposure and increased after recovery periods), this was during the exposure and the recovery period
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, since juveniles are often recognized as the most sensitive life-stages of fish species towards chemical stressors
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Overall, there was little indication of significant responses towards the stressor 'Roundup48'. The experimental period was relatively short-termed (acute to sub-chronic test design), and thus a population relevant effect could not be extrapolated by the results of this experiment.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Principally, biomarkers of stress could be taken as general indicators of toxic action of a test compound that could have an effect at higher levels of organisation, e.g. population level. The degree of uncertainty for the extrapolation to population level, which is relevant for ERA is unknown. The results could be taken as a hint that the substance is detoxified by the test organisms, which could lead to highly reactive Oxygen species.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The test substance was Roundup containing POEA. The surfactants are likely to cause a significant portion of the observed effects
2 Do the tested concentrations relate to predicted environmental concentrations?	The concentrations tested were far above expectable in the environment but at the suppliers' recommended concentrations in flooded rice cultures in tropical regions. The concentrations within the ERA in Europe are derived from non-rice cultures and thus not transferable without further considerations to the tested scenario..

3 Have parameters influencing the endpoints been considered adequately?	None
Concluding weight of evidence/proposed action	**There was little indication of adverse effects of the tested formulation on biomarker concentrations in different tissues of *Rhamdia quelen* at elevated concentrations compared to predicted environmental concentrations after spray application of Glyphosate. The findings support the classification of Glyphosate even in a formulation containing the potentially more toxic POEA as non to moderately toxic towards fish. Nevertheless, it cannot be distinguished between the effect of POEA and glyphosate.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Kreutz et al. (2011)

glyphecotox_434 Fish	**Kreutz, L.C., Barcellos, L.J.G., de Faria Valle, S., de Oliveira Silva, T., Anziliero, D., dos Santos, E.D., Pivato, M., Zanatta, R.**	**2011**	**Altered haematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate**	**Fish Shellfish Immunol 29 (4):694-7. DOI: 10.1016/j.fsi.2010 .06.003.**

Reliability	
Purpose of the study Description of endpoints	The impact of sublethal doses of glyphosate was tested on haematological and immunological responses of Silver catfish fingerlings Number of erythrocytes, lymphocytes, thrombocytes, total leukocytes, immature circulating cells, phagocytic index of coelomic cells, lysozyme, total peroxidase, bacteria agglutination, bactericidal activity, haemolytic activity, in serum
Test compound, application procedure, exposure period	commercial available glyphosate (N-phosphonomethyl glycine, 360 mg/L) 10% of the LC_{50} after 96 hours of silver catfish was tested = 0.730 mg a.i./L, static exposure Exposure period for haematological parameters was 96 hours, immunological endpoints were measured after 24 hours and 10 days
Experimental approach, Statistics, test environment	7-10 fish per tank, triplicate tanks, but replication used for statistics was 7 (fish individuals per treatment)
Test organisms	Male and female fingerlings of silver catfish (*Rhamdia quelen)*, 18±8 g weight for immunological studies, juveniles of 80-100 g for haematological studies,
Biological effects	Total leukocytes, lymphocytes, thrombocytes and erythrocytes counts were significantly lower ($p < 0.01$), and the number of circulating immature cells were significantly higher ($p < 0.01$) Haematocrit, monocytes and neutrophil, as well as glucose and total plasma proteins were not different between the groups significant reduction ($p < 0.05$) of phagocytic index after 24h, no effect after 10 days No effect on bactericidal activity Natural bacterial agglutination titer measured against formalin-inactivated pathogenic A. hydrophila was significantly lower ($p < 0.05$) in glyphosate exposed fingerlings, either at 24 h or 10 days Serum lysozyme: lowered after 10 days, myeloperoxidase: lowered after 24 h, natural complement haemolytic activity: no effect

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The threshold for considering observed effects as significant was set to 1% Type I-error probability, which points together with the numerous positive and negative significant deviations from the control level to a low variability of measurements and thus a high reliability of the statistics.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	A common methodology of transferring concentrations of immunological relevant blood cells to higher levels of organisation and thus to draw conclusions for an environmental risk on population level is scarcely available. Assuming large interspecies differences in critical concentrations leading to an effect on an individual and the lack of a well-established reference system for Rhamdia quelen, there is no indication of the results of this study for an ERA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, for glyphosate without surfactant addition.
2 Do the tested concentrations relate to predicted environmental concentrations?	In the of expectable concentrations (PEC surface water about 0.5 mg a.i./L
3 Have parameters influencing the endpoints been considered adequately?	No, but conditions have been held sufficiently constant amongst the experimental units.
Concluding weight of evidence/proposed action	**The study could be taken as supplementary information that environmentally relevant concentrations of glyphosate could induce subtle changes of the haematological status of fish. It could not be assessed if those changes have the potential to affect the health status of an individual and thus to cause effects relevant for a whole population.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Kreutz et al. (2008)

glyphecotox_436 Fish	**Kreutz, L.C., Barcellos, L.J.G., Silva, T.O., Anziliero, D., Martins, D., Lorenson, M., Marteninghe, A., da Silva, L.B.**	**2008**	**Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings**	**Fish Shellfish Immunol 30 (1):51-7. DOI: 10.1016/j fsi.2010.09.012.**

Reliability	
Purpose of the study Description of endpoints	The mortality (LC_{50}) caused by different pesticides (amongst them the herbicide glyphosate) was determined for silver catfish (*Rhamdia quelen)* fingerlings Mortality after 96 h
Test compound, application procedure, exposure period	Glyphosate as Roundup (N-phosphonomethylglycine), (360g L^{-1}) 2, 4, 8, 16, 32 mg a.i./L under static conditions
Experimental approach, Statistics, test environment	210 fingerlings uniformly distributed in 21 40-L plastic aquaria 5 concentrations, 3 replicates per treatment; 96 hours exposure period During acclimatisation period, 20 % water exchange per day, after treatmet exchange was stopped
Test organisms	*Rhamdia quelen* (silver catfish) fingerlings, 60-day-old mixed-sex fingerlings weighing between 2 and 4g

Biological effects	LC_{50} after 96 hours = 7.3 (6.5 – 8.2) mg a.i./L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The 50% lethal dose was deduced from a 'useful' and usual statistical design and could thus be considered as relevant for the population of silver catfish as other acute mortality studies
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Yes, as mortality under laboratory conditions and acute exposure is commonly agreed
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The 50% lethal dose was deduced from a 'useful' and usual statistical design and could thus be considered as relevant for the population of silver catfish as other acute mortality studies
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, most probably the Roundup formulation contained POEA as surfactants, which could also explain the elevated toxicity of the test item compared to other studies with different fish species reported in the literature (cited herein).
2 Do the tested concentrations relate to predicted environmental concentrations?	Not relevant, since that was an acute dose-response test design to derive an LC50 from a Probit distribution.
3 Have parameters influencing the endpoints been considered adequately?	Conditions in the test containers have been maintained at non-harmful ranges.
Concluding weight of evidence/proposed action	**The LC_{50} for the exposure of *R. quelen* reported here is far below toxicities reported from other acute studies with fish under laboratory conditions. This is most probably due to the composition of the tested formulation of glyphosate (Roundup), as discussed by the authors as well. The study can be seen as additional evidence of enhanced toxicity caused by the POEA in glyphosate formulations. However, the study is not suited to trigger the aquatic risk assessment of glyphosate.**
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Folmar et al. (1979)

glyphnosubm_012	Folmar, LC, Sanders HO, Julin AM	1979	Toxicity of the Herbicide Glyphosate and Several of Its Formulations to Fish and Aquatic Invertebrates.	Archives of Environmental Contamination and Toxicology 8: 269-278

Reliability

Purpose of the study Description of endpoints	Comparison of the acute toxicity of the technical grade glyphosate, the formulated herbicidal product 'Roundup' and the tallow amine surfactant. Mortality of rainbow trouts, fathead minnows, channel catfish, bluegills, determination of LC_{50} 24 and 96 hours after exposure. Additionally midges, scuds, daphnids were tested (also after 48h). The paper is mainly referred in the POEA section of the literature survey in DocM for the comparison between surfactant POEA and the active substance glyphosate. The year of publication was beyond the 10-years scope of the literature collection and thus discarded by the notifier. Here, the acute laboratory part of the study is reported only with no consideration of temperature effects and other aspects covered by this publication. Additional tests regarded avoidance behaviour, reproductive potential and stream drift of different organisms.
Test compound, application procedure, exposure period	Technical glyphosate (Isopropylamine salt, 480.42 g/L), Roundup with surfactant (360.32 g/L), surfactant Protocol: Methods recommended for static toxicity testing (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975) Static exposure of fish and amphibians for 24 and 96 hours
Experimental approach, Statistics, test environment	The exact number and spacing of concentrations tested in the acute studies were not reported in the publication 10 fish per test concentration have been tested at 12°C (O. mykiss) and 22°C water temperature (other species). Methods of Litchfield and Wilcoxon to derive LC50's (and (EC50's for invertebrates)
Test organisms	Rainbow trout (*Oncorhynchus mykiss*, synonym Salmo gairdneri), Fathead minnows (*Pimephales promelas*), Channel catfish (*Ictalurus punctatus*), Bluegills (*Lepomis macrochirus*)
Biological effects	The paper reports acute LC_{50}-values for four fish species (in mg/L)

	Glyphosate acid		*Roundup*		*POEA*	
Species	24h	96h	24h	96h	24h	96h
Oncorhynchus mykiss	140.0	140.0	8.3	8.3	2.1	2.0
Pimephales promelas	97.0	97.0	2.4	2.3	1.4	1.0
Ictalurus punctatus	130.0	130.0	13.0	13.0	18.0	13.0
Lepomis macrochirus	150.0	140.0	6.4	5.0	3.0	3.0

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints

Biological Relevance

1 Is an appropriate test species/ life-stage(s) studied?	Yes, mainly standard test species have been tested
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The endpoint mortality in acute studies with single species is of biological significance indeed and a widely accepted assessment aspect for the aquatic environment.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes, see above points 1 and 2

Environmental Relevance

1 Is the substance tested representative and relevant for the substance being assessed?	The comparison between the POEA surfactant, technical grade glyphosate and the formulated product Roundup touches the key concern on the use of the herbicide glyphosate. It allows for factoring out the toxicity of the two components of a mixture surfactant and active ingredient.

2 Do the tested concentrations relate to predicted environmental concentrations?	The tested concentrations were not reported in the publication, which is a strong deficit and could be regarded the only reason to reject the revision of the results.
3 Have parameters influencing the endpoints been considered adequately?	PH and temperature were tested systematically. At suboptimal conditions of pH and temperatures the toxicity of the surfactant and Roundup in particular. was increased.
Concluding weight of evidence/proposed action	**The paper is considered one of the key publications on the enhancing effect of adding tallow amine surfactants to glyphosate-based herbicides and could not be ignored even in a recent risk assessment. Since the concentration series and the spacing factors were not described appropriately, the study has formally a low reliability. Nevertheless, it could be shown that most of the toxicity of the product was due to the POEA. Nowadays, products formulated by means of POEA are not expected to be neither notified nor registered in the future. The publication supports this practice.** Note: **the review in glyphnosubmit_540 falsely reports that here the glyphosate acid was tested, whereas the IPA salt was applied.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Evrard et al. (2010)

glyphecotox_367 Fish	**Evrard, E., Marchand, J., Theron, M., Pichavant-Rafini, K., Durand, G., Quiniou, L., Laroche, J.**	**2010**	**Impacts of mixtures of herbicides on molecular and physiological responses of the European flounder *Platichthys flesus***	**Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 152 (3):321-331. DOI: 10.1016/j.cbpc.2010.05.009.**

Reliability

Purpose of the study Description of endpoints	Effects of glyphosate (Roundup Ultra) and its first metabolite AMPA on the physiological response of the European flounder (*Platichthys flesus*) using genetic transcription patterns Real-time PCR assays were conducted on several candidate transcripts identified by the SSH assay (mRNA analysis) as indicators of liver injury Nine gene transcripts in liver were analysed: betaine homocysteine methyltransferase (BHMT) transcript; apolipoprotein E1 transcript; chemotaxin (LECT2) transcript; α-2-macroglobulin transcript; anti thrombin III transcript; C1 inhibitor precursor (C1Inh) transcript; ubiquitin transcript; ATP synthase Fo subunit 6 transcript; cytochrome B transcript. Blood parameters and the physiological 'condition factor'
Test compound, application procedure, exposure period	Measured mean concentrations [nominal] over 62 days of exposure G-tank: Roundup Ultra, with unknown contents and identities of surfactants and the glyphosate salt *0.16 µg/L* [2 µg/L] plus AMPA *2.27 µg/L [2 µg/L]* GAMA2-tank Glyphosate 0.15 µg L [1.25 µg/L]+ AMPA 1.53 µg/L [1.25 µg/L]+ mecoprop 0.27 µg/L − [0.5 µg/L]+ acetochlor 0.36 µg/L [0.5 µg/L] + 2,4D 0.23 µg/L [0.5 µg/L]
Experimental approach, Statistics, test environment	3 replicate fish per treatment were taken from one of the three tanks (control, G, GAMA2) One sampling prior exposure, 3 sampling dates after exposure 15, 32, 62 days after treatment
Test organisms	European flounder (*Platichthys flesus*)

Biological effects	Results reported here only for G-tank: BHMT, Apolipoprotein E1, Chemotaxin, macroglobulin and ATPase were highly significantly altered 62 days after exposure at 0.16 µg glyphosate/L No impacts on physiological indices
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The extrapolation from gene transcript alteration to populations of corresponding fish species or even all fish should be proven by experiments that address the question of population vitality at the same test concentrations which is not discussed by the authors
3 Is the ecotoxicological manifestation level appropriate for the assessment?	See point 2 above
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Two different difficulties with the test diminish the relevance of the test for an ERA: a complex mixture was tested and the composition regarding surfactants is unknown. The combination AMPA + glyphosate could be taken as representative for glyphosate because AMPA is the main metabolite and the measured concentration of glyphosate is assigned the relevant final concentration
2 Do the tested concentrations relate to predicted environmental concentrations?	Conc. are far below highest expectable PECinis and thus of high environmental relevance.
3 Have parameters influencing the endpoints been considered adequately?	No particular test design to check for influencing parameters
Concluding weight of evidence/proposed action	**Main finding: Low concentrations of glyphosate are suited to alter the gene expression patterns of the liver of European flounders.** **There are many uncertainties in transferring the results of this study to populations of fish, which is the relevant organisational level for an environmental risk assessment. A simple and a complex mixture has been tested so far, that causes a limited use of the results.**
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2**

Cavalcante et al. (2008)

glyphecotox_316	**Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H.**	**2008**	**Genotoxic effects of Roundup® (R) on the fish *Prochilodus lineatus***	**Mutation Research-Genetic Toxicology and Environmental Mutagenesis 655 (1-2):41-46. DOI 10.1016/j mrgentox.2008.06.010.**

Reliability	
Purpose of the study Description of endpoints	The aim of this work was to evaluate the genotoxic effects of Roundup®® in P. lineatus acutely exposed to the herbicide for differentperiods, using the comet assay, micronucleus test and the occurrence of erythrocytic nuclear abnormalities (ENAs).
Test compound, application procedure, exposure period	Roundup®® (360 g glyphosate L−1 or 41% of glyphosate, Monsanto Brazil ThisRoundup®® concentration Test concentration not directly stated. corresponds to 75% of the LC50 of this herbicide to P. lineatus The 96 h-LC50 of Roundup® was 13.69 mg L−

Experimental approach, Statistics, test environment	Cell viability assay for erythrocytes and gill cells using the trypan blue exclusion method; Alkaline comet assaywas performed according to Singh et al. and Speit and Hartmann with some modifications as described by Vanzella et al. Two- tailed Student t test. Differences between meanswere considered significant when $p < 0.05$.
Test organisms	Juveniles of Prochilodus lineatus (Valenciennes, 1847), with 9.6±5.4 g and 9.7±1.81cm (mean±S.D., N= 50), were supplied by the Hatchery Station of Londrina State University.
Biological effects	In the micronucleustest micronucleus (MN) and erythrocytic nuclear abnormalities (ENA) were not significantly different from the respective negative controls. Comet assay showed significant effects towards DNA damage in erythrocytes.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The test species are considered of temperate to sub-tropical origin. Indication of species variability for the standard in ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Cell viability, nuclear abnormalities dependent on repair mechanisms
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Traditionally, survival, growth and reproduction of individuals are chosen as endpoints of the classic laboratory tests for ecotoxicity. No mortality assessed, low relevance for traditional ERA
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation , the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Testing exceeded environmentally realistic concentrations.
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	**Physiological study with the commercial formulation . No distinction between the activie substance and surfactants.**
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Langiano et al. (2008)

glyphecotox_452	**Langiano, V.dC., Martinez, C.B.R.**	**2008**	**Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus***	**Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 147 (2):222-231**

Reliability	
Purpose of the study Description of endpoints	Toxicity of Roundup® to P. lineatus and to evaluate the responses of this fish at biochemical, physiological and histological levels, after acute exposure to sub-lethal concentrations of the herbicide
Test compound, application procedure, exposure period	7.5 and 10 mg L−Roundup®
Experimental approach, Statistics, test environment	Parameteres observed mortality ans histological alterations , physiology, Student's t-test, ANOVA
Test organisms	Neotropical fish *Prochilodus lineatus*

Biological effects	Exposure to sub-lethal concentrations of Roundup® promoted an increase in plasma glucose, indicating a typical response to stress. The induction of liver catalase activity indicates the activation of antioxidant defenses, probably due to increased hydrogen peroxide generation. Roundup® exposure also induced a variety of liver histological alterations that might impair normal organ functioning. 96 h-LC50 of Roundup® was 13.69 mg L−
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	The test species are considered of temperate to sub-tropical origin. Indication of species variability for the standard in ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Parameteres observed mortality ans histological alterations
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Traditionally, survival, growth and reproduction of individuals are chosen as endpoints of the classic laboratory tests for ecotoxicity
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	Testing exceeded environmentally realistic concentrations
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	*Prochilodus lineatus* is more sensitive to Roundup® than rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar).* Physiological study with the commercial formulation . No distinction between the activie substance and surfactants
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Ferreira et al. (2010)

glyphecotox_376	**Ferreira, D., da Motta, A.C., Kreutz, L.C., Toni, C., Loro, V.L., Barcellos, L.J.G.**	**2010**	**Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals**	**Chemosphere 79 (9):914-921. DOI: 10.1016/ j.chemosphere.2010.03.024.**

Reliability	
Purpose of the study Description of endpoints	Verification whether MP, Gly, and Teb are potential oxidative stress inducers in R. quelen, and whether their effects could provoke histopathological changes in the liver of this fish species.
Test compound, application procedure, exposure period	Commercial formulation containing the herbicide glyphosate (N-phosphonomethylglycine). 6.6% of the LC50–96h, as previously determined by Kreutz et al. (2008 (glyphosate based herbicide (1.21 mg L_1 of Roundup®™).)
Experimental approach, Statistics, test environment	Physiological study evaluating oxidative stress, enzymitic repsonses, ANOVA
Test organisms	*R. quelen*

Biological effects	Survival rate eas not altered at taht concentrations. Glyphosate containing product did not alter reactive substances in liver, but decrease in CAT activity,no visible histological changes
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	American species. Indication of species variability for the standard in ERA.
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	No visible histological changes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	No visible histological changes. Traditionally, survival, growth and reproduction of individuals are chosen as endpointsf the classic laboratory tests for ecotoxicity
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formualtion not stated. No information about surfactants.
2 Do the tested concentrations relate to predicted environmental concentrations?	0.185 mg /l probably realitsic worse case concentrations
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	No visible histological changes, Indication for general fitness
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Haller et al. (2003)

glyphecotox_399	**Haller, W.T., Stocker, R.K.**	**2003**	**Toxicity of 19 adjuvants to juvenile Lepomis macrochirus (bluegill sunfish)**	**Environmental Toxicology and Chemistry 22 (3):615-619**

Reliability	
Purpose of the study Description of endpoints	Nineteen adjuvants, many used as surfactants for aquatic herbicide applications, were applied in static bioassay to bluegill sunfish (Lepomis macrochirus) for 96 h to determine median lethal concentrations (LC50).
Test compound, application procedure, exposure period	MON 0818
Experimental approach, Statistics, test environment	Surfactants are added to the tank mix as a percentage (v/v) of the total volume, in contrast to herbicide application rates,
Test organisms	bluegill sunfish
Biological effects	Ethoxylated tallow amine products were the most toxic, having LC50 values of 1.6 and 2.9 ppm Seven alcohol/glycol-based surfactants had 96-h LC50 values of 4.0 to 11.6 ppm polysiloxane- or siliconebased surfactants had toxicities of 18.1 to 29.7 ppm limonene- based products had LC50 values of 10.2 and 30.2 ppm.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Lepomis macrochirus*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	mortality

3 Is the ecotoxicological manifestation level appropriate for the assessment?	While toxicity of adjuvants has not been a focus of concern for aquatic applications, the data reported here will give resource managers guidance into the acute toxicities of some of the commercially available adjuvants and assist in the development of invasive plant management programs with an acceptable margin of safety.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	POEA: MON 0818
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Monsanto's MON 0818 and Entry II are 68 to 73% and 35% ethoxylated tallow amine surfactants, respectively, that have been used in glyphosate formulations. The material safety data sheet for MON 0818 lists 96-h toxicity to bluegill sunfish at 1.3 ppm, similar to the 1.6-ppm LC_{50} obtained in this study.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Zhidenko et al. (2007)

glyphecotox_670	**Zhidenko, A.A., Kovalenko, Y.M.**	**2007**	**The influence of Roundup® on the dynamics of histological changes in organs of carps**	**Hydrobiological Journal 43 (2):93-99**

Reliability	
Purpose of the study Description of endpoints	Identification of the dynamics of histological parameters in carp organism under the action of Roundup® (0.004 mg/dm3) and their possible influence on functional deviations in fish were the aim of this study.
Test compound, application	Roundup®
procedure, exposure period	
Experimental approach, Statistics, test environment	Histological observations
Test organisms	Two-year-old carps (*Cyprinus carpio L.)* weighing 200–300 g
Biological effects	Action of Roundup® at its 0.004 mg/dm3 contents in water environment leads to ambiguous alterations in organs of carp. The least deviations have taken place in the brain and gills, insignificant abnormalities were in the intestine and the greatest were in the muscles and liver; the latter organs are the most sensitive. Histologic changes in the liver of carp, which are connected with the granular and vacuolar-drop dystrophy, lead to the death of hepatocytes and to necrotic changes and, as a consequence, to the functional liver failure and to the formation of bilestones. The muscle fiber hypotrophy under the influence of Roundup® leads to destructive changes in skeletal muscles.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Cyprinus carpio L*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Histologic changes in the liver of carp, which are connected with the granular and vacuolar-drop dystrophy, lead to the death of hepatocytes and to necrotic changes and, as a consequence, to the functional liver failure.

3 Is the ecotoxicological manifestation level appropriate for the assessment?	Histological observations are Indication for general fitness. Survival, growth and reproduction of individuals are chosen as endpoints of the classic laboratory tests for ecotoxicity
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Although not specified precisely, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Environmentally realistic concentrations have been used (0.004 mg/l)
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Action of Roundup® and environmentally realistic concentrations leads to alterations in organs of carp which might lead to functional changes in organ function.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Ortiz-Ordoñez et al. (2003)

glyphecotox_532	**Ortiz-Ordoñez, E., Uría-Galicia, E., Ruiz-Picos, R.A., Duran, A.G.S., Trejo, Y.H., Sedeño-Díaz, J.E., López-López, E.**	**2011**	**Effect of Yerbimat Herbicide on Lipid Peroxidation, Catalase Activity, and Histological Damage in Gills and Liver of the Freshwater Fish *Goodea Atripinnis***	**Archives of Environmental Contamination and Toxicology 61 (3):443-452. DOI: 10.1007/s00244-011-9648-0.**

Reliability	
Purpose of the study Description of endpoints	Determination o facute toxicity and , evaluate biochemical parameters changes due to exposure to Yerbimat.
Test compound, application procedure, exposure period	In Mexico, one of the most widely used glyphosate-based herbicides is Yerbimat, which has agricultural as well as aquatic weed control applications
Experimental approach, Statistics, test environment	– static bioassay at 96 h (LC50) – chronic exposure (75 days) – Probit Analysis v.1.5 software.
Test organisms	*Goodea atripinnis* 6.0 ± 0.5 cm standard length and 3.0 ± 0.5 g weight)
Biological effects	The 96-h LC50 value was 38.95 ± 0.33 mg/L. Yerbimat induced significant decreases in CAT activity in the gills of 9.88 and 53.3% at 1/10 of the LC50 nd 1/5 of the LC50, respectively, compared to the control group. Hypertrophy was evidenced by loss of the normal structure of the gills, and gill filaments were nflamed due to the abnormal size of the cells at 30–75 days of exposure, hepatic cells displayed increasing vacuolation, in which vacuoles increased in both number and size, and nuclei were displaced toward the cell periphery.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Mexican fish species
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Histological alterations in the gills and liver that might impair normal organ functioning

3 Is the ecotoxicological manifestation level appropriate for the assessment?	Mortalitiy and biochemical alterations, Indication for general fitness
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation, probably containing relevant toxic surfanctants , probably POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	close to those environmental values estimated
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Biochemical damage, as evidenced by high LPX and CAT inhibition in gill tissue, was apparent following chronic Yerbimat exposure, indicative of damage due to oxidative stress, might lead to cellular damage and death
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Cattaneo et al. (2003)

glyphecotox_315	**Cattaneo, R., Clasen, B., Loro, V.L., de Menezes, C.C., Pretto, A., Baldisserotto, B., Santi, A.L., de Avila, L.A.**	**2011**	**Toxicological Responses of *Cyprinus carpio* Exposed to a Commercial Formulation Containing Glyphosate**	**Bulletin of Environmental Contamination and Toxicology 87 (6):597-602. doi: 10.1007/s00128-011-0396-7.**

Reliability	
Purpose of the study Description of endpoints	The effects of commercial glyphosate herbicide formulation on the activity of acetylcholinesterase (AChE) enzyme and oxidative stress were studied in Cyprinus carpio
Test compound, application procedure, exposure period	Roundup® (648 g/L of isopropylamine salt of Glyphosate, 480 g/L of acid equivalent Ghyphosate and 594 g/L of inert ingredients), at concentrations of 0 (without herbicide), 0.5, 2.5, 5.0 and 0.0 mg/L.
Experimental approach, Statistics, test environment	Exposition for 96 h to 0.0, 0.5, 2.5, 5.0 and 10.0 mg/L and then allowed to equal recovery period in water without herbicide. Tissues samples (brain and muscle) were obtained, two-way ANOVA followed by Tukey–Kramer multiple range tests.
Test organisms	*Cyprinus carpio*
Biological effects	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	AChE in brain and muscle. The inhibition by glyphosate might lead to an accumulation of acetylcholine, causing the stimulation of the receptors.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Indication for general fitness
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial herbicide formulation containing the active ingredient glyphosate. It also contains the surfactant, POEA, which is known to be more toxic than glyphosate to fish.
2 Do the tested concentrations relate to predicted environmental concentrations?	Probably exceed worse case concentrations.
3 Have parameters influencing the endpoints been considered adequately?	nd

Concluding weight of evidence/proposed action	Short-term exposure can affect their physiological conditions, nevertheless no discrimination between glyphosate and POEA possible.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Modesto et al. (2003)

glyphecotox_511	**Modesto, K.A., Martinez, C.B.R.**	**2010**	**Effects of Roundup® Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity**	**Chemosphere 81 (6):781-787. DOI: 10.1016/j.chemosphere.2010.07.005**
Reliability				
Purpose of the study Description of endpoints	The objective of this work was to evaluate its effects on hematological and biochemical parameters of P. lineatus.			
Test compound, application procedure, exposure period	Roundup® Transorb_ (480 g glyphosate/ L at two nominal concentrations 1 and 5 mg /L was used)			
Experimental approach, Statistics, test environment	Blood samples for hematological analysis, liver for antioxidants analysis, and brain and muscle for acetylcholinesterase (AChE) determination			
Test organisms	Neotropical fish Prochilodus lineatus.			
Biological effects	No fish mortality in any of the experimental groups Hematologic alterations appeared only after 96 h exposure, when fish showed an increase in the hematocrit and in the number of both red and white blood cells, lipid peroxidation (LPO) returned to control levels after 24 and 96 h exposure to RDT			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Mortality and enzymativ parameters			
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	The exposure to RDT for 96 h led to an inhibition of AChE in brain and muscle but at rates which may not be considered a life-threatening situation.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Indication for general fitness			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup® Transorb is a commercial formulation probably containing surfactants. Limited validity regarding effects of Glyphosate that does not contain the same surfactant.			
2 Do the tested concentrations relate to predicted environmental concentrations?	Exceeding the predicted concentrations			
3 Have parameters influencing the endpoints been considered adequately?	nd			

Concluding weight of evidence/proposed action	Hematological parameters in fish can significantly change in response towards chemical stressors; however, these alterations are non-specific to a wide range of substances. after 24 and 96 h the antioxidant defenses were apparently enough to combat ROS, preventing the occurrence of oxidative damage. The exposure to RDT for 96 h led to an inhibition of AChE in brain and muscle but at rates which may not be considered a life-threatening situation.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Evrard et al. (2003)

glyphecotox_367	**Evrard, E., Marchand, J., Theron, M., Pichavant-Rafini, K., Durand, G., Quiniou, L., Laroche, J.**	**2010**	**Impacts of mixtures of herbicides on molecular and physiological responses of the European flounder Platichthys flesus**	**Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 152 (3):321-331. DOI: 10.1016/j.cbpc.2010.05.009.**

Reliability	
Purpose of the study Description of endpoints	The effects of a simple mixture of a glyphosate-based formulation and AMPA and of a more complex mixture of herbicides (glyphosate/AMPA/ mecoprop/acetochlor/2,4D) were explored on the molecular and physiological responses of the European flounder *Platichthys flesus*
Test compound, application procedure, exposure period	Roundup® Ultra solution contains the monoisopropylamine salt of N glyphosate and surfactants that were not identified in terms of chemical composition and concentration on the product label. The corresponding percentage of Roundup® solution was 0.0055% in the glyphosate/AMPA tank (G tank) nominal concentrations of 2 µg L–1 glyphosate (from Roundup® solution) and 2 µg L–1 AMPA; this was known as the G tank
Experimental approach, Statistics, test environment	Flounder were sampled after 0, 15, 32 and 62 days of exposure Suppression subtractive hybridization, mRNA expression analysis, Blood samples and physiological measurements, Principal Component Analysis (PCA).
Test organisms	Juvenile flounders *P. flesus* (n=300, length=7–12 cm)
Biological effects	Thus, no significant difference was detected in the variation patterns of physiological parameters between contaminated and control fishes during the experiment; expression of three markers among the nine tested, namely BHMT, apolipoprotein E1 and chemotaxin, was altered by both types of pesticide mixture. these genes being implicated in stress response, but also in multiple biochemical pathways linked to the responses to abiotic and biotic factors of the experimental environment (light, salinity, social interaction, feeding…).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	European flounder *Platichthys flesus*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	level of assessment is not appropriate for population level effectsn LC_{50} stated..

3 Is the ecotoxicological manifestation level appropriate for the assessment?	Traditionally, survival, growth and reproduction of individuals are chosen as endpoints of the classic laboratory tests for ecotoxicity
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation Roundup® Ultra
2 Do the tested concentrations relate to predicted environmental concentrations?	Environmentally relevant concentrations of herbicides (4 µg/L)
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Significant alterations of liver gene expressions were detected for contaminated vs control fishes, particularly at the levels of methionine metabolism, lipid transport and metabolism, immunity and respiratory chain
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Smith et al. (2003)

glyphecotox_602	**Smith, B.C., Curran, C.A., Brown, K.W., Cabarrus, J.L., Gown, J.B., McIntyre, J.K., Moreland, E.E., Wong, V.L., Grassley, J.M., Grue, C.E.**	**2004**	**Toxicity of four surfactants to juvenile rainbow trout: Implications for use over water**	**Bulletin of Environmental Contamination and Toxicology 72 (3):647-654. DOI 10.1007/s00128-004-0292-5.**

Reliability	
Purpose of the study Description of endpoints	Comparison of 4 surfactants using effect on survival and behaviour as endpoints
Test compound, application procedure, exposure period	R-11, Li700, HaSTEN, Agri DEX
Experimental approach, Statistics, test environment	96h static acute test(USEPA 1996)
Test organisms	*Onccorhynchus mykiss*
Biological effects	Erratic swimming, , onbotom gilling, inability to maintain horizontal orinetation R11: LC50 96h = 6ppm Li700: LC50 96h = 17ppm HASTEN: LC50 96h = 74ppm Agri DEX : LC50 96h = 271ppm
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Onccorhynchus mykiss*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Specific Surfactant toxicity has limited validity regarding effects of products with different surfactants. Nevertheless, shows significance to evaluate on product level.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Surfactant toxicity was assessed
2 Do the tested concentrations relate to predicted environmental concentrations?	No MW stated

3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Surfactant apos envrionmental hazard, displaying non-specific narcosis .
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Kreutz et al. (2003)

glyphecotox_436	**Kreutz, L.C., Barcellos, L.J.G., Silva, T.O., Anzilierol, D., Martins, D., Lorenson, M., Marteninghe, A., da Silva, L.B.**	**2008**	**Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings**	**Fish Shellfish Immunol 30 (1):51-7. DOI: 10.1016/j fsi.2010.09.012.**
Reliability				
Purpose of the study Description of endpoints	Investigate the acute toxicity and the lethal concentration (LC50) of four herbicides, two fungicides and two insecticides to silver catfish fingerlings			
Test compound, application procedure, exposure period	Roundup® , 540-2160g/ha			
Experimental approach, Statistics, test environment	For the LC_{50} determinations, 210 fingerlings were uniformly distributed in 21 40-L plastic aquaria, keeping fish density below or equal to 1g /L, according to the Brazilian Association for Technical Rules (ABNT). Each product was tested using 5 to 6 different concentrations, with 3 repetitions each.			
Test organisms	*Rhamdia quelen*			
Biological effects	$96hLC_{50}$ 7.3mg L-1; 6.5–8.3; Lethargy, swimming at the water surface and erratic swimming (mainly vertical swimming) were the main behavioral changes observed.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	*Rhamdia quelen*			
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Mortality was observed and LC_{50} determined.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup® Transorb is a commercial formulation probably containing surfactants. Limited validity regarding effects of Glyphosate that does not contain the same surfactant.			
2 Do the tested concentrations relate to predicted environmental concentrations?	Recommended application rates were tested, pobabyl exceeding the predicted environmental concentration.			
3 Have parameters influencing the endpoints been considered adequately?	nd			
Concluding weight of evidence/proposed action	The 96-h LC_{50} determined for the glyphosate-based herbicide Roundup®→, in ***R. quelen*** (7.3mg L^{-1}) was much lower than that for the active substance glyphosate itself.			
Type of information (Critical, supporting, low weight)	**Supporting information**			

Consideration/concluding score	UBA2

- **B.9.13 9.2 Invertebrates (KIIA 8.16)**

Dutra et al. (2011)

glyphecotox_121	Dutra, B.K., Fernandes, F.A., Failace, D.M., Oliveira, G.T.	2011	Effect of Roundup®(R) (glyphosate formulation) in the energy metabolism and reproductive traits of *Hyalella castroi* (Crustacea, Amphipoda, Dogielinotidae)	Ecotoxicology 20: 255-263

Reliability	
Purpose of the study Description of endpoints	The objective of this investigation was to examine the effects of Roundup® (glyphosate formulation) on the biochemical composition, levels of lipoperoxidation, Na^+/K^+ ATPase activity and reproductive traits in the *Hyalella castroi*.
Test compound, application procedure, exposure period	Roundup®, glyphosate formulation
Experimental approach, Statistics, test environment	In the laboratory, the animals were kept in aquariums under controlled conditions for 7 days, and after this period they were exposed to 0.36, 0.52, 1.08 and 2.16 mg/l of glyphosate for 7 days. After the period of exposure the animals were immediately frozen for determination of glycogen, proteins, lipids, triglycerides, cholesterol, levels of lipoperoxidation, and Na^+/K^+ATPase activity. The number of reproductive pairs, ovigerous females and eggs in the marsupium(brood pouch) was counted in each day.
Test organisms	*Hyalella castroi*
Biological effects	All concentrations of Roundup® induced significant decreases in all biochemical parameters and Na^+/K^+ATPase activity, and significant increase in lipoperoxidation levels. No mating pairs, ovigerous females, or eggs in the marsupium were observed in the groups treated with the pesticide; these animals did not pair in the laboratory during all time of treatment. Survival rate 48% at 2.16 mg/l of glyphosate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Physiologigal parameters and reproductive parameters
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	animals did not pair in the laboratory during all time of treatment-7 changes in trophic structure of limnic environments
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Survival rate 48% at 2.16 mg/l of glyphosate.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	higher than predicted environmental concentrations
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Pyhsiological study, including survival EC50 approx. at 2.16 mg/l of glyphosate. changes in trophic structure of limnic environments

Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Achiorno et al. (2008)

glyphecotox_110	**Achiorno, C.L., de Villalobos, C., Ferrari, L.**	**2008**	**Toxicity of the herbicide glyphosate to Chordodes nobilii (Gordiida, Nematomorpha)**	**Ecotoxicology (2011) 20:255–263**
Reliability				
Purpose of the study Description of endpoints			The objective of this study is to evaluate the effect of different concentrations of glyphosate (technical grade and formulated product) on *Chordodes nobilii* (Gordiida, Nematomorpha).	
Test compound, application procedure, exposure period			Gyphosate, technical grade, 95% (w/v) (Gly), Roundup®, 35.2% (w/v) (formulatedGly).	
Experimental approach, Statistics, test environment			Bioassays were performed with embryos and larvae(preparasitic stages), andadults (postparasitic stage). Test organisms were exposed for a short period of time to concentrations ranging between 0.1 and8 mg a.e. l_1 of glyphosate (technical and formulated).	
Test organisms			*C. nobilii*.	
Biological effects			Embryo development was not inhibited, decrease in the infective capacity of larvae, Adult exposed for 96 h to 1.76 mg /L formulated Gly shown a mortality of 50%.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			No traditional test species, but The comparison between the POEA surfactant, technical grade glyphosate and the formulated product Roundup touches the key concern on the use of the herbicide glyphosate.	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?			Adult exposed for 96 h to 1.76 mg /L formulated Gly shown a mortality of 50%.	
3 Is the ecotoxicological manifestation level appropriate for the assessment?			Mortality tested	
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				Commercial product for mortality parameter
2 Do the tested concentrations relate to predicted environmental concentrations?				Exceed predicted environmental concentrations.
3 Have parameters influencing the endpoints been considered adequately?				nd
Concluding weight of evidence/proposed action	The minimum concentration tested(0.1 mg a.e. l_1 Gly.) decreased larval infectivity. Thisvalue is below the guidance level for glyphosate in freshwatersystems (0.24 mg l_1 Gly), established to protect the aquatic biota in Argentina. .			
Type of information (Critical, supporting, low weight)	**supporting**			
Consideration/concluding score	**UBA2**			

Brausch, J.M., Smith, P.N. (2007)

glyphecotox_113	**Brausch, J.M., Smith, P.N.**	**2007**	**Toxicity of three polyethoxylated tallowamine surfactant formulations to laboratory and field collected fairy shrimp,** *Thamnocephalus platyurus*	**Archives of Environmental Contamination and Toxicology 52 (2):217-221. DOI 10.1007/s00244-006-0151-y.**

Reliability	
Purpose of the study Description of endpoints	The objective of this study was to evaluate the toxicity(48-h LC_{50}) of three POEA surfactants to a freshwatermacroinvertebrate potentially exposed to POEA as it entersthe environment.
Test compound, application procedure, exposure period	POEA surfactant formulations (98.6%, 99.8%, and 99.4% pure forT-5, T-10, and T-15, respectively)
Experimental approach, Statistics, test environment	Three different POEA formulations were used for testing with average oxide: tallowamine ratios of 5:1 (Surfonic_ T-5 Surfactant), 10:1 (Surfonic_ T-10 Surfactant), and 15:1 (Surfonic_ T-15 Surfactant). Serial dilutions of a stock solution with final nominal concentrations of 0.01, 0.1, 1, 10, 100, 1,000, 10,000 µg/L for all three formulations of POEA were used as treatment levels. Each formulation was testedon three different strains of shrimp consisting of five acute toxicity tests (L1, C1, C2, G1, and G2) and replicated three times for a total of 15 toxicity tests per formulation.
Test organisms	*Thamnocephalus platyurus* (Crustacea,Anostraca)
Biological effects	All POEA formulations were found to be extremely toxic to *T. platyurus* with 48-h LC50 concentrations as low as 2.01 µg/ L for 15:1. POEA toxicity increased as the tallowamine chain length was reduced, whereas the oxide chain length appeared to only slightly increase toxicity
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	three strains of *T. platyurus*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-/-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	POEA was very toxic to *T. platyurus* with average 48-h LC50s of 2.01, 2.70, and 5.17 µg/L for POEA surfactantshaving an oxide:tallowamine ratio of 15:1, 10:1, and 5:1,respectively. Some deficiencies in data reporting
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Brausch et al. (2007)

glyphecotox_114	**Brausch, J.M., Blake, B., Smith, P.N.**	**2007**	**Acute and Sub-Lethal Toxicity of Three POEA Surfactant Formulations to** *Daphnia magna.*	**Bulletin of Environmental Contamination and Toxicology. Volume: 78 Issue: 6 Pages: 510-514**

Reliability	
Purpose of the study Description of endpoints	In this study, *Daphnia magna* was used to examine the lethal and sub-lethal toxicity of three POEA formulations consisting of 5:1, 10:1, and 15:1 average oxide:tallowamine.
Test compound, application procedure, exposure period	POEA formulations consisting of 5:1, 10:1, and 15:1 average oxide:tallowamine.
Experimental approach, Statistics, test environment	48h , test conc: 0,01- 10µg/L
Test organisms	*Daphnia magna*
Biological effects	All formulations inhibited growth at concentrations between 100 and 500 µg/L The formulation consisting of 10:1 was the most acutely toxic with a 48-h LC50 value of 97.0 µg/L and 15:1 was least toxic at 849.4 µg/L.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	surfactant
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	yes

Concluding weight of evidence/proposed action	POEA was very toxic to *Daphnia magna* POEA 15:1= 0.85 mg/L, POEA 10:1=0.097 mg/L POEA 5:1= 0.18mg/L
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Le et al. (2010)

glyphecotox_122	**Lee, T.H., Lim, E.S., Lee, S.K., Choi, Y.W., Kim, Y.H., Min, J.**	**2010**	**Effects of glyphosate and methidathion on the expression of the Dhb, Vtg, Arnt, CYP4 and CYP314 in** *Daphnia magna*	**Chemosphere 79: 67-71**

Reliability	
Purpose of the study Description of endpoints	In this study, the expression of five stress responsive genes was quantified and analyzed using a semiquantitativeRT-PCR to study the changes in their expression in *Daphnia magna* after exposure to known pesticides, glyphosate and methidathion.
Test compound, application procedure, exposure period	Glyphosate , FLUKA, probably technical, not stated
Experimental approach, Statistics, test environment	Standard US EPA protocol (2002) to determine the lethal endpoint caused byGlyphosate, concentrations: 190, 202, 214, and 234 mg/L, for 24 h probit method

Test organisms	*Daphnia magna*
Biological effects	LC_{50}=234 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Not clarified, probably glyphosate technical.
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	-/-
Concluding weight of evidence/proposed action	Tested substance not specified
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Bringolf et al. (2007)

glyphecotox_119	**Bringolf, R.B., Cope, W.G., Mosher, S., Barnhart, M.C., Shea, D.**	**2007**	**Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of** *Lampsilis siliquoidea* **(Unionidae)**	**Environmental Toxicology and Chemistry Volume: 26 Number: 10 Pages: 2094-2100**

Reliability		
Purpose of the study Description of endpoints	the toxicity of several forms of glyphosate, its formulations, and a surfactant (MON 0818) used in several glyphosate formulationswas determined for early life stages of Lampsilis siliquoidea, a native freshwater mussel.	
Test compound, application procedure, exposure period	Roundup®, its active ingredient, the technical-grade isopropylamine (IPA) salt of glyphosate, IPAalone, and MON 0818 (the surfactant in Roundup® formulations)	
Experimental approach, Statistics, test environment	Acute and chronic toxicity tests were performed with a newly established American Society of Testing and Materials (ASTM) standard guide for conducting toxicitytests with freshwater mussels.	
Test organisms	*Lampsilis siliquoidea* (Unionidae)	
Biological effects	EC_{50} values 48h (mg/L)acute Glyphosate technical >200 (glochidia) Glpyhosate IPA=5 (glochidia) Aquastar® >148(glochidia) Roundup®=2.9(glochidia) MON0818 =0.5(glochidia)	EC_{50} values 28days (mg/L)chronic Glyphosate technical = not applicable Glpyhosate IPA=4.8 Aquastar® =43.8 Roundup®=3.7 MON0818 =1.7
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?	Acute and chronic toxicity tests were performed with a newly established American Society of Testing and Materials (ASTM) standard guide for conducting toxicitytests with freshwater mussels.	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		
3 Is the ecotoxicological manifestation level appropriate for the assessment?		

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	See above
2 Do the tested concentrations relate to predicted environmental concentrations?	
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	MON 0818 was most toxic of the compounds tested and the 48-h median effective concentration (0.5 mg/L) for L. siliquoidea EC50 values are taken into account.
Type of information (Critical, supporting, low weight)	**Critical**
Consideration/concluding score	**UBA1**

Tsui, M.T.K., Chu, L.M. (2004)

glyphecotox_018	**Tsui, M.T.K., Chu, L.M.**	**2004**	**Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures**	**Arch. Environm. Contam. Toxicol.46, 316-323**

Reliability	
Purpose of the study Description of endpoints	In this study, the water-only acute toxicity of three formulations based on glyphosate (Rodeo, Roundup® Biactive, and Roundup®) were compared using a water-column organism (cladoceran: Ceriodaphnia dubia) and a benthic organism (amphipod: Hyalella azteca). In addition, Roundup® Biactive® and Roundup® were spiked into a clean sediment which was amended with appropriateamounts of peat moss to study the effect of different organic carbon levels (0, 0.4, 1.2, and 2.1%) on their sediment toxicity, with C. dubia exposed to overlying water or porewater prepared from the contaminated sediments.
Test compound, application procedure, exposure period	Rodeo (i.e., isopropylamine salt of glyphosate 53.8%) Roundup® (i.e., isopropylamine salt of glyphosate 41%, 0-20 POEA) Roundup®Biactive® (i.e., isopropylamine salt of glyphosate 41%, surfactant)
Experimental approach, Statistics, test environment	USEPA guideline 2000
Test organisms	*Ceriodaphnia dubia, Hyalella azteca*
Biological effects	The concentration units for the glyphosate-based herbicides were based on the acid equivalent concentration (of glyphosate acid) throughout the study.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulations probably showing differences between the inclsion of toxic and less toxic surfactants.
2 Do the tested concentrations relate to predicted environmental concentrations?	
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	EC50 values are taken into account

Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Chen et al. (2004)

glyphecotox_120	**Chen, C.Y., Hathaway, K.M., Folt, C.L.**	**2004**	**Multiple stress effects of Vision (R) herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands**	**Environmental Toxicology and Chemistry 23 (4):823-831**

Reliability		
Purpose of the study Description of endpoints	As part of a multiple-tier research program, interactions of the herbicide Visiont (glyphosate) with two stressors, pHand food level, were examined. Effects of the formulated product Vision were tested at two test concentrations (0.75 and 1.50 mgacid equivalent/L), two pH levels (pH 5.5 and 7.5), and under high and low food concentrations.	
Test compound, application procedure, exposure period	Glyphosate (356 g acid equivalent/L) in the form of an isopropylamine salt as well as polyethoxylated tallowamine surfactant (MON 0818) at a concentration equivalent to 15% by volume.	
Experimental approach, Statistics, test environment	S. vetulus, survival, reproduction, and development time were measured; SAS LIFETESTt (SAS, Ver 8,Cary, NC, USA). Pairwise comparisons of survival responses in each treatment were made using a log-rank test.	
Test organisms	*Simocephalus vetulus*	
Biological effects	Between 0.75 to 1.5 mg a.e./l	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		*Simocephalus vetulus,*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?		Survival and reproduction
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Vision®Commercial formulation containing POEA. Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?		
3 Have parameters influencing the endpoints been considered adequately?		nd
Concluding weight of evidence/proposed action		EC50 values are taken into account.
Type of information (Critical, supporting, low weight)		**supporting**
Consideration/concluding score		**UBA2**

Mensah et al. (2011)

glyphecotox_123	**Mensah, P.K., Muller, W.J., Palmer, C.G.**	**2011**	**Acute toxicity of Roundup® herbicide to three life stages of the freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae)**	**Physics and Chemistry of the Earth, Parts A/B/C 36 (14–15):905-909**

Reliability		
Purpose of the study Description of endpoints	The toxicity of the herbicide Roundup® was assessed using three different life stages of the freshwater shrimp Caridina nilotica, a prevalent species in South African freshwater ecosystems.	
Test compound, application procedure, exposure period	Roundup® active ingredient: 360 g glyphosate (glycine) a.e./L (contains 480 g isopropylamine salt of glyphosate/L, registered and distributed by Monsanto South Africa (Pty) Ltd.),	
Experimental approach, Statistics, test environment	ANOVA, Concentrations used were 0,1.7, 2.6, 4.1, 6.4 and 8 mg/L for the neonates (<7 days post hatching(dph)); 0, 1.7, 2.6, 4.1, 6.4, 8 and 10 mg/L for juveniles (>7 dphand < 20 dph); 0, 5.4, 8.4, 13.1, 20.5, 32 and 50 mg/L for adults(>40 dph).	
Test organisms	*Caridina nilotica (*Decapoda: Atyidae) is the most common of four indigenous freshwater caridean species found in the South Africa	
Biological effects	LC50 mg/L 48h neonates = 4.45 LC50 mg/L 48h juvenile = 9.39 LC50 mg/L 48h adults=37.12	LC50 mg/L 96h neonates = 2.54 LC50 mg/L 96h juvenile = 6.96 LC50 mg/L 96h adults=25.507
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		mortality
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation containing POEA,Roundup®. Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.	
2 Do the tested concentrations relate to predicted environmental concentrations?		
3 Have parameters influencing the endpoints been considered adequately?		
Concluding weight of evidence/proposed action	The neonates of *C. nilotica* were found to be most sensitive with a mean 96 h $LC_{50}$of 2.5 mg/L,	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Zeynep SARIGÜL11 (2009)

glyphecotox_124	**Zeynep SARIGÜL11**	**2009**	**Acute Toxicity of the Herbicide Glyphosate on** *Daphniamagna**	**JOURNAL OF AGRICULTURAL SCIENCES 2009, 15 (2) 204-208**

Reliability	
Purpose of the study Description of endpoints	In this study, median lethal concentrations (LC50) of herbicide Roundup, which contains 48% glyphosate, on Daphnia magna for 24 and 48 hours were determined.
Test compound, application procedure, exposure period	Roundup®
Experimental approach, Statistics, test environment	The experiment has been conducted with the method of static bioassay on two series; five different concentrations (0.0115; 0.018; 0.021; 0.028; 0.032) and one control group have been used. The LC50 values have been calculated with the method of probit analysis.
Test organisms	***Daphniamagna***
Biological effects	Experimental results showed that the concentration of the glyphosatewhich killed 50 % of Daphnia magna was 0.019 mg/L (95% confidence interval=0.012 mg/L–0.024 mg/L) for 24 hours, but the concentration of the glyphosate which killed 50 % of Daphniamagna was 0.012 mg/L (%95 confidence interval=0.001 mg/L-0.016 mg/L) for 48 hours
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	Oxygen low

Concluding weight of evidence/proposed action	EC50 values taken in to account for the formulation
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Conners, D.E., Black, M.C. (2004)

glyphecotox_325	**Conners, D.E., Black, M.C.**	**2004**	**Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia : Unionidae) exposed singly and in combination to chemicals used in lawn care**	**Archives of Environmental Contamination and Toxicology 46 (3):362-371. DOI 10.1007/s00244-003-3003-z.**

Reliability	
Purpose of the study Description of endpoints	In this study, we evaluated the lethal and genotoxiceffects of chemicals used in lawn care on an early life stage offreshwater mussels (Utterbackia imbecillis).
Test compound, application procedure, exposure period	glyphosate isopropylamine salt (Roundup; 18.0% active ingredient; Monsanto Company)
Experimental approach, Statistics, test environment	Johnson et al. (1993).
Test organisms	Gravid adult *U. imbecillis* mussels (average length 54.7 mm, averageheight 26.9 mm)
Biological effects	LC_{50} 18.3 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*U. imbecillis* mussels
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Parameter observed mortality
3 Is the ecotoxicological manifestation level appropriate for the assessment?	mortality

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation. Commercial formulation. The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concnetrations might be lower (for one indication per area).
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	LC 50 taken into account
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Frontera et al. (2011)

glyphecotox_378	**Frontera, J.L., Vatnick, I., Chaulet, A., Rodriguez, E.M.**	**2011**	**Effects of Glyphosate and Polyoxyethylenamine on Growth and Energetic Reserves in the Freshwater Crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae)**	**Archives of Environmental Contamination and Toxicology 61 (4):590-598. DOI 10.1007/s00244-011-9661-3.**

Reliability	
Purpose of the study Description of endpoints	Sublethal effects of a 50-day exposure to glyphosate acid and polyoxyethylenamine (POEA), both alone and in a 3:1 mixture, on the growth and energeticreserves in muscle, hepatopancreas and hemolymph ofgrowing juvenile crayfish were examined.

Test compound, application procedure, exposure period	All stock solutions of glyphosate (as acid) and POEA(99.8% purity; Sigma, St. Louis, Missouri) were preparedweekly by dissolving the appropriate amount of the chemicals in distilled water.	
Experimental approach, Statistics, test environment		
Test organisms	*Advanced juvenile C. quadricarinatus*	
Biological effects	No mortality was observed in any of the experimentalgroups during the experiment (glyphosate at a concentration of 22.5 mg/l., POEA (polyoxyethylene amine) at a concentrationof 7.5 mg/l.; a mixtureof 15 mg/l (3.75 mg/l POEA and 11.25 mg/l glyphosate and a mixtureof 30 mg/l (7.5 mg/l POEA and 22.5 mg glyphosate). Other physiological parameters like oxygen consumption, glycogen levels or body-weight gain were affected.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?	yes	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Mortality was not affected	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no	
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?	Commerciral formulation	
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concnetrations might be lower (for one indication per area).	
3 Have parameters influencing the endpoints been considered adequately?		
Concluding weight of evidence/proposed action	Physiollogical traits are affected, which might affect fitness. The decrease ofboth glycogen and lipid reserves, as observed in the mixture,could have lead to lower protein levels and decreasedsomatic growth in juvenile crayfish *C. quadricarinatus.*	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Mottiera (2013)

	Mottiera, Bouchartb, Serpentinia, Lebela, Jhac, Costil	**2013**	**Effects pof glyphosate –based herbicides on embryo-larval development and metamorphosis in the Pacific oyster,** *Crassostrea gigas*	**Aquatic Toxicology 128-129 (2013),67-78**

Reliability	
Purpose of the study Description of endpoints	In this context, the present study aimed to assess the toxicity of glyphosate, its by-product, aminomethylphosphonic acid (AMPA) and two commercial formulations, Roundup Express® (REX) and Roundup Allées et Terrasses® (RAT), containing glyphosate as the active ingredient, on the early life stages of the Pacific oyster, Crassostrea gigas.
Test compound, application procedure, exposure period	Roundup Express® 7.2 g/l Glyphosate +POEA(R_{EX}), Roundup Allées et Terrasses® 4.4 g/L+POEA(R_{AT}) glyphosate (97% purity) AMPA (97.5%purity)

Experimental approach, Statistics, test environment	For both endpoints, the nominal concentrations correspondingto 0.1, 1, 100 and 10,000 g L−1 of the chemicals (i.e. glyphosateand AMPA) were verified (in duplicate) by ultraperformance liquidchromatography (UPLC) and fluorometric detection (in accordancewith NF ISO 21458) Embryotoxicity bioassay and experimental design: AFNOR procedure (AFNOR XP- T90-382) published in 2009. Regarding the differences between the nominaland
Test organisms	Pacific oyster, *Crassostrea gigas*
Biological effects	The EC_{50} values were 27.1 and 46.1 mg/Lfor glyphosate and its metabolite, respectively for the parameter development and for both glyphosate and AMPA LC50 >100mg/L. Rex and Rat were more toxic than the active ingrdient , probably due to the surfactants. EC_{50} development REX= 1.1 mg/L, RAT=2.0mg/L

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Pacific oyster, *Crassostrea gigas* Embryotoxicity bioassay
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	Predicted environmental concnetrations might be lower (for one indication per area).
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	the embryos and 48 h D-shaped larvae were more sensitive than 21 days larvae LC_{50} taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Dominguez-Cortinas et al. (2008)

glyphecotox_347	**Dominguez-Cortinas, G., Saavedra, J.M., Santos-Medrano, G.E., Rico-Martinez, R.**	**2008**	**Analysis of the toxicity of glyphosate and Faena® using the freshwater invertebrates** ***Daphnia magna*** **and** ***Lecane quadridentata***	**Toxicological & Environmental Chemistry 90 (2):377 - 384**

Reliability	
Purpose of the study Description of endpoints	Therefore, the aim of the present contribution was to perform an ecotoxicologicalassessment of both glyphosate and its commercial formulation Faena using twoooplanktonic invertebrates: the rotifer *Lecane quadridentata*, and the cladoceran *Daphniamagna.*
Test compound, application procedure, exposure period	Glyphosate and Faena_ of the highest purity available (Sigma Co., St. Louis, MO, USA).
Experimental approach, Statistics, test environment	Statistica 5.0
Test organisms	*Daphnia magna* and *Lecane quadridentata*

Biological effects	LC_{50} 48h *L. quadridentata* Active ingredient =150 Faena®=13.1	LC_{50} 48h *Daphnia magna* Active ingredient=146 Faena®=7.9
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		mortality
3 Is the ecotoxicological manifestation level appropriate for the assessment?		mortality
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Active ingredient
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered adequately?		ndn
Concluding weight of evidence/proposed action	The LC_{50} values show that this freshwater rotifer is 11-foldmore susceptible to the commercial formulation (Faena) than to the active ingredient(glyphosate). This effect might be due to the synergistic activity of other components of the industrial formulation that increase the toxicity of the compound. Daphnia magna is almost 20-fold moresusceptible to Faena than to glyphosate. LC_{50} taken into account. EC50 (esteases activity)of glyphosate is 1500-fold smaller than the LC50.	
Type of information (Critical, supporting, low weight)		
Consideration/concluding score		

Demetrio et al. (2012)

glyphecotox_342	**Demetrio, P.M., Rossini, G.D.B., Bonetto, C.A., Ronco, A.E.**	**2012**	**Effects of Pesticide Formulations and Active Ingredients on the Coelenterate Hydra attenuata (Pallas, 1766)**	**Bulletin of Environmental Contamination and Toxicology 88 (1):15-9. doi:10.1007/s00128-011-0463-0.**

Reliability		
Purpose of the study Description of endpoints	The objective of the study is toassess and compare the acute effects on H. attenuata exposedto the active ingredients and commercial formulations ofglyphosate, cypermethrin, and chlorpyrifos.	
Test compound, application procedure, exposure period	Glyphosate (Technical Grade) were obtained from Gleba S.A. Roundup ®Max (74.4% glyphosate) was obtained from Monsanto S.A.	
Experimental approach, Statistics, test environment	probit model (Finney 1971) with a specific software (Probit USEPA version 1.5)	
Test organisms	*Hydra attenuata*	
Biological effects	LC50 glyphosate a.i (mg/l) =18.2	LC50 RoundupMax® (mg/l) =21.8
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		mortlaity
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Active ingredient versus formulation
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered adequately?		nd

Concluding weight of evidence/proposed action	With glyphosate, higher and significant effects were detected for the formulation at lower concentrations,with a reversal of the behavior at higher concentrations.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Melnichuk et al. (2007)

glyphecotox_501	**Melnichuk, S.D., Scherban, Y.P., Lokhanskaya, V.I.**	**2007**	**Effects of Fakel herbicide on vital activity of *Ceriodaphnia affinis* in acute and chronic experiments**	**Hydrobiological Journal 43 (6):83-91. doi: 10.1615/HydrobJ.v43.i6.70.**

Reliability		
Purpose of the study Description of endpoints	The aim of this work was to study the influence of Fakel herbicide on the vital activity parameters of *Ceriodaphnia affinis* and to evaluate the toxicity of this herbicide	
Test compound, application procedure, exposure period	Fakel herbicide is produced as the 36% (in acid equivalent)aqueous solution of the 48%isopropylamine salt of glyphosate	
Experimental approach, Statistics, test environment	concentrations from 0.001 up to 200 mg/dm3 were studied in acuteexperiments and of concentrations from 0.001 up to 10 mg/dm3 – in chronic experiments	
Test organisms	*Ceriodaphnia affinis*	
Biological effects	LC_{50} Fakel 48h= 13.6 mg/L(dm3)	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?	Comercial formulation	
2 Do the tested concentrations relate to predicted environmental concentrations?	At concentration of 1.0–0.01 mg/dm3, the herbicidereduces productivity of *Ñ. affinis* by 21–23% in each of generations	
3 Have parameters influencing the endpoints been considered adequately?	nd	
Concluding weight of evidence/proposed action	.Fakel herbicide exerted the greatest inhibitory influence on number of young per broodat concentration of 10 mg/dm3.	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Akcha et al. (2012)

glyphecotox_273	**Akcha, F., Spagnol, C., Rouxel, J.**	**2012**	**Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos**	**Aquatic toxicology (Amsterdam, Netherlands) 106-107:104-13. doi:10.1016/j.aquatox.2011.10.018**

Reliability	
Purpose of the study Description of endpoints	The embryotoxic effects of these herbicides were studied throughvarious embryo-larval bioassays.
Test compound, application procedure, exposure period	Roundup Express® Glyphosate a.s.
Experimental approach, Statistics, test environment	Test concentration 0.5; 1.0; 1.5; 2.5; 5.0 µga.s./L; one-way ANOVA
Test organisms	Mature oysters
Biological effects	Significant differences were highlighted in terms of D-larvae abnormalities ($p < 0.001$at exposure to glyphosate at concentrations of 5 µg /L leads to a significant increase in oyster embryo abnormalities versus the control. embryo-larval bioassays showed Roundup to have no embryotoxic effects, even at the highest testedconcentration of 5 µg of equivalent glyphosate /L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The commercial formulation did not appear to be more toxic than glyphosate – the active substance –
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	EC50 (estimated) Glyphosate a.s= 2.5**µg**/L.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

- **B.9.13 9.3 Algae and aquatic plants (KIIA 8.16)**

Ray et al. (2008)

glyphecotox_561	**Ray, P., Sushilkumar, Pandey, A.K.**	**2008**	**Deleterious effect of herbicides on waterhyacinth biocontrol agents** *Neochetina bruchi* **and** *Alternaria alternata*	**Biocontrol Science and Technology 18 (5):523-533. Doi 10.1080/09583150802001734.**

Reliability	
Purpose of the study Description of endpoints	Laboratory experiments were conducted to determine the toxic effect of herbicides on the insect biocontrol agent, the waterhyacinthweevil, Neochetina bruchi Hustache, and phytopathogen, Alternaria alternata,with two commonly used herbicides, glyphosate and 2,4-dichlorophenoxy aceticacid at three recommended doses.
Test compound, application procedure, exposure period	three recommended (labelled) doses, i.e. 0.89, 1.12and 1.34 ppm ai glyphosate
Experimental approach, Statistics, test environment	nd
Test organisms	*nd*
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Waterhycinth in India considered as target species
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Romero et al. (2011)

glyphecotox_578	**Romero, D.M., Rios de Molina, M.C., Juarez, A.B.**	**2011**	**Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of** *Chlorella kessleri*	**Ecotoxicol Environ Saf 74 (4):741-7. DOI: 10.1016/j.ecoenv.2010.10.034.**

Reliability	
Purpose of the study Description of endpoints	The aim of this work is to study the toxicity of the Herbicide glyphosate and to provide evidence of metabolic altera- Tions related to oxidative stress induced in at olerant strain of C. kessleri by exposure to a commercial formulation of glyphosate. For this purpose parameters related to metabolic damage were measured.
Test compound, application	Commercially available herbicide used in this study was
procedure, exposure period	48%(p/v)Glyphosate (isopropylaminesaltofN-phosphonomethylglycine) ATANORs (Atanor,Munro, Buenos Aires province,Argentina) and the surfactant was alkylaryl polyglycolether 50%IMPACTOs (AGROASISTS.R.L.,Argentina).
Experimental approach, Statistics, test environment	The experimental treatments were prepared according to algal growth inhibition test standards (USEPA, 2002). concentrationsof 40, 50,60,and70mgL_1 of glyphosate
Test organisms	C. kessleri (Trebouxiophyceae, Chlorophyta
Biological effects	Algal cell density and dry weight were statistically significant diminished with respect to the control values for concentrations of at least 60 mgL_1 of glyphosate, where the numbe rof cells was approximately one-third that of the control culture(Table 1). The EC50-96 h estimated by LinearInterpolation Methodsoft ware was 55.62 (53.08–57.56)mgL_1.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product containing surfactant alkylaryl polyglycolether. Although not specified precisely, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	High than predicted PEC vaues in RA
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	TheEC50-96h obtainedfor C. kessleri was higher than those used in risk assessment.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Debenest et al. (2010)

glyphecotox_340	**Debenest, T., Silvestre, J., Coste, M., Pinelli, E.**	**2010**	**Effects of Pesticides on Freshwater Diatoms**	**In Reviews of Environmental Contamination and Toxicology, edited by D. M. Whitacre. Springer New York. pp 87-103. DOI: 10.1007/978-1-4419-1352-4_2.**

Reliability		
Purpose of the study Description of endpoints	Book chapter which provides a broad bibliographical review of articlesthat address the effects of pesticides and certain other xenobiotics on diatoms. In thisreview, we emphasize the following targets of pesticide action: (i) cytology and cellultrastructure, (ii) cell metabolism, and, finally, (iii) effects on community speciescomposition.	
Test compound, application procedure, exposure period	nd	
Experimental approach, Statistics, test environment	nd	
Test organisms	*nd*	
Biological effects	nd	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?		nd
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		nd
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered adequately?		nd
Concluding weight of evidence/proposed action	No details about Glyphosate in particular.	
Type of information (Critical, supporting, low weight)	**low weight**	
Consideration/concluding score	**UBA3**	

Inderjit, I., Kaushik, S. (2010)

glyphecotox_412	**Inderjit, I., Kaushik, S.**	**2010**	**Effect of herbicides with different modes of action on physiological and cellular traits of** *Anabaena fertilissima*	**Paddy and Water Environment 8 (3):277-282. DOI: 10.1007/s10333-010-0208-4.**

Reliability		
Purpose of the study Description of endpoints	Comparative study designed to examine toxicity of propanil, pretilchlor and glyphosate on physiological and cellular characteristics of *A. fertilissima.*	
Test compound, application procedure, exposure period	nd	
Experimental approach, Statistics, test environment		
Test organisms	*A. fertilissima.*	
Biological effects	nd	

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Coording to Algae Base (http://www.algaebase.org/search/species/detail/?species_id=40443) species distribution in North America only.
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Romero et al. (2011)

glyphecotox_578	**Romero, D.M., Rios de Molina, M.C., Juarez, A.B.**	**2011**	**Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of *Chlorella kessleri***	**Ecotoxicol Environ Saf 74 (4):741-7. DOI: 10.1016/j.ecoenv.2010.10.034.**

Reliability	
Purpose of the study Description of endpoints	The aim of this work is to study the toxicity of the herbicide glyphosate and to provide evidence of metaboli calterations related to oxidative stress induced in a tolerant strain of C. kessleri by exposure to a commercial formulation of glyphosate.
Test compound, application procedure, exposure period	ATANOR, 48% IPA salt
Experimental approach, Statistics, test environment	Parameters related to metabolic damage (biomass, growth rate, chlorophyll content and protein content), lipid peroxidation (malondialdehyde content) and antiox- idant response (catalase and superoxide dismutase activities and reduced glutathione level) were measured.
Test organisms	Chlorella kessleri
Biological effects	EC50 = 55.6 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Although not specified precisely, the tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd

Concluding weight of evidence/proposed action	The freshwater species Chlorella in Europe according to Algae Base((http://www.algaebase.org/search/species/detail/?species_id=40443) species distribution in South America, Asia, in Europe in Romania and Spain. Tolerant strain used .
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Ma et al. (2002)

glyphecotox_476	**Ma, J., Xu, L., Wang, S., Zheng, R., Jin, S., Huang, S., Huang, Y.**	**2002**	**Toxicity of 40 herbicides to the green alga** *Chlorella vulgaris*	**Ecotoxicology and Environmental Safety 51 (2):128-132. DOI 10.1006/eesa.2001.2113**

Reliability		
Purpose of the study Description of endpoints	Work reported effect of 40 hebicieds on the green algae *Chlorella vulgaris*	
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.	
Experimental approach, Statistics, test environment	Initial cell conc.: $8x\ 10^5$ /ml. Linear regression for EC_{50} calculation, 5000 lx/cm2, duration 96h	
Test organisms	*Chlorella vulgaris*	
Biological effects	EC_{50}= 5 mg/L	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Yes, technical ingedient.
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered adequately?		nd
Concluding weight of evidence/proposed action	Presented EC_{50} values will be taken into account.	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Ma et al. (2006)

glyphecotox_474	**Ma, J., Wang, S., Wang, P., Ma, L., Chen, X., Xu, R.**	**2006**	**Toxicity assessment of 40 herbicides to the green alga** *Raphidocelis subcapitata*	**Ecotoxicology and Environmental Safety 63 (3):456-462. DOI 10.1016/j.ecoenv.2004.12.001**

Reliability	
Purpose of the study Description of endpoints	The effects of 40 herbicides with nine modes of action on the green alga *Raphidocelis subcapitata* were studied by 96-h acutetoxicity tests.
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.
Experimental approach, Statistics, test environment	Initial cell conc.: 5x $10^{5/ml,}$ Linear regression for EC50 calculation, 5000 lx/cm2, duration 96h
Test organisms	*R. subcapitata*
Biological effects	EC50= 5.5 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical ingedient.
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd

Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account. Freshwater species , taxonomic synonym *Pseudokirchneriella subcapitata*
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ma, J. (2002)

glyphecotox_471	**Ma, J.**	**2002**	**Differential sensitivity to 30 herbicides among populations of two green algae** *Scenedesmus obliquus* **and** *Chlorella pyrenoidosa*	**Bulletin of Environmental Contamination and Toxicology 68 (2):275-281**

Reliability	
Purpose of the study Description of endpoints	Effect of different herbiceds on the fgreen algae *Scenedesmus obliquus.*
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.
Experimental approach, Statistics, test environment	Initial cell conc.: 4x $10^{5/ml,}$ Linear regression for EC50 calculation, 5000 lx/cm2, duration 96h
Test organisms	*Scenedesmus obliquus and Chlorella pyrenoidosa*
Biological effects	EC_{50}= 56 mg/L

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical ingredient.
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account. EC_{50}= 56 mg/L
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ma et al. (2006)

glyphecotox_473	**Ma, J., Lin, F., Wang, S., Xu, L.**	**2006**	**Toxicity of 21 herbicides to the green alga** *Scenedesmus quadricauda*	**Environmental Contamination and Toxicology 71 (3):594-601. DOI 10.1007/s00128-003-8521-x.**
Reliability				
Purpose of the study Description of endpoints			In the present study, 21 herbicides have been tested to examine their effect on the green alga *Scenedesmus quadricauda* and thencompare their differential sensitivity three other green algae, *Scenedesmusobliqnus, Chlorella vulgaris* and *Chlorella pyrenoidosa.*	
Test compound, application procedure, exposure period			Glyphosate 95%, technical product.	
Experimental approach, Statistics, test environment			Initial cell conc.: 4x 10^{5}/ml, Linear regression for EC50 calculation, 5000 lx/cm2, duration 96h	
Test organisms			***Scenedesmus quadricauda***	
Biological effects			EC50= 70mg/L	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?				nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				Yes, technical ingredient.
2 Do the tested concentrations relate to predicted environmental concentrations?				nd
3 Have parameters influencing the endpoints been considered adequately?				nd
Concluding weight of evidence/proposed action			Presented EC50 values will be taken into account.Freshwater species occurin g amongst othersin Sout Europe. EC_{50}= 70.5 mg/L	
Type of information (Critical, supporting, low weight)			**supporting**	
Consideration/concluding score			**UBA2**	

Ma et al. (2001)

glyphecotox_477	**Ma. J., Liang, W., Xu, L., Wang, S., Wei, Y., Lu, J.**	**2001**	**Acute toxicity of 33 herbicides to the green alga** *Chlorella pyrenoidosa*	**Bull Environ Contam Toxicol 66 (4):536-41**

Reliability		
Purpose of the study Description of endpoints	s. above	
Test compound, application procedure, exposure period	Glyphosate 95%, technical product.	
Experimental approach, Statistics, test environment	Duration 96h, ECso values were calculated using linear regression analysisof transformed pesticide concentration as natural logarithm data versus percent inhibition (Ma et al. 2001), inital cell concentration: $6x10^5$ cells/ml.	
Test organisms	***Chlorella pyrenoidosa***	
Biological effects	EC_{50}= 3.5mg/L	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Yes, technical ingredient.
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered adequately?		nd
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account. EC_{50}= 3.5 mg/L	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Tsui, M.T.K., Chu, L.M. (2003)

glyphecotox_195	**Tsui, M.T.K., Chu, L.M.**	**2003**	**Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors**	**Chemosphere 52: 1189–1197.**

Reliability	
Purpose of the study Description of endpoints	In this study,the acute toxicity of technical-grade glyphosate acid,isopropylamine (IPA) salt of glyphosate,Roundup® and its surfactant polyoxyethylene amine (POEA) to Microtox bacterium (*Vibrio fischeri*),microalgae (*Selenastrum capricornutum* and *Skeletonema costatum*),protozoa (*Tetrahymena pyriformis* and *Euplotes vannus)* and crustaceans (*Ceriodaphnia dubia* and *Acartia tonsa*) was examined and the relative toxicity contributions of POEA to Roundup® were calculated.
Test compound, application procedure, exposure period	Glyphosate acid (CAS: 1071-83-6; P97% purity) Polyoxyethylene amine (POEA) (CAS: 61791-26-2;100% a.i.) Roundup® (commercial grade; 41% a.i.) Isopropylamine (IPA) salt of glyphosate (CAS: 38641-94-0; 56.8% a.i.)

Experimental approach, Statistics, test environment	ASTM (1994), Absorbance at 680nm, The IC50 (or median growth inhibition concentration)and 95% confidence interval were calculated byprobit analysis for the growth inhibition test (Finney,1971).	
Test organisms	Algae, *Selenastrum capricornutum* (UTEX 1648, Freshwater) and *Skeletonema costatum*(UTEX LB2038, Marine)	
Biological effects	Generally,the toxicity order of the chemicals was: POEA> Roundup®_ > glyphosate acid >IPA salt of glyphosate,while the toxicity of glyphosate acid was mainly due to its high acidity. In contrast,microalgae and crustaceans were 4–5 folds more sensitive to Roundup®_ toxicity than bacteria and protozoa. Except photosynthetic microalgae,POEA accounted for more than 86% of Roundup®_ toxicity and the toxicity contribution of POEA was shown to be species-dependent.	
	Selenastrum capricornutum 96 h IC_{50} Glyphosate acid = 24.7 mg AE/l IPA salt of glyphosate= 41.0 mg AE/l POEA=3.92 mg AE/l Roundup®= 1.85 mg AE/l	*Skeletonema costatum* 96 h IC_{50} Glyphosate acid = 2.27 mg AE/l IPA salt of glyphosat= 5.89 mg AE/l POEA =3.35 mg AE/l Roundup®=1.85 mg AE/l
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		yes
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered adequately?		nd
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account.	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Perez et al. (2011)

glyphecotox_540	**Perez, G.L., Vera, M.S., Miranda, L.A.**	**2011**	**Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems**	**In Herbicides and Environment, edited by Kortekamp. Croatia. InTech. Chapter 16. pp 343 - 368.**

Reliability	
Purpose of the study Description of endpoints	Revision of their toxicity to non-target species of algae, aquatic plants, protozoa,crustaceans, molluscs, fish and amphibians. In addition, we describe the importance of eachgroup of organisms in the functioning and health of aquatic ecosystems.
Test compound, application procedure, exposure period	nd
Experimental approach, Statistics, test environment	nd
Test organisms	nd
Biological effects	nd
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Review chapter in book.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Cedergreen, N., Streibig, J.C. (2005)

glyphecotox_319	**Cedergreen, N., Streibig, J.C.**	**2005**	**The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard**	**Pest Management Science 61 (12):1152-1160. Doi 10.1002/Ps.1117**

Reliability	
Purpose of the study Description of endpoints	In this study the toxicity of herbicides to aquatic plants and algae and relate it to environmental herbicide concentrations and exposure scenarios, herbicide formulation and mode of action was evaluated. This was done experimentally for ten herbicides, using the aquatic macrophyte *Lemna minor* L. and the green alga *Pseudokirchneriella subcapitata* (Korshikov) Hindak, supplemented with a database study comprisingalgae toxicity data for 146 herbicides.
Test compound, application procedure, exposure period	Roundup® 360 g/L
Experimental approach, Statistics, test environment	The algae test is described by Arensberg *et al.*and Mayer *et al.*20 and is coherent with the ISO standards. Initial densitiy: 10 000 cells /ml
Test organisms	*P. subcapitata*.
Biological effects	EC_{50} = 270 mg a.s./L EC_{50} formulation = 64.7 mg/L
Test organisms	*Lemna minor* L.
Biological effects	EC_{50} = 46.9 mg a.s./L $EC_{50=}$ formulation = 11.2 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	yes

3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Turgut, C., Fomin, A. (2002)

glyphecotox_128	**Turgut, C., Fomin, A.**	**2002**	**Sensitivity of the rooted macrophyte *Myriophyllum aquaticum* (Vell.) Verdcourt to seventeen pesticides determined on the basis of EC50**	**Bulletin of Environmental Contamination and Toxicology 69 (4):601-608**

Reliability	
Purpose of the study Description of endpoints	Sensitivity of the rooted macrophyte *Myriophyllum aquaticum* (Vell.) to seventeen pesticides was determined on the basis of EC50.
Test compound, application procedure, exposure period	Probably commercial product with 36 % a.s, not clarified.
Experimental approach, Statistics, test environment	Liqid growth medium , 5 replicates , 7-8 concentrations
Test organisms	*Myriophyllum aquaticum*
Biological effects	EC50 (mg/L)= 2.0 (fresh weight) EC50 (mg/L)= 0.22 (chl a)
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Probably commercial product with 36 % a.s
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	Sucrose added
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account. Pigment content was more senstive endpoint than other parameters.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Sobrero et al. (2007)

glyphecotox_125	**Sobrero, M.C., Rimoldi, F., Ronco, A.E.**	**2007**	**Effects of the glyphosate active ingredient and a formulation on *Lemna gibba* L. at different exposure levels and assessment end-points**	**Bulletin of Environmental Contamination and Toxicology 79: 537-54**

Reliability	
Purpose of the study Description of endpoints	The sensitivity of a local clone of the macrophyte *Lemna gibba* L. to glyphosate active principle and Roundup® Max formulation was studied in standardized laboratory conditions
Test compound, application procedure, exposure period	testing both the active ingredient, a.i. (glyphosate acid, technical grade, 95%w/w)and the commercial formulation (Roundup®1Max, 70.7%w/w a.i. as acid),
Experimental approach, Statistics, test environment	Herbicide phytotoxicity was assessed on growth rate(GR) measured at 2, 5, 7 and 10 days of exposure, and alsoon frond growth (FG), frond number per colony (FNC),total chlorophyll content (TCC) and root length measuredat 7 and 10 days.
Test organisms	L. gibba
Biological effects	EC_{50} (mg a.s./L)= 20.5 (growth) EC_{50} (mg/L)= 11.6

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	Presented EC50 values will be taken into account.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

- **B.9.13 9.4 Sediment-dwelling organisms (KIIA 8.16)**

Contardo-Jara et al. (2009)

glyphecotox_326	**Contardo-Jara, V., Klingelmann, E., Wiegand, C.**	**2009**	**Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes**	**Environ Pollut 157 (1):57-63. DOI: 10.1016/j.envpol.2008.07.027.**

Reliability	
Purpose of the study Description of endpoints	The bioaccumulation potential of glyphosate and the formulation Roundup Ultra, as well as possible effects on biotransformation and antioxidant enzymes in Lumbriculus variegatus were compared by four days exposure to concentrations between 0.05 and 5 mg L1 pure glyphosate and its formulation

Test compound, application procedure, exposure period	Glyphosate (N-(phosphonomethyl)glycine) was obtained from Dr. Ehrenstorfer (Augsburg, Germany) with 98 0.5% certified purity. The used Roundup Ultra solution (Monsanto Co, St. Louis, MO, USA) contains the monoisopropylamine salt of N-(phosphonomethyl)-glycine (360 g L1) and surfactants of
Experimental approach, Statistics, test environment	The bioaccumulation of glyphosate in *L. variegatus* was studied after four days exposure with renewal of the exposure medium after two days.
Test organisms	*Lumbriculus variegatus*
Biological effects	The bioaccumulation factor (BCF) varied between 1.4 and 5.9 for the different concentrations, and was higher than estimated from log Pow.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	yes

Concluding weight of evidence/proposed action	
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

- **B.9.13 9.5 Microcosm or mesocosm study (KIIA 8.16)**

Vera et al. (2010)

glyphecotox_129	**Vera, M.S., Lagomarsino, L., Sylvester, M., Perez, G.L., Rodriguez, P., Mugni, H., Sinistro, R., Ferraro, M., Bonetto, C., Zagarese, H., Pizarro, H.**	**2010**	**New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems**	**Ecotoxicology, 19:710-721**

Reliability	
Purpose of the study Description of endpoints	The experiment was carried outover 42 days in ten outdoor mesocosms of different typology: ‘‘clear’’ waters with aquatic macrophytes and/ormetaphyton and ‘‘turbid’’ waters with great occurrence of phytoplankton or suspended inorganic matter.
Test compound, application procedure, exposure period	The herbicide Roundup®was added at 8 mg L-1 of the active ingredient (glyphosate)in in five mesocosms while five were left as controls(without Roundup®addition).

Experimental approach, Statistics, test environment	The ten mesocosms (depth: 1.2 m; area:25 m2), constructed in an area of approximately 1 ha, wereBuilt. The bottom of eachexcavation was covered with soil from places nearby toprovide sediments to each environment (Fig. 1). Finally,they were filled with well water and were left to evolve. Kruskal–Wallisnon-parametric ANOVA
Test organisms	periphyton
Biological effects	Roundup®produced a clear delay in periphytic colonization in treated mesocosms and values of the periphytic mass variables (dry weight, ash-free dry weight and chlorophyll a) were always higher in control mesocosms. Despite the mortality of algae, mainly diatoms, cyanobacteria was favored in treated mesocosms. It was observed that glyphosate produced a long term shift in the typology of mesocosms, ‘‘clear’’ turning to ‘‘turbid’’, which is consistent with the regional trend in shallow lakes in the Pampa plain of Argentina.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	algal groups
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Diatoms (Bacillariophyceae) appeared to be the most affected by the herbicide, Cyanobacteria, on the other hand, emerged enhanced in number in treated mesocosms.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	joint effects
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product with surfactant
2 Do the tested concentrations relate to predicted environmental concentrations?	Might exceed the predicted envirnonmental concnetrations.
3 Have parameters influencing the endpoints been considered adequately?	It is important to point out that the toxicity is produced by the joint effect of both glyphosate and POEA, which is the surfactant of the commercial formulation Roundup®whose toxicity was shown to be higher than glyphosate.
Concluding weight of evidence/proposed action	Changes in community structure
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Perez et al. (2007)

glyphecotox_539	**Perez, G.L., Torremorell, A., Mugni, H., Rodriguez, P., Solange Vera, M., do Nascimento, M., Allende, L., Bustingorry, J., Escaray, R., Ferraro, M., Izaguirre, I., Pizarro, H., Bonetto, C., Morris, D.P., Zagarese, H.**	**2007**	**Effects of the herbicide Roundup® on freshwater microbial communities: a mesocosm study**	**Ecol Appl 17 (8):2310-22**

Reliability	
Purpose of the study Description of endpoints	Effect of the commercial formulation Roundup® using artificial earthen mesocosms.
Test compound, application procedure, exposure period	Roundup®
Experimental approach, Statistics, test environment	The herbicide was added at three doses: a control (without Roundup®) and two treatments of 6 and 12 mg/L of the activeingredient (glyphosate).
Test organisms	Phytoplancton and periphyton community

Biological effects	Roundup® affected the structure of phytoplankton and periphyton assemblages. Total micro- and nanophytoplankton decreased in abundance in treated mesocosms. In contrast, the abundance of picocyanobacteria increased by a factor of about 40. Primary production also increased intreated mesocosms (roughly by a factor of two). Similar patterns were observed in theperiphytic assemblages, which showed an increased proportion of dead : live individuals andincreased abundances of cyanobacteria (about 4.5- fold).	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.	
2 Do the tested concentrations relate to predicted environmental concentrations?	nd	
3 Have parameters influencing the endpoints been considered adequately?	nd	
Concluding weight of evidence/proposed action	Presented EC_{50} values will be taken into account. 6mg/l elicitated a change in community structure.	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

- **B.9.13 9.6 Summary of the relevant literature on aquatic organisms**

Aquatic organisms are considered to be exposed to glyphosate containing plant protection products via spray drift, runoff and drainage as a consequence of use near aquatic environments. Aquatic algae and macrophytes are especially vulnerable to the impact of glyphosate due to their physiological similarity to terrestrial plants.

For the group of algae, a comprehensive database of nearly 30 peer-reviewed papers was submitted by the notifier. The notifier considered five publications (Sobrero et al. 2007; Sanchez et al. 2007; Turgut et al. 2011 and Vera et al. 2010) and considered one publication to be rated in category "Klimisch 2" (Klimisch 1997) and annotated with minimal remarks, whereas the remaining were considered as not acceptable for risk assessment. The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. Out of the submitted publications, 15 studies were recognized as supporting information (category UBA2) and are reviewed here. Endpoints deriving out of these publications are listed in the table below.

The peer reviewed open literature about toxicity on algae provides a wide range of EC50 and IC50 values for algae treated with glyphosate (technical grade). The EC50 values range from 2.3 mg/l for *Skeletonema costatum* (Tsui, 2003) to 70 mg/L for *Scenedesmus quadricauda* (Ma, 2006) and the marine diatom *Skeletonema costatum* seems to be the most sensitive species towards glyphosate. Regarding macrophytes, similar EC50 values compared to algae are reported in the peer reviewed open literature . IC50 and EC50 values ranged from 0.22 mg a.s./L for *Myriophyllum aquaticum* (Turgut & Fomin, 2002) to 46.9 mg/L for *Lemma minor* (Cedergreen & Streibig, 2005).

Beside single species tests, a few studies were performed focusing on the natural aquatic community in order to assess indirect effects towards algae. Mesocosm studies showed differences at 6 mg glyphosate

containing product/L in the structure of phytoplancton and periphyton assemblages in treated mesocosms compared to controls. Total micro- and nanophytoplancton decreased in abundance, whereas the abundance of picocyanobacteria increased (Perez, 2007). Similar effects were observed by Vera et al. (2010), who could also show that despite the mortality of algae, mainly diatoms, cyanobacteria were favored in treated mesocosms. However, it must be considered that in both studies commercial products containing surfactants were used, and therefore the toxicity is determined by the joint effect of both glyphosate and the surfactants of the commercial formulations. Commercial products containing specific formulation ingredients additionally to the active ingredient were shown to be more toxic towards algae than glyphosate acid (Cedergreen & Streibig, 2005; Tsui, 2003). There was no critical data in the open literature that could be directly included in an environmental risk assessment for the active substance glyphosate. Endpoints reported have been detected inthe same magnitude or it was not possible to distinguish between the effects of the technical glyphosate and the surface-active substances added to the commercial formulations in the experimental designs used.

Table B.9.13-3: Effects values of algae and aquatic plants in per-reviewed literature

Species	Substance	Study type	EC50 (mg/L)	Reference (internal tag)
Algae				
Chlorella vulgaris	Glyphosate 95%, technical product.	96h	5	Ma, J.,2002; glyphecotox_476
Raphidocelis subcapitata	Glyphosate 95%, technical product.	96h	5.5	Ma, J.,2002; glyphecotox_474
Scenedesmus obliquus	Glyphosate 95%, technical product.	96h	56	Ma, J., 2002; glyphecotox_471
Scenedesmus quadricauda	Glyphosate 95%, technical product.	96h	70	Ma, J.,2006; glyphecotox_473
Chlorella pyrenoidosa	Glyphosate 95%, technical product.	96h	3.5	Ma. J.,2001; glyphecotox_477
Chlorella kessleri	ATANOR	96h	55.62	Romero, et al., 2011 glyphecotox_578
Pseudokirchneriella subcapitata	Glyphosate 95%, technical product.	48h	270	Cedergreen, N., Streibig, J.C., 2005; glyphecotox_319
	Roundup 360 g/L		64.7	
Periphyton	Commercial product with surfactant	Mesocsm, 42days	8mg/l Changes in community structure	Vera, M.S.,, 2010; glyphecotox_129
Periphyton, Phytoplancton	Roundup®	Mesocsm, 11days	6 mg/L Changes in community structure	Perez, G.L.,2007; glyphecotox_539
Selenastrum capricornutum	Glyphosate acid	96h	24.7 mg a.e./L	Tsui, M.T.K2003 glyphecotox_195
	IPA salt of glyphosate		41.0 mg a.e./L	
	POEA		3.92 mg a.e./L	
	Roundup®		1.85 mg a.e./L	
Skeletonema costatum	Glyphosate acid	96h	2.27 mg a.e./L	Tsui, M.T.K., 2003 glyphecotox_195
	IPA salt of glyphosate		5.89 mg a.e./L	
	POEA		3.35 mg a.e./L	
	Roundup®		1.85 mg a.e./L	

Macrophytes				
Myriophyllum aquaticum	Commercial product, 36% a.s.	14 days	2.0 (fresh weight) 0.22 (chl a)	Turgut & Fomin, 2002, glyphecotox_128
Lemna minor L.	Glyphosate 95%, technical product.	7 days	46.9	Cedergreen, N., Streibig, J.C., 2005; glyphecotox_319
	Roundup 360 g/L		11.2	
Lemna minor L.	Glyphosate 95%, technical product.	10 days	20.5	Sobrero, M.C.,2007; glyphecotox_125
	Roundup1Max, 70.7%w/w a.i. as acid		11.6	

For the group of aquatic invertebrates, a comprehensive database of 42 peer-reviewed papers was submitted by the notifier. The notifier considered three publications (Bringolf et al. 2007; Chen et al. 2004 and Mensah et al. 2011) to be rated in category "Klimisch 2" (Klimisch 1997) and annotated with minimal remarks, whereas the remaining were considered as not acceptable for risk assessment. The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. Out of the submitted publications, 18 studies were recognized as supporting information (category UBA2) and are reviewed here. Endpoints deriving out of these publications are listed in the table below.
Most of the cited studies were performed with formulated products and not with the active ingredient alone. Those studies, which investigated the effect of glyphosate itsef or the Glyphosate IPA-salt obtained LC50 values ranging from 49.3 mg acid equivalents /L for the marine copepod Acartia tonsa to 415 mg acid equivalents /L for the cladoceran Ceriodaphnia dubia (Tsui, 2003; Le, 2010; Tsui et al., 2004; Dominguez-Cortinas et al., 2008; Bringolf et al., 2007; Mottiera et al., 2013; Frontera, 2011; Dominguez-Cortinas, 2008). However, mores sensitive species like the coelenterate Hydra attenuata showed lower sensibility and LC50 values were determined to be 18.2 mg/L for the active ingredient glyphosate. These organisms are generally not considered in Tier 1 risk assessment, but is was shown that they are exposed to toxicants to a higher extent due its anatomical and physiological structure (Demetrio, 2012). Moreover, sublethal effects were observed at much lower concentrations of glyphosate in comparison to lethal effects (Mottiera, 2013).

In general, the formulations are of higher ecotoxicological relevance than the active ingredient glyphosate itself. One of the main commercial formulations is Roundup ®, which in addition to the active ingredient glyphosate contains polyoxyethoxylated alkylamines (POEA) as a surfactant. A few studies investigate the effects of the formulation versus the surfactant POEA. These studies have shown that formulations containing POEA are several times more toxic (3 to 5 fold more toxic than Roundup®) to aquatic invertebrates than the active ingredient glyphosate acid or formulations without POEA. For more details concerning surfactant ingredients and their toxicity to aquatic organisms please refer to chapter 0.
There was no critical data that could directly be included in the environmental risk assessment for the active substance glyphosate.

Table B. 9.13-4: Effects values for aquatic invertebrates exposed to glyphosate acid or formulated products with glyphosate. Endpoints published in peer- reviewed open literature

Species	Test item	Study type	LC50 (mg a.e./L)	Reference (internal tag)
Crustaceans				
Daphnia magna	glyphosate acid	48h (mortality)	234	**Le**, T.H.,2010; glyphecotox_122
C. quadricarinatus	glyphosate acid	50days	>33	Frontera, J.L.,2011; glyphecotox_378
Daphnia magna	glyphosate acid	48h (mortality)	146	Dominguez-Cortinas, G.,2008; glyphecotox_347
	Faena®		7.9	
Lecane quadridentata	glyphosate acid	48h (mortality)	150	
	Faena®		13.1	

Species	Test item	Study type	LC50 (mg a.e./L)	Reference (internal tag)
Hyalella castroi	Roundup®	7days (survival estimated)	2.16	Dutra, B.K.,, 2011; glyphecotox_121
Chordodes nobilii	Roundup®	96h (mortality)	1.76	Achiorno, C.L.,2008; glyphecotox_110
Caridina nilotica	Roundup®	48h (mortality)	neonates = 4.45	Mensah, P.K.,2011; glyphecotox_123
			juvenile = 9.39	
			adults=37.12	
		96h (mortality)	neonates = 2.54	
			juvenile = 6.96	
			96h adults=25.507	
Daphnia magna	Roundup®	48h (mortality)	0.019	Sarigül Z.,2009; glyphecotox_124
Simocephalus vetulus	Vision®	48h (mortality)	0.75 to 1.5 a.e.	Chen, C.Y.,2004; glyphecotox_120
Ceriodaphnia affinis	Fakel herbicide	48	43.6	Melnichuk, S.D.,2007; glyphecotox_501
Thamnocephalus platyurus	POEA 15:1	48h (mortality)	2.01	Brausch, J.M.,2007, glyphecotox_113
	POEA 10:1		2.70	
	POEA 5:1		5.17	
Daphnia magna	POEA 15:1	48h (mortality)	0.85	Brausch, J.M.,2007; glyphecotox_114
	POEA 10:1		0.097	
	POEA 5:1		0.18	
Ceriodaphnia dubia	Glyphosate IPA-salt	48h (mortality)	415	Tsui, M.T.K., 2003 glyphecotox_195
	Roundup®		5.4	
	POEA		1.2	
Acartia tonsa	Glyphosate IPA-salt	48h (mortality)	49.3	Tsui, M.T.K., 2003 glyphecotox_195
	Roundup®		1.77	
	POEA		0.57	
Ceriodaphnia dubia	Rodeo®	48h (mortality)	415 a.e.	Tsui, M.T.K., 2004; glyphecotox_018
	Roundup Bioactive®		81.5 a.e.	
	Roundup®		5.7 a.e.	
Hyalella azteca	Rodeo®	48h (mortality)	347a.e.	
	Roundup Bioactive®		120 a.e.	
	Roundup®		1.5 a.e.	
Nonarthropoda				
Lampsilis siliquoidea	Glyphosate technical	48h (mortality)	>200	Bringolf, R.B.,, 2007; glyphecotox_119
	Glpyhosate IPA		5	
	Aquastar®		>148	
	Roundup®		2.9	
	MON0818		0.5	
Utterbackia imbecillis	Roundup®	24h (mortality)	18.3	Conners, D.E.,2004; glyphecotox_325
Hydra attenuata	glyphosate (as acid)	96h (mortality)	18.2	Demetrio, P.M.,,2012; glyphecotox_342
	RoundupMax® (74.4% glyphosate)		21.8	

Species	Test item	Study type	LC50 (mg a.e./L)	Reference (internal tag)
Mature oysters	Glyphosate technical		0.002 (larval development)	Akcha, F.,2012; glyphecotox_273
Crassostrea gigas	Glyphosate technical	48h (mortality)	>100	Mottiera, A., 2013
	AMPA		>100	
	Roundup Express®		8.5	
	Roundup Allées et Terrasses®		7.9	
Crassostrea gigas	Glyphosate technical	48h (larval development)	27.1	
	AMPA		46.1	
	Roundup Express®		1.1	
	Roundup Allées et Terrasses®		2.0	

For the group of aquatic vertebrates, a database of more than 60 peer-reviewed publications were submitted by the notifier. The notifier considered seven publications (Filizadeh et al. 2011; Guilherme et al. 2012; Hued et al. 2012; Kelly et al. 2010; Salbego et al. 2010; Benck Soso et al. 2007 and Tierny et al. 2006) and all seven were rated in category "Klimisch 3" (Klimisch 1997). The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. Out of the submitted publications, 24 studies were recognized as supporting information (category UBA2) and are reviewed here.

In the environmental risk assessment of pesticides, the group of aquatic vertebrates is mainly assessed by the results of acute, early life stage or full-life cycle effect studies on laboratory level with the choise of survival, growth and reproduction of individuals as endpoints. This is mainly due to the fact that on higher tiers of the aquatic assessment procedure in semi-field mesocosm studies plankton-dominated aquatic communities are tested that would be strongly disturbed by the presence of fish.

Nevertheless, recent research is focused on endpoints on sub-organismic level, such as indicators of metabolic, haematological and reproduction alterations caused by glyphosate formulations. Varous studies deal with sub-lethal endpoints such as histological alterations of gill, liver and further organ tissues, such as neurotoxic endpoints and genetic biomarkers (Guilherme et al., 2010, Salbego et al., 2010; Soso et al., 2007; De Menezes et al., 2011; Kreutz et al., 2011; Cavalcante et al., 2008; Ferreira et al., 2010; Cattaneo et al., 2011; Modesto et al., 2010; De Menezes et al., 2011).

In a few studies (Evrard et al., 2010; Langiano et al., 2008) histological alterations in the gills and liver or in liver gene expressions or in methionine metabolism, lipid transport and metabolisms related to oxidative stress were observed. Most of these endpoints measured can be taken as early warning indicators of genotoxic and oxidative stress at the individual level but could not be used for in traditional environmental risk assessment, which takes into account the populations levels. Moreover, a few alterations like the enhancement of stress related genes and enzymes are of general character since linked to the metaboolic repsonse towards abiotic and biotic factors of the experimental environment. In most cases they are not considered to be life-threatening or have evident effects on population level. In cases where strong histologic changes were observed, which might lead to impaired organ functioning (e.g Zhidenko et al. 2007; Ortiz-Ordoñez et al., 2003;), the commercial formulation tested was likely to contain POEA as surfactant. The toxicological studies testing the the commercial formulation Roundup® are of limited validity regarding effects of glyphosate-based formulations that do not contain POEA. Although Roundup as the most important herbicide formulation world-wide has been tested frequently, most of the authors have not been stated exactly the contents of acid equivalents, POEA or other surfactants in the formulation used. Concerns on side-effects of glyphosate formulations containing POEA as surfactants raised in

particular early studies (Folmar et al., 1979, Smith et al., 2004, Haller et al., 2003), wheras testing on technical grade glyphosate have seldom been conducted. One example for a test with glyphosate technical is the study by Tierney et al. (2006), who evaluated the effect of relatively low doses of glyphosate on the olfactorial sense of salmons.

None of the studies that were evaluated in detail reported the statistical power of the respective test design. This poses a common difficulty in classifying the validity of tests of highly variable biological systems, even conducted under formally unified laboratory conditions regarding the influence of the environment. The minimal detectable difference between a treatment and a control group depends on the number of replicates and the variability amongst them.

There were no acute mortality endpoints on fish reported in the peer-reviewed open literature that raise particular new concerns compared to the standard studies submitted with the notification of the active substance glyphosate. Most studies were conducted with commercially available formulations that did not allow for keeping apart the effects of the parent active substance glyphosate, its metabolites and the surfactants.

- ***References***

Bringolf, R.B., Cope, W.G., Mosher, S., Barnhart, M.C., Shea, D.; Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae), 2007; Environmental Toxicology and Chemistry Volume: 26 Number: 10 Pages: 2094-2100

Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H.; Genotoxic effects of Roundup (R) on the fish *Prochilodus lineatus* (2008) Mutation Research-Genetic Toxicology and Environmental Mutagenesis 655 (1-2):41-46. DOI 10.1016/j.mrgentox.2008.06.010.

Cattaneo, R., Clasen, B., Loro, V.L., de Menezes, C.C., Pretto, A., Baldisserotto, B., Santi, A.L., de Avila, L.A. Toxicological Responses of *Cyprinus carpio* Exposed to a Commercial Formulation Containing Glyphosate (2011) Bulletin of Environmental Contamination and Toxicology 87 (6):597-602. doi: 10.1007/s00128-011-0396-7.

Cedergreen, N., Streibig, J.C. The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard; 2005; Pest Management Science 61 (12):1152-1160. Doi 10.1002/Ps.1117

Chen, C.Y., Hathaway, K.M., Folt, C.L. Multiple stress effects of Vision (R) herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands; 2004; Environmental Toxicology and Chemistry 23 (4):823-831

De Menezes, C.C., da Fonseca, M.B., Loro, V.L., Santi, A., Cattaneo, R., Clasen, B., Pretto, A., Morsch, V.M.; Roundup Effects on Oxidative Stress Parameters and Recovery Pattern of *Rhamdia quelen* (2011) Archives of Environmental Contamination and Toxicology 60 (4):665-671. DOI: 10.1007/s00244-010-9574-6.

Demetrio, P.M., Rossini, G.D.B., Bonetto, C.A., Ronco, A.E.; Effects of Pesticide Formulations and Active Ingredients on the Coelenterate *Hydra attenuata* (Pallas, 1766); 2012; Bulletin of Environmental Contamination and Toxicology 88 (1):15-9. doi:10.1007/s00128-011-0463-0.

Dominguez-Cortinas, G., Saavedra, J.M., Santos-Medrano, G.E., Rico-Martinez, R., Analysis of the toxicity of glyphosate and Faena® using the freshwater invertebrates *Daphnia magna* and *Lecane quadridentata*; 2008; Toxicological & Environmental Chemistry 90 (2):377 – 384.

Evrard, E., Marchand, J., Theron, M., Pichavant-Rafini, K., Durand, G., Quiniou, L., Laroche,

J.Impacts of mixtures of herbicides on molecular and physiological responses of the European flounder Platichthys flesus (2010) Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 152 (3):321-331. DOI: 10.1016/j.cbpc.2010.05.009.

Filizadeh, Y., Islami, H.R.; Toxicity determination of three sturgeon species exposed to glyphosate (2001) Iranian Journal of Fisheries Sciences 10 (3):383-392.

Ferreira, D., da Motta, A.C., Kreutz, L.C., Toni, C., Loro, V.L., Barcellos, L.J.G.; Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals (2010) Chemosphere 79 (9):914-921. DOI: 10.1016/j.chemosphere.2010.03.024.

Folmar, LC, Sanders HO, Julin AM; Toxicity of the Herbicide Glyphosate and Several of Its Formulations to Fish and Aquatic Invertebrates (1979) Archives of Environmental Contamination and Toxicology 8: 269-278

Guilherme, S., Gaivão, I., Santos, M.A., Pacheco, M.; European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® a glyphosate-based herbicide (2010) Mutagenesis 25 (5):523-530. DOI: 10.1093/mutage/geq038.

Haller, W.T., Stocker, R.K. Toxicity of 19 adjuvants to juvenile *Lepomis macrochirus* (bluegill sunfish) (2003) Environmental Toxicology and Chemistry 22 (3):615-619.

Hued, A.C., Oberhofer, S., de los Ángeles Bistoni, M., Exposure to a Commercial Glyphosate Formulation (Roundup) Alters Normal Gill and Liver Histology and Affects Male Sexual Activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes) (2012) Archives of Environmental Contamination and Toxicology 62 (1):107-117. DOI: 10.1007/s00244-011-9686-7.

Kelly, D.W., Poulin, R., Tompkins, D.M., Townsend, C.R.; Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival (2010) Journal of Applied Ecology Volume: 47 Issue: 2 Pages: 498-504 Url: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2010.01791.x/pdf DOI: 10.1111/j.1365-2664.2010.01791.x ISSN: 1365-2664 (online).

Kreutz, L.C., Barcellos, L.J.G., de Faria Valle, S., de Oliveira Silva, T., Anziliero, D., dos Santos, E.D., Pivato, M., Zanatta, R. Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate (2011) Fish Shellfish Immunol 29 (4):694-7. DOI: 10.1016/j.fsi.2010.06.003.

Le, T.H., Lim, E.S., Lee, S.K., Choi, Y.W., Kim, Y.H., Min, J.; Effects of glyphosate and methidathion on the expression of the Dhb, Vtg, Arnt, CYP4 and CYP314 in Daphnia magna; 2010;Chemosphere 79: 67-71

Langiano, V.dC., Martinez, C.B.R.; Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*; (2008)Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 147 (2):222-231

Ma, J., Lin, F., Wang, S., Xu, L.; Toxicity of 21 herbicides to the green alga *Scenedesmus quadricauda;* 2006; Environmental Contamination and Toxicology 71 (3):594-601. DOI 10.1007/s00128-003-8521-x.

Modesto, K.A., Martinez, C.B.R. Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity (2010) Chemosphere 81 (6):781-787. DOI: 10.1016/j.chemosphere.2010.07.005

Mensah, P.K., Muller, W.J., Palmer, C.G.; Acute toxicity of Roundup® herbicide to three life stages of the freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae); 2011; Physics and Chemistry of the Earth, Parts A/B/C 36 (14–15):905-909

Mottiera, A., Kientz-Bouchart V., Serpentinia, A., Lebela, J.M.; Jhac,A.N., Costil, K.; Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, *Crassostrea gigas*; 2013; Aquatic Toxicology 128– 129 (2013) 67–78.

Ortiz-Ordoñez, E., Uría-Galicia, E., Ruiz-Picos, R.A., Duran, A.G.S., Trejo, Y.H., Sedeño- Díaz, J.E., López-López, E. Effect of Yerbimat Herbicide on Lipid Peroxidation, Catalase Activity, and Histological Damage in Gills and Liver of the Freshwater Fish *Goodea Atripinnis* (2011) Archives of Environmental Contamination and Toxicology 61 (3):443-452. DOI: 10.1007/s00244-011-9648-0.

Perez, G.L., Torremorell, A., Mugni, H., Rodriguez, P., Solange Vera, M., do Nascimento, M., Allende, L., Bustingorry, J., Escaray, R., Ferraro, M., Izaguirre, I., Pizarro, H., Bonetto, C., Morris, D.P., Zagarese, H.; Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study; 2007; Ecol Appl 17 (8):2310- 22.

Salbego, J., Pretto, A., Gioda, C.R., de Menezes, C.C., Lazzari, R., Radunz Neto, J., Baldisserotto, B., Loro, V.L.; Herbicide formulation with glyphosate affects growth, acetylcholinesterase activity, and metabolic and hematological parameters in piava (*Leporinus obtusidens*) (2010) Arch Environ Contam Toxicol 58 (3):740-5. DOI: 10.1007/s00244-009-9464-y.

Soso, A.B., Barcellos, L.J.G., Ranzani-Paiva, M.J., Kreutz, L.C., Quevedo, R.M., Anziliero, D., Lima, M., da Silva, L.B., Ritter. F., Bedin, A.C., Finco, J.A.; Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundia (*Rhamdia quelen*) (2007) Environmental Toxicology and Pharmacology 23 (3):308-313. DOI 10.1016/j.etap.2006.11.008.

Smith, B.C., Curran, C.A., Brown, K.W., Cabarrus, J.L., Gown, J.B., McIntyre, J.K., Moreland, E.E., Wong, V.L., Grassley, J.M., Grue, C.E. Toxicity of four surfactants to juvenile rainbow trout: Implications for use over water (2004) Bulletin of Environmental Contamination and Toxicology 72 (3):647-654. DOI 10.1007/s00128- 004-0292-5.

Tierney, K.B., Ross, P.S., Jarrard, H.E., Delaney, K.R., Kennedy, C.J.; Changes in juvenile coho salmon electro-olfactogram during and after short-term exposure to current-use pesticides (2006) Environ Toxicol Chem 25 (10):2809-17.

Tsui, M.T.K., Chu, L.M.; Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors; 2003;Chemosphere 52: 1189–1197.

Tsui, M.T.K., Chu, L.M.; Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors; 2003; Chemosphere 52: 1189–1197.

Tsui, M.T.K., Chu, L.M.; Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures; 2004; Arch. Environm. Contam. Toxicol.46, 316-323

Turgut, C., Fomin, A.; Sensitivity of the rooted macrophyte *Myriophyllum aquaticum* (Vell.) Verdcourt to seventeen pesticides determined on the basis of EC50; 2002; Bulletin of

Environmental Contamination and Toxicology 69 (4):601-608

Vera, M.S., Lagomarsino, L., Sylvester, M., Perez, G.L., Rodriguez, P., Mugni, H., Sinistro, R., Ferraro, M., Bonetto, C., Zagarese, H., Pizarro, H. New evidences of Roundup (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems; 2010; Ecotoxicology, 19:710-721

Zhidenko, A.A., Kovalenko, Y.M.; The influence of Roundup on the dynamics of histological changes in organs of carps (2007) Hydrobiological Journal 43 (2):93-99

- **B.9.13.10 Effects on amphibians**

For the group of amphibians, a comprehensive database of 85 peer-reviewed papers was collected by the notifier. Out of the submitted, 20 studies were recognized by the notifier as relevant for full evaluation. Further 54 papers were cited in the text and considered supporting the submission, but were not submitted with the application, mostly because of publishing date older than 10 years.
Moreover, further publications have been submitted, but were not assigned to one of the areas of assessment by the notifier. After UBA screening, further 28 papers were identified for the assessment of glyphosate effects on amphibians. Critical and relevant studies are summarized below.

Howe et al. (2004)

glyphecotox_025	**Howe, C.M., Berrill, M., Pauli, B.D., Helbing, C.C., Werry, K., Veldhoen, N.**	**2004**	**Toxicity of glyphosate-based pesticides to four North American frog species**	**Environmental Toxicology and Chemistry 23 (8):1928-1938**
Reliability				
Purpose of the study Description of endpoints	The study was designed to compare the acute and chronic toxicities of six glyphosate formulations, the technical-grade glyphosate and a polyethoxylated tallow amine surfactant to tadpoles of four species of North American frogs. The sensitivity of different tadpole stages towards glyphosate was examined. Acute studies: Mortality, survival Chronic studies: Forelimb emergence = time to reach Gosner stage 42, Total length, body length, tail length, visible tail damage and maximum tail height were recorded, snout-vent-length of metamorphs; gonadal histology to determine sex ratios			
Test compound, application procedure, exposure period, protocol	Acute studies static exposure for 96h, expressed as mg test item/L and as formulation glyphosate acid equivalents to enable direct comparisons between the different mixtures of ingredients of the different formulations (FAE: It is assumed 'that the surfactant does not contain glyphosate acid, so the FAE used for the surfactant refers to the calculated amount of glyphosate acid in its formulation equivalent, assuming the surfactant component to be approximately 15%. To calculate the FAE in each glyphosate herbicide formulation, the values published by Giesy et al. (2000, glyphnosubm_050) were followed Thus, for a glyphosate-based formulation of 1.0, the FAE is 0.31, and the surfactant is 0.15. In other words, 1 mg of the formulation is assumed to contain 0.31 mg of glyphosate acid equivalent and approximately 0.15 mg of POEA.' Chronic studies Exposure period 42d, static renewal: weekly spiking of the test items (6 application dates), then rearing in clean water until day 70. Non-GLP			

Experimental approach Statistical design, test environment	Acute studies 20 tadpoles at Gosner-stage 25 were used per treatment-replicates for the formulation comparison and 10 tadpoles at Gosner stage 20 and 25 for the stage-comparison with Roundup Original®, 3 replicates per concentration were tested Test items: Roundup Original®, Glyphosate technical, POEA, Roundup Biactive®, Touchdown®, Glyfos BIO®, Glyphos AU®, Roundup Transorb®; at least four concentrations up to 18 mg FAE/L were tested to determine LC50 and confidence itervals Determination of LC_{50} for 24 and 96h exposure by trimmed Spearman-Karber method Chronic studies test items 1.8 FAE glyphosate technical/L, 0.6 and 1.8 FAE polyethoxylated tallowamine surfactant (POEA)/L, 0.6 and 1.8 FAE Roundup Original/L, 0.6 and 1.8 FAE Roundup Transorb/L
Test organisms	Acute studies *Rana pipiens*, *Rana sylvatica*, *Bufo americanus*, *Rana clamitans* Chronic studies *Rana pipiens*
Biological effects	Acute studies Not all treatment showed sufficient mortality to calculate proper LC50. From the published paper: Table 2. Acute toxicity (median lethal concentration values [LC50] with 95% confidence intervals in parentheses) obtained in 24-h and 96-h exposures of four amphibian species exposed to glyphosate-based herbicides, glyphosate technical material, and polyethoxylated tallowamine surfactant (POEA) at two life stages[a]

			LC50			
			24 h		96 h	
Species	Gosner stage	Compound	mg/L	mg FAE/L	mg/L	mg FAE/L
Rana pipiens[b]	25	Roundup Original®	11.9 (11.2–12.6)	3.7 (3.5–3.9)	9.2 (NR)	2.9 (NR)
R. pipiens[c]	20	Roundup Original	>25.8	>8	20.9 (19.8–21.9)	6.5 (6.1–6.8)
R. sylvatica[c]	25	Roundup Original	18.1 (16.7–19.6)	5.6 (5.2–6.1)	16.5 (15.7–17.4)	5.1 (4.9–5.4)
R. sylvatica[c]	20	Roundup Original	>25.8	>8	>25.8	>8
Bufo americanus[c]	25	Roundup Original	13.5 (NR)	4.2 (NR)	<12.9	<4
B. americanus[c]	20	Roundup Original	>25.8	>8	25.8 (NR)	8 (NR)
R. clamitans[d]	25	Roundup Original	6.6 (6.1–7.1)	2.0 (1.9–2.2)	6.5 (6.0–7.0)	2.0 (1.9–2.2)
R. clamitans[c]	20	Roundup Original	>25.8	>8	22.8 (21.2–24.5)	7.1 (6.6–7.6)
R. clamitans[d]	25	Glyphosate technical	>38.9	>17.9	>38.9	>17.9
R. clamitans[d]	25	POEA	1.1 (1.1–1.2)	2.4 (2.2–2.5)	1.1 (1.0–1.1)	2.2 (2.1–2.4)
R. clamitans[d]	25	Roundup Biactive®	>57.7	>17.9	>57.7	>17.9
R. clamitans[d]	25	Touchdown®	>57.7	>17.9	>57.7	>17.9
R. clamitans[d]	25	Glyfos BIO®	>57.7	>17.9	>57.7	>17.9
R. clamitans[d]	25	Glyfos AU®	29.1 (28.1–30.2)	9.0 (8.7–9.4)	28.6 (27.6–29.6)	8.9 (8.6–9.2)
R. clamitans[d]	25	Roundup Transorb®	7.4 (6.9–7.9)	2.3 (2.2–2.4)	7.2 (6.8–7.7)	2.2 (2.1–2.4)

[a] Roundup Original, Roundup Biactive, and Roundup Transorb from Monsanto (St. Louis, MO, USA); Touchdown from Syngenta (Wilmington DE, USA); Glyfos BIO and Glyfos AU from Cheminova (Wayne, NJ, USA). FAE = formulation glyphosate acid equivalents; NR = 95% confidence intervals not reliable.
[b] 2000 Chronic study.
[c] 1994 Study.
[d] 2001 Study.

Earlier stages were slightly less sensitive than Gosner stage 25-individuals
Chronic studies
38 % mortality in control aquaria undermines experiment validiy
POEA<1.8, Roundup Original ® 0.6 and 1.8 and Roundup Transorb ® showed significant tail damages and reduced tail lengths. NO effects with glyphosate alone.
POEA containing formulations showed displaced sex ratios towards intersex individuals.
The lengths and percent surviving tadpoles to reach Gosner stage 42 as well as the days to reach stage 42 were significantly altered by most of the treatments except of glyphosate technical. However, results were not strictly dose-dependent. |
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	It was necessary to measure the developmental endpoints on juvenile tadpole stages. It was proven in this experiment that earlier tadpole stages were less sensitive, which is quite contrary to common expectations that earlier stages should be more sensitive towards chemical stress.
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Yes

3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	The intersex-hypothesis has been intensively criticised by the notifier (weaknesses in histological and statistical analysis), which can be only partly agreed by RMS. There was no statistical procedure described for this endpoint, so that it could be seen as a qualitative measure. Other endpoints have been measured and analysed adequately and are considered by RMS appropriate growth and developmental indicators of toxic stress.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Since there was a full-factorial design with glyphosate technical, the POEA surfactants and diverse formulations containing both glyphosate and surfactants, the study is of high environmental relevance.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The LC_{50}s of the tested formulations were mainly around 5 mg FAE/L, which is in the range of the relevant aquatic endpoints for the environmental risk assessment of glyphosate.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	There was a very high control mortality of 38% that was tried to eliminate by the authors by regular water exchange. The ammonia level was presumably too high, as well as the density of tadpoles per area most probably was.
Concluding weight of evidence/proposed action	Important data are presented that prove the high toxic potential of polyethoxylated tallow amines to different species of amphibians. The POEA treatment showed the highest toxicity in the acute tests. Dependent on the formulation that will be assessed by an ERA, the enhanced toxicity by surfactant additives should be considered and could be referenced to this well conducted and informative publication. Can be used for aquatic assessments. The chronic studies have several weaknesses (statistics, control survival rates, dose-response-relationship). Nevertheless, the results indicate several open questions in the assessment of chronic exposure of amphibian to formulated glyphosate products. None of the effects was observed in the treatments with glyphosate alone
Type of information (Critical, supporting, low weight)	**Critical, supporting**
Consideration/concluding score	**UBA1, also for assessment of surfactand effects (POEA)**

Thompson et al. (2004)

glyphecotox_038	**Thompson, D.G., Wojtaszek, B.F., Staznik, B., Chartrand, D.T., Stephenson, G.R.**	**2004**	**Chemical and biomonitoring to assess potential acute effects of Vision (R) herbicide on native amphibian larvae in forest wetlands**	**Environmental Toxicology and Chemistry, Vol. 23, No. 4, pp. 843–849, 2004**

Reliability	
Purpose of the study Description of endpoints	Chemical and biological monitoring studies were conducted following operational forest herbicide spray programs in Ontario, Canada. Magnitude of contamination by a glyphosate herbicide formulation (Vision) was investigated in 51 different wetlands. Wetlands were classified as oversprayed, adjacent, or buffered.
Test compound, application procedure, exposure period	Vision, Glyphosate product identical to Roundup Original, Monsanto. Aerial herbicide treatments of conifer crop trees. Percent mortality at 48 h posttreatment was calculated as response variable.
Experimental approach, Statistics, test environment	Not clear how many sites have replicates within blocks. Larval condition was observed and recorded periodically at approximately 6, 24, 48, and 96 h following herbicide applicationsData for each larval test species were pooled across years and mean mortality rates were calculated for each wetland classification (oversprayed, adjacent, or buffered)
Test organisms	*Rana pipiens* and *Rana clamitans* larvae (Gosner 25)

Biological effects	The mean glyphosate concentrations recorded were: buffered wetlands : 0.03 mg a.e./L adjacent wetlands: 0.18 mg a.e./L.. oversprayed wetlands: 0.33 mg a.e./L; maximum of 1.95 mg a.e./L. Mean mortality rates leopard frog larvae < 15% in all wetland types. No significant differences in mean mortality rates were observed for leopard frog larvae exposed to different glyphosate concentrations under adjacent, buffered, or oversprayed wetland scenarios. Green frog larvae showed higher mean mortality rates of 10, 26, and 36% in adjacent, buffered, and oversprayed wetlands, respectively. These differences were not statistically significant. RMS remark: sites/years/blocks were pooled. No separation of different factors possible (time/site/block and glyphosate concentration).
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Active ingredient: yes, but applied as formulated product Lead formulation: no (Vision® with 15% POEA)
2 Do the tested concentrations relate to predicted environmental concentrations?	yes (no overspray of wetlands in the European Union, though)
3 Have parameters influencing the endpoints been considered adequately?	Not conclusive (pooling of several monitoring years/no single data available)
Concluding weight of evidence/proposed action	No data available on the variance of larval response allocated to site/year/block factors. Pooling of results on biological and chemical responses over sites and years may lead to misleading interpretation of results. Effects of glyphosate application at site level with direct comparison of sprayed/not sprayed wetland not reported.
Type of information (Critical, supporting, low weight)	**Supporting/low weight**
Consideration/concluding score	**UBA2**

Edge et al. (2011)

glyphecotox_043	**Edge, C.B., Gahl, M.K., Pauli, B.D., Thompson, D.G., Houlahan, J.E.**	**2011**	**Exposure of juvenile green frogs (Lithobates clamitans) in littoral enclosures to a glyphosate-based herbicide**	**Ecotoxicology and Environmental Safety 74, 1363–1369 doi:10.1016/j.ecoenv.2011.04.020**
Reliability				
Purpose of the study Description of endpoints	Juvenile green frogs(*Lithobates clamitans)* were exposed to two concentrations (2.16 and 4.27 kg a.e./ha) of a glyphosate formulation (VisionMax®), under typical application scenarios in Canadian forestry. survival, body condition, liver somatic index, observed rate of *Batrachochytrium dendrobatidis* infection.			

Test compound, application procedure, exposure period	Enclosures: half of the enclosure was terrestrial and the other half aquatic Each herbicide treatment was comprised of two spray applications. One application was made by spraying the formulated product using a backpack sprayer (Flowmaster, Root-Lowell Manufacturing, Lowell, MI, USA) to one-half of the wetland, while an equivalent second spray application was made directly to the enclosure using a small plant-misting bottle. Environmentally observed concentration (EOC): 0.55 mg a.e./L (upper 99th centile of concentrations measured in Thompson et al., 2004). Predicted maximum environmental concentration(PMEC): 2.89 mg a.e./L
Experimental approach, Statistics, test environment	s.a. Following herbicide applications, animals were counted 1, 4, 7and 14 days after treatment (DAT) to determine survival. On DAT 14, SVL was measured and all animals were weighed.On DAT 14 all animals were euthanized. All animals were dissected, livers were removed. Liver somatic index (LSI) was calculated by dividing wet liver mass by wet body mass and multiplying by100. All animals were examined for Bd infection. Differences in arcsinesquareroot transformed proportional survival data/split-plot analysis of variance (ANOVA), with treatment rate as between subject factor,and side (control or treatment) as the within subject factor on DAT 14.
Test organisms	Juvenile green frogs(*Lithobatesclamitans)*
Biological effects	No significant difference in survival between treated and control wetlands. After 14 days, no difference in body condition between wetland sides and no relationship between the measured glyphosate application rate and body condition was observed No significant difference in the number of animals infected with Bd. "marginally significant" negative relationship between the measured glyphosate application rate and the frequency of Bd infection. RMS: Difficulties in the determination of the glyphosate concentrations in the wetlands. Option for use of nominal concentrations discussed.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	VisionMax co-formulation ingredients not known
2 Do the tested concentrations relate to predicted environmental concentrations?	-/-
3 Have parameters influencing the endpoints been considered adequately?	-/-

Concluding weight of evidence/proposed action	Coformulants not known. Glyphosate was not tested alone. Product was applied to soil and water. Exposure pattern not clear
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Wojataszek et al. (2004)

glyphecotox_044	**Wojtaszek, B.F., Staznik, B., Chartrand, D.T., Stephenson, G.R., Thompson, D.G..**	**2004**	**Effects of Vision (R) herbicide on mortality, avoidance response, and growth of amphibian larvae in two forest wetlands**	**Environmental Toxicology and Chemistry, Vol. 23, No. 4, pp. 832–842, 2004**
Reliability				

<table>
<tr><td>Purpose of the study
Description of endpoints</td><td>The effects of Visiont (glyphosate, 356 mg acid equivalents (a.e.)/L) on mortality, avoidance response, and growth of larval amphibians (<i>Rana clamitans</i> and <i>Rana pipiens</i>) were investigated.
In situ enclosures deployed in two forest wetlands of northern Ontario, Canada.</td></tr>
<tr><td>Test compound, application procedure, exposure period, protocol</td><td>Visiont (glyphosate, 356 mg acid equivalents (a.e.)/L) similar formulation to Roundup Original ® with 15% POEA
Twenty-four in situ enclosures were positioned at each site. Thirteen enclosures used. The amount of formulated product required to achieve the desired nominal concentrations was based on enclosure volume.</td></tr>
<tr><td>Experimental approach Statistical design, test environment</td><td>General Linear Model procedure (SAS), variance analysis on 96-h mortality least-squares means for each combination of site and species. Preplanned comparisons for differences between mean mortality observed in untreated controls and in replicate enclosures treated at the 1.43 mg a.e./L (RMS: PEC overspray Canada)..</td></tr>
<tr><td>Test organisms</td><td>Free-swimming <i>Rana pipiens</i> and <i>Rana clamitans</i> larvae (Gosner 25 at time of herbicide application)</td></tr>
<tr><td>Biological effects</td><td>
Table 4. Toxicity of Vision® (Monsanto, Winnipeg, MB, Canada) to <i>Rana clamitans</i> and <i>Rana pipiens</i> larvae. Ninety-six-hour lethal concentration (LC) point estimates at 10 and 50% mortality were estimated from predictive models derived from analysis of deviance. Ninety-five percent confidence limits about point estimates are given in parentheses. Both experimental sites were located approximately 80 km northeast of Sault Ste. Marie, Ontario, Canada. Site A: 46°53′20″N, 84°7′45″W; Site B: 47°02′04″N, 84°23′06″W.
<table>
<tr><th>Site</th><th>Species</th><th>LC10 (mg a.e.^a/L)</th><th>LC10 (mg Vision/L)</th><th>LC50 (mg a.e./L)</th><th>LC50 (mg Vision/L)</th></tr>
<tr><td>A</td><td><i>Rana clamitans</i></td><td>1.78
(0.99, 2.86)</td><td>5.74
(3.19, 9.23)</td><td>4.34
(3.05, 6.02)</td><td>14.0
(9.84, 19.4)</td></tr>
<tr><td></td><td><i>Rana pipiens</i></td><td>7.30
(3.83, 9.54)</td><td>23.6
(12.4, 30.8)</td><td>11.47
(9.50, 14.5^b)</td><td>37.0
(30.6, 46.8^b)</td></tr>
<tr><td>B</td><td><i>R. clamitans</i></td><td>1.20^c
(0.84, 1.60)</td><td>3.87
(2.71, 5.16)</td><td>2.70
(2.06, 3.67)</td><td>8.71
(6.65, 11.8)</td></tr>
<tr><td></td><td><i>R. pipiens</i></td><td>3.26
(1.66, 3.61)</td><td>10.5
(5.36, 11.6)</td><td>4.25
(2.45, 7.10)</td><td>13.7
(7.90, 22.9^b)</td></tr>
</table>
[a] a.e. = glyphosate acid equivalents.
[b] Above range of concentrations tested.
[c] Below expected environmental concentration (EEC) of 1.43 mg a.e./L.
As the aurthors state:‘Experimental site and biotic/abiotic factors therein, such as pH and suspended sediments, substantially affected the expression of Vision herbicide toxicity in the amphibian larvae tested.’</td></tr>
<tr><td colspan="2">Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints</td></tr>
</table>

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Yes
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	Yes.

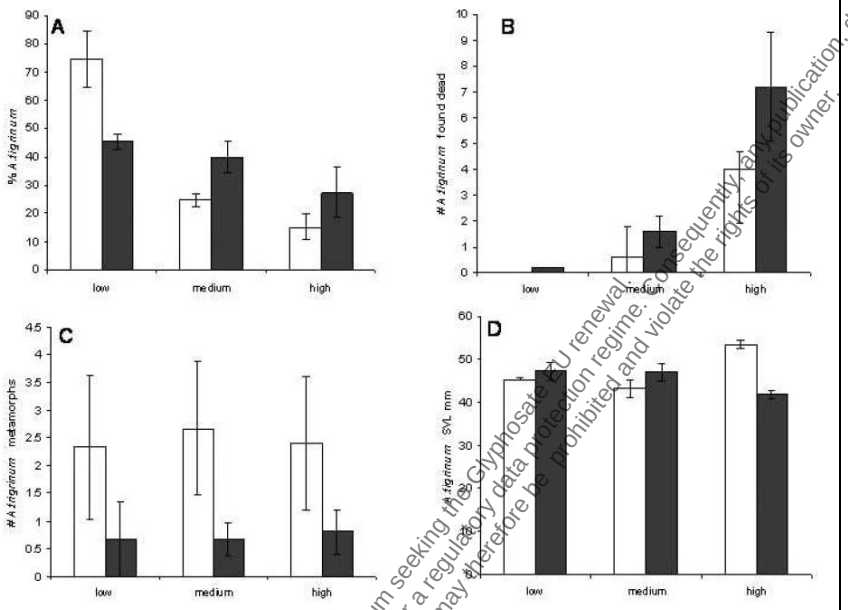
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, probably for all formulations containing POEA. Can not be used for the assessment of glyphosate technical/glyphosate acid, although all endpoints are given in acid equivalents.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Comparable high PECsurface water reported in the paper due to watershed overspray practice in Canada.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Other parameters influencing the response have been measured, but were not directly considered e.g. in the assessment of difference in toxicity beween sites.
Concluding weight of evidence/proposed action	The study presents acute mortalities for two amphibian species. The data can be employed in the assessment of formulations containing POEA as surfactants. No data for glyphosate technical or glyphosate acid deducible from the tested formulation. Lead formulation for EU renewal of glyphosate approval contains no POEA

Type of information (Critical, supporting, low weight)	**Critical/Supporting**
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Brodman et al. (2010)

glyphecotox_048	**Brodman, R., Newman, W.D., Laurie, K., Osterfeld, S,, Lenzo, N.**	**2010**	**Interaction of an Aquatic Herbicide and Predatory Salamander Density on Wetland Communities**	**Journal of Herpetology, Vol. 44, No. 1, pp. 69–82, 2010 DOI: 10.1670/08-320.1**

Reliability	
Purpose of the study Description of endpoints	Replicated field experiment in constructed ponds to test for both the effects of the glyphosate formulation Accord® and predator (Tiger Salamanders, *Ambystoma tigrinum*) density on amphibians and aquatic invertebrates. Behavior assays of salamander larvae to investigate predator-prey relationships.
Test compound, application procedure, exposure period, protocol	5% herbicide mixture of Accord and 3% Cide-Kick II (aquatic surfactant) Active substance(s): Glyphosate. Surfactant: a nonylphenolpolyethylene NPE-based product (wetting agent Cide-Kick II ®) no a.s. loading reported
Experimental approach Statistical design, test environment	a) Outdoor experimental ponds b) Behavioural assays were conducted ex vivo under laboratory conditions Ponds: 6 x 6 m, volume: 24 m^3, depth: 0.67 m; exposure 18th May until end of June 2006 (ca. 1 ½ month), repeated in the year 2007 (started on 14th May, duration not stated); Activity and feeding assays: pyrex containers (7 cm high x 20 cm wide x 20 cm long) Microhabitat assay: plastic containers (26 cm high x 23.5 cm wide x 33 cm long) filled to a depth of 16 cm with dechlorinated tap water. The containers were partitioned into two equal chambers using a plastic mesh with 1.5-cm openings. One chamber had a 2-cm layer of pondweed, leaf litter, and algae, the other chamber was left empty. For the activity, behaviour, feeding and microhabitat assay, samples were collected once a week and assessments were conducted in the afternoon and early evening (13:00 – 20:00h). Snout vent length (SVL) of *A. tigrinum* larvae, amphibian and invertebrate density, species richness and diversity, mortality, metamorphosis, number of movements (position of the head), distance moved by the larvae, feeding activity (predation rate, prey preference), stomach content of dead larvae, behaviour (aggression, percentage of time in vegetation and time separated).
Test organisms	Experimental pond communities containing tadpoles of different species of amphibians: *Ambystoma tigrinum* *Rana pipiens* *Rana clamitans* *Bufo americanus* Aquatic invertebrates as natural inhabitants *Ambystoma tigrinum* larvae (mesocosm study):mean size of 32.1 mm SVL19 (3 size classes "< 25 mm", "25 – 35 mm" and ">35 mm"), age not specified Laboratory assays conducted with larvae of *A. tigrinum*: not precisely stated, for microhabitat assay approximately the same age with SVL differences ranging from 5 – 10 mm.

Biological effects	From the paper:  FIG. 1. The effect of herbicide treatment on the mean percentage of *Ambystoma tigrinum* larvae (A) captured, (B) number found dead, (C) number of metamorphs, and (D) SVL (mm) per pond, at three initial salamander densities (low, medium, high). Light bars indicate control ponds, and dark bars indicate herbicide treated ponds. Error bars indicate the standard error of the mean. Effects of glyphosate formulation on salamander is density dependent. Herbicide treatment and salamander density have an overall effect on tadpoles (interaction between herbicide treatment and initial density also significant). Significant effect of herbicide treatment on the SVL of Northern Leopard Frog tadpoles. SVL and number of metamorphic Northern Leopard Frogs were greater in herbicide-treated ponds.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	No. Formulation with nonylphenolpolyethylene NPE-based surfactant
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Difficult to determine (loading not determined, no analytics)
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Abiotic parameters (pH, alkalinity, nitrate, nitrite and dissolved oxygen) in outdoor ponds were monitored on week 1, 3, 5 and 7, but not entirely reported
Concluding weight of evidence/proposed action	Formulated product not relevant for current assessment (glyphosae + NPE). Glyphosate was ot tested per se.
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Cauble & Wagner, (2005)

glyphecotox_049	**Cauble, K., Wagner, R.S.**	**2005**	**Sublethal effects of the herbicide glyphosate on amphibian metamorphosis and development**	**Bulletin of Environmental Contamination and Toxicology, 75, 429-435**

Reliability	
Purpose of the study Description of endpoints	Effects of chronic exposure to Roundup ® were investigated at non-acute levels in a static renewal test on *Rana cascadae* larval metamorphosis and development. Larvae were evaluated on a daily basis for 43 days for mortality, feeding behaviour, swimming activity, morphological abnormalities and behavioural alterations.
Test compound, application procedure, exposure period, protocol	Roundup ® (50.2% glyphosate isopropylamine salt), specific product not reported! RMS: Roundup ® Original? Seven larvae per treatment were exposed to five replicates of each treatment of 0, 1, and 2 mg glyphosate/L (nominal) and a control.
Experimental approach Statistical design, test environment	Static renewal (7 day intervals). Duration of study: 43 d chronic 5 replicates per concentration, Organisms per replicate: 7; Feeding: Not stated Larvae were evaluated on a daily basis for 43 days for mortality (time to death), feeding behaviour (feeding or not feeding), swimming activity (high, medium, slow), morphological abnormalities (edema, lesions, bent tail) and behavioural alterations (head out of water, erupted forelimbs, erupted hind limbs, emersion from water). Mean dry mass was compared using Student's t-test; differences among replicates and treatments were evaluated using one-way ANOVA followed by Tukey-Kramer Multiple comparison tests with NCSS as post-hoc.
Test organisms	*Rana cascadae*
Biological effects	Tadpoles were continuously exposed to concentrations of 1 and 2 mg glyphosate a.e./L along with an untreated control in a static renewal system with weekly renewals. Glyphosate concentrations were measured and mean measured levels were similar to nominal concentrations. At the highest concentration tested (1.94 mg glyphosate/L, mean measured), no individuals survived until end of the exposure. The 48 hour LC50 value for *R. cascadae* is reported to be 3.2 mg a.e/L. Exposure to 1 mg glyphosate/L resulted in earlier metamorphosis and smaller size for Roundup ®, when compared to the control.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Unfortunately, the product assessed is not specified (Roundup®..). Therefore, no precise assignment is possible.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Control survival, the loading rate, water quality parameters, and the water temperature during the exposures not reported

Concluding weight of evidence/proposed action	Unfortunately, the precise formulation identification is not possible. Moreover, experimental details are missing (e.g. mortalities in controls). Supporting for fromulations containing POEA (LC50 same range as other publications with Glyphosate+POEA formulations).
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Dinehart et al. (2010)

glyphecotox_064	**Dinehart, S.K., Smith, L.M., McMurry, S.T., Smith, P.N., Anderson, T.A., Haukos, D.A.**	**2010**	**Acute and chronic toxicity of Roundup WeatherMAX® and Ignite® 280 SL to larval *Spea multiplicata* and *S. bombifrons* from the Southern High Plains, USA**	**Environmental Pollution 158 (8):2610-2617. DOI 10.1016/j.envpol.2010.05.006.**

Reliability	
Purpose of the study Description of endpoints	Acute and chronic effects of two herbicide formulations (Roundup WeatherMAX®, active ingredient glyphosate; and Ignite 280 SL® (IG), active ingredient glufosinate) to larvae of New Mexico spadefoot toads and Plains spadefoot toads. It was desired to compare for differences between typical populations of croplands and grasslands. Here only setup and results of tests with glyphosate are discussed (not glufosinate). Body weight and survival rates of amphibian larvae
Test compound, application procedure, exposure period, protocol	Roundup WeatherMAX, 48.8 % glyphosate in potassium salt form, 51.2% 'other ingredients' Non-GLP, but ASTM Guideline for acute toxicity tests with aquatic organisms, including amphibians was used. 80% of water was changed after 4 days to maintain normal range ammonia concentrations Acute studies 48h static exposure, 168h post-exposure period Chronic studies Static-renewal exposure for 30 days
Experimental approach Statistical design, test environment	Aquaria of 18.95 L, containing 15 L aged tap water, water quality was monitored Acute study Test concentrations: WM 0.75, 1.5, 2.25, 3, 4.5, 6, 7.5,10 mg glyphosate/L, 9 tadpoles in each of three replicate containers of each treatment Non-normal distribution of weights: Wilcoxon-two-sample test; 48 and 216 hour LC50 by Probit-analysis Chronic study Test concentrations: 2.0 and 2.8 mg acid equivalents of glyphosate T-Tests for weight differences, GLM to analyse survival data-series, percent survival as response variable, treatment, landuse, species as independent variables
Test organisms	larval *Spea multiplicata*, New Mexico spadefoot toads and larval *Spea bombifrons,* Plains spadefoot toads, all at Gosner stages 29-30

Biological effects	Acute studies: Most sensitive LC_{50} 48h = 1.85 mg ae/L and LC_{50} 216h = 1.65 mg ae/L for *S. bombifrons*, no significant differences between the individual origins crop- or grassland nor between the species in survival rates and body weights Chronic studies none of all spadefoots tested chronically survived the longer than 12 days of exposure, while control mortality was very low. The statistical comparison of the factor-combinations did not reveal clear answers. From the published paper (modified): Table 3: Acute toxicity of RoundupWeatherMAX (WM) to larval Spea multiplicata and S. bombifrons (New Mexico and Plains spadefoot, respectively) from playa wetlands embedded in cropland or grassland. Both 48- and 216-h (i.e., including post-exposure mortality) LC values and associated 84% confidence intervals were calculated via probit analysis. From the paper:

	n	WM LC_{50} values (84% confidence intervals), mg glyphosate acid equivalents (ae) [a]	
		48-h	216-h
S. bombifrons			
Grass	208	2.03 (1.90–2.16)^{Bc*}	1.99 (1.85–2.13)^{Bc*}
Crop	175	1.85 (1.62–2.06)^{Bc*}	1.65 (1.42–1.87)^{Ba*}
S. multiplicata			
Grass	80	2.30 (2.06–2.55)^{Bc*}	1.93 (1.68–2.20)^{Bc*}
Crop	113	2.11 (1.85–2.41)^{Bc*}	2.11 (1.85–2.41)^{Bc*}

RMS: The use of the generalised linear statistical method remained unclear, so was the use of the replication in the whole study. It could not be understood if the data was analysed as a time series, since time was not taken as an explanatory factor or as covariable in the analysis.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, larvae of toads should be more sensitive towards aquatic exposure due to gill breathing
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes, survival of juveniles.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, probably for all formulations containing adjuvants of relevant toxicity in similar amounts. Not to be used for the assessment of glyphosate technical/glyphosate acid, although all endpoints are given in acid equivalents.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The authors state that predicted environmental concentrations of glyphosate were modelled up to 2.8 mg ae/L due to direct overspray.
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	The authors adjust and maintain normal range conditions for the tadpoles, regarding water quality, nutrients and volume of the containers. The systems were stable
Concluding weight of evidence/proposed action	In conclusion, the study presents slightly differing acute mortalities between the species and land use type, which can be assessed togheter with other glyphosate formulations containing surfactants of relevant toxicity like the one tested in this study. No clear interaction between the species, origin from crop- or grassland and treatments on survival could be shown. The chronic data was insufficiently analysed, or it was ambiguously described.
Type of information (Critical, supporting, low weight)	**Supporting/critical**
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Edginton et al. (2004)

glyphecotox_066	Edginton, A.N., Sheridan, P.M., Stephenson, G.R., Thompson, D.G., Boermans, H.J.	2004	Comparative effects of pH and Vision (R) herbicide on two life stages of four anuran amphibian species	Environmental Toxicology and Chemistry 23/4, 815–822

Reliability

Purpose of the study Description of endpoints	Product Vision® and the concurrent factor pH were tested to determine their effects on early life-stage anurans. Mortality and the prevalence of malformations
Test compound, application procedure, exposure period, protocol	Vision® (glyphosate-based formulation with15% (weight:weight) polyethoxylated tallow amine surfactant blend)
Experimental approach	Ninety-six-hour laboratory static renewal studies under a central composite
Statistical design, test environment	rotatable design. Generalized linear models.
Test organisms	Embryonic and larval life stages (Gosner 25) of *Rana clamitans*, *R. pipiens*, *Bufo americanus*, and *Xenopus lae*vis
Biological effects	Significant interaction of pH with Vision® concentration in all eight models. The toxicity of Vision® was amplified by elevated pH. Larvae of *B. americanus* and *R. clamitans* were 1.5 to 3.8 times more sensitive than their corresponding embryos, whereas *X. laevis* and *R. pipiens* larvae were 6.8 to 8.9 times more sensitive. From the published paper:

Table 2. Comparative sensitivity of the embryonic and larval stages (Gosner 25) of four anuran species to Vision® (Monsanto Canada, Winnipeg, MB, Canada) at representative pH levels of 6.0 and 7.5. Based on these lethal concentration (LC) estimates, the embryo models produced values greater than those of the larvae and, in general, Vision was more toxic at pH 7.5 than at pH 6.0. The asterisks denote a point estimate at or below the expected environmental concentration of 1.4 mg acid equivalents (a.e.)/L

Species	Life stage[a]	pH	96-h LC10 (mg a.e./L) (95% confidence interval)	96-h LC50 (mg a.e./L) (95% confidence interval)
Xenopus laevis	Embryo	6.0	6.2 (4.7, 7.4)	15.6 (12.7, 23.0)
		7.5	4.0 (3.1, 4.7)	7.9 (7.2, 8.7)
	Larvae	6.0	1.99 (1.7, 2.0)	2.1 (2.0, 2.7)
		7.5	0.85 (0.55, 0.87)*	0.88 (0.84, 0.92)*
Bufo americanus	Embryo	6.0	2.2 (0, 3.8)	4.8 (4.0, 5.7)
		7.5	4.3 (0, 7.5)	6.4 (5.8, 7.0)
	Larvae	6.0	2.1 (1.8, 3.9)	2.9 (2.3, 10.5)
		7.5	1.2 (1.0, 1.4)*	1.7 (1.5, 1.9)
Rana clamitans	Embryo	6.0	2.6 (0, 6.0)	5.3 (3.9, 9.2)
		7.5	2.8 (2.2, 3.8)	4.1 (3.4, 6.4)
	Larvae	6.0	2.1 (1.7, 2.5)	3.5 (3.0, 4.6)
		7.5	0.89 (0.70, 1.1)*	1.4 (1.2, 1.7)*
Rana pipiens	Embryo	6.0	13.1 (12.8–13.3)	15.1 (14.0–17.5)
		7.5	6.7 (6.3–6.9)	7.5 (7.0–9.0)
	Larvae	6.0	1.1 (1.0–1.3)*	1.8 (1.5–2.2)
		7.5	0.83 (0.71–0.92)*	1.1 (0.96–1.14)*

[a] Embryo = Gosner 8 to Gosner 25; larvae = Gosner 25.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints

Biological Relevance

1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance

1 Is the substance tested representative and relevant for the substance being assessed?	Formulated product Vision® contains POEA. Glyphosate alone was not tested.

2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	Results Vision® can be used for the assessment of glyphosate formulations with POEA
Type of information (Critical, supporting, low weight)	**Critical/Supporting**
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Jayawardena et al. (2011)

glyphecotox_074	**Jayawardena UA, Navaratne AN, Amerasinghe PH, Rajakaruna RS**	**2011**	**Acute and chronic toxicity of four commonly used agricultural pesticides on the Asian common toad, *Bufo melanostictus* Schneider.**	**Journal of the National Science Foundation of Sri Lanka 39: 267-276. doi: 10.4038/jnsfsr.v39i3.3631.**

Reliability	
Purpose of the study Description of endpoints	Acute and chronic effects of the formulation Roundup ® containing the active ingredient glyphosate on juvenile Asian common toads (*Bufo melanostictus*) were tested. Recorded were survival (LC_x), snout-vent length, time to forelimb emergence (TE_{50}), body weight of the tadpoles. A tropical, Sri-Lankan scenario was aimed to be represented by the study.
Test compound, application procedure, exposure period, protocol	Commercial Roundup® formulation with a.i. Glyphosate and possibly containing POEA. Exposure acute study: 9.50, 11.25, 15.00, 18.75 and 25.0 ppm. Exposure in chronic experiment: series of 0.25, 0.50, 0.75 and 1.00 ppm of glyphosate were applied (ppm equals mg a.i./L at an assumed density of the solution of 1). In the chronic study the medium was renewed every week, exposure semi-static. Non-GLP
Experimental approach Statistical design, test environment	2 L glass tanks per treatment and each of 3 egg clutch replicates; Replication was not used in the Probit analysis to find the LCx after an F-test on variance differences. Pearson correlation between growth parameters and treatments. 3 replicates for comparison of body weights, snout-vent lengths and TE50.
Test organisms	20 five-days post-hatch tadpoles per tank; Acute measurements (mortality) at 48 h after exposure. Chronic measurements at 10 days post-hatch, 30 days post-hatch and metamorphic tadpoles.
Biological effects	LC_{50} after 48h: 45.94 mg /L. Not clear if product or active substance are meant. Most sensitive survival endpoint (chronic study): 1 ppm glyphosate treatment to metamorphs. Significant overall impact of glyphosate concentrations on mean body weight, SVL and TE_{50} (chronic study), ANOVA, no post-hoc tests applied or explained in the text. Other malformations not quantitatively analyzed.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

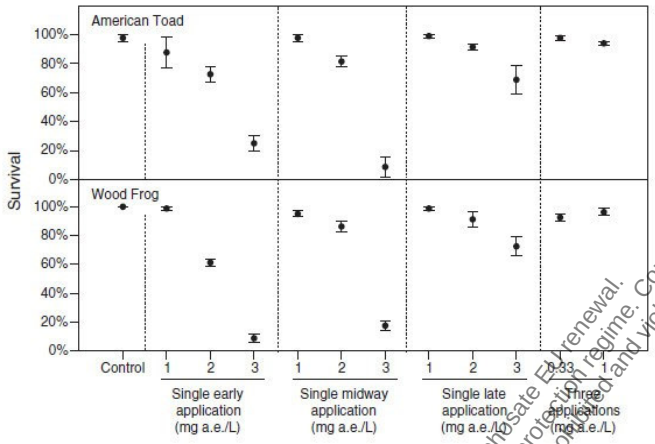
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes
2 Is the magnitude of effects of biological significance?	Statistics: It remained widely unclear if post-hoc tests after the ANOVA on an overall effect of the test concentrations were applied as indicated by the asterisks in figure 1 vs. no indication in table 3.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	A variety of endpoints was assessed, which is appropriate for refined considerations of the most sensitive and relevant endpoint

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The toxicity of surfactant is know to interfere with the toxicity of the active substance and may contribute majorly to the overall effect of a formulation. Unfortunately, the glyphosate formulation used is not identifiable
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	There was no measurement of the environmental conditions in the test tanks, in particular during the chronic study that lasted at least for about 50 days until metamorphosis (figure 1, TE_{50}). Crowding stress with succeeding experimental duration and toxic metabolism products would probably cause a treatment-related bias in the data. However, mortality and malformation rates of the controls were negligible low and statistically significant different from the treatments.
Concluding weight of evidence/proposed action	Many details of the experimental procedures, the use of statistics and the identity of the tested substances are lacking. The study is therefore considered not applicable for a specific use in ERA.
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

Jones, et al. (2010)

glyphecotox_075	**Jones, D.K., Hammond, J.I., Relyea, R.A.**	**2010**	**Roundup® and amphibians: The importance of concentration, application time, and stratification**	**Environmental Toxicology and Chemistry, Vol. 29, No. 9, 2016–2025, DOI: 10.1002/etc.240**

Reliability	
Purpose of the study Description of endpoints	Role of application amount, timing, and frequency using outdoor mesocosm communities containing larval amphibians (*Rana sylvatica* and *Bufo americanus*) and using a commercial formulation of the herbicide glyphosate (Roundup Original MAX®) were assessed. Survival day 18.
Test compound, application procedure, exposure period, protocol	Roundup Original MAX®, authors state Glyphosate isopropylamine salt (glyphosate-Ipa), Surfactant reported not to be POEA (*pers. comm*.Monsanto). Purity: 48.7% active ingredient. RMS: surfactant not known/ Formulation not correctly reported? Roundup Original MAX ® is a potassium salt formulation according to MSDS Monsanto Company
Experimental approach Statistical design, test environment	Mesocosm; Duration of study: 18 days 750-L cattle watering tanks filled with 542 L of well water. Addition of the 2 test species was defined as day 0. Total of 12 treatments including 9 treatments with 3 different concentrations of single applications on day 0, on day 7 and on day 14, respectively, 2 treatments with 2 concentrations of multiple applications on day 0, 7 and 14, and 1 control treatment. Test concentrations: 1 x 1 mg a.e./L, 1 x 2 mg a.e./L, 1 x 3 mg a.e./L, 3 x 0.33 mg a.e./L and 3 x 1 mg a.e./L. 4 replicates /20 tadpoles of each of species in every mesocosm. No additional feeding
Test organisms	*Rana sylvatica* (Ranidae; wood frog) Gosner stage 26 *Bufo americanus* (Bufonidae; American toad) Gosner stage 25

<table>
<tr><td>Biological effects</td><td colspan="2">Quoted from article
 
Fig. 1. Survival of American toad and wood frog tadpoles when exposed to varying Roundup Original MAX concentrations (mg a.e. of glyphposate/L) at different times (day 0, 7, or 14). Data points represent mean survival (± standard error) for all four replicates. Survival was recorded on day 18 following experimental takedown.

Exposures of up to 3 mg acid equivalent (a.e.)/L caused substantial amphibian death. However, the amount of death was considerably higher when the herbicide was applied earlier in the experiment than later in the experiment. Single, large applications (at different times) had larger effects on tadpole mortality and growth than multiple, small applications (of the same total amount).
Effects on mass were also dependent from application time and gyphosate concentration.
RMS: From the results
- an overall NOEC of 1 mg a.e./L is postulated
- lowest LC50 is 2.10 mg a.e./L (2.00, 2.19) for *Bufo americanus*
From the published paper (modified):
Table 3. Results of probit analyses used to estimate the LC10, LC50 values (mg a.e. /L)lethal concentrations that cause 10, 50% mortality) for Roundup Original Max® in outdoor mesocosms at three application times. Means are followed by 84% confidence intervals; non-overlapping confidence intervals are significantly different (p<0.05).
<table>
<tr><th>Species</th><th>Application</th><th>LC10</th><th>LC50</th></tr>
<tr><td>Wood frog</td><td>Early (day 0)</td><td>1.45 (1.29, 1.57)</td><td>2.10 (2.00, 2.19)</td></tr>
<tr><td></td><td>Midway (day 7)</td><td>1.56 (1.07, 1.84)</td><td>2.44 (2.15, 2.79)</td></tr>
<tr><td></td><td>Late (day 14)</td><td>2.02 (1.47, 2.34)</td><td>4.27 (3.47, 7.42)</td></tr>
<tr><td>American toad</td><td>Early (day 0)</td><td>0.99 (0.42, 1.35)</td><td>2.31 (1.86, 3.06)</td></tr>
<tr><td></td><td>Midway (day 7)</td><td>1.67 (0.72, 2.01)</td><td>2.30 (1.84, 2.89)</td></tr>
<tr><td></td><td>Late (day 14)</td><td>1.98 (1.49, 2.28)</td><td>3.93 (3.33, 5.83)</td></tr>
</table></td></tr>
<tr><td colspan="3">Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints</td></tr>
<tr><td colspan="3">Biological Relevance</td></tr>
<tr><td colspan="2">1 Is an appropriate test species/ life-stage(s) studied?</td><td>yes</td></tr>
<tr><td colspan="2">2 Is the magnitude of effects of biological significance?</td><td>yes</td></tr>
<tr><td colspan="2">3 Is the ecotoxicological manifestation level appropriate for the assessment?</td><td>yes</td></tr>
<tr><td colspan="3">Environmental Relevance</td></tr>
<tr><td colspan="2">1 Is the substance tested representative and relevant for the substance being assessed?</td><td>Not clear. No POEA are included in the formulation. Nevertheless, results point at a significant toxicity of the surfactant. The surfactant might belong to the so-called group of POEA-similar surfactant classes.
Roundup Original MAX® contains a potassium salt of glyphosate and not the IPA salt as stated in the paper?</td></tr>
</table>

2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	Formulation ingredient undisclosed. No separate test of glyphosate as acid or salt
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Jones, et al. (2011)

glyphecotox_076	**Jones, D.K., Hammond, J.I., Relyea, R.A.**	**2011**	**Competitive stress can make the herbicide Roundup® more deadly to larval amphibians**	**Environmental Toxicology and Chemistry, 30/2, 446-454 DOI: 10.1002/etc.384**

Reliability	
Purpose of the study Description of endpoints	To explore how the natural stress of competition might interact with a glyphosate-based herbicide. Outdoor mesocosms containing three tadpole species exposed to a factorial combination of three glyphosate concentrations (0, 1, 2, or 3mg acid equivalent (a.e.)/L of the commercial formulation Roundup Original MAX1) and three tadpole densities (low, medium, or high). growth, mortality
Test compound, application procedure, exposure period, protocol	Roundup Original MAX®, authors state Glyphosate isopropylamine salt (glyphosate-Ipa), Surfactant reported not to be POEA (*pers. comm*.Monsanto). Purity: 48.7% active ingredient. RMS: surfactant not known/ Formulation not correctly reported? Roundup Original MAX ® is a potassium salt formulation according to MSDS Monsanto Company Exposure: approx 22-23 days
Experimental approach Statistical design, test environment	12 treatments including 3 different concentrations of single glyphosate applications and 1 untreated control crossed with 3 tadpole densities (low, medium, or high). Test concentrations: 1, 2 and 3 mg a.e./L glyphosate (a.e. = acid equivalent). 2 replicates per treatment Organisms per replicate: Low density: 20 of each of the three species Medium: 40 of green and gray tree frog and 20 of bullfrog High: 60 of green and gray tree frog and 20 of bullfrog multivariate analysis of variance (MANOVA).
Test organisms	*Rana catesbeiana* (bullfrog) *Rana clamitans* (green frog) *Hyla versicolor* (gray tree frog) Age of test organisms at study initiation: early stage appr. Gosner 25

Biological effects	The LC50 values for the tested species reflected a competition effect; LC50 values were similar at low and medium densities, but both were different from the LC50 values at high tadpole density Lowest LC50 reported for Bullfrog at high densities = 1.61 mg a.e./L (1.52, 1.70) From the paper (modified): Table 3. Results of species-specific probit analyses used to estimate the lethal concentrations of Roundup Original MAX1 (Monsanto) that cause 10, 50% death (LC10 and LC50 respectively). Estimates are based on outdoor mesocosm experiments that crossed four concentrations of Roundup (0, 1, 2, or 3 mg acid equivalent/L) with three levels of tadpole competition. Means are followed by 84% confidence intervals; nonoverlapping confidence intervals are significant. All estimates adjust for low amounts of mortality in the controls.

Species	Competition	LC10	LC50
Gray tree frog	Low	1.41 (0.81, 1.70)	2.04 (1.70, 2.35)
	Medium	1.85 (0.00, 2.20)	2.29 (1.56, 10.8)
	High	1.00 (0.53, 1.29)	1.71 (1.36, 2.07)
Green frog	Low	1.26 (0.42, 1.68)	2.58 (2.07, 3.86)
	Medium	1.84 (1.09, 2.13)	2.35 (1.98, 2.84)
	High	1.58 (1.25, 1.78)	2.18 (1.99, 2.37)
Bullfrog	Low	1.38 (0.69, 1.72)	2.18 (1.77, 2.63)
	Medium	1.63 (0.59, 1.91)	2.12 (1.70, 2.58)
	High	1.18 (1.06, 1.28)	1.61 (1.52, 1.70)

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Not clear. No POEA are included in the formulation. Nevertheless, results point at a significant toxicity of the surfactant. The surfactant might belong to the so-called group of POEA-similar surfactant classes. Roundup Original MAX® contains a potassium salt of glyphosate and not the IPA salt as stated in the paper?
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	Formulation ingredient undisclosed. No separate test of glyphosate as acid or salt
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Lajmanovich et al. (2003)

glyphecotox_078	**Lajmanovich, R.C., Sandoval, M.T., Peltzer, P.M.**	**2003**	**Induction of mortality and malformation in Scinax nasicus tadpoles exposed to glyphosate formulations**	**Bull. Environ. Contam. Toxicol. 70, 612–618 DOI: 10.1007/s00128-003-0029-x**

Reliability	
Purpose of the study Description of endpoints	Tadpoles of *Scinax nascius* were exposed under laboratory conditions to GLYFOS®, a formulation containing glyphosate at nominal test concentrations of 3.07, 3.84, 4.8, 6 and 7.5 mg formulation/L. A negative control (artificial pond water) was prepared in parallel. Ten tadpoles were exposed in three replicates in the control and at each treatment level. All tadpoles were observed at daily intervals for the 96 hour study duration with mortality recorded. At the end of exposure, surviving tadpoles were fixed in formalin solution and examined for morphological changes.
Test compound, application procedure, exposure period, protocol	Glyfos® 48% glyohosate
Experimental approach Statistical design, test environment	See above
Test organisms	*Scinax nascius* tadpoles
Biological effects	Larval malformations were minimal at 3.07 mg/L when tadpoles were exposed for one day, whereas an increased malformation was observed at levels of 7.5 mg Glyfos®/L. The 96 hour LC50 value for tadpoles of *Scinax nascius* exposed to Glyfos® was 2.64 mg formulation/L (nominal) with 95% confidence interval of 2.19 to 2.84 mg/L
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Glyphos® contains with very high probabiliy POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants.
Type of information (Critical, supporting, low weight)	**Supporting/critical**
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Lajmanovich et al. (2011)

glyphecotox_080	**Lajmanovich, R.C., Attademo, A.M., Peltzer, P.M., Junges, C.M., Cabagna, M.C.**	**2011**	**Toxicity of Four Herbicide Formulations with Glyphosate on Rhinella arenarum (Anura: Bufonidae) Tadpoles: B-esterases and Glutathione S-transferase Inhibitors**	**Arch Environ Contam Toxicol, 60, 681–689 10.1007/s00244-010-9578-2**

Reliability	
Purpose of the study Description of endpoints	tadpoles *Rhinella arenarum* were exposed to different concentrations of Roundup Ultra-Max (ULT), Infosato (INF), Glifoglex, and C-K YUYOS FAV. Tadpoles were exposed at thefollowing concentrations (acid equivalent [ae]): 0 (control), 1.85, 3.75, 7.5, 15, 30, 60, 120, and 240 mg ae/L for 6–48 h (short-term). Mortality was recorded. Acetylcholinesterase (AChE), butyrylcholinesterase (BChE), carboxylesterase (CbE), and glutathione S-transferase (GST) activities were measured among tadpoles sampled from those treatments that displayed survival rates >85%.
Test compound, application procedure, exposure period, protocol	Roundup Ultramax®: commercial grade, 74.7% a.i. No POEA Infosato, Glifoglex, C-K Yuyos FAV: commercial grade, 48% a.i., each, co-formulants undisclosed 48 h
Experimental approach Statistical design, test environment	Larvae were exposed in glass tanks (12.5 cm diameter × 13.5 cm height) filled with 1 L of DTW (deionised tap water?). Whole tadpoles were homogenized in 0.1% triton X-100, 25 mM Tris-HCL (pH 8.0) Replicates per concentration: 3; Organisms per replicate: 7
Test organisms	*Rhinella arenarum*
Biological effects	Forty-eight-hour LC_{50} for *R. arenarum* tadpoles in the static tests ranged from ULT = 2.42 to FAV = 77.52 mg ae/L. For all CF-GLY, the LC50 values stabilized at 24 h of exposure. Enzyme activity measues possibly biased by high mortalities
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Acute endpoint: yes
2 Is the magnitude of effects of biological significance?	Acute endpoint: yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Acute endpoint: yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup UltraMAX® is stated not to contain POEA. Test results indicate the formulation contains surfactants with toxicity similar to POEA. Other formulation employed with unknown co-formulants. Far lower toxicities than the product above.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Not conclusively

Concluding weight of evidence/proposed action	Acute endpoints reliable, reporting of experimental details not exaustive, formulations partly unknown
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Relyea (2005)

glyphecotox_083	Relyea R.A.	2005	The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities	Ecology Letters 9 (10):1157-1171. DOI 10.1111/j.1461-0248.2006.00966.x.

Reliability	
Purpose of the study Description of endpoints	Effects of glyphosate as Roundup ® on total and functional group based species richness of zooplankton, periphyton, invertebrates and amphibians; biomass of functional groups; abundance of individual species.
Test compound, application procedure, exposure period, protocol	Amongst three other pesticides, the active ingredient glyphosate was tested using a commercial Roundup® formulation containing polyethoxylated tallowamines as surfactants. A single concentration of 3.8 mg a.i./L was used, corresponding to the maximum recommended application rate of 6.4 mL Roundup® (25.2 % a.i.)/m². Exposure: 13 d approx.. Non-GLP
Experimental approach Statistical design, test environment	An artificial assemblage of several specimens of limnic vertebrate and invertebrate organisms (Spotted salamander, Diving beetle, Dragonfly, Damselfly, Backswimmer, Water bug, Wood frog, Leopard frog, American toad, Gray tree frog, Spring peeper, Snail, Cladoceran, Copepod) was added to experimental ponds of 1000L volume. Controls and treatments were 6-fold replicated and sampled once on day 13 of the experiment.
Test organisms	See above
Biological effects	Effects at 3.8 mg glyphosate/L. Total species richness decreased by 22 % compared to control (statistically different at 5 % error probability). The test item caused a decrease in large herbivore richness. A significant decrease of the abundance of individual species was seen for the copepod *Eurytemora affinis* The amphibian species tested were affected by the Roundup treatment. Effects of glyphosate as Roundup ® on total and functional group based species richness of zooplankton, periphyton, invertebrates and amphibians; biomass of functional groups; abundance of individual species.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Although the author stated that the species compete with each other at the respective trophic level, the communities were not tested at an equilibrium state. The test item was applied immediately after completion of the experimental setup by introducing the vertebrate specimens to the ponds. It should be seen as a 'combined single species approach' and not as a community level study and could serve as an estimate of acute to subchronic toxicity under realistic exposure conditions.
2 Is the magnitude of effects of biological significance?	There is no indication of the numbers that form the basis of the statistical analysis, i.e. no recovery rates of the previously introduced individuals were given by the author. It remains unclear why it was necessary to conduct multivariate pre-testing and data conversions.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Generally, the abundance of persisting or newly hatched individuals is an appropriate level of investigation for the semi-field ecosystem level.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The active compound Glyphosate was tested in a formulation possibly containing POEA, representing a common practice of enhancing the surfactant characteristics of a formulation. Does not resemble the lead formulation for EU assessment of reneval of approval for glyphosate as active substance
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The tested concentration was deduced by the assumption of direct overspray at the recommended field rate, and is thus considered a realistic and possible worst-case.

3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Obviously, there the author did not measure any environmental parameters, nor considered them fort the interpretation of results. Indirect effects within the newly established community were discussed.
Concluding weight of evidence/proposed action	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants. Not relevant for the risk assessment of glyphosate due to weakness in methodological accuracy.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Relyea (2005)

glyphecotox_085	**Relyea R.A.**	**2005**	**The lethal impact of roundup on aquatic and terrestrial amphibians**	**Ecological Applications, 15(4), 2005, pp. 1118–1124 DOI: 10.1890/04-1291**

Reliability	
Purpose of the study Description of endpoints	Aquatic: Communities of three species of North American tadpoles in outdoor pond mesocosms that contained different types of soil and Roundup as a direct overspray. Terrestrial: three species of juvenile (post-metamorphic) anurans to a direct overspray of Roundup in laboratory containers. Aquatic: survival after 21 days exposure Terrestrial: survival after 24h
Test compound, application procedure, exposure period, protocol	Roundup® "Weed and Grass Killer" concentrated lawn and garden formulation. Active substance(s): Glyphosate isopropylamine salt (glyphosate-IPA), 25.2% glyphosate. Adjuvant/Surfactant: suspected POEA. Aquatic: Experimental units were 1200-L cattle watering tanks filled with 1000 L of well water. Each tank was treated with no soil, sand or loamy soil. After inoculation of mesocosm and addition of test species, ponds were applied with test item or water (control) two day later. Survival was recorded 21 days later at termination of experiments. Terrestrial: Post-metamorphic animals were placed in 10-L plastic tubs that were lined with damp water towels. Subsequently, replicates were treated with glyphosate or water (control) and survival was recorded 24 h later.
Experimental approach Statistical design, test environment	Aquatic: total of 6 treatments including 3 soil treatments (i.e. no soil, 19 L sand, 19 L loam soil) crossed with 1 glyphosate and 1 control treatment. Test concentrations: 3.8 mg a.s./L (corresponding to 1.6 mL a.s./m2), 5 replicates. Notifier: Correcting for density, nominal tested concentrations become 1.37 mg GlyIPA/L and 4.17 mg GlyIPA/L or 1.01 mg glyphosate a.e./L and 3.09 mg glyphosate a.e./L. RMS: only one concentration tested, though. Recalculation to 3.09 mg glyphosate a.e./L? Terrestrial: 1 glyphosate and 1 control treatment: 1.6 mL a.s./m2, 4 replicates Rate corresponds to 16,000 mL a.s./ha. Approx. 4 to 5 times higher than applied in Europe. Organisms per replicate: Aquatic: 20 tadpoles of each of the 3 species in every mesocosm. Terrestrial: 7 juvenile frogs/toads per experimental unit separated by species.
Test organisms	Aquatic experiments: *Rana pipiens* (leopard frog), *Bufo americanus* (toad), and *Hyla versicolor* (gray tree frogs). Terrestrial experiments: *Rana sylvatica* (wood frog), *Bufo woodhousii fowleri* (Fowler's toad), and *Hyla versicolor* (gray tree frogs)

Biological effects	Aquatic: After three weeks, Roundup at tested concentrations resulted in a mortality of 96–100% of larval amphibians (regardless of soil presence). Terrestrial: After one day, Roundup at tested concentrations resulted in a mortality of 68–86% of juvenile amphibians

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The active compound Glyphosate was tested in a formulation possibly containing POEA. Does not resemble the lead formulation for EU assessment of reneval of approval for glyphosate as active substance
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Terrestrial:1.6 mL a.s./m2. Rate corresponds to 16,000 mL a.s./ha. Approx. 4 to 5 times higher than applied in Europe. Aquatic: overspray scenario, not appropriate for evaluation of intended uses in Europe
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	The study confirms the relatively high toxicity of glyphosate preparations possibly mediated by POEA surfactants.
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Relyea (2012)

glyphecotox_088	**Relyea R.A.**	**2012**	**New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology.**	**Ecological Applications 22/2, 634-647 DOI: 10.1890/11-0189.1**

Reliability	
Purpose of the study Description of endpoints	Outdoor mesocosms with simple wetland communities containing leaf litter, algae, zooplankton, and three species of tadpoles (wood frogs *Rana sylvatica* or *Lithobates sylvaticus*, leopard frogs *R. pipiens* or *L. pipiens* and American toads *Bufo americanus* or *Anaxyrus americanus*.
Test compound, application procedure, exposure period, protocol	Roundup Original MAX® Glyphosate 540 mg a.e./L; Adjuvant/Surfactant: Undisclosed Duration of study: 21 days
Experimental approach Statistical design, test environment	Factorial combination of herbicide concentrations (0, 1, 2, or 3 mg acid equivalents [a.e.]/L of Roundup Original MAX) crossed with three predator-cue treatments (no predators, adult newts *Notophthalmus viridescens*) or larval dragonflies *Anax junius*).
Test organisms	*Rana sylvatica* (wood frog), *Rana pipiens* (northern leopard frog), *Bufo americanus* (American toad), early stage appr. Gosner 25

Biological effects	From the published paper: TABLE 2. Estimated LC50 values (i.e., the concentration required to kill 50% of a population) for three species of tadpoles when exposed to a range of concentrations of glyphosphate (as the commercial formulation Roundup Original MAX) in the presence of three caged-predator environments. Species / Caged predator / LC50 / 84% CI Wood frog / no predator / 2.95 / - caged newt / 2.63 / 2.45, 2.84 caged dragonfly / 3.09 / 2.75, 3.80 Leopard frog / no predator / 2.91 / 2.74, 3.03 caged newt / 3.02 / caged dragonfly / 3.26 / 3.02, 3.90 American toad / no predator / 2.46 / 2.27, 2.69 caged newt / 2.44 / 2.26, 2.64 caged dragonfly / 2.82 / 2.66, 2.96 *Notes:* Estimates are followed by 84% confidence intervals; nonoverlapping confidence intervals are significant at approximately $\alpha = 0.05$ (Payton et al. 2003). In two cases, indicated by dashes, the confidence interval could not be estimated due to the distribution of the data. Interactions between pesticide and predator effects. Surprisingly, in presence of predators, the LC50 increases.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The active compound Glyphosate was tested in a formulation possibly containing surfactants with similarl toxicity as POEA. Does not resemble the lead formulation for EU assessment of reneval of approval for glyphosate as active substance
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Aquatic: overspray scenario, not appropriate for evaluation of intended uses in Europe
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence/proposed action	The study confirms the relatively high toxicity of glyphosate preparations mediated by surfactants.
Type of information (Critical, supporting, low weight)	**Supporting**
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Lajmanovich et al. (2010)

glyphecotox_448	**Lajmanovich, R.C., Peltzer, P.M., Junges, C.M., Attademo, A.M., Sanchez, L.C., Bassó, A.**	**2010**	**Activity levels of B-esterases in the tadpoles of 11 species of frogs in the middle Paraná River floodplain: Implication for ecological risk assessment of soybean crops**	**Ecotoxicology and Environmental Safety 73 (7):1517-1524. DOI: 10.1016/j.ecoenv.2010.07.047.**

Reliability	
Purpose of the study Description of endpoints	Determination of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and carboxylesterases (CbEs) activities in 11 anuran species in the Parana River floodplain, Brasil
Test compound, application procedure, exposure period, protocol	-/-
Experimental approach Statistical design, test environment	-/-
Test organisms	-/-
Biological effects	-/-
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	-/-
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	-/-
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?	-/-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	-/-
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	-/-

Concluding weight of evidence/proposed action	**The study is not relevant for ERA of glyphosate because the substance was not applied nor monitored explicitly. Most likely, the notifier assigned erroneously a high relevance to the study.**
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3**

McDaniel et al. (2008)

glyphecotox_496	**McDaniel, T.V., Martin, P.A., Struger, J., Sherry, J., Marvin, C.H., McMaster, M.E., Clarence, S., Tetreault, G.**	**2008**	**Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (Rana pipiens) and green frogs (Rana clamitans) from areas of intensive row crop agriculture**	**Aquatic Toxicology 88 (4):230-42. DOI: 10.1016/j.aquatox.2008.05.002.**

Reliability	
Purpose of the study Description of endpoints	The occurrence of potential endocrine effects in amphibians inhabiting farm ponds and agricultural drains in intensive row crop agriculture areas of southwestern Ontario was assessed. Effects were compared to amphibians from two agricultural reference sites as well as four non-agricultural reference sites. Blood samples were taken from northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) for analysis of circulating sex steroids and vitellogenin-like protein (Vtg-lp), a biomarker of exposure to environmental estrogens. Gonads were histologically examined for evidence of abnormalities.
Test compound, application procedure, exposure period	A suite of different pesticides were found at the field sites. The applied products are not known
Experimental approach, Statistics, test environment	The relationships between the proportion of males with TOFS/circulating sex steriods and a broad suite of pesticide residues and nutrients concentrations in waterwere explored using Partial Least Squares (PLS). PLSwas used to correlate biological endpoints [independent variable] (Y) with multivariate contaminants components (X), consisting of pesticide residues and nutrient concentrations.
Test organisms	northern leopard frogs *(Rana pipiens)* and green frogs *(Rana clamitans)*
Biological effects	The occurrence of testicular ovarian follicles (TOFS) in male R. pipiens was significantly higher (42%; $p < 0.05$) at agricultural sites. The proportion of testicular oocytes did correlate with a mixture of pesticides and nutrients, particularly atrazine and nitrate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	not conclusive
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	A mixture was assessed. No allocation of effects to single substances possible. Applied products not known
2 Do the tested concentrations relate to predicted environmental concentrations?	Field assessment. Glyphosate Level of Detection far higher than for all other measured pesticides.
3 Have parameters influencing the endpoints been considered adequately?	The parameters were assessed in the survey only as aggregate exposure of all employed agricultural substances. No further discrimination possible.
Concluding weight of evidence/proposed action	Study is a field survey on the impact of an exposure to the sum of possible substances employed in agricultural management on amphibian biomarkers and development. The investigation of the effects of single substances was not part of the study. The impact of glyphosate cannot be determined.
Type of information (Critical, supporting, low weight)	**Low weight**
Consideration/concluding score	**UBA3 (since monitoring)**

B.9.13 10.1 Summary of the relevant literature on amphibians

Since the 2001 EU glyphosate evaluation, a number of acute and chronic amphibian toxicity studies with glyphosate and commercial glyphosate-based formulations have been published. For this review of the literature, acute studies are considered to be 96 hours or less. Chronic studies did mostly evaluate lethality, though, coupled with the investigations of glyphosate formulations on weight and/or performance at metamorphosis. Only few studies assessed the toxicity of glyhosate formulation on terrestrial stages of amphibians.

Most of the acute and chronic amphibian study results are from laboratory toxicity tests. However, some of the studies were performed in the field using in situ enclosures or field mesocosms to assess impacts under representative natural conditions, and chemical and biological monitoring studies conducted under conditions directly relevant to product use. Studies were evaluated based on criteria of reliability and relevance/adequacy for risk assessment.

A significant amount of research exists on the toxicity of glyphosate and several glyphosate- based formulations for amphibian.
Acute studies with glyphosate acid and glyphosate IPA for sensitive Gosner stage 25 tadpoles show medial lethal values comparable to values obtained with fish in regulatory studies and in the literature. The LC50 values for amphibian exposed to glyphosate and its salts range from >17.9 to >466 mg a.s./L (see table below).

Table B.9.13-5: Effect values reported in peer reviewed literature for amphibians: glyphosate acid and salts of glyphosate

Species	Substance	Study duration (h)	LC_{50} (mg a.s./L)	Reference
Amphibians				
Crinia insignifera tadpole	Glyphosate acid	96	103.2	Bidwell and Gorrie 1995 glyphnosubm_023
Crinia insignifera adult	Glyphosate acid	96	75.0	Bidwell and Gorrie 1995 glyphnosubm_023
Litoria moorei tadpoles	Glyphosate acid	48	81.2	Mann and Bidwell 1999 glyphnosubm_024
Litoria moorei tadpoles	Glyphosate acid	48	121.0	Mann and Bidwell 1999 glyphnosubm_024
Crinia insignifera adult	Glyphosate acid	48	83.6	Mann and Bidwell 1999 glyphnosubm_024
Rana clamitans	Glyphosate IPA	96	>17.91	Howe et al., 2004 glyphecotox_025
Lymnodynastes dorsalis tadpoles	Glyphosate IPA	48	>400.0	Mann and Bidwell 1999 glyphnosubm_024
Litoria moorei tadpoles	Glyphosate IPA	48	>343.0	Mann and Bidwell 1999 glyphnosubm_024
Crinia insignifera tadpole	Glyphosate IPA	48	>466.0	Mann and Bidwell 1999 glyphnosubm_024
Heleioporus eyrei tadpole	Glyphosate IPA	48	>373.0	Mann and Bidwell 1999 glyphnosubm_024

The effects of different glyphosate-based formulations on amphibian survival have been evaluated on almost 30 species of amphibians (e.g. Howe et al., 2004; Cauble and Wagner, 2005; Dinehart et al., 2010; Edginton et al., 2004; Jayawardena et al., 2001; Jones et al., 2010 and 2011; Relyea 2012).

The medial lethal concentration for amphibian exposed to formulation of glyphosate containing specific surfactant classes are far lower than for glyphosate acid or its salts (see Table B.9.13-6). The surfactants displaying a high toxicity in glyphosate-based formulations belong usually to the classes of polyoxyethoxylated alkylamines (POEA; e.g. ethoxylated tallow- and cocoamines), or are e.g. fatty nitrogen derivate etheramine. For the implications resulting from these obervations, please see chapter B.9.13 16.1.

Table B.9.13-6: Effect values reported in peer reviewed literature for amphibians: glyphosate formulations and surfactants; GLY: glyphosate; POEA: polyoxyethoxylated alkylamine; w: with; w/o: without

Species	Substance	Study duration (hours or days)	LC_{50} (mg a.e./L)	Reference
Amphibians				
Rana pipiens; Gosner 25	Roundup Original GLY w POEA	96 h	2.9	Howe et al., 2004 glyphecotox_025
Rana sylvatica, Gosner 25		96 h	5.1	
Bufo americanus; Gosner 25		24 h	4.2	
		48 h	<4.0	
Rana clamitans Gosner 25		48 h	2.0	
Rana clamitans Gosner 25	POEA	48 h	2.2	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	Roundup Bioactive ® GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	Touchdown ® GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana clamitans Gosner 25	Glyfos BIO ® GLY w/o POEA	48 h	> 17.9	Howe et al., 2004 glyphecotox_025
Rana cascadae tadpole	Roundup Original? GLY w POEA	48 h	3.2	Cauble and Wagner, 2005 glyphecotox_049
Spea bombifrons Gosner 29	Roundup WeatherMAX® GLY w/o POEA; w surfactants of POEA similar toxicity	48 h	1.9	Dinehart et al., 2010 glyphecotox_064
Spea multiplicata Gosner 29		48 h	2.1	
Xenopus laevis Gosner 25	Vision® GLY w POEA	96 h	0.9	Edginton et al., 2004 glyphecotox_066 [a)]
Bufo americanus Gosner 25		96 h	1.7	
Rana clamitans Gosner 25		96 h	1.4	
Rana pipiens Gosner 25		96 h	1.1	
Scinax nascius Gosner 25	Glyfos® GLY w POEA?	96 h	2.6 [b)] 1.3	Lajmanovich et al., 2003 glyphecotox_078

Species	Substance	Study duration (hours or days)	LC_{50} (mg a.e./L)	Reference
Rhinella arenarum Gosner 25	Roundup UltraMAX® GLY w/o POEA; w surfactants of POEA similar toxicity	48 h	2.4	Lajmanovich et al. 2011 glyphecotox_080
Rana sylvatica Gosner 25	Roundup Original MAX® GLY w/o POEA w surfactants of POEA similar toxicity	21 d	2.9	Relyea, 2012 glyphecotox_088
Rana pipiens Gosner 25		21 d	2.9	
Bufo americanus Gosner 25		21 d	2.5	
Rana sylvatica Gosner 26	Roundup Original MAX®, GLY w/o POEA; w surfactants of POEA similar toxicity	18 d	2.1	Jones et al., 2010 glyphecotox_075 [c]
Bufo americanus Gosner 26		18 d	2.3	
Species	**Substance**	**Study duration (hours or days)**	**LC_{50} (mg a.e./L)**	**Reference**
Amphibians				
Rana catesbeiana Gosner 25	Roundup Original MAX®, GLY w/o POEA; w surfactants of POEA similar toxicity	23 d	2.2	Jones et al., 2011 glyphecotox_076 [d]
Rana clamitans Gosner 25		23 d	2.6	
Hyla versicolor Gosner 25		23 d	2.0	

a) Values reported for test series with pH 7.5; lower toxicity at pH 6.0
b) Value refer to mg formulation/L
c) Values reported for early application day 0; lower toxicity with split applications
d) Values reported for low animal density (single species); higher toxicity if kept at higher densities

Comparable to the findings regarding glyphosate as salt or acid, also the range in LC50 values reported for tadpoles when exposed to formulations of glyphosate is comparable to the range of LC50 values reported for fish. A first mechanistic explanation proposed why fish and tadpoles have very similar acute sensitivities to the surfactants that are added to glyphosate- based formulations relates to the toxic mode of action of surfactants. Increasing the permeability of cell membranes, addition of surfactants result in loss of osmotic or ionic stability at the gill. Consequently, the mode of action of surfactants to aquatic organisms could explain why the range of sensitivities for amphibians and fish in acute tests are similar when exposed to comparable glyphosate-based formulations. It should be noted here that only few data assessing the effect of glyphosate and glyphosate-based products on terrestrial stages of amphibians. Studies with other products (Brühl et al. 2013; Belden et al. 2010) have shown that terrestrial stage of amphibians do experience environmental concentration far higher than a medial lethal rate (LR50) at authorized field uses. Therefore, if the acute risk for the aquatic stages of amphibian seem to be covered by a proper assessment of the risk to fish, this is not the case for the terrestrial stages. The risk assessment for bird and mammals has long been taken also as protective for amphibian in terrestrial environments. Since it has been shown by Brühl et al. (2013) that juvenile amphibian exposed to other products die at authorized field rates - and for some products even at 1/10 of field rates - the conclusions of the risk assessment for birds and mammals for a specific product do not cover necessarily the risk of exposed terrestrial amphibian stages.

Further studies evaluated were performed in the field in controlled enclosures (Thompson et al., 2004; Edge et al., 2011; Wojtaszek et al., 2004) employing specific formulations for forest applications with overspray scenarios for surface water ponds. These studies were not performed with dose response design and report amphibian survival rates and other parameters at given concentrations supposed to be environmentally relevant. Since exact exposure scenario was not always quantifiable and mean lethal concentrations mostly not reached in the chosen study design, these results are not directly utilizable for the ecotoxicological

assessment of glyphosate formulations for amphibians.

Regarding chronic toxicity enpoints that do not relate merely to long-term effects on amphibian survival rate, Cauble and Wagner (2005) studied the effects of glyphosate formulations on larval methamorphosis. In glyphosate treatments (1 mg a.e./L), there are indications of earlier metamorphosis and smaller size of Rana cascadae when compared to the control.

Also Howe et al. (2004) monitored in lab studies several chronic enpoints (e.g. forelimb emergence, tail damage and maximum tail height, snout-vent-length of metamorphs; gonadal

histology to determine sex ratios). Significant tail damages and reduced tail lengths were recorded in treatments with the Roundup Original ® formulation and in treatments with the surfactant POEA. No effects on chronic endpoints were determined when the amphibian were exposed to glyphosate alone. POEA containing formulations showed displaced sex ratios towards intersex individuals. Again, this was not observed in treatment with glyphosate technical. However, results were not always strictly dose-dependent.

The studies by Cauble and Wagner (2005) and Howe et al. (2004) have been criticized by the Notifier as regards to experimental and/or reporting deficiencies. Not all critical points are shared by RMS. RMS believes that the findings pointing at chronic toxicity of surfactants in glyphosate-based formulations are not exaustively resolved by a critique of the study set up. Even if the cited studies suffer from experimental difficulties, the results indicate effects of ethoxylated surfactants on amphibian metamorphosis. The implications of these findings for the potential registration of glyphosate-based formulation with surfactants of significant toxicity are discussed in chapter B.9.13.16.

The lead formulation for the assessment of glyphosate as active substance does not contain surfactants of overt toxicity.

- ***References***

Belden, J., McMurry, S., Smith, L. & Reilley, P. (2010): Acute toxicity of fungicide formulations to amphibians at environmentally relevant concentrations. Environ. Toxicol. Chem. 29, 2477–2480

Bidwell, J.R., Gorrie, J.R. (1995): Acute Toxicity of a Herbicide to Selected Frog Species. Technical Series 79, Departement of Environmental Protection, Western Australia.

Brühl C. A., Schmidt, T., Pieper, S., Alscher, A. (2012): Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? Nature Scientific Reports 3, Article number 1135

Cauble, K., Wagner, R.S. (2005): Sublethal effects of the herbicide glyphosate on amphibian metamorphosis and development. Bulletin of Environmental Contamination and Toxicology 75, 429-435

Dinehart, S.K., Smith, L.M., McMurry, S.T., Smith, P.N., Anderson, T.A., Haukos, D.A. (2010): Acute and chronic toxicity of Roundup WeatherMAX® and Ignite® 280 SL to larval *Spea multiplicata* and *S. bombifrons* from the Southern High Plains, USA. Environmental Pollution 158/8, 2610-2617

Edge, C.B., Gahl, M.K., Pauli, B.D., Thompson, D.G., Houlahan, J.E. (2011): Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide. Ecotoxicology and Environmental Safety 74, 1363–1369

Edginton, A.N., Sheridan, P.M., Stephenson, G.R., Thompson, D.G., Boermans, H.J. (2004): Comparative effects of pH and Vision (R) herbicide on two life stages of four anuran

amphibian species. Environmental Toxicology and Chemistry 23/4, 815–822

Howe, C.M., Berrill, M., Pauli, B.D., Helbing, C.C., Werry, K., Veldhoen, N.(2004):Toxicity of glyphosate-based pesticides to four North American frog species. Environmental Toxicology and Chemistry 23/8, 1928-1938

Jayawardena U.A., Navaratne A.N., Amerasinghe P.H., Rajakaruna R.S. (2011): Acute and chronic toxicity of four commonly used agricultural pesticides on the Asian commontoad, *Bufo melanostictus* Schneider. Journal of the National Science Foundation of Sri Lanka 39, 267-276

Jones, D.K., Hammond, J.I., Relyea, R.A. (2010): Roundup® and amphibians: The importance of concentration, application time, and stratification. Environmental Toxicology and Chemistry 29/9, 2016–2025

Jones, D.K., Hammond, J.I., Relyea, R.A. 01.01.2011 Competitive stress can make the herbicide Roundup® more deadly to larval amphibians. Environmental Toxicology and Chemistry, 30/2, 446-454

Mann, R.M., Bidwell, J.R. (1999): The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs . Archives of Environmental Contamination and Toxicology 36, 193-199

Relyea, R.A. (2012): New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology. Ecological Applications 22/2, 634-647

Thompson, D.G., Wojtaszek, B.F., Staznik, B., Chartrand, D.T., Stephenson, G.R. (2004): Chemical and biomonitoring to assess potential acute effects of Vision (R) herbicide on native amphibian larvae in forest wetlands Environmental Toxicology and Chemistry 23/4, pp. 843–849

Wojtaszek, B.F., Staznik, B., Chartrand, D.T., Stephenson, G.R., Thompson, D.G. (2004): Effects of Vision (R) herbicide on mortality, avoidance response, and growth of amphibian larvae in two forest wetlands. Environmental Toxicology and Chemistry, 23/4,832–842

B.9.13.11 Bees

For a detailed description and evaluation of acceptability and validity of the study please refer to Vol. 3, chapter B.9.4.

B.9.13.12 Effects on other arthropod species (KIIA 8.16)

For the group of terrestrial non-target arthropods (NTA), a database of 31 publications was collected by the notifier. The notifier considered none publications to be rated to be acceptable for risk assessment. The submitted publications were also evaluated by zRMS and have been assigned according to an UBA screening. 11 studies were recognized as information with low weight (category UBA3) and 7 publications (Bueno et al., 2011; Benamu et al., 2010; Evans et al., 2010; Michalkova et al., 2009; Schier A., 2006, Renaud et al., 2004; Santos et al., 2010) have been considered as supportive information (UBA2).

Addison, P.J., Barker, G.M. (2006)

glyphecotox_266	**Addison, P.J., Barker, G.M.**	**2006**	**Effect of various pesticides on the non-target species *Microctonus* hyperodae, a biological control agent of *Listronotus bonariensis***	**Entomologia Experimentalis Et Applicata 119 (1):71-79**

Reliability	
Purpose of the study Description of endpoints	Four experiments were conducted to investigate the effects of various pesticides that are commonly used in the pastoral environments of L. Bonariensis and M. hyperodae
Test compound, application procedure, exposure period	glyphosate (Roundup®, Monsanto Co., St. Louis, MO, USA), or the adjuvant Silwett L-77m(Pulse, EI DuPont de Nemours and Co. Inc., Wilmington, DE, USA) were mixed with water according to their label recommendations
Experimental approach, Statistics, test environment	Field experiment
Test organisms	*Microctonus hyperodae*
Biological effects	Silwett L-77, an organo-silicone copolymer penetrant and surfactant, was the only treatment to significantly increase M. hyperodae mortality compared to that of the water-treated controls. The herbicidal products had no demonstrable effect.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	no
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Species are not relevant for central zone, no data presented for glyphosate
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Albajes et al. (2011)

glyphecotox_274	**Albajes, R., Lumbierres, B., Pons, X.**	**2011**	**Albajes, R., Lumbierres, B., Pons, X.**	**Biological Control 59 (1):30-36. DOI 10.1016/j.biocontrol.2011.03.008.**

Reliability	
Purpose of the study Description of endpoints	The study aimed to compare arthropod densities in GMcorn plots treated with a broad-spectrum herbicide or with a conventional selective pre-emergence treatment.
Test compound, application, exposure, protocol	MON 78044 at 3 l/ha

Experimental approach, Statistical design, test environment	Field experiment, Each year, the number of predators and main herbivore prey (leafhoppers, aphids and phytophagous thrips) were counted: ANOVA
Test organisms	Orius spp., Nabis sp.
Biological effects	Authors conclude that no significant changes in heteropteran predator densities may be expected from moderate alterations in weeds arising from the deployment of herbicide- tolerant corn varieties and that leafhoppers are probably the herbivore prey that most influences Orius spp. densities in corn in our study area.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	no significant changes observed, GM corn tested
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Bueno et al. (2011)

glyphecotox_305	**Bueno, A.F., Bueno, R.C.O.F., Parra, J.R.P., Vieira, S.S.**	**2011**	**Effects of pesticides used in soybean crops to the egg parasitoid** *Trichogramma pretiosum*	**Ciencia Rural 38 (6):1495-1503**

Reliability	
Purpose of the study Description of endpoints	This research aimed to study the effects of different insecticides, herbicides and fungicides on eggs, larvae and pupae of *Trichogramma pretiosum*
Test compound, application procedure, exposure period, protocol	glyphosate 960 grams ha-1 (Gliz® 2000 milliliters ha-1); glyphosate 972 grams ha-1 (Roundup® Ready® 2000 milliliters ha-1); glyphosate 960 grams ha-1 (Roundup® Transorb® 1500 milliliters ha-1); glyphosate 960 grams ha-1 (Roundup® Original® 2000 milliliters ha-1);
Experimental approach, Statistical design, test environment	Laboratory: Cardboard squares (1cm2) with approximately 250 *A. kuehniella* eggs each were offered for 24 hours to recently emerged *T. pretiosum* females in vials. Then, these cards were transferred to vials and kept until the time after parasitism was sprayed with the treatments that were: 72 hours (eggs), 144 hours (larvae), 192 hourspupae) (MANZONI et al., 2007).
Test organisms	*Trichogramma pretiosum*
Biological effects	glyphosate 960.0 (Gliz® and Roundup® Transorb®), was classified as harmless to all imature *T. pretiosum* stages.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Reduction of parasitism viability comparared to the untreated was 100 % for Roundup® Ready.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial products
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	Glyphosate (Roundup® Ready) 972 grams ha-1 were classified as harmfull for T. pretiosum eggs and harmless to the other parasitoid stages . Glyphosate 960 (Roundup® Original) was classified as slightly harmful for eggs and harmless for pupae of the parasitoid .
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Lipok, J. (2009)

glyphecotox_462	**Lipok, J.**	**2009**	**Dual action of phosphonate herbicides in plants affected by herbivore-Model study on black bean aphid Aphis fabae rearing on broad bean Vicia faba plants**	**Ecotoxicology and Environmental Safety 72 (6):1701-1706. DOI 10.1016/j.ecoenv.2009.03.007.**

Reliability	
Purpose of the study Description of endpoints	The paper describes the sensitivity of blackaphid *A.fabae* towards tested herbicides as the nutrients and the influence of herbicide-treated plants of broad bean *V. faba* L. on the host plant choice and population development of black aphid *A.fabae*. The combined effect of sublethal doses of herbicides and presence of aphids on the growth of broad bean plants was also investigated.
Test compound, application procedure, exposure period, protocol	Each of four tested compounds was studied at three concentrations: 15, 1.5 and 0.015mM using two means of treatment. Pure glyphosate (N-phosphonomethylglycine) was obtained by the author via laboratory procedure: from commercial Roundup®s 360 SL(Monsanto, MO,U SA) formulation by dissolving in water and maintaining the pH of the solution to 1.5–2.0 with hydrochloric acid. This resulted incrystallisation of the pure herbicide. Its structure and purity were confirmed using 1H, 13C and 31P NMR spectroscopy. The retention ime of this substance incapillary electrophoresis was the same as the retention time of glyphosate standard obtained from Monsanto.
Experimental approach, Statistical design, test environment	The experimental system was composed of phosphonate herbicides, broad bean *Vicia faba* (L.) plants and blackbean aphid *Aphis fabae (Scopoli*). Two mean of herbicide application, namely standard spraying and direct introduction of the herbicide into stem via glass capillary, were examined.
Test organisms	*Aphis fabae , Vicia faba*

Biological effects	Reaction of aphids towards artificial diet supplemented with herbicides The insects, which were settled on the artificial diet supplemented with glyphosate, tended to escape from the membranes or were dead at the second or fourth day of experiment. Studies on aphids cultured on artificial diet supplemented with herbicides revealed that application of which decreased the number of aphids on treated plants, influence negatively the insect development most likely exhibiting weak insecticidal activity.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Authors state that the herbicides decreased the rate of growth and development of the aphid populations, most probably by exhibiting weak insecticidal activity.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Active ingredient
2 Do the tested concentrations relate to predicted environmental concentrations?	15, 1.5 and 0.015mM
3 Have parameters influencing the endpoints been considered adequately?	
Concluding weight of evidence/proposed action	Number of aphids accompanied with treated plants cannot be used for risk assessment, no effects observed
Type of information (Critical, supporting, low weight)	**low weight)**
Consideration/concluding score	**UBA3**

Evans et al. (2010)

glyphecotox_147	**Evans, S.C., Shaw, E.M., Rypstra, A.L.**	**2010**	**Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival**	**Ecotoxicology 19: 1249-1257**

Reliability	
Purpose of the study Description of endpoints	Study quantifies the effects of a commercial formulation of a glyphosate-based herbicide on the activity of three predatory arthropod species that inhabit agricultural fields in the eastern United States. Authors measured the survival of the most common species.
Test compound, application procedure, exposure period, protocol	commercially formulated herbicide solution (Buccaneer Plus) containing 41% (480 g/l) glyphosate (N-(phosphonomethyl)glycine) isopropylamine salt and 59% other ingredients, including a polyethoxylated tallowamine (POEA) surfactant
Experimental approach, Statistical design, test environment	We tested the reactions of the wolf spider, Pardosa milvina, to either direct application (topical) or contact with a treated substrate (residual). We quantified the reactions of a larger wolf spider, Hogna helluo, and a ground beetle, Scarites quadriceps, to a compound (topical plus residual) exposure.
Test organisms	wolf spider *Pardosa milvina*, wolf spider, *Hogna helluo*, ground beetle, *Scarites quadriceps*
Biological effects	Exposure of terrestrial arthropods to glyphosate-based herbicides affects their behaviour and long-term survival.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Pardosa spp.*
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Results suggest that herbicides can affect arthropod community dynamics separate from their impact on the plant community and may influence biological control in agroecosystems.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Activity metrics recorded,
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	commercially formulated product containing POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	12 g/l of the glyphosate salt, higher than the expected drift rates
3 Have parameters influencing the endpoints been considered adequately?	Laboratory approach
Concluding weight of evidence/proposed action	No endpoints on mortality. Tested concentrations higher thatn expected drift rates. But authors demonstrate that arthropod predators inhabiting agroecosystems around the world exhibit subtle shifts in behaviour and reproduction during or after exposure to herbicide.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Griesinger et al. (2010)

glyphecotox_148	**Griesinger, L.M., Evans, S.C., Rypstra, A.L.**	**2010**	**Effects of a glyphosate-based herbicide on mate location in a wolf spider that inhabits agroecosystems**	**Chemosphere 84: 1461 - 1466**

Reliability	
Purpose of the study Description of endpoints	The aim of this study was to examine effects of a commercial formulation of a glyphosate-based herbicide on the ability of males to find females.
Test compound, application procedure, exposure period, protocol	Roundup®_ II Original. This herbicide is manufactured by Monsanto, St Louis, MO, USA (United States Patent US4528023). As provided, this herbicide contains 41% (480 g L_1) glyphosate (N-(phosphonomethyl)glycine) isopropylamine salt and 59% other ingredients, including a polyethoxylated tallowamine (POEA) surfactant. For the pitfall experiment, we diluted it with distilled water to a concentration of 2.5% (12 g L_1 of the glyphosate salt).
Experimental approach, Statistical design, test environment	Field experiment , Pitfall experiment, In one pair of treatments we applied 5 lL of either distilled water or herbicide solution to the filter paper inside the vial with the female. In another two treatments, we applied 0.926 mL of either distilled water or herbicide solution to the ring of filter paper surrounding the cup.
Test organisms	wolf spider, *Pardosa milvina*
Biological effects	Traps with herbicide on the filter paper inside with the female captured fewer males.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?	wolf spider, *Pardosa milvina*	
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Our experiments suggest that a commercial formulation of a glyphosate-based herbicide affects mate location in a wolf spider that is common in agroecosystems where these chemicals are routinely applied. however, the circumstances under which these effects influence population viability, community structure, and/ or the food web remain to be explored	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Reduction of the efficacy of natural infochemicals important to mate location in *Pardosa milvina*has probably minor impact on population and communities of spiders.	
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Commercial product containing POEA
2 Do the tested concentrations relate to predicted environmental concentrations?		Drift rates are predicted to be lower.
3 Have parameters influencing the endpoints been considered adequately?		nd
Concluding weight of evidence/proposed action	Behavioral study , with POEA containing product. Tested concentrations probably higher than drift rates.	
Type of information (Critical, supporting, low weight)	**low weight**	
Consideration/concluding score	**UBA3**	

Michalková, V., Pekár, S. (2009)

glyphecotox_506	**Michalková, V., Pekár, S.**	**2009**	**How glyphosate altered the behaviour of agrobiont spiders (Araneae: Lycosidae) and beetles (Coleoptera: Carabidae)**	**Biological Control 51 (3):444-449**
Reliability				
Purpose of the study Description of endpoints	Aim of the study was to assess the effect of the Roundup® residues on the predatory, defensive, locomotory and reproductive behaviour of epigeic spiders and carabid beetles			
Test compound, application procedure, exposure period, protocol	Roundup® Biaktiv (Monsanto; glyphosate, IPA 480 g l_1). The formulation wasdiluted in water using a rate (1:25) recommended for use in cereals. A piece (5 _ 5.5 cm) of the filter paper (Whatman 2R/80 g) wasdipped into the solution and gave rise to two different residues: freshand 1-day old, thepapers were rolled to form a tube and inserted into 10 ml glass tube.Inside of the paper roll a spider or a beetle was kept for 2 h to maximiseits contact with the residues.			
Experimental approach, Statistical design, test environment	Locomotory and reproductive behaviour of epigeic spiders and carabid beetles. Specimens of *Pardosa* Agricola (Araneae: Lycosidae) and *Poecilus cupreus* (Coleoptera: Carabidae) were exposed for 2 h to the fresh and 1-day old residues of Roundup® Biaktiv (Monsanto, IPA 480 g/l).			
Test organisms	*Pardosa* and *Poecilus*			

Biological effects	Capture and consumption of flies by *Pardosa* spiders did not differ between spiders exposed to any herbicide residues and the control surface, *Pardosa* spiders ran slightly slower after being exposed to herbicide residues but the difference was not significant, But *Poecilus* beetles exposed to both types of herbicide residues moved significantly slower than those exposed to the control surface, no effects on avoidance and defence, no qualitative difference in mating behaviour.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?	*Pardosa* and *Poecilus* are standard test species in RA	
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Roundup® Bioaktiv thus appears to be harmless to lycosid spiders and only slightly harmful to carabid beetles. The biological control potential of both predators should not be reduced directly by the application of Roundup® Bioaktiv.	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd	
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?		Recommended in field rate was used.
3 Have parameters influencing the endpoints been considered adequately?		nd
Concluding weight of evidence/proposed action	*Pardosa* and *Poecilus* are standard test species in RA, predation rate, locomotion speed, avoidance, defence and mating behavior nor standard parameters. Biological control potential of the two species should not be directly reduced following herbicide application in the field.	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Schier, A. (2006)

glyphecotox_595	**Schier, A.**	**2006**	**Field study on the occurrence of ground beetles and spiders in genetically modified, herbicide tolerant corn in conventional and conservation tillage systems**	**Journal of Plant Diseases and Protection. Special Edition XX:101-113**
Reliability				
Purpose of the study Description of endpoints	The objective of this study was to analyse and compare the impact on weed control and arthropod abundance of conventional and conservation tillage methods under different herbicide regimes. The study was conducted between 2002 and 2005 on continuously planted Roundup® Ready® (RR) corn.			
Test compound, application procedure, exposure period, protocol	MON 78044 (Glyphosate, 360g/l) , Roundup® Ready®			
Experimental approach, Statistical design, test environment	field experimental design, Pitfall traps were used to survey populations of soil dwelling arthropods			
Test organisms	soil dwelling arthropods			
Biological effects	The results of this multi year study indicate that the combination of conserva-tion tillage and herbicide tolerant corn has a positive impact on biodiversity			

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	soil dwelling arthropods
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product with unknown surfactant chemistry included. Therfore limited validity for other products and the active substance glyphosate itself.
2 Do the tested concentrations relate to predicted environmental concentrations?	Not stated.
3 Have parameters influencing the endpoints been considered adequately?	Field study with climatic extremes and uncertainties.
Concluding weight of evidence/proposed action	Field study with climatic extremes and uncertainties. The results of this multi year study indicate that total abundance of ground beetles and spiders were not affected due to reduced soil tillage combined with glyphosate treatment.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Ainsworth, N. (2003)

glyphecotox_272	**Ainsworth, N.**	**2003**	**Integration of herbicides with arthropod biocontrol agents for weed control**	**Biocontrol Science and Technology 13 (6):547-570. Doi 10.1080/0958315031000151819.**

Reliability	
Purpose of the study Description of endpoints	This literature review first considers the direct toxic effects of herbicides and surfactants on biocontrol agents.
Test compound, application procedure, exposure period, protocol	nd
Experimental approach, Statistical design, test environment	nd
Test organisms	arthropods
Biological effects	Glyphosate had low, if any, direct toxicity to several biocontrol agents (Ding et al ., 1998;Boersma & Ireson, 1999; Lindgren et al. , 1999; Hayes, 2000b). However, Searle et al. (1990) reported some toxicity to mites, which increased when extra surfactant was added.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	No information relevant for risk assessment
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Renaud et al. (2004)

glyphecotox_570	**Renaud, A., Poinsot-Balaguer, N., Cortet, J., Le Petit, J.**	**2004**	**Influence of four soil maintenance practices on Collembola communities in a Mediterranean vineyard**	**Pedobiologia 48 (5-6):623-630..**

Reliability	
Purpose of the study Description of endpoints	Influence of a) postemergence herbicide with glyphosate; (b) postemergence and pre-emergence herbicides with glyphosate, terbuthylazine, diuron and oryzalin; (c) natural flora and (d) tillage to a depth of10–15 cm was studied.
Test compound, application procedure, exposure period, protocol	Not stated
Experimental approach, Statistical design, test environment	Vineyard called ‘‘le Domaine de Donadille’’ situated at Rodilhan, The vineyard was planted with 15–20 year old Syrah variety vine plants in a silt-clay soil. Sampling took place between December 2000 and June 2002; no samples were taken in summer due to drought. On each sampling date, six soil samples were taken from the central inter-row space of each treatment plot.
Test organisms	Collembola
Biological effects	The postemergence herbicide glyphosate treatment practice and the natural flora practice practice favoured the development of epigeic and hemiedaphic species,due to preservation of the weed cover. C. denticulate and *L. cyaneus* were favoured in tillage practice.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Abundance and species diversity were assessed.
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	no
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Probably commercial product, no information
2 Do the tested concentrations relate to predicted environmental concentrations?	Glyphosate (15 l /ha)
3 Have parameters influencing the endpoints been considered adequately?	Wheatear and rainfall influence was discussed.
Concluding weight of evidence/proposed action	Total abundance was highest in natural flora practice and in the practice with a postemergence herbicide. Glyphosate treatment weed cover was preserved.

Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Wrinn et al. (2012)

glyphecotox_650	**Wrinn, K.M., Evans, S.C., Rypstra, A.L.**	**2012**	**Predator cues and an herbicide affect activity and emigration in an agrobiont wolf spider**	**Chemosphere. doi: 10.1016/j.chemosphere.2011.12.030.**

Reliability	
Purpose of the study Description of endpoints	Exploration how Buccaneer_ Plus, a common herbicide similar to Round-up_ (active ingredient glyphosate), affected the interactions between intraguild predators.
Test compound, application procedure, exposure period, protocol	BuccaneerPlus, also known as Roundup® II original, created by the Monsanto Company, St. Louis, Missouri, USA (United States Patent US4528023). This herbicide contains the active ingredient glyphosate (480 g L_1) in the form of isopropylamine salt, and an added polyethoxylated tallowamine (POEA) surfactant, diluted it to 2.5%, which was within the manufacturer's recommended levels of 0.625–5%, spray rate of 127.4 mL m_2 (or 15.3 kg a.i. ha_1 of glyphosate), which was the minimum necessary to gain a complete and uniform coverage of the areas for the laboratory container with filter paper.
Experimental approach, Statistical design, test environment	Arthropods were collected within 3 d after herbicide application in the field, and were not used in experiments until 2 months after the date of last herbicide application. Laboratory arena for exposing Pardosa milvina to herbicide and/or predator cues. Filter paper pieces with herbicide or water are alternated by those with predator cues (*Hogna helluo or Scarites quadriceps*) or blank paper.
Test organisms	The focal species for these experiments was *Pardosa* milvina (Araneae: Lycosidae), a numerically dominant, epigeal generalist arthropod predator in agricultural fields throughout eastern North America
Biological effects	Predator cues and herbicide led to a decrease in movement by *P. milvina*. However, although *H. helluo* cues alone decreased movement, S. quadriceps cues only decreased movement when combined with herbicide.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Pardosa milvina*
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product with POEA
2 Do the tested concentrations relate to predicted environmental concentrations?	Tested concentration probably higher than the expected drift rate. application rate was higher than that which would likely be found in a real situation
3 Have parameters influencing the endpoints been considered adequately?	spray rate was not properly controlled
Concluding weight of evidence/proposed action	Authors conclude that predation risk andmherbicide application likely interact to affect the movement of a major arthropod predator.
Type of information (Critical, supporting, low weight)	**low weight**

Consideration/concluding score	**UBA3**

Benamu et al. (2010)

glyphecotox_146	**Benamu, M.A., Schneider, M.I., Sanchez, N.E.**	**2010**	**Effects of the herbicide glyphosate on biological attributes of Alpaida veniliae (Araneae, Araneidae), in laboratory**	**Chemosphere 78 (7):871-6.**

Reliability	
Purpose of the study Description of endpoints	The purpose of this study was to address the effects of glyphosate on some biological attributes of *A. veniliae*, in laboratory.
Test compound, application procedure, exposure period, protocol	ANOVA, Glifoglex 48_ (48% glyphosate, Gleba SA, Buenos Aires, Argentina) was used in toxicity bioassays. Fresh solutions with 192 mg L_1 a.i. (maximum field registered nominal concentration) (CASAFE, 2007).
Experimental approach, Statistical design, test environment	Solutions were prepared using acetone (Analytical Grade) as solvent to assure the evaporation of herbicide solution, considering that spiders avoid feeding on wet preys. The exposure route was by ingestion ‘‘through the treated prey” and the chronic toxicity was analyzed. The prey (*M. domestica* adults) was treated by dipping during 20 s according to Schneider et al. (2009), and dried under fume cupboard.
Test organisms	Arthropod predator *Alpaida veniliae* (Araneae, Araneidae) is one of the most abundant orb web weaver spiders of Argentinia.
Biological effects	Results of this study showed no lethal direct effects of Glifoglex_ on this spider, but it is the first report in literature about sublethal effects of this herbicide on a spider´s biological attributes. Negative effects on prey consumption, web building, fecundity, fertility and developmental time of progeny were observed.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	web weaver spiders of Argentinia
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Sublethal effects of glyphosate in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of A. veniliae. Females poorly fed will be affected in their survival, fecundity and fertility, therefore, natural populations of this spider would be seriously affected in its capacity to grow and persist in natural conditions
3 Is the ecotoxicological manifestation level appropriate for the assessment?	prey consumption, web building, fecundity, fertility and developmental time of progeny were analysed, no lethal or reproductive endpoint.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	In Off field expected drift values are lower.
3 Have parameters influencing the endpoints been considered adequately?	Deficiencies are discussed, parameter (sublethal effects)not reliable for RA, argentinian species.
Concluding weight of evidence/proposed action	Authors conclude that sublethal effects are relevant from an ecological point of view, since the reduction of the arthropod performance may create risks to arthropod biodiversity conservation in agroecosystems.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Castilla et al. (2010)

glyphecotox_311	**Castilla, A.M., Dauwe, T., Mora, I., Malone, J., Guitart, R.**	**2010**	**Nitrates and Herbicides Cause Higher Mortality than the Traditional Organic Fertilizers on the Grain Beetle,** *Tenebrio molitor*	**Bulletin of Environmental Contamination and Toxicology 84 (1):101-105. DOI 10.1007/s00128-009-9883-5.**

Reliability	
Purpose of the study Description of endpoints	The present laboratory study determined mortality of adult beetles (Tenebrio molitor) rates of under different pesticide treatments (a mixture of glyphosate and 2,4-D)
Test compound, application procedure, exposure period, protocol	Mixture of two types of herbicides: 1 L of the isopropylamine salt of Glyphosate (Lograd, Masso Division Agro), 36% p/v (360 g/L), and 100 cm3 of2,4-D (Agrodan), 80%, in 4 L of water.
Experimental approach, Statistics, test environment	Beetles were placed in manufactured soft aluminium open boxes (16 9 11 9 3 cm).
Test organisms	Grain Beetle, *Tenebrio molitor*
Biological effects	Using a binary mixture makes it difficult to deduce the individual effect of each herbicide to the insect.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The individual effect s of each herbicide to the insect cannot be assigned.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations?	nd
3 Have parameters influencing the endpoints been considered adequately?	nd

Concluding weight of evidence/proposed action	Herbicide mixture, deficiencies in test design,
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Bernard et al. (2010)

glyphecotox_296	**Bernard, M.B., Cole, P., Kobelt, A., Horne, P.A., Altmann, J., Wratten, S.D., Yen. A.L.**	**2010**	**Reducing the Impact of Pesticides on Biological Control in Australian Vineyards: Pesticide Mortality and Fecundity Effects on an Indicator Species, the Predatory Mite Euseius victoriensis (Acari: Phytoseiidae)**	**Journal of Economic Entomology 103 (6):2061-2071. Doi 10.1603/Ec09357.**

Reliability	
Purpose of the study Description of endpoints	Laboratory bioassays on detached soybean, Glycine max (L.) Merr., leaves were used to test pesticides on a key Australian predatory mite species Euseius victoriensis (Womersley) in "worst-case scenario" direct overspray assays

Test compound, application procedure, exposure period, protocol	Glyphosate (360 g/liter) Roundup® (Nufarm Australia) 2.187 g /L in 400 ml	
Experimental approach, Statistics, test environment	Zero- to 48-h-old juveniles, their initial food, and water supply were sprayed to runoff with a Potter tower; Cumulative mortality was assessed 48 h, 4 d, and 7 d after spraying., Fecundity was assessed for 7 d from start of oviposition	
Test organisms	*Euseius victoriensis*	
Biological effects	Glyphosate had no signiÞcant effects on mortality (Tukey b; Table 2), or fecundity (F_1.6285; df _ 6, 20; P _ 0.191; Table 2) compared with the control	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		Australien mite
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?		No effects
3 Is the ecotoxicological manifestation level appropriate for the assessment?		nd
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?		Infield concentrations used.
3 Have parameters influencing the endpoints been considered adequately?		nd
Concluding weight of evidence/proposed action	Test species not relevant for Europe, no effects detected	
Type of information (Critical, supporting, low weight)	**low weight**	
Consideration/concluding score	**UBA3**	

Santos et al. (2010)

glyphecotox_591	**Santos, M.J.G., Soares, A.M.V.M., Loureiro, S.**	**2010**	**Joint effects of three plant protection products to the terrestrial isopod** *Porcellionides pruinosus* **and the collembolan** *Folsomia candida*	**Chemosphere 80 (9):1021-1030.**
Reliability				
Purpose of the study Description of endpoints	Determination of the effects of 3 products on the avoidance response pattern of *P. pruinosus* and in the reproductive output of F. *candida*; secondly to predict the response patterns for mixture exposures using the CA and IA conceptual models for the two test-species.			
Test compound, application procedure, exposure period, protocol	Commercial formulations: (ROUNDUP®_ with 360 g AI/L, and which contains glyphosate-isopropylammonium (45%), surfactant (16%) and water 42.5%)), The nominal concentrations: 0.5 to 54.5 mg kg_1 dry soil in the avoidance experiment and between 0.1 andm 2mg kg_1 dry soil in the reproduction test;			
Experimental approach, Statistics, test environment	The avoidance tests conducted with *P. pruinosus* were performed based on a methodology by Loureiro et al. (2005), consisting in exposing 10 isopods in a plastic box (14.3 cm _ 9.3 cm _ 4.7 cm height) divided in two sections, one with the control soil and the other with the test soil. After 24 and 48 h the number of animals in each side of the test-box was counted and mortality was registered. The experimental procedure for the reproduction test with the springtail F. candida was performed accordingly to the ISO 11267 protocol (ISO, 1999).			

Test organisms	terrestrial isopod *Porcellionides pruinosus* and the collembolan *Folsomia candida Miguel* J.
Biological effects	The exposure resulted in a clear avoidance response in the higher concentrations (73% avoidance at 17.4 mg kg_1) although a small decrease in the degree of avoidance response was reflected in the highest concentration. EC_{50} values (mg kg_1 dry soil) and 95% confidence intervals (CI) for the effects of m single exposure on the reproductive output of *Folsomia candida* exposed for 28 d on LUFA 2.2 soil= 0.33 (mg kg_1 dry soil (0.18–0.48). For the effect of single exposure pesticide on the avoidance behaviour of *Porcellionides pruinosus* exposed for 48 h on LUFA 2.2 soil teh AC_{50} = 40 mg /kg dry soil

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Yes, standard test species
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to predicted environmental concentrations?	0.33 mg/kg dry soil corresponding to approximately 250 g /ha in the top 5 cm soil 40 mg/kg dry soil corresponding to approximately 30kg/ha
3 Have parameters influencing the endpoints been considered adequately?	nd
Concluding weight of evidence/proposed action	ER_{50} reproduction at approx.12xPEC AC_{50} for avoidance at approx 10xPEC
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

- **B.9.13 12.1 Summary of the relevant literature on on other arthropod species**

For the group of terrestrial non-target arthropods (NTA), a database of 31 publications was collected by the notifier. The notifier considered none of the publication as acceptable for risk assessment. The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening (please refer for detailed description to the document on the Evaluation of peer-reviewed literature regarding ecotoxicity). From this screening, 11 studies were recognized as information with low weight (category UBA3) and 7 publications (Bueno et al., 2011; Benamu et al., 2010; Evans et al., 2010; Michalkova et al., 2009; Schier, 2006, Renaud et al., 2004; Santos et al., 2010) have been considered as supporting information (UBA2).

Indirect effects on beneficial arthropod communities take place within treated areas and are principally due to vegetation changes subsequent to herbicide application. These vegetation changes, mainly decompostion of plant cover, might result in a drastic reduction of the habitats of beneficial and other non-target arthropod communities and a loss of their refuges from predators.In a multiyear study using pitfall trapping to collect mobile arthropod species on the soil surface, the combination of conservation tillage and herbicide treatment had less impact on biodiversity than conventional ploughing (Schier, 2006). However, conservation tillage without the use of glyphosate is not practiced, due to the upcoming weed pressure on culture crops. It is not possible to identify the effects of glyphosate applications in the perfomed studies, When collembolan populations were assessed in field plot experiments in Mediterranean vineyards (Renaud et al.. 2004) the result suggested apparently that plant protection products containing glyphosate

favored the occurrence of epigeic and hemiedaphic species due to the preservation of decaying organic material on the soil surface compared to tillage practice. RMS considers it misleading to confuse the effects of tillage practice vs. non- tillage practice with the effects of an application of glyphosate without proper negative control. In a laboratory study it could be shown that reproductive capacity of the collembolan species Folsomia candida was not influenced by the application of glyphosate containing plant protection product when applied at relevant environmental concentrations (Santos et al., 2010).

Arthropods in their natural environment can be exposed directly to pesticides after the application due to residues on food or due to contact with contaminated surfaces (such as plants, soil, surrounding substrate).

Risk analysis is currently based on so called beneficial arthropods which are important in the biological control of agronomic pests, typically through predation or parasitism including beetles, mites, wasp and spider. Tests are performed on glass plates or on extended laboratory tests with a 2 dimensional exposure on leaf substrates testing the formulated product for the determination of the median lethal dose (LD50) and/or median effect on reproduction. Thereby test species were selected more for practical reasons because of their utility in agricultural production and feasibility in experimental setups than on the basis of their ecological relevance. At the same time effects on various developmental stages of arthropods, physiology, and behavior or prey consumption are not given consideration in traditional risk assessment. Bueno et al., (2011) could show that glyphosate containing products can be harmfull towards egg stages of Trichogramma, whereas at other parasitoid stages the same product was harmless. Sublethal effects of glyphosate were assessed in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of a web weaver spider (Alpaida veniliae) in Argentina (Benamu et al., 2010) and on wolf spiders in north America (Evans et al., 2010). The authors concluded that the exposure to glyphosate containing products affects the behavior of the animals and their capacity to grow and persist in agroecosystems. In contrast, short term exposures (2h and one-day residues) of spiders and carabid beetles, respectively Pardosa agricola and Poecilus cupreus, did not affect mating or avoidance of the arthorpods, but (only) slightly slower movement (Michalkova et al., 2009).

These effects together with the indirect effects of herbicide treatment on the vegetation of their habitat receive less attention even though they might have implications for the success of survival and reproduction.

- ***References***

Benamu, M.A., Schneider, M.I., Sanchez, N.E.; Effects of the herbicide glyphosate on biological attributes of *Alpaida veniliae* (Araneae, Araneidae), in laboratory (2010): Chemosphere 78/7, 871-6. DOI: 10.1016/j.chemosphere.2009.11.027.

Bueno, A.F., Bueno, R.C.O.F., Parra, J.R.P., Vieira, S.S. (2008): Effects of pesticides used in soybean crops to the egg parasitoid *Trichogramma pretiosum.* Ciencia Rural 38/6, 1495-1503

Evans, S.C., Shaw, E.M., Rypstra, A.L. (2010): Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. Ecotoxicology 19, 1249-1257

Michalková, V., Pekár, S. (2009): How glyphosate altered the behaviour of agrobiont spiders (Araneae: Lycosidae) and beetles (Coleoptera: Carabidae). Biological Control 51/3, 444-449

Renaud, A., Poinsot-Balaguer, N., Cortet, J., Le Petit, J.(2004): Influence of four soil maintenance practices on Collembola communities in a Mediterranean vineyard. Pedobiologia 48/5-6, 623-630. DOI 10.1016/j.pedobi.2004.07.002.

Santos, M.J.G., Morgado, R., Ferreira, N.G.C., Soares, A.M.V.M., Loureiro, S.(2011): Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem.

Ecotoxicology and Environmental Safety 74/7, 1994-2001. DOI: 10.1016/j.ecoenv.2011.06.003.

Schier, A. (2006): Field study on the occurrence of ground beetles and spiders in genetically modified, herbicide tolerant corn in conventional and conservation tillage systems. Journal of Plant Diseases and Protection. Special Edition XX:101-113

B.9.13.13 Effects on earthworms

Among soill organisms, eathworms are standard organsims in the ERA as the have a potential role in the formation and maintenance of fertile soils. Besides laboratory studies submitted for the application for Renewal of Approval (AIR 2) following international guidelines, additional 21 references "open literature" has been submitted dealing with soil organsims in gerneral. Within these references 5 studies (Casabe et al., 2007; Correia et al., 2012; Kaneda et al., 2009; Verrel et al., 2004 and Yasmin et al., 2003) focusing on earthworms have been considered to represent supporting information for risk assessment.

Kaneda et al. (2009)

glyphecotox_419	**Kaneda, S., Okano, S., Urashima, Y., Murakami, T., Nakajima, M.**	**2009**	**Effects of herbicides, glyphosate, on density and casting activity of earthworm, Pheretima (Amynthas) carnosus**	**Japanese Journal of Soil Science and Plant Nutrition 80:469-476, inc. English translation**
Reliability				
Purpose of the study Description of endpoints		Direct effects of herbicide application on the mortality, behavior, and body weight of earthworms were studied in a pot test.		
Test compound, application procedure, exposure period, protocol		Roundup® (ingredient: 41% glyphosate isopropylamine salt;		
Experimental approach Statistical design, test environment		herbicide was applied several years, The pplication amount was 0.33 L of a 100-fold dilution per square meter, as recommended bythe manufacturer, throughout the test period. The relationship between the earthworm habitat density and the amount of castings produced on the surface was evaluated via simple linear regression		
Test organisms		*Pheretima (Amynthas) carnosus*		
Biological effects		It is considered that herbicide application in no-tillage field did not directly affect the mortality and behavior of Pheretima (Amynthas) carnosus, but instead affected the casting production rate indirectly via changes in soil moisture and litter amount.		
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				yes
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				The conclusion from this study is only valid for glyphosate formulations that contain POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?				nd

Concluding weight of evidence/proposed action	**Not considered because of deficiencies in translation**
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Casabe et al. (2007)

glyphecotox_309	**Casabe, N., Piola, L., Fuchs, J., Oneto, M.L., Pamparato, L., Basack, S., Gimenez, R., Massaro, R., Papa, J.C., Kesten, E.**	**2007**	**Ecotoxicological Assessment of the Effects of Glyphosate and Chlorpyrifos in an Argentine Soya Field**	**Journal of Soils and Sediments 7 (4):232-239. DOI 10.1065/jss2007.04.224.**

Reliability	
Purpose of the study Description of endpoints	Authors performed field-laboratory study on an Argentinem soya field sprayed with glyphosate and chlorpyrifos under controlled conditions. GLY reduced cocoon viability, decreasing the number of juveniles. Moreover, earthworms avoided soils treated with GLY and a reduction in the feeding activity under laboratory and field conditions.
Test compound, application procedure, exposure period, protocol	Roundup® 1440 g a.s./ha, inc. analytic
Experimental approach Statistical design, test environment	In laboratory assays, Eisenia fetida Andrei were exposed to soil samples (0–10 cm depth) collected between the rows of soya. Endpoints linked to behavior and biological activity (reproduction, avoidance behavior and bait-lamina tests) and cellular/subcellular assays (Neutral Red Retention Time – NRRT; DNA damage – Comet assay) were tested.
Test organisms	Eisenia fetida Andrei
Biological effects	behavior and biological activity (reproduction, avoidance behavior and bait-lamina tests) and cellular/subcellular assays (Neutral Red Retention Time – NRRT; DNA damage – Comet assay) were tested.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	**Detailed study, will be considered.**
Type of info. (Critical, supporting, low weight)	**supporting**

Consideration/concluding score	**UBA2**

Correia, F.V., Moreira, J.C. (2010)

glyphecotox_171	**Correia, F.V., Moreira, J.C.**	**2010**	**Effects of glyphosate and 2,4-D on earthworms (Eisenia foetida) in laboratory tests**	**Bull. Environ.Contam.Toxicol. DOI10.1007/s00128-010-0089-7**
Reliability				
Purpose of the study Description of endpoints			Long-term exposure (56 days) to soil contaminated with glyphosate demonstrated a toxic effect on normal development and reproduction of *Eisenia foetida*, indicating that this substance may have significant toxic effects on soil biota. Study describes results of a 56d Reproduction test with *Eisenia fetida* Andrei. Earthworms kept in glyphosate treated soil were classified as alive in all evaluations, but showed gradual and significant reduction in mean weight (50%) at all test concentrations.	
Test compound, application procedure, exposure period, protocol			Glyphosate 99.7% from SIGMA Aldrich	
Experimental approach Statistical design, test environment			1, 10, 100, 500, 1000 mg/kg Soil representative for Brazil	
Test organisms			*Eisenia foetida*	
Biological effects			Morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate (1000 mg/kg).	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				Reduction in mean weight (50%) at all test concentrations.
3 Is the ecotoxicological manifestation level appropriate for the assessment?				nd
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				nd
Concluding weight of evidence			**Study will be considered.**	
Type of info. (Critical, supporting, low weight)			**supporting**	
Consideration/concluding score			**UBA2**	

Verrell, P., Van Buskirk, E. (2004)

glyphecotox_640	**Verrell, P., Van Buskirk, E.**	**2004**	**As the worm turns: Eisenia fetida avoids soil contaminated by Glyphosate-based herbicide**	**Bulletin of Environmental Contamination and Toxicology 72 (2):219-224. DOI 10.1007/s00128-003-9134-0.**
Reliability				
Purpose of the study			Laboratory acute experiments designed to test acute	
Description of endpoints			effects on E. fetida . Exposure to nominal concentration influences the activity of worms, as they emerged onto the surface within 2 h in all seven replicates exposed to nominal concentrations.	
Test compound, application procedure, exposure period, protocol			Ortho Ground clear vegetation Killer (5% glyphosate as IPA salt)	
Experimental approach Statistical design, test environment			Not similar to standard Nominal to 1/ 10.000, no statistics	
Test organisms			*Eisenia foetida*	
Biological effects			After 48 h animals were found to be buried in the soil. Authors suggest that acute exposure to concentrations of Groundclear recommended for application may comprise the survival of earthworms even though is direct toxicity appears low.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?				nd
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				nd
Concluding weight of evidence			**Study will be considered. No GLP, no OECD, no standard method, but results and conclusion shown credibly.**	
Type of info. (Critical, supporting, low weight)			**supporting**	
Consideration/concluding score			**UBA2**	

Yasmin, S., D'Souza, D. (2003)

glyphecotox_304	**Yasmin, S., D'Souza, D.**	**2003**	**Effect of Pesticides on the Reproductive Output of Eisenia fetida**	**Journal of Agricultural and Food Chemistry 51 (15):4268-4272. Doi 10.1021/Jf034018f.**
Reliability				
Purpose of the study Description of endpoints			Effects of glyphosate on growth and reproduction of the earthworm species, *Eisenia fetida* was tested.	
Test compound, application procedure, exposure period, protocol			Glycel 41% S.L. 2 mg /kg soil and 8 mg/kg soil	

Experimental approach Statistical design, test environment	similar to standard procedure, no statistic
Test organisms	*Eisenia fetida*
Biological effects	Earthworm weight was significantly reduced due to its exposure to 8mg /kg soil glyphosate In contrast, glyphosate did not have a significant effect on the reproduction of *E. fetida.*
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	No GLP, no OECD, no standard method, but results and conclusion shown credibly.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Moreno et al. (2009)

glyphecotox_517	**Moreno et al.**	**2009**	**Rainfed olive farming in south-eastern Spain: Long-term effect of soil management on biological indicators of soil quality**	**Agriculture Ecosystems & Environment 131 (3-4):333-339. DOI 10.1016/j.agee.2009.02.011.**
Reliability				
Purpose of the study Description of endpoints			The elimination of weeds with herbicides reduced the microbial functional diversity in covered soil but did not affect the other microbiological parameters.	
Test compound, application procedure, exposure period, protocol			Field study design lasting over 40 years	
Experimental approach Statistical design, test environment			Field study design lasting over 40 years No statistics	
Test organisms			Bacterial 16S rRNA soil DNA extracts	
Biological effects				
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?				nd
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				not assessable
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				not assessable

3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	not assessable
Concluding weight of evidence	**Supporting evidence**
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2**

Negga et al. (2011)

glyphecotox_523	**Negga et al.**	**2011**	**Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in Caenorhabditis elegans**	**NeuroToxicology 32 (3):331-341. DOI: 10.1016/j.neuro.2011.02.002.**

Reliability	
Purpose of the study Description of endpoints	Toxicology studies determining whether exposure to our pesticides of interest could induce regionally specific neurodegeneration.
Test compound, application procedure, exposure period, protocol	Touchdown Hitech formulation with [52.3% glyphosate] from Syngenta AG, Wilmington, DE. Exposure 30 min an 24h.
Experimental approach Statistical design, test environment	No eco -toxicological standard methods
Test organisms	*Caenorhabditis elegans* (N2) and NW1229worms
Biological effects	Studies demonstrate that C. elegans are vulnerable to glyphosate-containing herbicides and Mn/Zn-EBDC-containing fungicides at environmentally relevant concentrations, suggesting that these worms are a valuable and viable model system for future testing involving these pesticides. Studies demonstrate that C. elegans are vulnerable to glyphosate-containing herbicides at environmentally relevant concentrations in terms of neurotoxicity.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial formulation
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	
Type of info. (Critical, supporting, low weight)	**Not relevant**
Consideration/concluding score	**UBA3**

Jeffrey et al. (2010)

	Jeffrey D. Weidenhamer & Ragan M. Callaway	**2010**	**Direct and Indirect Effects of Invasive Plants on Soil Chemistry and Ecosystem Function**	**Journal of Chemical Ecology 36 (1):59-69. DOI 10.1007/s10886-009-9735-0.**
Reliability				
Purpose of the study Description of endpoints			The literature review indicates that invasive species can alter the biogeochemistry of ecosystems, that secondary metabolites released by invasive species may play important roles in soil chemistry as well as plant-plant and plantmicrobe interactions.	
Test compound, application procedure, exposure period, protocol			nd	
Experimental approach			Review article	
Test organisms				
Biological effects			Herbicides used to control invasive species can impact plant chemistry and ecosystems in ways that have yet to be fully explored.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				Yes, the literature review indicates that invasive species can alter the biogeochemistry of ecosystems
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				nd
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				nd
Concluding weight of evidence				
Type of info. (Critical, supporting, low weight)				
Consideration/concluding score			**UBA 2 relevant**	

Druart et al. (2010)

glyphecotox_353	Druart et al.	2010	Towards the development of an embryotoxicity bioassay with terrestrial snails: Screening approach for cadmium and pesticides	Journal of Hazardous Materials 184 (1-3):26-33. DOI 10.1016/j.jhazmat.2010.07.099

Reliability	
Purpose of the study Description of endpoints	Description of the method to assess the embryotoxicity of chemicals on *Helix aspersa*. This terrestrial gastropod is already the subject of a standardized test with snail eggs.
Test compound, application procedure, exposure period, protocol	Roundup® Biovert 360 (360 g/l glyphosate; Monsanto Europe S.A.), No, no standard test
Experimental approach Statistical design, test environment	Yes, EC50=18 mg /L POEA influence
Test organisms	Helix aspersa
Biological effects	Glyphosate and its formulations or its associated adjuvants was toxic to snail embryos at lower concentrations than the recommended application concentrations for agriculture. The authors hypothesized that the surfactant polyoxyethylene amine (POEA, also called MON 818) contained in Roundup®, improved the transfer of glyphosate, by interacting with the plasma membrane. Another hypothesis is that the POEA is in fact the compound mainly responsible for the toxicity of Roundup® and could even be more toxic than the Roundup® itself . It appears necessary to assess the risk of the final product (which will be applied to crops) and not only of the active ingredient individually.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	POEA influence
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	lower concentrations than the recommended application concentrations for agriculture were used
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	
Concluding weight of evidence	**Relevant information about formulations containing POEA.**
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA 2**

B.9.13 13.1 Summary of the relevant literature on earthworms

Among soil organisms, eathworms are standard organisms in the risk assessment as they have an important role in the formation and maintenance of fertile soils. Besides laboratory studies submitted for the application for the renewal of approval of the active substance glyphosate following international guidelines, additional 21 references have been submitted dealing with soil organsims in general. Within these references, 5 studies (Casabe et al., 2007; Correia et al., 2012; Kaneda et al., 2009; Verrel et al., 2004 and Yasmin et al., 2003) focusing on earthworms have been considered to represent supporting information for risk assessment.

In the risk assessment for acute effects on soil organisms, behaviour is not included as a sensitive endpoint. However, these responses might also have negative consequences, e.g. – when worms move to the surface of contaminated soil- exposure to predators or to detrimental light. It could be shown that the activity of worms was influenced by the exposure to environmentally relevant concentration of commercial formulation of glyphosate (Verrel and Buskirk, 2004). The worms emerged onto the surface within 2 h after exposure. Nevertheless, after 48 h animals were found to be buried in the soil again. Authors concluded that acute exposure to the glyphosate containing plant protection product may compromise the survival of earthworms even though its direct toxicity appears low (Verrel & Buskirk, 2004).

Effects on reproduction were examined by Casabé et al. (2007), Kaneda et al. (2009) and Yasmin et al. (2006) using commercial formulations with the recommended application rates. It is concluded that the observed responses will not impact the population of earthworm in nature.

However, it can not be excluded that with repeated applications of glyphosate containing plant protection products during the season or year by year will have negative effects on the biotic soil community. It is considered that herbicide application did not directly affect the mortality or reproduction but instead the biological activity of the animals.

In a reproduction test with Eisenia fetida, which was conducted with the active substance glyphosate itself (Correia et al., 2012), earthworms were kept in treated soil and were classified as alive after the evaluation period, but showed significant reduction in mean weight at all test concentrations. Moreover morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate (1000 mg/kg). Further behavioural abnormalities were described in terms of reduced casting production (Kaneda et al., 2009), reduced cocoon viability, a reduction in the feeding activity (Casabé et al., 2007) or reduced body weight (Yasmin et al., 2006). However, the test rates were similar or above the one tested in the offically submitted studies, so that the outcome of the risk assessment for earthworm did not change.

References

Casabe, N., Piola, L., Fuchs, J., Oneto, M.L., Pamparato, L., Basack, S., Gimenez, R., Massaro, R., Papa, J.C., Kesten, E. (2007): Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. Journal of Soils and Sediments 7/4, 232-239. DOI 10.1065/jss2007.04.224

Correia, F.V., Moreira, J.C. (2010): Effects of glyphosate and 2,4-D on earthworms (*Eisenia foetida*) in laboratory tests. Bull. Environ. Contam. Toxicol. DOI 10.1007/s00128- 010-0089-7

Kaneda, S., Okano, S., Urashima, Y., Murakami, T., Nakajima, M. (2009): Effects of herbicides, glyphosate, on density and casting activity of earthworm, *Pheretima (Amynthas) carnosus*. Japanese Journal of Soil Science and Plant Nutrition 80, 469- 476

Verrell, P., Van Buskirk, E.(2004): As the worm turns: *Eisenia fetida* avoids soil contaminated by a glyphosate-based herbicide. Bulletin of Environmental Contamination and Toxicology 72/2,

219-224. DOI 10.1007/s00128-003-9134-0

Yasmin, S., D'Souza, D.(2007): Effect of pesticides on the reproductive output of *Eisenia fetida*. Bull Environ Contam Toxicol 79/5, 529-32. DOI: 10.1007/s00128-007-9269-5

B.9.13.14 Effects on soil non-target micro-organisms

For the group of soil non-target micro-organisms, a database of 99 publications was collected by the notifier. The notifier considered 21publications to be necessary to be described in the literature review. The submitted publications were also evaluated by zRMS and have been assigned according to an UBA screening. Most of the studies submitted by the notifier dealt with the rhizobia of glyphosate-resistant crops and were therefore not assignable for ERA in the European Union. However, after screening 28 studies were recognized as informative with low weight (category UBA3), 18 publications have been considered as supportive information (UBA2) and only one publication from Cycon & Kaczynska (2004) has been classified as UBA1 (critical data, high weight of evidence in risk assessment). In this study, performed according to the OECD guidelines 216 and 217, the authors applied glyphosate at the field rate of 4.5 mg/kg of soil (PEC) as well as at a 5-fold higher concentration (22.5 mg/ kg of soil). After 1, 7, 14 and 28 days of incubation, soil respiration rates (SIR – Substrate Induced Respiration) and the amounts of nitrate did not significantly differ from control soil.

Accinelli et al. (2002)

glyphecotox_265	**ACCINELLI C., SCREPANTI C., DINELLI G., VICARI A.**	**2002**	**SHORT-TIME EFFECTS OF PURE AND FORMULATED HERBICIDES ON SOIL MICROBIAL ACTIVITY AND BIOMASS**	**Intern. J. Environ. Anal. Chem., (82): No. 8–9, pp. 519–527**
Reliability				
Purpose of the study Description of endpoints	Investigate short-time effects of glyphosate and gluphosinate-ammonium on soil microbial activity. Pure and formulated herbicides were tested. Endpoints: soil respiration & soil dehydrogenase activity (DH)			
Test compound, application procedure, exposure period, protocol	Glyphosate: Roundup® Bioflow (31% a.i. SL) Gluphosinate-ammonium: Basta (11.33% a.i. SL) 2, 20 and 200 mg a.i. g/ soil = 1X, 10X and 100X (multiple value with respect to the recommended agricultural rate) RQ: a soil layer of 1 cm was considered 20-days incubation period. Non-GLP			
Experimental approach Statistical design, test environment	Three-way ANOVA was employed to test at, each time interval, the significance of soil microbial activity and biomass C in soil samples receiving separately different application rates of the six pure and formulated herbicides with respect to the untreated soil			
Test organisms	Sandy loam: from Experimental Farm of the University of Bologna at Ozzano (Bologna, Italy), from the top 20 cm of a field with no previous pesticide history.			
Biological effects	Both pure and formulated glyphosate and glufosinate-ammonium determined a rapid and significant increase of soil respiration compared with the untreated soil.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	These results further support the absence of adverse effects of glyphosate and glufosinate-ammonium on soil microbial population (as previously reported by other authors) The paper focuses on ecosystem function and do not inform on ecosystem structure diversity
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Araujo et al. (2003)

glyphnosubm_151	**Araujo, A.S.F., Monteiro, R.T.R., Abarkeli, R.B..**	**2003**	**Effect of glyphosate on the microbial activity of two Brazilian soils**	**Chemosphere 52 (5):799-804. Doi 10.1016/S0045-6535(03)00266-2.**

Reliability	
Purpose of the study Description of endpoints	Study in vitro, changes in the microbial activity of 2 typical Brazilian soils, with and without applied glyphosate. Endpoints: soil respiration (evolution of CO2), fluorescein diacetate (FDA), plate counts of bacteria, actinomycetes and fungi
Test compound, application procedure, exposure period, protocol	Glyphosate (technical glyphosate) 2.16 mg glyphosate kg/soil 32 days No-GLP
Experimental approach Statistical design, test environment	Comparison of soils with 11 years of application of glyphosate with soils without reported history of glyphosate Soils were sampled from surface layer up to a depth of 10 cm.
Test organisms	- Microcosms - 2 types of soil (Hapludult and Hapludox Brazilian soils) with different histories of glyphosate application

Biological effects	increase of 10–15% in the CO_2 evolved and a 9–19% increase in FDA hydrolyses in the presence of glyphosate Community shift: number of actinomycetes and fungi had increased while the number of bacteria showed a slight reduction long-term effects of repeated application (six and eleven years) showed an increase in the microbial activity compared to soils with no reported application of glyphosate, showing that repeated application lead to increased microbial activity due the utilization of glyphosate as an available substrate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	-
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	glyphosate was biodegraded by soil microorganisms with the formation AMPA, and that the herbicide had positive effect on the soil microbial activity in short- and long-term. Detection of a community shift, was not discussed.
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Cycon, M., Kaczynska, A. (2004)

glyphecotox_331	**Cycon, M., Kaczynska, A.**	**2004**	**Effects of selected pesticides on soil microbial activity in nitrogen and carbon transformation**	**Pestycydy 1/2:113-120**
Reliability				
Purpose of the study Description of endpoints			Investigate the effects of selected fungicides (dithianon, procymidone), herbicides (glyphosate, Hnuron) and insecticides (lambda-cyhalothrin, diazinon) on microbial activity measured by SIR and the level of nitrification in sandy-loam soil during the 28d. Endpoints: soil microbial activity (SIR: Substrate-Induced Respiration) and nitrogen transformation	
Test compound, application procedure, exposure period, protocol			Glyphosate : 360 g dm-3 Used concentrations [mg/ kg of soil]: PEC: 4.5 and 5xPEC: 22.5 The OECD Guidelines No 216 and 217	

Experimental approach Statistical design, test environment	Soil was collected from the top 20 cm layer from an agricultural plot in Pszczyna, South of Poland ANOVA + Turkey HSD (post hoc comparison)	
Test organisms	Refer to paper	
Biological effects	Application of above-mentioned pesticides at their recommended field rates did not have any effect on soil microbial activity and nitrogen transformation	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?		yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?		yes
Concluding weight of evidence	Application of glyphosate at recommended field rates did not have negatively effect on soil microbial activity measured by SIR and nitrogen transformation 28 days treatment. However, it is impossible to draw a general conclusion regarding the effect of glyphosate on soil microorganisms because a number of factors influence on the activity of this agrochemical in soil ecosystem, therefore estimation of two parameters only may be not adequate in some situations	
Type of info. (Critical, supporting, low weight)	Critical data, high weight of evidence in RA	
Consideration/concluding score	UBA1	

Gomez et al. (2009)

glyphecotox_391	**Gomez, E., Ferreras, L., Lovotti, L., Fernandez, E.**	**2009**	**Impact of glyphosate application on microbial biomass and metabolic activity in a Vertic Argiudoll from Argentina**	**European Journal of Soil Biology 45 (2):163-167**
Reliability				
Purpose of the study Description of endpoints	To evaluate the effect of increasing doses of glyphosate on biomass, metabolic activity and metabolic quotient of soil microbiota under controlled conditions in a soil with a long history of glyphosate. Endpoints: carbon from microbial biomass (C-MB), microbial respiration rate (MR), metabolic quotient (qCO2), and dehydrogenase activity (DA) at day 4 and day 45			
Test compound, application procedure, exposure period, protocol	Commercial formulation of glyphosate (48%) 0.48, 0.96, 1.92 and 3.84 L a.i ha-1 Analysis of repeated measures; Means comparisons Duncan test			
Experimental approach Statistical design, test environment	25°C and 75% of water holding capacity.			
Test organisms	Vertic Argiudoll (Argentina)			

Biological effects	C-MB: significantly lower in the highest doses at day 4 and 45 MR: significant differences over the time but not between doses qCO2: significant differences between doses after both 4d and 45d DA: significantly higher in the treatments with glyphosate at day 4.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Refer to paper
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Refer to paper
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Refer to paper
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Refer to paper
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	The results of this study demonstrate an initial inhibitory effect that affected the microbial cells, which showed to be temporary, indicating that no harmful effects should be expected in the short-term when glyphosate is applied at doses equivalent or higher than those usually applied in the field.
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Haney et al. (2002)

glyphecotox_400	**Haney, R.L., Senseman, S.A., Hons, F.M.**	**2002**	**Effect of Roundup® ultra on microbial activity and biomass from selected soils**	**Journal of Environmental Quality 31 (3):730-735**

Reliability	
Purpose of the study Description of endpoints	To determine the effect of Roundup® Ultra on soil microbial biomass and activity Endpoints: C- and N-mineralization and soil microbial biomass
Test compound, application procedure, exposure period, protocol	Roundup® Ultra [Monsanto, St. Louis, MO]; (480 g a.i. L-1) 234 mg active ingredient kg-1 soil based on an assumed 2-mm glyphosate–soil interaction depth
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Nine soils from Georgia and Texas were used Variation in pH, soil organic C, clay content
Biological effects	Cumulative C- mineralization and-mineralization increased for all treatments with RU Strong linear relationships between C & N mineralized (slope= 3) Glyphosate C to N ratio of 3:1 => strongly suggest that RU was the direct cause of the enhanced microbial activity
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes

3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Roundup® Ultra appeared to be rapidly degraded by soil microbes regardless of soil type or organic matter content, and increased their population and activity even at high application rates, without adversely affecting microbial activity
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Haney et al. (2002)

glyphecotox_401	**Haney, R.L., Senseman, S.A., Krutz, L.J., Hons, F.M.**	**2002**	**Soil carbon and nitrogen mineralization as affected by atrazine and glyphosate**	**Biology and Fertility of Soils 35 (1):35-40**

Reliability	
Purpose of the study Description of endpoints	Atrazine alone and atrazine plus glyphosate were added to soil to determine their effect on soil microbial activity Endpoints: C and N mineralization (Cmin, Nmin)
Test compound, application procedure, exposure period, protocol	Roundup® Ultra (480 g active ingredient l–1) + Atrazine (1/2 w/w) 2× (188 mg kg^{-1}), 4× (376 mg kg^{-1}) and 6× (564 mg kg^{-1}) assuming a 2-mm soil penetration depth for glyphosate 56 days of incubation
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Weswood silt loam
Biological effects	Atrazine plus glyphosate stimulated microbial activity more than atrazine alone The addition of glyphosate with atrazine significantly increased C mineralization in all treatments compared with atrazine alone
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Refer to paper

Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Hart, M.M et al. (2009)

glyphecotox_404	**Hart et al.**	**2009**	**Separating the effect of crop from herbicide on soil microbial communities in glyphosate-resistant corn**	**Pedobiologia 52 (4):253-262**
Reliability				
Purpose of the study Description of endpoints			To examine the effect of both the transgenic corn and the use of glyphosate on two groups of rhizosphere microbes, denitrifying bacteria and fungi. Endpoints: qPCR, t-RFLP based on DNA	
Test compound, application procedure, exposure period, protocol			Roundup®(1.8kgha-1 atrazine) conventional herbicides : isoxaflutone + atrazine (79 + 800 g aiha-1)	
Experimental approach Statistical design, test environment			Fully factorial, field study where the effects of crop type and herbicide treatment on microbe numbers and diversity were separated. Measurement of the numbers and community composition of two soil rhizosphere microbes to determine if their communities were affected by: (1) glyphosate-resistant corn versus conventional corn and (2) glyphosate vs conventional herbicides (isoxaflutole & atrazine).	
Test organisms			Experimental field located at the Elora Research Station of the University of Guelp (Canada) Conostogo silt loam soil	
Biological effects			we found neither crop type (transgenic or conventional) nor herbicide (glyphosate or conventional) affected rhizosphere denitrifying or fungal communities. results showed that seasonality was a significant determinant of denitrifier and fungal abundance	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species, life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?			na	
Concluding weight of evidence			Neither GR corn nor glyphosate had significant impacts on the denitrifying bacteria and fungi in this study	
Type of info. (Critical, supporting, low weight)			Supporting information	
Consideration/concluding score			**UBA2/3**	

Kyaw, K.M., Toyota, K. (2007)

glyphecotox_446	Kyaw, K.M., Toyota, K.	2007	Suppression of nitrous oxide production by the herbicides glyphosate and propanil in soils supplied with organic matter	Soil Science and Plant Nutrition 53 (4):441-447

Reliability		
Purpose of the study Description of endpoints	Investigate the impact of two herbicides, a commercial formulation of glyphosate (Roundup®) and propanil (DCPA), on nitrous oxide (N2O) production and soil respiration in two different soils (Tyatkone and Miura) amended with rice straw and chitine Endpoints: N2O production rates	
Test compound, application procedure, exposure period, protocol	Roundup® (41% a.i., 59% water and surfactant, Nissan Chemical, Tokyo, Japan) Application: 2 L a.i. ha^{-1}, 10 cm soil layer 6-week incubation	
Experimental approach Statistical design, test environment	Refer to paper	
Test organisms	Refer to paper	
Biological effects	Application of glyphosate AND propanil: Suppress cumulative N2O production in both types of amended soils Decrease N_2O production in rice straw amended soil (< 25%)	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?		
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?		
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no	
Concluding weight of evidence	herbicides used in this study had no severely adverse effects on the overall soil microbial community.	
Type of info. (Critical, supporting, low weight)	**low weight in RA, not assignable**	
Consideration/concluding score	**UBA3**	

Lupwayi, N.Z., et. al (2004)

glyphecotox_467	Lupwayi et. al	2004	Soil microbial biomass and diversity after herbicide application	Canadian Journal of Plant Science 84 (2):677-685

Reliability	
Purpose of the study Description of endpoints	Greenhouse and field experiments were conducted to investigate the effects of herbicides on soil microbial biomass, bacterial diversity and community structure Endpoints: Microbial biomass: microbial C Bacterial diversity: Biolog method Community structure: specific patterns of substrate utilization by bacteria (CLPP) => Shannon index, Evenness
Test compound, application procedure, exposure period, protocol	Glyphosate IPA (900 g a.i. /ha) Glufosinate ammonium (500 g a.i. /ha) 0, 1, 2, 3 and 4 wk after treatment
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Gray Luvisolic soil
Biological effects	Microbial C increased/Shannon index was lower In all experiments, examination of microbial community structure revealed herbicide-induced shifts in microbial composition even when diversity indices among treatments were not different
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no
Concluding weight of evidence	Herbicides applied once at recommended rates did not have significant or consistent effects on microbial C or diversity indices However, shifts in microbial community structures were sometimes evident where microbial C and diversity were not different. Such shifts can lead to successions in the microbial communities that could have longterm effects on soil biological processes. Therefore, it is important to incorporate measures of microbial diversity and composition in herbicide ERA studies.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Malkomes, H.-P. (2007)

glyphecotox_481	Malkomes, H.-P.	2007	Influence of differently formulated glyphosate herbicides and a herbicidal reference compound on microbial activities in soil	Nachrichtenbl. Deut. Pflanzenschutzd. 59 (6):124-132
Reliability				
Purpose of the study Description of endpoints			Investigate under laboratory conditions the effects of differently formulated glyphosate herbicides on biomass-related microbial activities and carbon and nitrogen mineralization in two soils with and without lucerne meal amendment. Endpoints: dehydrogenase, substrate-induced short-term respiration	
Test compound, application procedure, exposure period, protocol			Basamid Granulat: 0,24 g/kg Dazomet: 0,23 g/kg Herbogil Liquide 7,86 µl/kg Dinoterb : 1,96 mg/kg Roundup® Ultra:6,67 µl/kg Roundup® Ultragran 4 kg/ha Touchdown 7,28 µl/kg Glyphosat (Isopropylamin-Salz) 2,4 mg/kg Glyphosat (Na-Salz) 2,4 mg/kg Glyphosat (-Trimesium) 2,4 mg/kg	
Experimental approach Statistical design, test environment			Refer to paper	
Test organisms				
Biological effects			The various glyphosate treatments (formulation, dosage) only sometimes had small effects on the endpoints. Only the nitrogen mineralization was increased for some time by the higher dosage whereas the relation of carbon to nitrogen mineralization was diminished. Sodium and isopropylamine salts of glyphosate sometimes acted little stronger than the trimesium compound When the soil was stressed by a preceding fumigation no further additional effects occurred by glyphosate.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				no
Concluding weight of evidence			Independently from the tested formulations the overall effects of field-related dosages of glyphosate induced only relatively small effects on the investigated microbial activities in the soil.	
Type of info. (Critical, supporting, low weight)			**Supporting information**	
Consideration/concluding score			**UBA2**	

Mijangos, I., et al. (2009)

glyphecotox_508	**Mijangos, et al.**	**2009**	**Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies**	**Soil Biology & Biochemistry 41 (3):505-513**
Reliability				
Purpose of the study Description of endpoints			study the short-term effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions (triticale versus a mixture of triticale and pea) by cultivation-dependent (Biolog Ecoplates_) and –independent (PCR-DGGE) methodologies Endpoints: potentially mineralizable nitrogen, ammonium content, community-level physiological profiles using Biolog EcoplatesTM, DNA microbial biomass and genotype diversity by means of PCR-DGGE	
Test compound, application procedure, exposure period, protocol			Roundup® Plus 15 and 30 days	
Experimental approach Statistical design, test environment			factorial treatments that included two different compositions of forage plant species (triticale versus a mixture of triticale and pea) and two concentrations of glyphosate (50 and 500 mg active ingredient kg-1 soil, as a commercial formulation, Roundup® Plus)	
Test organisms			pot study carried out with soil collected from the top layer (0–30 cm) of natural grassland located in Derio (Basque Country, northern Spain)	
Biological effects			15 days: stimulation of the activity and functional diversity (glyphosate acting as an available source of C, N and P.) 30 days: inconsistent response to glyphosate addition Shift in the carbon utilization pattern as a result of herbicide treatment, which again suggests a non-target effect of glyphosate on the rhizosphere soil microbial community BiologTM was more sensitive than PCR-DGGE to detect changes in soil microbial communities	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				
3 Is the ecotoxicological manifestation level appropriate for the assessment?				
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?			no	

Concluding weight of evidence	Glyphosate was quickly used by soil microorganisms as a source of nutrients which resulted in a stimulation of the activity and functional diversity of the cultivable portion of the heterotrophic soil microbial community. effects on the rhizosphere soil microbial community which were, interestingly, more enhanced in triticale than in ''triticaleþ pea'' pots. Biolog was more sensitive to detect changes in soil microbial communities induced by glyphosate and plant composition than PCR-DGGE.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Ratcliff et al. (2006)

glyphecotox_560	**Ratcliff, A.W., Busse, M.D., Shestak, C.J.**	**2006**	**Changes in microbial community structure following herbicide (glyphosate) additions to forest soils**	**Applied Soil Ecology 34 (2-3):114-124**
Reliability				
Purpose of the study Description of endpoints			To examine changes in community structure by PLFA and C utilization analyses, supported by a coarse-level comparison of bacteria and fungi by epifluorescent microscopy and traditional culturing techniques. Our objective was to determine whether glyphosate results in short-term changes, either deleterious or beneficial, in forest soil microbial communities Endpoints: Total and culturable bacteria, fungal hyphal length, bacterial:fungal biomass, carbon utilization profiles (BIOLOG), bacterial and fungal phospholipid fatty acids (PLFA)	
Test compound, application procedure, exposure period, protocol			Roundup® Field rate of 5 kg a.i. ha_1 and 100x field rate 1, 3, 7 and 30 days	
Experimental approach Statistical design, test environment			Factorial treatments including 3 levels of glyphosate (0, 50, and 5000 mg a. i./kg soil) and four sampling dates (1, 3, 7, and 30d)	
Test organisms			Clay loam and a sandy loam forest soil (0 to 15 cm depth from two ponderosa pine) plantations in northern California	
Biological effects			Endpoints not affected at field rate application High concentration of glyphosate (100x field rate) altered the bacterial community in both soils: Increase of generalist bacteria Community shifted from fungal dominance to equal ratio	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				

Concluding weight of evidence	No major changes in microbial community structure assessed by C utilization, PLFA, and standard cultural and microscope methods were detected in forest soils following the addition of the recommended field-rate concentration of glyphosate commercial formulation of glyphosate has a benign affect on community structure when applied at the recommended field rate, and produces a non- specific, short-term stimulation of bacteria at a high concentration.
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Zabaloy et al. (2008)

glyphecotox_663	**Zabaloy, M.C., Garland, J.L., Gomez, M.A.**	**2008**	**An integrated approach, to evaluate the impacts of the herbicides glyphosate, 2,4-D and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina**	**Applied Soil Ecology 40 (1):1-12.**
Reliability				
Purpose of the study Description of endpoints			Investigate the impact of postemergence herbicides on soil microbial communities Endpoints: - culturable aerobic heterotrophic bacterial (AHB) density - substrate-induced respiration (SIR) - dehydrogenase activity (DHA) - fluorescein diacetate (FDA) hydrolysis - functional richness (biolog)	
Test compound, application procedure, exposure period, protocol			glyphosate (N-(phosphonomethyl)glycine), soluble concentrate (48% a.i.) Application: 10X recommended field rate: 150 mg a.i. kg-1 Incubation: 3 weeks	
Experimental approach Statistical design, test environment			Refer to paper	
Test organisms			Typic Argiudoll, Typic Haplustoll and Petrocalcic Paleustoll (Argentina)	
Biological effects			(1) early stimulation of SIR and AHB; (2) dissimilar response in the soils for FDA and DHA (3) transient increase in functional richness.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				no

Concluding weight of evidence	The addition of these herbicides at a dose 10 times higher than the normal field application rates caused minor changes to soil microbial activity, bacterial density and functional richness. The specific changes varied among herbicides, with the effects of glyphosate most pronounced.
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Lancaster et al. (2006)

glyphecotox_449	**Lancaster, S.H., Haney, R.L., Senseman, S.A., Hons, F.M., Chandler, J.M.**	**2006**	**Soil microbial activity is affected by Roundup® WeatherMax and pesticides applied to cotton (Gossypium hirsutum)**	**J Agric Food Chem 54 (19):7221-6**

Reliability	
Purpose of the study Description of endpoints	Evaluate the influence of glyphosate-based cotton pest management systems on soil microbial activity. Endpoints: C and N mineralization Soil microbial biomass (chloroform-fumigation-incubation method)
Test compound, application procedure, exposure period, protocol	Roundup®: WeatherMAX, Monsanto Co., St. Louis, MO Application rate: 152.7 µg a.i./kg soil trifluralin, aldicarb, and mefenoxam + pentachloronitrobenzene with or without glyphosate (applied as Roundup® WeatherMax). 1 control and 1 treatment with only Roundup® WeatherMax
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Weswood clay loam collected from a bermuda grass pasture and a fallow field previously planted with cotton
Biological effects	Soils treated with glyphosate alone exhibited greater cumulative C mineralization 30 days after treatment than all other treatments The addition of Roundup® WeatherMax reduced C mineralization in soils treated with fluometuron, aldicarb, or mefenoxam + PCNB formulations. These results indicate that glyphosatebased herbicides alter the soil microbial response to other pesticides
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The tested formulation is likely to content POEA as surfactant. This causes limited validity regarding effects of Glyphosate that does not contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	no

Concluding weight of evidence	For all variables measured, pasture soil exhibited greater microbial activity and biomass than cultivated soil. Cumulative C mineralization after 30 days was greater in soils treated only with glyphosate as compared to all other treatments. Nitrogen mineralization was greater in soils that had been treated with applications that included glyphosate as compared with soils that were not treated with glyphosate. Soil microbial biomass C increased relative to non-treated soils when glyphosate was applied alone Soil microbial biomass N was not affected soil microbial biomass measurements using the fumigation-incubation method are less sensitive than C and N mineralization measurements for detecting the influence of microbial activity
Type of info. (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Bennicelli et al. (2009)

glyphecotox_294	**Bennicelli, R.P., Szafranek-Nakonieczna, A., Wolinska, A., Stepniewska, Z., Bogudzinska, M.**	**2009**	**Influence of pesticide (glyphosate) on dehydrogenase activity, pH, Eh and gases production in soil (laboratory conditions)**	**International Agrophysics 23 (2):117-122**

Reliability	
Purpose of the study Description of endpoints	Determinate dehydrogenase activity (DHA) and soil gases (CO_2, N_2O) emission in soils enriched with glyphosate (1 µg and 10 µg g-1 of pesticide doses) during time (42 days), under lab conditions at 20°C. Endpoints: dehydrogenase activity (DHA)
Test compound, application procedure, exposure period, protocol	Glyphosate (Product ?) 0, 1 and 10 µg g-1 of soil
Experimental approach Statistical design, test environment	Refer to paper
Test organisms	Mollic Gleysols, Eutric Fluvisols and Terric Histosols taken from surface layer (0-20 cm)
Biological effects	The decrease of DHA activity was observed that depended on the pesticide dose Increase of the N2O concentration with growth of pesticide dose
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	

Concluding weight of evidence	1. Glyphosate caused an inhibition of DHA activity in all investigated soils up to 21st day. 2. CO2 formation increased in the case of Terric Histosols and Eutric Fluvisols, but decreased in the case of Mollic Gleysols. 3. Glyphosate caused an increase of N2O concentration in all investigated soils. 4. Eh, pH and CO2 concentration had high correlations with DHA activity.
Type of info. (Critical, supporting, low weight)	Supporting information
Consideration/concluding score	**UBA2**

Lancaster et al. (2010)

glyphecotox_450	**Lancaster, S.H., Hollister, E.B., Senseman, S.A., Gentry, T.J.**	**2010**	**Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate**	**Pest Manag Sci 66 (1):59-64**
Reliability				
Purpose of the study Description of endpoints	study the effect of one, two, three, four or five applications of glyphosate on soil microbial community composition and glyphosate mineralization and distribution of 14C residues in soil. Endpoints: - fatty acidmethyl esters - sequencing of 16S rRNA bacterial genes - cumulative percentage 14Cmineralized - Incorporation of 14C residues into soil microbial biomass			
Test compound, application procedure, exposure period, protocol	Glyphosate-isopropylammonium 480 g AE L^{-1} SL (Roundup® WeatherMAX; Monsanto Company, St Louis, MO) Applied in 3 mL solution (giving 33% v/v water content) at a rate of 49 µg AE g−1 soil to the soil surface			
Experimental approach Statistical design, test environment	At 2, 4, 6 and 8 weeks after the initial glyphosate applications, an additional 49 µg AE g−1 soil was added in 0.5 mL solution, to create soil samples that received one, two, three, four or five applications of glyphosate. Each treatment was replicated 4 times Endpoints measured 3, 7 and 14 days after the final glyphosate application to each treatment (DAA).			
Test organisms	Weswood silt loam with no record of glyphosate application during previous 2 years			
Biological effects	- Increase of gram-negative bacteria FAMES - Increase of the abundance of the gram-negative Burkholderia ssp sequences - Decrease of the cumulative percentage 14C mineralized 14 DAA when glyphosate was applied 4 or 5 times - Incorporation of 14C residues into soil microbial biomass was greater following five glyphosate applications than following the first application 3 and 7 DAA			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	yes			
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Refer to paper			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes			

Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?		Refer to paper
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?		yes
Concluding weight of evidence	Changes in the dissipation or distribution of glyphosate following repeated applications of glyphosate may be related to shifts in the soil microbial community composition	
Type of info. (Critical, supporting, low weight)	**Supporting information**	
Consideration/concluding score	**UBA2**	

Ruzkova et al. (2011)

glyphecotox_583	**Ruzkova, M., Ruzek, L., Vorisek, K., Vrablik, P., Musilova, D.**	**2011**	**Microbiological characterization of land set-aside before and after Roundup® desiccation**	**Plant Soil and Environment 57 (2):88-94**
Reliability				
Purpose of the study Description of endpoints			To describe the changes in the biological parameters under different soil management (chemical vs biological). Endpoints: microbial biomass, available organic carbon, basal respiration, metabolic quotient, biomass-specific available organic carbon, arylsulfatase activity, soil organic matter carbon and total nitrogen	
Test compound, application procedure, exposure period, protocol			Roundup® Biaktiv (5 l/ha)	
Experimental approach Statistical design, test environment			Refer to paper	
Test organisms			loamy luvic chernozem developed on carbonate loess with a 200 mm thick layer of arable top-soil. Formerly used in arable system until 1995, then changed into a land set-aside	
Biological effects			Repeated Roundup® desiccation caused a strong (highly significant) decrease of arylsulfatase activity (–28%), however highly significant increase of microbial biomass (+69%) and nitrate-nitrogen ratio (+86%) (=>decreased immobilization nitrates by the plants!!)	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				no
Concluding weight of evidence			Refer to paper	
Type of info. (Critical, supporting, low weight)			**Supporting information**	
Consideration/concluding score			**UBA2**	

Widenfalk et al. (2008)

glyphecotox_649	**Widenfalk, A., Bertilsson, S., Sundh, I., Goedkoop, W.**	**2008**	**Effects of pesticides on community composition and activity of sediment microbes - responses at various levels of microbial community organization**	**Environmental Pollution 152 (3):576-584**
Reliability				
Purpose of the study Description of endpoints	To assess whether sediment microbes were affected by exposure to the pesticides captan, glyphosate, isoproturon and pirimicarb at environmentally relevant and high pesticide concentrations, at both community and subcommunity (''species'') levels Endpoints: community-level: bacterial activity, fungal and total microbial biomass sub-community level: PLFA, 16S rRNA genotyping, T-RFLP			
Test compound, application procedure, exposure period, protocol	Glyphosate N-(phosphono-methyl)-glycine 150 and 150.000 µg/kg dw			
Experimental approach Statistical design, test environment	microcosms			
Test organisms	sediment from lake Erken, Sweden (relatively unaffected by agricultural activities)			
Biological effects	- Community-level: endpoints were not affected by pesticide exposure (bacterial activity was quantified too late? bacterial activity usually shows an almost instantaneous response to pesticide exposure…) - Sub-community level: significant shifts in bacterial community composition (as T-RFLP) at environmentally relevant concentrations => certain groups of bacteria were stimulated at low exposure concentrations?			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				yes
Concluding weight of evidence		The study showed that community-level end points failed to detect these changes, underpinning the need for application of molecular techniques in aquatic ecotoxicology.		
Type of info. (Critical, supporting, low weight)		**Supporting information**		
Consideration/concluding score		**UBA2**		

Liphadzi, K.B., et al. (2005)

glyphecotox_461	**Liphadzi, K.B., et al.**	**2005**	**Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosate-resistant cropping system**	**Weed Science 53 (4):536-545**

Reliability		
Purpose of the study Description of endpoints	Determine the response of soil microbial and nematode communities to different herbicides and tillage practices under a glyphosate-resistant cropping system. Endpoints: - soil microbial biomass (SMB) carbon determination - substrate-induced respiration (SIR) - BIOLOG substrate utilization - nematode populations	
Test compound, application procedure, exposure period, protocol	Glyphosate: ? Application rate: 1.12 kg ai ha-1, when weeds were 10-20 cm tall All glyphosate treatments received a second glyphosate application approximately 2 wk after the first application	
Experimental approach Statistical design, test environment	Conventional herbicides: - tank mixture of cloransulam plus S-metolachlor plus sulfentrazone for soybean - commercially available mixture of acetochlor and atrazine	
Test organisms	Report to paper	
Biological effects	- SMB carbon, SIR, and BIOLOG substrate utilization were not altered by glyphosate Nematode community response to the glyphosate treatment was similar under both conventional tillage and no-till environments. Total nematode densities were similar with the glyphosate and conventional herbicide treatments	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?		
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?		no
Concluding weight of evidence	soil health when glyphosate was applied in a glyphosate-resistant cropping system was similar to that of cropping systems that used conventional herbicides.	
Type of info. (Critical, supporting, low weight)	**Supporting information**	
Consideration/concluding score	**UBA2**	

Busse et al. (2001)

glyphnosubm_155	**Busse, M.D., A.W. Ratcliff, C.J. Shestak, and R.F. Powers**	**2001**	**Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities.**	**Soil Biology and Biochemistry 33:1777-1789.**
Reliability				
Purpose of the study Description of endpoints			Assess direct and indirect effect of glyphosate on soil microbial communities from pine plantation. Endpoints: Lab: soil bioassay at high concentrations Field: microbial biomass, respiration, metabolic diversity	
Test compound, application procedure, exposure period, protocol			Report to paper	
Experimental approach Statistical design, test environment			Refer to paper	
Test organisms			3 types of soil: clay, Fe, Al oxide content (northern California)	
Biological effects			Microbial respiration was unchanged at expected field concentrations and stimulated at conc. 100-fold greater	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?				yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?				
3 Is the ecotoxicological manifestation level appropriate for the assessment?				yes
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?				
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?				no
Concluding weight of evidence			Long-term, repeated applications had minimal effects on seasonal microbial characteristics, which was more a function of time of year and site. Tests in artificial media are of limited relevance for glyphosate Field rate applications of glyphosate should have little or no effect on soil microbial communities in ponderosa pine plantations.	
Type of info. (Critical, supporting, low weight)			**Supporting information**	
Consideration/concluding score			**UBA2**	

B.9.13 14.1 Summary of the relevant literature on soil non-target micro-organisms

Soil microorganisms play a very important role in soil fertility by assuming key ecological functions like matter decomposition and nutrient cycling. Therefore, information about how agricultural practices and especially pesticides significantly affect soil microorganisms is highly required in risk assessment. However, soil microbial diversity is extremely difficult to measure because of is high complexity (Tiedje et al. 1999). In practice, the ERA of soil non- target micro-organisms is hence often restricted to the measurement of impact of pesticides on soil functional diversity (i.e. carbon and nitrogen mineralization rates, respiration rate, enzyme activities...) or bacterial and fungal biomass.

In the case of the herbicide glyphosate, only few studies failed to detect significant effect on soil functional diversity after application of the herbicide (e.g. Liphadzi, et al. 2005). Zabaloy, et al. (2008) reported that "the addition of glyphosate at a dose 10 times higher than the normal field application rates caused minor changes to soil microbial activity, bacterial density and functional richness". In rare cases, inhibitory effects have also been reported. In a land set-aside in the western part of Prague (Czech Republic), Ruzkova et al. (2011) found that repeated application of Roundup® desiccation caused a significant increase of microbial biomass (+69 %), but also strongly decreased the immobilization of nitrates by the plants (nitrate-nitrogen ratio +86 %) as well as the arylsulfatase activity (–28 %).
In some studies, differences in microbial parameters are more a function of time and site quality than pesticides doses. For example, Gomez et al. (2009) detected significant differences in microbial respiration over the time but not between doses of applied glyphosate. In Hart et al. (2009), seasonality was a significant determinant of denitrifier and fungal abundance. Parallelly, Busse et al. (2001) found that variation in microbial community size, activity and metabolic diversity was more a function of time of year and land-use that herbicide treatment.

Nevertheless, glyphosate is an organophosphonate herbicide that can be easily used as a source of P, C or N by either by gram-positive or gram-negative bacteria (van Eerd et al., 2003). Therefore, in most studies, the application of glyphosate at expected or higher field concentration rates is correlated with an immediate and significant increase in soil respiration (Accinelli et al., 2002), microbial biomass (Lupwayi, N.Z., et al., 2004), C- and N- mineralizations (Lancaster et al., 2006; Haney et al., 2000a, 2002b). This stimulation of soil principal functional parameters is assumed to be linked to a rapid use of glyphosate as source of nutrients (Mijangos et al., 2009) usually correlated with a metabolisation of the pesticide. Araujo et al. (2003) demonstrated in two Brazilian soils a rapid biodegradation of glyphosate by soil microorganisms with the formation the metabolite AMPA, resulting in short- and long-term positive effect of the herbicide on the soil microbial activity (increase of 10–15 % in the CO2 evolved and a 9–19 % increase in FDA hydrolyses in the presence of glyphosate).

This potential use of glyphosate as a source of P, C or N by soil non-target micro-organisms is likely to induce a shift in their community structures. Ratcliff et al. (2006) detected a community shifted from fungal dominance to equal ratio with an enrichment of opportunistic cobiotrophic bacteria that use glyphosate as a nutrient and/or C source. Community shifts from bacterial to fungal dominance were also observed (Araujo et al., 2003). Lupwayi, et al. (2004) observed herbicide-induced shifts in microbial composition even when diversity indices among treatments did not differ. This study points out the importance to assess microbial diversity and composition when looking at the effects of pesticides on non-target micro-organisms. In microcosm experiments performed with sediment microbes, Widenfalk et al. (2008) focused their monitoring on various levels of microbial community organization. Community-level endpoints like bacterial activity, fungal and total microbial biomass were not affected by pesticide exposure, whereas endpoints recorded at the "sub-community level"
(e.g. Phospholipid Fatty-acid Analysis, 16S rRNA genotyping, T-RFLP) demonstrated significant shifts in bacterial community composition even at environmentally relevant concentrations. The same authors concluded that "Any shifts in community structure will, however, only have consequences on ecosystem function if the tolerant microorganisms cannot compensate for biogeochemical functions normally carried out by inhibited or eliminated microbial groups". Such community shifts coupled with a loss of function are clearly illustrated in Lancaster et al. (2006). The authors looked at how the combinations of pesticides may affect soil microbial activity differently than pesticides applied alone. They found that after 30 days, soils treated with glyphosate alone (applied as Roundup® WeatherMAX, Monsanto Co., St. Louis, MO) exhibited greater microbial biomass, cumulative C and N mineralization than all other treatments. However, the addition of "Roundup® WeatherMax" reduced C mineralization in soils treated with the pesticides fluometuron, aldicarb, or mefenoxam + pentachloronitrobenzene formulations. The authors concluded that glyphosate based herbicides might alter the soil microbial response to other pesticides.

Therefore, like stated in Lupwayi et al. (2004), community shifts could have longterm effects on soil biological processes and the relevance of microbial diversity and composition is of importance when assessing the impact pesticides on soil non-target micro-organisms.

Although the application of glyphosate seems to have no negative effects on microbial functions as they are defined at the moment in the risk assessment of soil non-target micro- organisms (C- and N-mineralisation), important communitiy shifts are observed. As stated in Lupwayi et al. (2004), these community shifts could have long-term effects on soil biological processes or impact other essential ecosystem services. Therefore, the relevance of both microbial diversity and composition is of main importance and should be included in future risk assessment looking at the impact pesticides on soil non-target micro-organisms.

References

Accinelli, C., Screpanti, C., Dinelli, G., Vicari, A.(2002): Short-time effects of pure and formulated herbicides on soil microbial activity and biomass. International Journal of Environmental Analytical Chemistry 82/8-9, 519-527

Araujo, A.S.F., Monteiro, R.T.R., Abarkeli, R.B. (2003): Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52 (5), 799-804

Busse, M.D., Ratcliff, A.W., Shestak, C.J., Powers, R.F. (2001): Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biology & Biochemistry 33 (12-13), 1777-1789

Cycon, M., Kaczynska, A. (2004): Effects of selected pesticides on soil microbial activity in nitrogen and carbon transformation. Pestycydy 1/2,113-120

Haney, R.L., Senseman, S.A., Hons, F.M. (2002a): Effect of roundup ultra on microbial activity and biomass from selected soils. Journal of Environmental Quality 31 (3), 730-735

Haney, R.L., Senseman, S.A., Krutz, L.J., Hons, F.M., Soil carbon and nitrogen mineralization as affected by atrazine and glyphosate, 2002b; Biology and Fertility of Soils 35 (1):35-40

Hart, M.M., Powell, J.R., Gulden, R.H., Dunfield, K.E., Pauls, K.P., Swanton, C.J., Klironomos, J.N., Antunes, P.M., Koch, A.M., Trevors, J.T.(2009): Separating the effect of crop from herbicide on soil microbial communities in glyphosate-resistant corn. Pedobiologia 52 (4), 253-262

Gomez, E., Ferreras, L., Lovotti, L., Fernandez, E. (2009): Impact of glyphosate application on microbial biomass and metabolic activity in a Vertic Argiudoll from Argentina. European Journal of Soil Biology 45 (2),163-167

Lancaster, S.H., Haney, R.L., Senseman, S.A., Hons, F.M., Chandler, J.M. (2006): Soil microbial activity is affected by Roundup WeatherMax and pesticides applied to cotton (*Gossypium hirsutum*). J Agric Food Chem 54 (19), 7221-6

Liphadzi, K.B., Al-Khatib, K., Bensch, C.N., Stahlman, P.W., Dille, J.A., Todd, T., Rice, C.W., Horak, M.J., Head, G. (2005): Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosate-resistant cropping system. Weed Science 53 (4), 536-545

Lupwayi, N.Z., Harker, K.N., Clayton, G.W., Turkington, T.K., Rice, W.A., O'Donovan, J.T., (2004): Soil microbial biomass and diversity after herbicide application. Canadian Journal of Plant Science 84 (2), 677-685

Mijangos, I., Becerril, J.M., Albizu, I., Epelde, L., Garbisu, C. (2009): Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies. Soil Biology & Biochemistry 41 (3), 505-513

Ratcliff, A.W., Busse, M.D., Shestak, C.J. (2006): Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Applied Soil Ecology 34 (2- 3), 114-124

Ruzkova, M., Ruzek, L., Vorisek, K., Vrablik, P., Musilova, D. (2011): Microbiological characterization of land set-aside before and after Roundup desiccation. Plant Soil and Environment 57 (2), 88-94

Tiedje J.M.; Asuming-Brempong S.; Nusslein K.; Marsh T.L.; Flynn S.J. (1999): Opening the black box of soil microbial diversity. Applied Soil Ecology, 13 (2), 109-122

Van Eerd, L.L., Hoagland, R.E., Zablotowicz, R.M., Hall, J.C (2003): Pesticide metabolism in plants and microorganisms. Weed Science 51, 472-495

Widenfalk, A., Bertilsson, S., Sundh, I., Goedkoop, W. (2008): Effects of pesticides on community composition and activity of sediment microbes - responses at various levels of microbial community organization. Environmental Pollution 152 (3), 576- 584

Zabaloy, M.C., Garland, J.L., Gomez, M.A. (2008): An integrated approach, to evaluate the impacts of the herbicides glyphosate, 2,4-D and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina. Applied Soil Ecology 40 (1), 1-12

B.9.13.15 Effects on other non-target organisms (flora and fauna)

For the group of terrestrial non-target plants (NTA), a comprehensive database of 87 peer- reviewed papers was collected by the notifier. The notifier considered one publication (Boutin et al., 2010) to be rated in category “Klimisch2” and annotated with minimal remarks, whereas the remaining were considered not acceptable for risk assessment.

The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. 27 studies were recognized as supporting information (category UBA2). Most of the cited studies were performed with formulated products than for the active ingredient alone. It is known that surfactants or additives form a significant amount of plant protection products. The function of these compounds is supposed to enhance the herbicidal activity of the active ingredient glyphosate by e.g. improving the dispersal and retention on the leaf surface or the glyphosate uptake. When considering that herbicide sensitivity among crops species of within the same crop can be extensive and that, depending on the species included in testing, conclusions regarding the phytotoxicity of any given herbicide may differ (White and Boutin 2007), it is essential for current regulatory ERA to take into account toxicity data considering the possible synergistic effects of the products in formulation in order to avoid underestimation of glyphosate containing products.

- **B.9.13 15.1 General**

Boutin et al. (2004)

glyphecotox_175	**Boutin, C., Elmegaard, N., Kjaer, C.**	**2004**	**Toxicity testing of fifteen non-crop plant species with six herbicides in a greenhouse experiment: Implications for risk assessment**	**Ecotoxicology Volume: 13 Issue: 4 Pages: 349-369**
Reliability				
Purpose of the study Description of endpoints	The objectives of this study were (1) to investigate the pattern of sensitivity of several types of plant species to six herbicides with different modes of action, and (2) to explore the feasibility of using non-crop plants commonly found in field boundaries as test species for herbicide risk assessment			
Test compound, application procedure, exposure period, protocol	Roundup® Bio (360 g/l glyphosate with 480 g glyphosate-isopropylaminsalt), Monsanto; Four dosages plus control were sprayed, 0.01, 0.1, 1 and 5 or 10 times recommended label rates for agricultural use in Canada and Denmark.			
Experimental approach, Statistical design, test environment	Greenhouse test, calculation of the EC50 the linear interpolation method for sublethal toxicity, also called the inhibition concentration approach (ICp) was used (as described in US EPA report EPA/600/4/-89-001 and 001A).			
Test organisms	Fifteen species were selected, 5 species from the Asteraceae family (daisy family), four from the Lamiaceae family (mint family), two from the Polygonaceae family (buckwheat family) and the rest from four other families.			
Biological effects	This paper presents the result of a greenhouse experiment where testing was performed with 15 non-crop plant species sprayed with 6 herbicides. EC50 values for non crop species range between 14 and 63 g a.s./ha.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Non crop species were tested			
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Authors states that the current suite of species prescribed in current guidelines will not be adequate for the protection of habitats, e.g., field margin species, in agricultural areas.			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Dry weight of aerial parts were determined as endpoint			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	All herbicides were used as formulated products thereby containing a number of surfactant compounds.			
2 Do the tested concentrations relate to predicted environmental concentrations?	See above			
3 Have parameters influencing the endpoints been considered adequately?	yes			
Concluding weight of evidence/proposed action	Study describes that field margin species may be not adequate protected and risk may be underestimated when non crop species are not tested for risk assessment.			
Type of information (Critical, supporting, low weight)	**supporting**			
Consideration/concluding score	**UBA2**			

White, A.L., Boutin, C. (2007)

glyphecotox_646	**White, A.L., Boutin, C.**	**2007**	**Herbicidal effects on nontarget vegetation: Investigating the limitations of current pesticide registration guidelines**	**Environmental Toxicology and Chemistry 26 (12):2634-2643**
Reliability				
Purpose of the study Description of endpoints			Several crops and wild plant species were grown under greenhouse conditions following standard protocol for phytotoxicity testing. Plants were sprayed with five different herbicides at the four- to six-leaf stage, and biomass was recorded at 28 d after spray.	
Test compound, application procedure, exposure period, protocol			Round-Up Original_ (Monsanto Canada, Mississauga, ON) containing 356 g ai/L glyphosate was applied. A nonionic surfactant (Agral 90_; Norac Concepts, Ottawa, ON, Canada) containing nonylphenoxy polyethyoxyethanol was added, Label rates (defined as grams of active ingredient applied per hectare) selected were	
Experimental approach, Statistical design, test environment			All species were exposed to a one-time herbicide application at the two- to six-leaf stage. At 28 d, visual observations were recorded, ANOVA	
Test organisms			10 different crop species were paired with closely related wild plant relatives found in field margin habitats in Eastern Ontario	
Biological effects			Results showed that current regulatory protocol will likely underestimate herbicide phytotoxicity if testing does not include data for the complete tank-mix formulation. The present study also showed that the range in herbicide sensitivity among cultivars of the same crop can be quite extensive and that, depending on the cultivar included in a risk assessment, conclusions regarding the phytotoxicity of any given herbicide may differ.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			two- to six-leaf stage	
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?			IC25 for *solanum lycopersicon* was determined 51 g a.s. /ha.	
3 Is the ecotoxicological manifestation level appropriate for the assessment, e.g. gene induction vs. apical endpoints like growth or reproduction?				
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			For all species for which it could be calculated, the IC25 was much lower for the formulated product than it was for the active ingredient alone, indicating that glyphosate is much less toxic to the species tested than the formulated product Round-Up Original.	
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?			Drift values would probably less than the label rate of 2,136 g ai/ha for Round-Up Original	
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?			yes	

Concluding weight of evidence/proposed action	This study extends the current interest by presenting three experiments highlighting some of the limitations to current phytotoxicity testing guidelines.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Boutin et al, (2010)

glyphecotox_173	**Boutin, C., White, A.L., Carpenter, D.**	**2010**	**Measuring variability in phytotoxicity testing using crop and wild plant species**	**Environmental Toxicology and Chemistry 29 (2):327-337. DOI: 10.1002/etc.30.**

Reliability	
Purpose of the study Description of endpoints	The study was conducted in greenhouse or growth chamber environments with plants growing individually in pots and harvested 28 d after spraying with two herbicides, glyphosate and atrazine, as formulated products.
Test compound, application procedure, exposure period, protocol	Round-Up Original1 or Vision1 (Monsanto Canada), both formulations containing 356 g/L glyphosate [N- (phosphonomethyl) glycine], 2,136 g a.i. ha_1 for glyphosate additionally Agral 901 (Norac Concepts
Experimental approach, Statistical design, test environment	At 28 d after herbicide exposure, all above-ground green plant material was harvested and placed in a forced-air dryer for a minimum of 72 h at approximately 708C for dry biomass determination, ANOVA
Test organisms	Eight different herbaceous broad-leaf species from four families and with different life spans were included in the ecotype variability experiment
Biological effects	It was shown that test conditions induced a large variability in a given species' response to herbicides. Both crops and wild plant species responded quite variably when they were tested in different seasons as well as when tested in a greenhouse or in growth chambers.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	IC25 values are stated, no EC 50 values
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	The HD calculated using species sensitivity distributions with the ecotype experiment data revealed that a factor of two generally separated the least sensitive and the most sensitive ecotypes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	Application rate is supposed to be above the predicted drift exposure
3 Have parameters influencing the endpoints been considered ?	Different prevailing conditions were discussed

Concluding weight of evidence/proposed action	The present study supports the inclusion of an uncertainty factor in risk assessments to account for the intrinsic variability in plant sensitivity to herbicides.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Dalton, R.L., Boutin, C. (2010)

glyphecotox_333	**Dalton, R.L., Boutin, C.**	**2010**	**Comparison of the effects of glyphosate and atrazine herbicides on nontarget plants grown singly and in microcosms**	**Environ Toxicol Chem 29 (10):2304-15. DOI: 10.1002/etc.277.**

Reliability	
Purpose of the study Description of endpoints	Objective of the present study was to compare the response of terrestrial and wetland plants to the herbicides glyphosate and atrazine when grown singly in pots versus under different microcosm conditions.
Test compound, application procedure, exposure period, protocol	Roundup® Original contains 356 g/L of the active ingredient glyphosate (N-(phosphonomethyl)glycine) in the form of its isopropylamine salt. The surfactant Agral1 90 (Syngenta Crop Protection), containing nonylphenoxy polyethoxy ethanol, was added to Roundup® Original solutions to give a concentration of 0.5% (v/v) as recommended on the product label, Application: 2136 g ai/ha for glyphosate.
Experimental approach, Statistical design, test environment	Greenhouse microcosm experiments were conducted for both a standard test period (28 d) and a longer test period (60 or 70 d).
Test organisms	Nine terrestrial and seven wetland plant species common to agroecosystems of Eastern Ontario and Western Quebec were selected.
Biological effects	Greenhouse microcosms were generally more sensitive than single-species tests. Plants grown for an extended test period or in seminatural field conditions were generally less sensitive to herbicides. Sensitivity was found to be dependent on interactions between species and test conditions. Changes in community structure were observed in herbicide-treated microcosms that would not be predicted from single-species testing.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment,	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations	Corresponding to In field application rate, not representing drift rate.
3 Have parameters influencing the endpoints been considered?	yes

Concluding weight of evidence/proposed action	Authors state that Single-species tests are useful because they are inexpensive, can demonstrate clear dose–response patterns uncomplicated by other factors influencing growth, and are able to provide a measure of the sensitivity of a given species to glyphosate and atrazine. However, they are unable to predict subtle changes in community structure that may have important long-term consequences.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Martin, M.L., Ronco, A.E. (2006)

glyphecotox_489	**Martin, M.L., Ronco, A.E.**	**2006**	**Effect of mixtures of pesticides used in the direct seeding technique on nontarget plant seeds**	**Bull Environ Contam Toxicol 77 (2):228-36. DOI: 10.1007/s00128-006-1054-3.**

Reliability	
Purpose of the study Description of endpoints	Assessment of effects on germination and root elongation of seeds exposed to Roundup® Max formulation of glyphosate hernbicide.
Test compound, application procedure, exposure period, protocol	Roundup® Max (74.4% glyphosate)
Experimental approach Statistical design,test environment	Germination test with 2,5 to 2500 mg/L, assessment points were seed germination and seedling root elongation , regression analysis
Test organisms	*Lactuca sativa, Brassica napus, allium cepa, medicago sativa, Lolium perenne*
Biological effects	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance	Yes, considering that the first days of seedling groth are often the most sensitive stage of plant development.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	IC50 values are given : L.sativa 9.89 as mg/l, L.perenne 15.31 mg/L, M.sativa: 56.31 mg/L, A.cepa: 131.8 mg/L, B.napus 1164.31 mg/L
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	No effect on seed germination were observed with any concentration for any tested species.
Concluding weight of evidence	
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Olszyk et al. (2004)

glyphecotox_529	**Olszyk, D.M., Burdick, C.A., Pfleeger, T.G., Lee, E.H., Watrud, L.S.**	**2004**	**Assessing the risk to non-target plants from herbicides**	**J Agric Meteorol 60 (4):221-242**

Reliability		
Purpose of the study Description of endpoints	Paper addresses current trends in general ERA of plants, herbicide use in general, problems of formulations etc. in US.	
Test compound, application procedure, exposure period, protocol	no endpoints, 10 years old	
Experimental approach, Statistical design, test environment	no	
Test organisms	no	
Biological effects	nd	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		no
2 Is the magnitude of effects of biological significance?		no
3 Is the ecotoxicological manifestation level appropriate for the assessment?		Review describes uncertainties s of phytotoxicity testing and gives recommendations for improvement.
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		no
2 Do the tested concentrations relate to predicted environmental?		no
3 Have parameters influencing the endpoints been?		no
Concluding weight of evidence/proposed action	Not relevant in terms of risk assessment, but review indicates limitations of ERA in general.	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Wagner et al. (2003)

glyphecotox_642	**Wagner, R., Kogan, M., Parada, A.M.**	**2003**	**Phytotoxic activity of root absorbed glyphosate in corn seedlings (Zea mays L.)**	**Weed Biology and Management 3:228-232**

Reliability		
Purpose of the study Description of endpoints	The purpose of the present study was to determine the relationship between the amount of glyphosate absorbed from roots, avoiding interaction of the herbicide with any substrate and its effect on plant growth. Also, the effects of glyphosate concentration and plant transpiration on herbicide's uptake were assessed.The treated plants presented a normal pattern of glyphosate allocation, with the apex the principal sink, accumulating more than 38% of mobilized glyphosate. When corn plants absorbed more than 0.6 mg they showed a decrease in growth. The relatively high glyphosate quantities allocated in the new leaves showed the relevance of the symplastic pathway in the translocation process for root absorbed glyphosate.	
Test compound, application procedure, exposure period, protocol	Commercial herbicide solution of glyphosate isopropylamine salt (0.36 kg ae L - 1) with [phosphonomethyl14C]-glyphosate (specific activity 1·0 GBq mmol-, determined to be98.5% pure by HPLC, International Isotope München)to obtain 2% of the radiolabeled glyphosate in a100 mg kg-1total glyphosate solution.Growth chamber experiments were conducted in order to study the absorption, translocationand activity of glyphosate when applied to roots with aqueous solution avoiding any glyphosate–substrate interaction	
Experimental approach, Statistics, test environment		
Test organisms	*Zea mays*	
Biological effects	A linear relationship was found between glyphosatesolution concentration and glyphosate uptake over therange of 2–30 mg L–1	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		nd
2 Is the magnitude of effects of biological significance?		Small amounts of glyphosate absorbed by corn root stimulates its growth; however, a very low increase in these amounts starts to produce phytotoxic effects.
3 Is the ecotoxicological manifestation level appropriate for the assessment?		no
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered?		nd
Concluding weight of evidence	Authors expect that if there is glyphosate available in the soil solution it could be absorbed from and root crop damagecould occur. Non target plant might therefore be exposed not only vial drift , but also via the soil.	
Type of info. (Critical, supporting, low weight)	**supporting**	

Consideration/concluding score	**UBA2**

Wibawa et al. (2009)

glyphecotox_647	**Wibawa, W., Bin Mohamad, R., Bin Puteh, A., Omar, D., Juraimi, A.S., Abdullah, S.A.**	**2009**	**Residual Phytotoxicity Effects of Paraquat, Glyphosate and Glufosinate-Ammonium Herbicides in Soils from Field-Treated Plots**	**International Journal of Agriculture and Biology 11 (2):214-216**

Reliability		
Purpose of the study Description of endpoints	Soil residual phytotoxicity of commonly used herbicides in plantation crops in Malaysia were investigated through bioassay	
Test compound, application procedure, exposure period, protocol	Roundup®R (360 g L-1glyphosate Monsanto)	
Experimental approach, Statistics, test environment	glyphosate (Round-upR)at 400, 800, 1200 and 1600 g a.i. ha-1 were applied to field plots of 5 x 20 m2.	
Test organisms		
Biological effects	Glyphosate, when applied to the field in Malaysia at rates with ranges inclusive of their field recommended rates did not leave residues in the soil, which may cause phytotoxic effect to the indicator plants, corn and cucumber	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		nd
2 Is the magnitude of effects of biological significance?		nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?		nd
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		nd
2 Do the tested concentrations relate to predicted environmental concentrations?		nd
3 Have parameters influencing the endpoints been considered?		nd
Concluding weight of evidence	Field experiment in Malaysia, environmental conditions not comparable.	
Type of info. (Critical, supporting, low weight)	**low weight**	
Consideration/concluding score	**UBA3**	

- **B.9.13 15.2 Ecological side effects (KIIA 8.16)**

Neumann et al. (2006)

	G. Neumann, S. KohlsE. Landsberg, K. Stock-Oliveria Souza, V. Römheld,	**2006**	**Relevance of glyphosate transfer to non-target plants via the rhizosphere**	**Zeitschrift für Pflanzenkrankheiten un d Pflanzenschutz, Sonderheft, ISSN 0938-9938**
Reliability				
Purpose of the study Description of endpoints			In nutrient solution, rhizobox and pot experiments authors show that foliar applied glyphosate to target plants is released into the rhizosphere after a fast translocation from shoots to roots.	
Test compound, application procedure, exposure period, protocol			Roundup®-Ultra (Monsanto, St. Louis, USA) was diluted as recommended by the manufacturer (1 l/200 l-1 deionized water) to obtain a glyphosate concentration of 28.4 mM. In the nutrient solution experiment, glyphosate was applied with 0 %, 5 %, 50 % and 100 % (v/v) of the recommended concentration. In the rhizobox experiment, 0 % and 100 % (v/v) were foliar applied.	
Experimental approach Statistical design, test environment			Seedlings were cultivated nutrient solution or planted into rhizoboxes. Measurements of 54Mn uptake and intracellular shikimate accumulation	
Test organisms			*Glycine max, Helianthus annuus*	
Biological effects			In the rhizosphere glyphosate can obviously be stabilized long enough to achieve negative effects on non-target plants. Such a negative side-effect is for example inhibited acquisition of micronutrients such as Mn, but also Zn, Fe and B, which are involved in plant own disease resistance mechanisms	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			yes	
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?			No , as effects are involved in plant own disease resistance mechanisms	
3 Is the ecotoxicological manifestation level appropriate for the assessment?			Authors predict an increase in disease problems, particularly on soils with low micronutrient availability as already reported in the USA due to Glyphosate transfer from target to non-target plants (e.g. from weed to trees in orchards)	
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?				Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?				yes
3 Have parameters influencing the endpoints been considered?				yes
Concluding weight of evidence			Not relevant for the traditional risk assessment, but important to improve management of glyphosate long-term applications in agricultural practice.	
Type of info. (Critical, supporting, low weight)			**supporting**	
Consideration/concluding score			**UBA2**	

Fernandez et al. (2009)

glyphecotox_375	**Fernandez, M.R., Zentner, R.P., Basnyat, P., Gehl, D., Selles, F., Huber, D.**	**2009**	**Glyphosate associations with cereal diseases caused by Fusarium spp. in the Canadian Prairies**	**Crop Science 47 (4):1574-1584. DOI 10.2135/cropsci2006.09.0596**

Reliability	
Purpose of the study Description of endpoints	This review deals primarily with the effects of tillage systems and glyphosate use on the development of FHB and CRR in wheat and barley in eastern Saskatchewan.
Test compound, application procedure, exposure period, protocol	Test compounds not stated, experimental units were selected randomly within Crop Districts in south-east and east-central Saskatchewan to represent the most common cropping practices in the area.
Experimental approach Statistical design, test environment	nd
Test organisms	*Fusarium* spp.
Biological effects	Glyphosate use was consistently associated with higher FHB levels caused by the most important Fusarium head blight pathogens, *Fusarium avenaceum* and *Fusarium graminearum*.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance to cause a (population) relevant effect?	Because of the close association between non cereal crops, reduced tillage and glyphosate use, it was not possible to completely separate the effects of these factors on *Fusarium* infections.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	No , glyphosate might cause changes in fungal communities, which are not assessed in current risk assessment
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations ?	nd
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Study established a relationship between previous glyphosate use and increased *Fusarium* infection of spikes and subcrown internodes of wheat and barley, or Fusarium colonization of crop residues.
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Piotrowicz-Cieslak et al. (2010)

glyphecotox_180	**Piotrowicz-Cieslak, A.I., Adomas, B., Michalczyk, D.J.**	**2010**	**Different Glyphosate Phytotoxicity of Seeds and Seedlings of Selected Plant Species**	**Polish Journal of Environmental Studies Volume: 19 Issue: 1 Pages: 123-129 Url:**
Reliability				
Purpose of the study Description of endpoints	The aim of this study was to compare the physiological responses of six plant species (popular crops or plants recommended as indicators of soil pollution) to a wide range of glyphosate concentrations. Percent germination, root length, seedling dry mass and myo-inositol content, as well as seedling leachate electroconductivity were determined in *Lepidium sativum, Sinapis alba, Sorghum saccharatum, Brassica napus, Lupinus luteus* and *Avena sativa*.			
Test compound, application procedure, exposure period, protocol	(Roundup® Ultra 360 SL containing 360 g/L active principle) at final concentrations: 1, 3, 7, 10, 40, 80, 120, 180, 240, 400, 750, 1000, 1500, 1700 or 2000 µM.			
Experimental approach Statistical design, test environment	PHYTOTOXKIT™ (MicroBio Test Inc., Belgium), variance (F test) for two factor experiments (split-plot). The mean values of the plots were compared using q SNK test (Student-Newman-Keuls).			
Test organisms	Seeds of oilseed rape (*Brassica napus*), white mustard (*Sinapis alba*), yellow lupin (*Lupinus luteus*), cress (*Lepidium sativum*), oats (*Avena sativa*) and sorghum (*Sorghum saccharatum*)			
Biological effects	Even the dose 7- fold lower than that recommended in agronomical practice (7 µM, i.e. 3.0 L /ha Roundup® Ultra 360 SL inhibited root growth in *B. napus* and *A. sativa*, while it did not suppress root elongation in *Lepidium sativum* and it even increased root length in *Sinapis alba*. For glyphosate concentrations within the range 1-40 µM the sharpest drop in root length occurred in *Sorghum saccharatum*, which confirms the value of this plant as a herbicide sensor plant in biotests.			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Germination test			
2 Is the magnitude of effects of biological significance	nd			
3 Is the ecotoxicological manifestation level appropriate for the assessment?	No, endpoints were stated at day 6: EC50 of root growth after six days for *Sinapis alba, Sorghum saccharatum, Brassica napus* and *Avena sativa* was 25, 22, 35 and 110 µM, respectively. In OECD 208 effects are usually determined between14 to 21 days.			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial Product			
2 Do the tested concentrations relate to predicted environmental concentrations	3.0 L /ha Roundup® Ultra			
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Not all indicator plants are equally suitable for analysis of biological activity of glyphosate residues.			
Concluding weight of evidence	Indication that glyphosate inhibits root growth.			
Type of info. (Critical, supporting, low weight)	**supporting**			
Consideration/concluding score	**UBA2**			

Eker et al. (2006)

Non-target plants	Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Romheld, V., Cakmak, I.	2006	Foliar applied glyphosate substantialla reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus L.*) plants	J. Agric. Food Chem. 54: 10019-10025

Reliability	
Purpose of the study Description of endpoints	To study the effect of glyphosate on shoot dry matter production, chlorophyll concentration, and the uptake, translocation, and tissue accumulation of Fe, Mn, Zn, and Cu in sunflower plants
Test compound, application procedure, exposure period	- Roundup Ultra [active ingredient (ai): 480 g L-1 N-[phosphonomethyl]glycine isopropylamine salt, Monsanto Co.] - Application: "subherbicidal rates of glyphosate": 1.25, 2.5, and 6% of the recommended application rate provided on the product label (equivalent to 0.39, 0.79, and 1.89 mM a.i., respectively). - sprayed on foliage in a volume of nearly 1.5 mL per plant
Experimental approach, Statistics, test environment	- Each treatment consisted of four independent replications, and each replication (pot) had two plants. - Statistics: Least significant difference (LSD) calculations were performed according to Student's t-test using MSTAT-C software
Test organisms	Helianthus annuus
Biological effects	- Reduction of the uptake and transport of Fe and Mn in plants. -glyphosate is antagonistic to the uptake, transport, and accumulation (tissue concentration) of Fe and Mn in sunflower plants
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	*Helianthus annuus*
2 Is the magnitude of effects of significance to cause a (population) relevant effect?	Effects might reduce fitness of plants or change sensitiveness towards pest organisms but willprobably not related to population effects towards non target plants
3 Is the ecotoxicological manifestation level appropriate for the assessment?	See above
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Roundup Ultra, commercial formulation containing probalby surfactants which can not completely separate from the effects of the active substance .
2 Do the tested concentrations relate to predicted environmental concentrations?	Concnetrations close to drift rates of glyphosate have been tested (up to 6% of the recommended application rate)
3 Have parameters influencing the endpoints been considered adequately?	yes
Concluding weight of evidence/proposed action	The results suggest that glyphosate residues or drift may result in severe impairments in Fe and Mn nutrition of nontarget plants, possibly due to the formation of poorly soluble glyphosate-metal complexes in plant tissues and/or rhizosphere interactions.
Type of information (Critical, supporting, low weight)	**Supporting information**
Consideration/concluding score	**UBA2**

Tesfamariam et al. (2006)

Non-target plants	**Tesfamariam T, Bott S, Cakmak I, Römheld V & Neumann G.**	**2009**	**Glyphosate in the rhizosphere – role of waiting times and different glyphosate binding forms in soils and phytotoxicity to non-target plants.**	**. European Journal of Agronomy 31:**
Reliability				
Purpose of the study Description of endpoints			Investigation whether plant residues of glyphosate-treated weeds or direct soil application of glyphosate bears an intoxication risk for subsequently cultivated	
Test compound, application procedure, exposure period			nd	
Experimental approach, Statistics, test environment			- Greenhouse studies on two soils with contrasting properties (acidic, sandy Arenosol, calcareous loess subsoil) - treated weed: model plant *Lolium perenne* - Treatments: direct soil application and treated-weeds	
Test organisms			*Helianthus annuus* seedlings	
Biological effects			- detrimental effectswere more pronounced after glyphosate weed application (90% biomass reduction of seedling) compared with direct soil application (55–70% biomass reduction) at waiting time 0 d. - increase in shikimate accumulation in theseedling root tissue - impairment of the manganese-nutritional status, still detectable after 21 d.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			*Helianthus annuus* seedlings	
2 Is the magnitude of effects of significance to cause a (population) relevant effect?			Indication for general fitness	
3 Is the ecotoxicological manifestation level appropriate for the assessment?			Probably would affect the population integrity in the end due to high biomass reduction.	
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			nd	
2 Do the tested concentrations relate to predicted environmental concentrations?			nd	
3 Have parameters influencing the endpoints been considered adequately?			dn	
Concluding weight of evidence/proposed action			Study shows an role of glyphosate in plant residues in determining the risk of non-target plant intoxication	
Type of information (Critical, supporting, low weight)			**Supporting information**	
Consideration/concluding score			**UBA2**	

B.9.13 15.3 Drift simulation

Ellis et al. (2003)

glyphecotox_362	**Ellis, J.M., Griffin, J.L., Linscombe, S.D., Webster, E.R.**	**2003**	**Rice (Oryza sativa) and corn (Zea mays) response to simulated drift of glyphosate and glufosinat**	**Weed Technology 17 (3):452-460**
Reliability				
Purpose of the study Description of endpoints	Field research was conducted during 3 yr to evaluate response of rice and corn to simulated drift rates representing 12.5, 6.3, 3.2, 1.6, and 0.8% of the usage rates of 1,120 g ai/ha glyphosate (140, 70, 35, 18, and 9 g/ha, respectively)			
Test compound, application procedure, exposure period, protocol	Drift rates represented 12.5, 6.3, 3.2, 1.6, and 0.8% of the usage rate of 1,120 g ai/ha glyphosate (140, 70, 35, 18, and 9 g/ha, respectively)			
Experimental approach, Statistics,	Early-postemergence applications were made to two- to three-leaf rice and six-leaf corn, and late-postemergence applications to rice at panicle differentiation and to corn at nine-leaf stage (1 wk before tasseling). ANOVA			
Test organisms	Rice and corn			
Biological effects	Glyphosate consistently reduced rice plant height when the two highest rates were applied early, and heading was delayed 2 to 5 d. In 2 of 3 yr, the highest rate of glyphosate reduced rice yield 99 and 67% when applied early and 54 and 29% when applied late. Germination of rice seeds from glyphosate-treated plants was reduced in 1 of 2 yr and for only the highest rate. Early application of glyphosate reduced corn yield an average of 22 to 78% for the three highest rates, but only for the highest rate at the late timing (33%).			
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?	Six-leaf growth stage was assessed after 28d similar to OECD227.			
2 Is the magnitude of effects of biological significance,	Injury greater 50 % was observed at realistic drift simulation (70 g a.s. /ha) for corn height, corn injury after 14 DAT. Recovery was observed for this parameter after 28d.			
3 Is the ecotoxicological manifestation level appropriate for the assessment	yes			
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?	Probably commercial product , but not stated			
2 Do the tested concentrations relate to predicted environmental concentrations ?	Yes, drift rates are simulated			
3 Have parameters influencing the endpoints been considered?	nd, Field study.			
Concluding weight of evidence/proposed action	Visual injury to both rice and corn associated with the lower herbicide, rates in some cases was minimal, but the negative effect on yield was significant. Visual injury alone, therefore, would not be a good indicator of potential yield loss from sublethal rates of glyphosate.			
Type of information (Critical, supporting, low weight)	**supporting**			
Consideration/concluding score	**UBA2**			

Blackburn, L.G., Boutin, C. (2003)

glyphecotox_172	Blackburn, L.G., Boutin, C.	2003	Subtle effects of herbicide use in the context of genetically modified crops: A case study with glyphosate (Roundup® (R))	Ecotoxicology 12 (1-4):271-285
Reliability				
Purpose of the study Description of endpoints			Paper presents results of literature review and results of a experiment performed with emphasis on non crop species	
Test compound, application procedure, exposure period, protocol			Roundup® Liquid	
Experimental approach, Statistics,			nd	
Test organisms			nd	
Biological effects			nd	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			yes	
2 Is the magnitude of effects of biological significance,			nd	
3 Is the ecotoxicological manifestation level appropriate for the assessment			Application affectd F1 generation os species from Poaceae family, Brassieae family members were affectd in root and shoot development, Fabaceae members were affectes serious at alls doses tested	
Environmental Relevance				
1 Is the substance tested representative and relevant for the substance being assessed?			Commercial product	
2 Do the tested concentrations relate to predicted environmental concentrations ?			89o ga.s /ha, this application rate is supposed to be above the exposure anticipated to result from drift at typical glyphosate use rates	
3 Have parameters influencing the endpoints been considered?			Lack of uniform solution, lack of green leaves, yes	
Concluding weight of evidence/proposed action			More powerful experiment with non crop species were conducted later by the author, which might overwrite the present .	
Type of information (Critical, supporting, low weight)			**low weight**	
Consideration/concluding score			**UBA3**	

Al-Khatib et al. (2003)

glyphecotox_276	**Al-Khatib, K., Claassen, M.M., Stahlman, P.W., Geier, P.W., Regehr, D.L., Duncan, S.R., Heer, W.F.**	**2003**	**Grain sorghum response to simulated drift from glufosinate, glyphosate, imazethapyr, and sethoxydim**	**Weed Technology 17 (2):261-265**

Reliability	
Purpose of the study Description of endpoints	Field experiments were conducted at four locations in Kansas in 1999 and 2000 to evaluate grain sorghum response to simulated drift rates of four herbicides. Imazethapyr, glufosinate, glyphosate, and sethoxydim were applied at 1/3, 1/10, 1/33, and 1/100 of the use rate when plants were 10 to 20 cm tall.
Test compound, application procedure, exposure period, protocol	Use rates were 1/100, 1/33, 1/10, and 1/3 of the recommended use
Experimental approach, Statistics,	
Test organisms	sorghum
Biological effects	Visible crop injury increased as rates of each herbicide increased. The highest rate of glyphosate resulted in injury at all sites in both years. Injury ranged from 64 to 99% 8 WAT not stetd if active ingriedien tor or commercial product,
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance,	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to predicted environmental concentrations ?	Yes, but at 1/ 100 and 1/33 drift rates, no significant effects observed.
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence/proposed action	No information about product, no endpoints, field experiment in Canada not reliable for RA
Type of information (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Felix et al. (2011)

glyphecotox_369	**Felix, J., Boydston, R., Burke, I.C.**	**2011**	**Potato Response to Simulated Glyphosate Drift**	**Weed Technology 25 (4):637-644. DOI: 10.1614/wt-d-11-00001.1.**

Reliability	
Purpose of the study Description of endpoints	Field studies were conducted in 2008 in Ontario, OR and Paterson, WA to determine the effect of simulated glyphosate drift on 'Ranger Russet' potato, including visual injury, shikimic acid accumulation, and tuber yield.

Test compound, application procedure, exposure period, protocol	Roundup® Original Max ®Glyphosate was applied at 8.5, 54, 107, 215, and 423 g ae ha21; which corresponds to 0.01, 0.064, 0.126, 0.254, and 0.5 of the lowestrecommended (846 g ha21) single application dose for glyphosate-resistant corn and sugar beet.
Experimental approach, Statistics,	Glyphosate was applied when potato plants were at 10-cm height, stolon hooking, tuber initiation, or bulking stage; ANOVA
Test organisms	Corn an sugar beet
Biological effects	The greatest visual foliar injury was observed when glyphosate was applied at a dose of 54 g ha21 or greater and potato plants were at the hooking stage.the lowest foliar injury was observed when glyphosate was applied to potato plants at the bulking stage. The I50 glyphosate dose at 42 d after treatment (DAT) was estimated to be 167 g ha21 for potatoes sprayed at the hooking stage
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	visual foliar injury data at 21 DAT
2 Is the magnitude of effects of biological significance,	The estimated I50 glyphosate doseat 21 DAT was lowest at hooking stage (80.3 g/ ha) followedby tuber initiation (156.4 g/ ha)
3 Is the ecotoxicological manifestation level appropriate for the assessment	80g/ha is the amount of glyphosate which can be predicted with an application rate of 2880 ga.s./ha.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	commercial product
2 Do the tested concentrations relate to predicted environmental concentrations ?	yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?	Field study
Concluding weight of evidence/proposed action	Significant effects at concentrations related to predicted driftl concentrations, EC50 values stated for 21DAT.
Type of information (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Deeds et al. (2006)

glyphecotox_341	**Deeds, Z.A., Al-Khatib, K., Peterson, D.E., Stahlman, P.W.**	**2006**	**Wheat Response to Simulated Drift of Glyphosate and Imazamox Applied at Two Growth Stages**	**Weed Technology 20:23-31**
Reliability				
Purpose of the study Description of endpoints			The objectives of this research were to determine the effects of simulated drift of glyphosate and imazamox applied at the jointing and flowering stages of winter wheat on growth, yield, and seed germination, and to determine the correlation between early injury symptoms and grain yield.	

Test compound, application procedure, exposure period, protocol	Roundup® Ultra Max ®Glyphosate at 1/1003, 1/333, 1/103, and 1/33 of usage rates of 840 g ae/ha glyphosate and 35 g/ha imzamox were applied individually to wheat in the early jointing or the early flower stages of growth. All glyphosate5 treatments included 2% ammonium sulfate by weight,	
Experimental approach, Statistics,	Wheat plants were observed for injury symptoms and recovery throughout the growing season, and visible injury ratings were determined 1, 2, and 4 wk after treatment (WAT) using a scale of 0 to 100, with 0 equal to no wheat injury and 100 equal to plant mortality; regression analyis	
Test organisms	Wheat varieties	
Biological effects	Glyphosate injury symptoms were noticeable on wheat plants within 4 to 7 d after treatment and peaked at 3 to 4 WAT. Symptom intensity differed depending on glyphosate rate and environmental conditions. Wheat injury ratings . Wheat injury ratings were highly correlated with yield reduction, and the correlation was more apparent between yield reduction and injury rating at 4 WAT than injury ratings at 1 and 2 WAT.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		Jointing an flowering stage
2 Is the magnitude of effects of biological significance,		no
3 Is the ecotoxicological manifestation level appropriate for the assessment		no
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations ?		yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?		Field experiment
Concluding weight of evidence/proposed action	No EC50 values calculated, but obviously ranging for visual injury between 0,05 and 0.25 of use rate (approx 40 to 210 g/ha).	
Type of information (Critical, supporting, low weight)	**supporting**	
Consideration/concluding score	**UBA2**	

Gilreath et al. (2001)

glyphecotox_385	**Gilreath, J.P., Chase, C.A., Locascio, S.J.**	**2001**	**Crop injury from sublethal rates of herbicide. I. Tomato**	**Hortscience 36 (4):669-673**
Reliability				
Purpose of the study Description of endpoints	The objectives of these studies were to evaluate the extent of phytotoxic injury and the effect on yield of fresh market tomato exposed at three stages of development to levels of glyphosate known to be sublethal.			
Test compound, application procedure, exposure period, protocol	Roundup® 4EC®; Monsanto Agricultural Products, St. Louis) were applied at three reproductive growth stages of 'Sunny' tomato. The active ingredient was applied at 0,1, 10, and 100 g·ha–1 in a volume of 234 L			
Experimental approach, Statistics,				
Test organisms	tomato			

Biological effects	Exposure to 60 to 100 g·ha–1 during the period 4 to 5.5 weeks after transplanting, just prior to bloom of the first cluster and during bloom, caused foliar injury and flower abscission, and reduced fruit set. Plants treated later were larger and more mature. They were less susceptibleto foliar injury	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		nd
2 Is the magnitude of effects of biological significance,		Yield reductions
3 Is the ecotoxicological manifestation level appropriate for the assessment		nd
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations ?		yes
3 Have parameters influencing the endpoints been considered adequately (e.g. pH, temperature, light conditions)?		Field study
Concluding weight of evidence/proposed action	Field study in Florida, not comparable, Nevertheless	
Type of information (Critical, supporting, low weight)	**low weight**	
Consideration/concluding score	**UBA3**	

Gove et al. (2007)

glyphecotox_394	**Gove, B., Power, S.A., Buckley, G.P., Ghazoul, J.**	**2007**	**Effects of herbicide spray drift and fertilizer overspread on selected species of woodland ground flora: comparison between short-term and long-term impact assessments and field surveys**	**Journal of Applied Ecology 44 (2):374-384. DOI 10.1111/j.1365-2664.2007.01261.x.**
Reliability				
Purpose of the study Description of endpoints			Six species of woodland plants were exposed to the herbicide glyphosate at concentrations equivalent to those measured in spray drift trials (1–25% of the full application rate) in short-term greenhouse and long-term field experiments.	
Test compound, application procedure, exposure period, protocol			Glyphosate (Egret, Cardel, France) comes in liquid form at a concentration of 360 g glyphosate/ L plus a polyoxyethylene amine surfactant; application rates vary between 2 and 10 L active ingredient (a.i.) /ha. The median application rate of 6 L (2160 g) a.i. /ha was chosen as the maximum dose rate (100%).	
Experimental approach, Statistics, test environment			A glyphosate dosing regime of 0, 0·06, 0·3, 0·6 and 1·5 L a.i./ ha (0%, 1%, 5%, 10% and 25% of the median field application rate) was followed, covering the range of doses measured in spray drift situations. Non-parametric Kruskal–Wallis test.	
Test organisms			Fourteen native woodland plant species	
Biological effects			This study has shown that herbicide concentrations as low as 1–5% of the median field application rate can have biologically significant effects on several woodland species, among them species of conservation value.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance?	The implication of the results is that the vigour and fitness of understorey plants in woodland margins may be affected by herbicide applications to adjacent agricultural land.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations?	yes
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Relevant for general risk assessment. Authors recommend the adoption of no-spray buffer zones of atm least 5 m to protect the majority of woodland species from the impacts of agrichemicals applied to adjacent land.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Pfleeger et al. (2008)

glyphecotox_544	**Pfleeger, T., Olszyk, D., Plocher, M., Yilma, S.**	**2008**	**Effects of low concentrations of herbicides on full-season, field-grown potatoes**	**Environmental Toxicology and Chemistry 30 (2):455-468. Doi 10.1002/Etc.394.**
			Reliability	
Purpose of the study Description of endpoints			Field trials were conducted to determine if potato (Solanum tubersum L.) vegetative growth and tuber yield and quality were affected by herbicides at below recommended field rates.	
Test compound, application procedure, exposure period, protocol			Commercial products, brand names were not stated. Herbicide characteristics are listed in Table 1. Herbicides were applied at 14 or 28 days after emergence (DAE) at 0.00056, 0.0032, 0.018, or 0.1 times the field application rate (FAR)	
Experimental approach Statistical design, test environment			Potatoes were grown in fields at the Oregon State University Horticulture Farmwith herbicides applied at below recommended field applicationrates 14 d after emergence (DAE) or at 28 DAE. ANOVA	
Test organisms			*Solanum tubersum*	
Biological effects			Tuber yield and quality parameters were more affected by lower herbicide rates than were plant height or injury.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				
Biological Relevance				
1 Is an appropriate test species/ life-stage(s) studied?			Mean plant height at time of spraying for 14 DAE plants in 2003, 2004, and 2005 was 0.213, 0.193, and 0.295 m, respectively. Mean plant height at time of spraying for 28 DAE plants in 2003, 2004, and 2005 was 0.393, 0.578, and 0.581 m, respectively.	

2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Glyphosate affected tuber production more in 2004 than in 2003.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Vegetative responses did not accurately predict yield and quality responses of tubers; therefore, reproductive responses should be considered in phytotoxicity test protocols for pesticide registration in the USA.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Unknown commercial product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Field study
Concluding weight of evidence	Reproductive responses should be considered in phytotoxicity test protocols for pesticide registration
Type of info. (Critical, supporting, low weight)	**low weight for ERA of glyphosate, critical for ERAin general**
Consideration/concluding score	**UBA2**

Pfleeger et al. (2011)

glyphecotox_179	**Pfleeger, T., Olszyk, D., Lee, E.H., Plocher, M.**	**2011**	**Comparing Effects of Low Levels of Herbicides on Greenhouse- and Field-Grown Potatoes (Solanum Tuberosum L.), Soybeans (Glycine Max L.), and Peas (Pisum Sativum L.)**	**Environmental Toxicology and Chemistry. Volume: 30 Issue: 2 Pages: 455-468 DOI: 10.1002/etc.394 ISSN: 1552-8618 (online)**

Reliability	
Purpose of the study Description of endpoints	Toxicology tests were conducted on potatoes, peas, and soybeans grown in a native soil in pots in the greenhouse and were compared to plants grown outside under natural environmental conditions to determine toxicological differences between environments, whether different plant developmental stages were more sensitive to herbicides, and whether these species were good candidates for plant reproductive tests.
Test compound, application procedure, exposure period, protocol	Roundup®, field application rate 832 g ha_1 a.i.; concentrations of 0.00000, 0.00056, 0.00320, 0.01800 and 0.10000_the FAR for each herbicide.
Experimental approach Statistical design, test environment	For potatoes, herbicide treatments were applied each year at tuber initiation and bulking (generally 14 or 28 d after emergence [DAE]).ANOVA,
Test organisms	*Pisum sativum, (Solanum tuberosum, Glycine max)*
Biological effects	The results indicate that potatoes were not more sensitive in either environment for the chemicals tested. potatoes exposed to glyphosate at different developmental stages had nonsignificant effects on tuber measures. However, vegetative measures were as sensitive or more sensitive when potatoes were exposed to glyphosate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Authors found visual injury not to be necessarily the most sensitive endpoint
2 Is the magnitude of effects of biological significance	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	Method was discussed including possible deficiencies

Concluding weight of evidence	General consideration for RA that ratio between greenhouse- and field-grown plants to be around 1.8. Results may be more or less sensitive than reality, and more restrictive regulations (safety factors) should be imposed to account for this variability.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Nandula et al. (2007)

glyphecotox_522	**Nandula, V.K., Reddy, K.N., Rimando, A.M., Duke, S.O., Poston, D.H.**	**2007**	**Glyphosate-resistant and -susceptible soybean (Glycine max) and canola (Brassica napus) dose response and metabolism relationships with glyphosate**	**Journal of Agricultural and Food Chemistry 55 (9):3540-3545. doi: 10.1021/jf0635681**

Reliability	
Purpose of the study Description of endpoints	Experiments were conducted to determine (1) dose response of glyphosate-resistant (GR) and -susceptible (non-GR) soybean [Glycine max (L.) Merr.] and canola (Brassica napus L.) to glyphosate
Test compound, application procedure, exposure period, protocol	Glyphosate-K was applied at 0.87, 1.73, 3.47, 6.93, 13.86, 27.72, 55.44, and 110.88 kg ae ha-1 to Asgrow 4603RR GR soybean and at 0.007, 0.015, 0.03, 0.06, 0.11, 0.22, 0.44, and 0.87 kg ha-1 to HBKC 5025 non-GR soybean.
Experimental approach Statistical design, test environment	Data were subjected to analysis of variance, and means were separated using Fisher's protected least significant difference (GR50 (glyphosate dose required to cause a 50% reduction in plant dry wt accumulation) values for GR and non-GR soybean and canola were calculated fitting nonlinear regression equations
Test organisms	Soybean and canola
Biological effects	GR50 non-GR soybean = 0.47 kg/ha, canola= 0.3 kg/ha
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Plants were subirrigated with water and fertilized as needed. Soybean plants at oneto two-trifoliate leaf (22 days old, 45 cm tall) growth stage and canola plants at four- to five-leaf (29 days old, 14 cm tall) growth stage were used for treatment.
2 Is the magnitude of effects of biological significance	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	No details about formulated product
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	The greenhouse was maintained at 25/20 ?C ((3 ?C) day/night temperature with natural light supplemented
Concluding weight of evidence	Additional information about endpoints for herbicidal product (GR50 non-GR soybean = 0.47 kg/ha, canola= 0.3 kg/ha)
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Koger et al. (2005)

glyphecotox_428	**Koger, C.H., Shaner, D.L., Krutz, L.J., Walker, T.W., Buehring, N., Henry, W.B., Thomas, W.E., Wilcut, J.W.**	**2005**	**Rice (Oryza sativa) response to drift rates of glyphosate**	**Pest Management Science 61 (12):1161-1167. Doi 10.1002/Pt.1113.**

Reliability	
Purpose of the study Description of endpoints	Greenhouse and field studies were conducted to investigate response of two rice varieties, Priscilla and Cocodrie, to sub-lethal rates of glyphosate in terms of injury, shikimate accumulation and yield.
Test compound, application procedure, exposure period, protocol	An isopropylamine salt of glyphosate (Roundup® Customn) was applied at 0, 26, 105 and 420 gAE ha−1 to 31- to 37-cm-tall plants in the three-leaf growth stage. A nonionic surfactant (Inducen, a mixture of alkylarylpolyoxyalkane ethers and free fatty acids) was added at 2.5ml liter−1 to each glyphosate solution
Experimental approach Statistic, test environment	In the greenhouse, more shikimate accumulated in Cocodrie than Priscilla at comparable glyphosate rates applied to plants at the three-leaf stage. In field studies, glyphosate was applied to both varieties when they were 74-cm tall and in the internode separation growth stage.
Test organisms	*Oryza sativa*

Biological effects	The highest rate of glyphosate reduced yield in Cocodrie by 92% whereas there was only a 60% yield reduction in Priscilla. The estimated IC50 of glyphosate on Cocodrie was 60 g ha−1 compared with 339 g ha−1 for Priscilla. The differences in the sensitivity of these two varieties to glyphosate may be related to the physiological state of the plants at the time of treatment. Both varieties were sprayed at internode elongation when the plants were 74cm tall.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	31- to 37-cm-tall plants in the three-leaf growth stage.
2 Is the magnitude of effects of biological significance	yes
3 Is the ecotoxicological effect appropriate for the assessment?	This research demonstrates that a drift event can be detected and any subsequent effect on rice yield can be measured, especially if the rice is exposed to sub-lethal rates of glyphosate at the beginning of the reproductive growth stage.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to predicted environmental concentrations	Yes,
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Visual injury was apparent by 7 DAT and was a better parameter than height reduction for confirming glyphosate exposure.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Brown et al. (2009)

glyphecotox_303	**Brown, L.R., Robinson, D.E., Young, B.G., Loux, M.M., Johnson, W.G., Nurse, R.E., Swanton, C.J., Sikkema, P.H.**	**2009**	**Response of Corn to Simulated Glyphosate Drift Followed by In-Crop Herbicides**	**Weed Technology 23 (1):11-16. Doi 10.1614/Wt-08-067.1**
Reliability				
Purpose of the study, Description of endpoints	Thirteen field experiments were conducted in Illinois, Indiana, Ohio, and Ontario from 2005 to 2007 to determine the effects of simulated glyphosate drift followed by in-crop applications of nicosulfuron/rimsulfuron plus dicamba/ diflufenzopyr or foramsulfuron plus bromoxynil plus atrazine on nontransgenic corn injury, height, stand count, shoot dry weight, and yield.			
Test compound, application procedure, exposure period, protocol	Glyphosate, Roundup® WeathermaxH, Monsanto Canada Inc., Glyphosate1 was applied to corn at the 4- to 5-leaf stage at 0, 10, 50, 100, and 200 g/ha, representing approximately 0, 1, 5, 10, and 20% of the recommended rate (1,000 g/ha), respectively, to simulate herbicide drift. Conventional cornherbicides consisting of nicosulfuron/rimsulfuron (25 g/ha) plus dicamba/diflufenzopyr2 (200 g/ha), or foramsulfuron3 (35 g/ha) plus bromoxynil3 (280 g/ha) plus atrazine4 (1,000 g/ha) were applied 2 to 5 d after the simulated glyphosate drift application.			

Experimental approach Statistic, test environment	randomized complete block design with four replications. Corn was planted
Test organisms	*corn*
Biological effects	Simulated glyphosate drift at 100 and 200 g/ha, resulted in 11 to 61% visual crop injury and a 19 to 45% decrease in corn height. Simulated glyphosate drift at 200 g/ha caused a reduction in shoot dry weight by 46%, stand count by 28% and yield by 49 to 56%.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance	nd
3 Is the ecotoxicological effect appropriate for the assessment?	No, as after glyphosate treatment additional herbicides were used.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product , plus additional herbicides
2 Do the tested concentrations relate to predicted environmental concentrations	yes
3 Have parameters influencing the endpoints been considered?	nd
Concluding weight of evidence	Glyphosate drift can result in an additive increase in crop injury from the application of in-crop herbicides in adjacent fields.
Type of info. (Critical, supporting, low weight)	Low weight
Consideration/concluding score	**UBA3**

B.9.13 15.4 Biochemical studies

Cruz-Hipolito et al. (2001)

glyphecotox_329	**Cruz-Hipolito, H., Rojano-Delgado, A., Dominguez-Valenzuela, J.A., Heredia, A., de Castro, M.D.L., de Prado, R.**	**2001**	**Glyphosate tolerance by Clitoria ternatea and Neonotonia wightii plants involves differential absorption and translocation of the herbicide**	**Plant and Soil 347 (1-2):221-230. doi:10.1007/s11104-011-0840-9.**

Reliability	
Purpose of the study Description of endpoints	The purpose of this work was to investigate the glyphosate tolerance mechanism for C. ternatea, N. wightii and an Amaranthus hybridus population susceptible to this herbicide in order to establish the basis for nontarget site-based mechanisms.
Test compound, application procedure, exposure period, protocol	The herbicide used was [14C]glyphosate-N phosphonomethyl) glycine of 52 mCi mmol−1 specific activity from American Radiolabeled Chemicals, Inc. (St.Louis, MO). Dose–response tests were conducted using the commercially formulated isopropylamine salt of glyphosate 360 g a.e. L−1 (Roundup® plus®).
Experimental approach	nd
Test organisms	Plants of C. ternatea, N. wightii and A. hybridus were sprayed with commercially formulated glyphosate at 500 g ae ha−1 as described above.

Biological effects	significant correlation between glyphosate differential absorption and epicuticular wax coverage has been found: high wax coverage leads to reduced glyphosate uptake. This provides new, solid evidence of the protective role of wax covering the lipid cuticle of higher plants
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	nd
3 Is the ecotoxicological manifestation level appropriate for the assessment?	nd
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	nd
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Physiological study not relevant in terms of risk assessment
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

McMullin et al. (2012)

glyphecotox_497	**McMullin, R.T., Bell, F.W., Newmaster, S.G.**	**2012**	**The effects of triclopyr and glyphosate on lichens**	**Forest Ecology and Management 264:90-97. doi: 10.1016/j foreco.2011.09.039.**
Reliability				
Purpose of the study Description of endpoints			Two commonly used silvicultural herbicides (triclopyr and glyphosate) were examined for their effects on lichens in northeastern Ontario.	
Test compound, application procedure, exposure period, protocol			Glyphosate was formulated as Vision_ at 368 g a.e. isopropylamine salt L_1 (1.0–3.0 kg a.e. ha_1).	
Experimental approach, Statistical design,			ANOVA	
Test organisms			Lichen cover was comprised primarily of Cladonia species in the ubgenus *Cladina* (reindeer lichens)	
Biological effects			Of eighteen lichen species treated in the glyphosate plots, eight species showed no reduction in abundance and 10 (56%) were negatively affected (Fig. 2b and d). Species most affected by glyphosate were *Cladonia uncialis*, *Bryoria furcellata,* and *T. granulosa*; with the latter two showing 100% mortality.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints				

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Lichens are not part of the current ERA procedure
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	Glyphosate reduced the abundance, respectively in 40% and 56% of the boreal forest lichen species treated in this study. For most species that decreased in abundance effects were minor, but three species where strongly affected.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Study describes ecotoxicological side effects towards lichens. Given the important functions of these two lichen species, their sensitivity to herbicide applications is relevant to forest managers.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Miteva et al. (2010)

glyphecotox_510	**Miteva, L.P.E., Ivanov, S.V., Alexieva, V.S.**	**2010**	**Alterations in glutathione pool and some related enzymes in leaves and roots of pea plants treated with the herbicide glyphosate**	**Russian Journal of Plant Physiology 57 (1):131-136. doi: 10.1134/s1021443710010188.**

Reliability	
Purpose of the study Description of endpoints	the changes in the endogenous level of glutathione (total and oxidized) and the activities of glutathione reductase (GR) and glutathione S_transferase (GST) after treatment with glyphosate were studied in pea plants (*Pisum sativum* L., cv. Skinado).
Test compound, application procedure, exposure period, protocol	10 mM glyphosate (Roundup®, produced by Monsanto, United States).
Experimental approach Statistical design, test environment	The plant were treated at the stage of the third leaf development. Root treatment was made with 0.01 mM solution of glyphosate.
Test organisms	Pisum sativum L., cv. Skinado
Biological effects	It was found that glyphosate application to leaves provoked strong enhancement in the GST activity in leaves, while its root application stimulated the enzyme activity in the roots. The general internal thiol–disulfide balance has a great influence on biochemical processes, including photo_ synthesis, photorespiration and gene expression in the plant cell
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Changes in the glutathione levels andGR activity observed with the progress of the oxidative stress in plants. Apparently, the inhibiting of the shikimic acid pathway by glyphosate induces nonspecifically the oxidative stress. Despite the activation of the antioxidant system, oxidative stress appears to be the major reason for the injuries of the plants.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	no
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	The conclusion from this study is only valid for glyphosate formulations that contain POEA.
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	The concentration used for the leaf treatment was calculated on the basis of the field rate of the herbicide
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Biochemical study defining the oxidative stress related to mode of action of glyphosate
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Moldes et al. (2008)

glyphecotox_515	**Moldes, C.A., Medici, L.O., Abrahao, O.S., Tsai, S.M., Tsai, S.M., Azevedo, R.A.**	**2008**	**Biochemical responses of glyphosate resistant and susceptible soybean plants exposed to glyphosate**	**Acta Physiologiae Plantarum 30 (4):469-479. doi: 10.1007/s11738-008-0144-8.**

Reliability	
Purpose of the study Description of endpoints	The effect of glyphosate application on chlorophyll level, lipid peroxidation, catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GOPX) and superoxide dismutase (SOD) activities, soluble amino acid levels and protein profile, in leaves and roots, was examined in two conventional (non-GR) and two transgenic (GR) soybean.
Test compound, application procedure, exposure period, protocol	Glyphosate (Agrisato 480 CS manufactured by ALKAGRO)
Experimental approach Statistical design, test environment	5-week-old plants were sprayed in an application chamber. The herbicide was diluted in water at 2:100 proportion and applied on the foliar surface
Test organisms	soybean cultivars
Biological effects	An improved adaptive capacity of the antioxidant pathway for detoxification of oxidative stress appears to be generated during glyphosate action, since CAT activity increased in roots of non-GR soybean cultivars. The total soluble amino acid content increased after glyphosate application, which might be responsible for reducing oxidative damage.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	The slight oxidative stress generated by glyphosate has no relevance to plant mortality.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	The slight oxidative stress generated by glyphosate has no relevance to plant mortality.

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Commercial product
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	nd
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	The objective of this work was to study biochemical parameters that may be affected in roots and leaves of soybean plants exposed to glyphosate, focusing on the antioxidant response and soluble amino acid content, thus evaluating possible biochemical markers for differential characterization of glyphosate-resistant and conventional soybean lines.
Type of info. (Critical, supporting, low weight)	**low weight**
Consideration/concluding score	**UBA3**

Alvarez-Moya et al. (2011)

glyphecotox_277	**Alvarez-Moya, C., Silva, M.R., Arambula, A.R.V., Sandoval, A.I., Vasquez, H.C., Montes, R.M.G.,**	**2011**	**Evaluation of genetic damage induced by glyphosate isopropylamine salt using Tradescantia bioassay**	**Genetics and Molecular Biology 34 (1):127-130**

Reliability	
Purpose of the study Description of endpoints	Various concentrations of a glyphosate isopropylamine salt were tested using two methods of genotoxicity assaying, viz., the pink mutation assay with Tradescantia (4430) and the comet assay with nuclei from staminal cells of the same plant.
Test compound, application procedure, exposure period, protocol	N-(phosphonomethyl)-glycine 96% (CAS No. 1071- 83-6, lot 09816 PE) was obtained from Aldrich. The evaluated concentrations were 0.7, 0.07, 0.007 and 0.0007 mM.
Experimental approach Statistical design, test environment	In this assay, color changes in cells from floral parts are used to determine mutational events, ANOVA
Test organisms	The *Tradescantia*, clone (4430) (hybrid T. *Subacaulis* X T. *hirsutiflora*), which is highly sensitive to environmental mutagens was used.
Biological effects	Isopropylamine possesses strong genotoxic activity, but its detection can vary depending on the test systems used.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	nd
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Authors believe that isopropylamine used in commercial farming can induce genetic damage, depending on the dose used and the physiological characteristics of the plants exposed to it
3 Is the ecotoxicological manifestation level appropriate for the assessment?	No, as genetic damage is not assessed in the current risk assessment.
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes: N-(phosphonomethyl)-glycine 96%
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	nd
Concluding weight of evidence	Authors believe that isopropylamine used in commercial farming can induce genetic damage, depending on the dose used and the physiological characteristics of the plants exposed to it.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

Pline et al. (2002)

glyphecotox_181	**Pline, W.A., Wilcut, J.W., Edmisten, K.L., Wells, R.**	**2002**	**Physiological and morphological response of glyphosate-resistant and non-glyphosate-resistant cotton seedlings to root-absorbed glyphosate**	**Pesticide Biochemistry and Physiology Volume: 72 Issue: 1 Pages: 48-58**

Reliability	
Purpose of the study	Studies were conducted to determine relative tissue sensitivity in glyphosate-
Description of endpoints	resistant (GR) and non-GRcotton seedlings to the herbicide glyphosate.
Test compound, application procedure, exposure period, protocol	100, 10, 1, 0.1, or 0lM technical grade glyphosate (N-(phosphonoethyl)glycine, 95% purity, Sigma, St. Louis, MO). These concentrations would correspond to 169, 1, 16.9, 1.69, 0.169, or 0ppm in a hydroponic solution, whereas a glyphosate application of 1:12kgaiha_1 would produce a 3.39 ppm concentration if the herbicide remained evenly distributed in the top 2.54 cm of the soil profile.
Experimental approach Statistical design, test environment	Cotton seedlings were grown in hydroponic solutions containing technical grade glyphosate to ensure constant exposure to glyphosate. non-linear regression analysis (Weibull model)
Test organisms	Seeds of Delta Pine & Land varieties ‘DP 5415’ (non-glyphosate resistant) and ‘DP 5415RR’ (GR)
Biological effects	Glyphosate inhibited the growth of non-GR cotton cotyledons, hypocotyls, and roots 50% at concentrations of 23, 69, and 27lM glyphosate, respectively. Additionally, glyphosate inhibited the development of lateral roots at concentrations of 0.01 or 0:1lM glyphosate greater, in GR and non-GR cotton, respectively. Lateral roots of GR and non-GR cotton inhibited by glyphosate appeared shorter and were surrounded by a thick layer of necrotic cells or root exudate which was not present in roots from plants grown in media not containing glyphosate.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	Cotyledon, hypocotyl, and root tissue from GR and non-GR plants
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	Because seedlings may come in contact with glyphosate, either applied foliarly or via root absorption from root exudates from neighboring sensitive species in a field situation, the potential for glyphosate to slow or inhibit seedling establishment may exist.
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, technical grade glyphosate (N-(phosphonoethyl)glycine, with 95% purity was tested.
2 Do the tested concentrations relate to predicted environmental concentrations	nd
3 Have parameters influencing the endpoints been considered	Controlled environment
Concluding weight of evidence	Even though no endpoints were stated, observed effects on root development are considered to have an effect on seedling emergence.
Type of info. (Critical, supporting, low weight)	**supporting**
Consideration/concluding score	**UBA2**

B.9.13 15.5 Summary of other non-target organisms (flora and fauna)

For the group of terrestrial non-target plants (NTP), a comprehensive database of 87 peer- reviewed papers was collected by the notifier. The notifier considered one publication from Boutin et al. (2010), measuring the variability in phytotoxicity testing using crop and wild plant species, to be rated in category "Klimisch2" (Klimisch, 1997) and annotated with minimal remarks. The remaining papers were considered not acceptable for risk assessment.

The submitted publications were also evaluated by RMS and have been assigned according to an UBA screening. From this screening, 27 studies were recognized as supporting information (category UBA2). Most of the cited studies were performed with formulated products rather

than with the active ingredient alone. Surfactants or additives may be contained in significant amounts in plant protection products. The function of these compounds is supposed to enhance the herbicidal activity of the active ingredient by e.g. improving the dispersal and retention on the leaf surface or the uptake of glyphosate. Considering that herbicide sensitivity among crops species or within the same crop can be extensive and that, depending on the species included in testing, conclusions regarding the phytotoxicity of any given herbicide may differ (White and Boutin 2007), it is essential for current regulatory risk assessment to take into account toxicity data on the possible synergistic effects of the products in the assessed formulation in order to avoid underestimation of the toxicity of glyphosate containing products.

The use of herbicides to control weeds in target areas may affect non-target terrestrial plants (NTP) also in off- field situation. Potentially at risk are -besides NTP-, non-target arthropods or birds and mammals that are dependent on these plants for food and shelter. The objective of the risk assessment towards NTP, especially for herbicides, is to ensure that they will not be harmed by unintended exposure due to drifting into the off- field area outside the intended spray zones. Under optimal spraying conditions and appropriate application techniques, total spray drift (the portion of herbicide achieving off-field area) was considered to range from (2.77 % to 29.2 % of the volume applied) depending on the crop to be sprayed (Ganzelmeier & Rautmann, 2000).

Several publications were evaluated that simulate glyphosate drift with different test organisms (Deeds et

al., 2006; Ellis et al., 2003; Felix et al., 2011; Gove et al., 2007; Koger et al., 2005; Nandula et al., 2007; Pfleeger et al., 2011). At tested rates corresponding to predicted environmental exposure, the authors detected visual injuries to test plants depending on test concentration, time of treatment, crop variety and experimental approach. Gove et al. (2007) even recommend the adoption of a buffer zones of at least 5 m to protect woodland species from the impacts of agrichemicals. Pfleeger et al. (2011) conducted toxicity test in greenhouse and under natural conditions and found that visual injury is not to be necessarily the most sensitive endpoint, but that reproductive endpoints in many cases were more sensitive that vegetative ones. Therefore, the proposes that more restrictive regulations (safety factors) should be imposed to account for the variability in sensitivity observed between greenhouse- and field-grown plants (Pfleeger et al. 2011). The study of Boutin et al. (2007) supports the inclusion of an uncertainty factor in risk assessments to account for the intrinsic variability in plant sensitivity to herbicides. It could be shown in Boutin et al.(2010), that crops and wild plant species responded quite variably when they were tested in different seasons as well as when tested under different environmental conditions. These findings are in line with uncertainties of phytotoxicity testing described by Olzyk et al. (2004), who addresses current trends in general risk assessment of plants in US.

More limitations to current phytotoxicty testing were described, taking into account that herbicides can influence plant communities in terms of species composition and diversity. Greenhouse microcosms were more sensitive than single-species tests and changes in community structure were observed in herbicide-treated microcosms that would not be predicted from single-species testing (Dalton and Boutin, 2010). The authors of this study concluded that even though single-species tests are useful as they can demonstrate clear dose– response patterns independently from other factors influencing growth, these test are unable to predict changes in community structure that may have long-term consequences. Additionally, it was shown that foliar applied glyphosate to target plants is released into the rhizosphere and might negatively affect non-target plants, disease problems and nutritional status (Neumann et al., 2006; Eker et al., 2006).

The decrease of certain plant species in agriculture landscape might associate with impacts on birds and mammals, as well as arthropods by influencing food resources or plant cover to reproduce or to hide from predators (Norris and Kogan 2005). Ecological side effects might even be stronger in diverse and species rich forest ecosystems. McMullin et al.(2012) showed that glyphosate reduced the abundance, respectively in 40 % and 56 % of the boreal forest lichen species. This study shows on the one hand that sensitivity to herbicide applications is relevant to forest managers, as for example lichen vegetation provides food, habitat for invertebrates and on the other hand highlights the limitations of current phytotoxicity testing by neglecting indirect effects or limiting species testing, which shall represent plant species in a whole ecosystem.

References

Boutin, C., White, A.L., Carpenter, D. (2010): Measuring variability in phytotoxicity testing using crop and wild plant species. Environmental Toxicology and Chemistry 29 (2), 327-337. DOI: 10.1002/etc.30

Deeds, Z.A., Al-Khatib, K., Peterson, D.E., Stahlman, P.W. (2006): Wheat Response to Simulated Drift of Glyphosate and Imazamox Applied at Two Growth Stages. Weed Technology 20, 23-31

Ellis, J.M., Griffin, J.L., Linscombe, S.D., Webster, E.R. (2003): Rice (*Oryza sativa*) and corn (*Zea mays*) response to simulated drift of glyphosate and glufosinate. Weed Technology 17 (3), 452-460

Felix, J., Boydston, R., Burke, I.C. (2011): Potato Response to Simulated Glyphosate Drift. Weed Technology 25 (4), 637-644. DOI: 10.1614/wt-d-11-00001.1

Ganzelmeier, H., Rautmann, D. (2000): Drift, drift-reducing sprayers and sprayer testing.

Pesticide Application, Aspects of Applied Biology 57

Gove, B., Power, S.A., Buckley, G.P., Ghazoul, J. (2007): Effects of herbicide spray drift and fertilizer overspread on selected species of woodland ground flora: comparison between short-term and long-term impact assessments and field surveys. Journal of Applied Ecology 44 (2), 374-384. DOI 10.1111/j.1365-2664.2007.01261.x

Koger, C.H., Shaner, D.L., Krutz, L.J., Walker, T.W., Buehring, N., Henry, W.B., Thomas, W.E., Wilcut, J.W.; (2005): Rice (*Oryza sativa*) response to drift rates of glyphosate. Pest Management Science 61 (12), 1161-1167. Doi 10.1002/Pt.1113

Nandula, V.K., Reddy, K.N., Rimando, A.M., Duke, S.O., Poston, D.H. (2007): Glyphosate- resistant and -susceptible soybean (*Glycine max*) and canola (*Brassica napus*) dose response and metabolism relationships with glyphosate.Journal of Agricultural and Food Chemistry 55 (9), 3540-3545. doi: 10.1021/jf0635681

Pfleeger, T., Olszyk, D., Plocher, M., Yilma, S. (2008): Effects of low concentrations of herbicides on full-season, field-grown potatoes. Environmental Toxicology and Chemistry 30 (2), 455-468. Doi 10.1002/Etc.394

White, A.L., Boutin, C. (2007): Herbicidal effects on non-target vegetation: Investigating the limitations of current pesticide registration guidelines. Environmental Toxicology and Chemistry 26 (12), 2634-2643

B.9.13.16 Surface active substances in glyphosate-based formulations

Paganelli et al. (2010)

glyphnosubm_244	**PAGANELLI, A., GNAZZO, V., ACOSTA H., LOPEZ, S.L., CARRASCO, A.E.**	**2010**	**GLYPHOSATE-BASED HERBICIDES PRODUCE TERATOGENIC EFFECTS ON VERTEBRATES BY IMPAIRING RETINOIC ACID**	**CHEMICAL RESEARCH IN TOXICOLOGY (23): 1586-1595**
Reliability				
Purpose of the study Description of endpoints	To conduct an embryological approach to explore the effects of low doses of glyphosate in development - EP: neural crest markers			
Test compound, application procedure, exposure period, protocol	- Roundup Classic ® (48% (w/v) glyphosate salt); Glyphosate - 1/3000, 1/4000, and 1/5000-dilutions of Roundup Classic® prepared in 0.1× MBS (modified Barth's saline) - Treatments were performed from the 2-cell stage - 0.5 or 1 µM Ro-415253 was added at the 9-cell stage - Embryos were incubated in 0.1 x MBS. Cyclopamine was used at 100 µM concentration in 0.1 x MBS and was applied from the 2-cell stage until fixation. Embryos were fixed in MEMFA when sibling controls reached the desired stage.			
Experimental approach Statistical design, test environment	Refer to the study			
Test organisms	*Xenopus laevis* Chicken embryos			

Biological effects	Relevant experimental set up for ecotoxicological assessment: Effects on eggs Effects were detected in glyphosate-based formulations. Please refer also to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	partly
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	partly
3 Is the ecotoxicological manifestation level appropriate for the assessment?	partly
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	-/-
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Romano et al. (2010)

glyphnosubm_249	**ROMANO, R.M. ROMANO, M.A. BERNARDI, M.M. FURTADO, P.V. OLIVEIRA, C.A.**	**2010**	**PREPUBERTAL EXPOSURE TO COMMERCIAL FORMULATION OF THE HERBICIDE GLYPHOSATE ALTERS TESTOSTERONE LEVELS**	**ARCHIVES OF TOXICOLOGY (84): 309-317**

Reliability	
Purpose of the study Description of endpoints	To evaluation the endocrine disruption potential of glyphosate formulation by assessment of rats prepubertal reproductive development. EP: progression of puberty, body development, hormonal production of testosterone, estradiol and corticosterone, and morphology of the testis
Test compound, application procedure, exposure period, protocol	Roundup Transorb purity: 480 g/L of glyphosate (648 g/L as isopropylamine salt) Duration of study: From postnatal day (PND) 23 until PND53 Dose levels: Control group – deionized water; 5, 50 or 250 mg/kg of body weight of glyphosate-Roundup Transorb Administration by gavage Dosing volume: 0.25 mL/100 g of body weight, Application time: between 7 and 8 a.m. each day

Experimental approach Statistical design, test environment	Animals per dose group: 4 treatment groups, 17 animals per group Animal selection No mention of avoiding selection of siblings within the same group to control for possible litter effects Administration: The glyphosate-Roundup Transorb was diluted in a watery suspension and administered once a day, by gavage; Dosing volume: 0.25 mL/100 g of body weight, Application time: between 7 and 8 a.m. each day
Test organisms	Wistar rats
Biological effects	Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = 85.8 ± 2.8 µm; 5 mg/kg = 71.9 ± 5.3 µm; 50 mg/kg = 69.1 ± 1.7 µm; 250 mg/kg = 65.2 ± 1.3 µm) and increased the luminal diameter ($P < 0.01$; control = 94.0 ± 5.7 µm; 5 mg/kg = 116.6 ± 6.6 µm; 50 mg/kg = 114.3 ± 3.1 µm; 250 mg kg = 130.3 ± 4.8 µm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups ($P <0.001$; control = 154.5 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50mg/dL = 84.5 ± 12.2 ng/dL; 250mg/kg = 76.9 ± 14.2 ng/dL).

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Romano et al. (2012)

glyphnosubm_250	**ROMANO, M.A., ROMANO, R.M., SANTOS, L.D., WISNIEWSKI, P., CAMPOS, D.A., DE SOUZA, P.B., VIAU, P., BERNARDI, M.M., NUNES, M.T., DE OLIVIERA, C.A.**	**2012**	**GLYPHOSATE IMPAIRS MALE OFFSPRING REPRODUCTIVE DEVELOPMENT BY DISRUPTING GONADOTROPIN EXPRESSION**	**ARCHIVES OF TOXICOLOGY (86) 4: 663-673**

Reliability	
Purpose of the study Description of endpoints	To investigate the effect of gestational maternal glyphosate exposure (50 mg/kg, NOAEL for reproductive toxicity) on the reproductive development of male offspring. EP: sexual behavior, partner preference; serum testosterone concentrations, estradiol, FSH and LH; mRNA and protein content of LH and FSH; sperm production and morphology of the seminiferous epithelium; weight of the testes, epididymis and seminal vesicles. The growth, the weight and age at puberty of the animals were also recorded.
Test compound, application procedure, exposure period, protocol	Roundup Transorb purity: 480 g/L of glyphosate (648 g/L as isopropylamine salt) Duration of exposure: From gestational day 18 to postnatal day (PND) 5 Dose levels: Control group – deionised water; 50 mg/kg bw of glyphosate
Experimental approach Statistical design, test environment	2 treatment groups Administration: Roundup Transorb was diluted in a watery suspension and administered once a day by gavage from Gestation Day 18 to Post Natal day 5; Dosing volume: 0.25 mL/100 g bw, Application time: between 7 and 8 a.m. each day Statistics: First the Kolmogorov–Smirnov tests for normality and the Bartlett test for homoscedasticity. For analysis of body growth the multi-way analysis of variance for repeated measures MANOVA) by a general linear model (GLM) was used. Weights were compared between different groups and ages, considering the expected changes with age. The sexual behavior and day of PPS were compared among the groups using the Mann–Whitney *U* test. Weights of seminal vesicle (drained and undrained) were compared by paired Student's *ttest.* All other parameters were analyzed by Student's *t-test*. Statistical differences were considered significant when the value of P was < 0.05. Values were expressed as means and the standard error of the mean (±SEM) for parametric and interquartile ranges of nonparametric analysis.
Test organisms	Wistar rats
Biological effects	Increases in sexual partner preference scores and latency time to the first mount; testosterone and estradiol serum concentrations; mRNA expression and protein content in the pituitary gland and the serum concentration of LH; sperm production and reserves; and height of the germinal epithelium of seminiferous tubules. Early onset of puberty but no effect on the body growth of the animals
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol 1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Benachour et al. (2007)

glyphnosubm_237	**BENACHOUR, N. SIPAHUTAR, H. MOSLERNI, S. GASNIER, C. TRAVERT, C. SERALINI, G. E.**	**2007**	**TIME- AND DOSE-DEPENDENT EFFECTS OF ROUNDUP ON HUMAN EMBRYONIC AND PLACENTAL CELLS.**	**ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY (53) 126-133**

Reliability	
Purpose of the study Description of endpoints	To test the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. EP: cell viability, aromatase activity inhibition
Test compound, application procedure, exposure period, protocol	Test item: Roundup Bioforce® and glyphosate Active substance(s): Glyphosate Purity: Glyphosate: not reported Roundup Bioforce® : 360 g/L acid glyphosate (equivalent to 480 g/L of isopropylamine salt of glyphosate)
Experimental approach, Statistical design, test environment	*Please refer to the study*
Test organisms	Human: Human embryonic kidney (HEK) 293 cell line (ECACC 85120602), choriocarcinoma-derived placental JEG3 cell line (ECACC 92120308) Horse: Equine testis (aromatase activity inhibition)
Biological effects	The median lethal dose (LD_{50}) of Roundup with embryonic cells is 0.3% within 1 h in serum-free medium, and it decreases to reach 0.06% (containing among other compounds 1.27 mM glyphosate) after 72 h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1-2%, i.e., with 21-42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Benachour et al. (2009)

glyphnosubm_238	**BENACHOUR, N. SERALINI, G. E.**	**2009**	**GLYPHOSATE FORMULATIONS INDUCE APOPTOSIS AND NECROSIS IN HUMAN UMBILICAL, EMBRYONIC, AND PLACENTAL CELLS**	**CHEMICAL RESEARCH IN TOXICOLOGY (22) 97-105**

Reliability	
Purpose of the study Description of endpoints	To evaluate the toxicity of four glyphosate (G)-based herbicides in Roundup formulations, from 10(5) times dilutions, on three different human cell types. The formulations have been compared to glyphosate alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA (Polyethoxylated tallowamine) EP: Cell viability, membrane damage, apoptosis induction, cell morphology
Test compound, application procedure, exposure period, protocol	Test item: Glyphosate, Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus®; AMPA Active substance(s): Glyphosate Purity: Glyphosate and AMPA: not reported Roundup Express®: 7.2 g/L (R7.2) Bioforce® or Extra 360: 360 g/L (R360) Grands Travaux®: 400 g/L (R400) Grands Travaux plus®: 450 g/L (R450) Dose levels: Roundup formulations, glyphosate, AMPA and POEA: 14 concentrations ranging from 10 ppm to 2 % Additional AMPA concentrations: 4, 6, 8 and 10% POEA concentrations. 1 and 5 ppm Combined exposures of G, AMPA and POEA mixtures: For the two cell lines, the first mixture was the combination of glyphosate (0.4999%) with POEA (0.0001%); the second was the combination of glyphosate (0.4%) with AMPA (0.1%), and the third was AMPA (0.4999%) plus POEA (0.0001%). Combined exposures of G, AMPA and POEA mixtures: For the primary HUVEC cells, the first mixture was glyphosate (0.04999%) with POEA (0.0001%); the second was glyphosate (0.04%) with AMPA (0.01%), and the third was AMPA (0.04999%) plus POEA (0.0001%).

Experimental approach, Statistical design, test environment	MTT assay: Assessment of cell viability ToxiLight® assay: Bioluminescent assay for quantitative measurement of cell membrane damage Caspase-Glo® 3/7 assay Assessment of caspase activity or apoptosis induction Microscopy: Assessment of cell viability due to cell morphology Statistics: All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01.
Test organisms	Human embryonic kidney 293 cell line (ECACC 85120602) Human choriocarcinoma-derived placental JEG3 cell line (ECACC 92120308)
Biological effects	All R formulations: - cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage - induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells Glyphosate provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and possibly amplify toxicity induced by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Tested concentrations are far below agricultural recommendations and corresponds to low levels of residues in food or feed.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Gasnier et al. (2009)

glyphnosubm_239	**GASNIER, C., DUMONT, C., BENACHOUR, N., CLAIR, E., CHAGNON, M. C., SERALINI, G. E**	**2009**	**GLYPHOSATE-BASED HERBICIDES ARE TOXIC AND ENDOCRINE DISRUPTORS IN HUMAN CELL LINES**	**TOXICOLOGY (262)3:184-191**

Reliability	
Purpose of the study Description of endpoints	To study xenobiotic toxicity, to four different formulations and to glyphosate EP: cytotoxicity(3 assays : Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ERα, ERβ) and anti-androgenic effects (on AR) using gene reporter tests. Androgen to estrogen conversion by aromatase activity and mRNA
Test compound, application procedure, exposure period, protocol	Test item:Glyphosate, Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus® Purity: Glyphosate: not reported Roundup Express®: 7.2 g/L (R7.2) Bioforce® or Extra 360: 360 g/L (R360) Grands Travaux®: 400 g/L (R400) Grands Travaux plus®: 450 g/L (R450) Dose levels: Glyphosate: not reported Roundup Express®: 7.2 g/L Bioforce® or Extra 360: 360 g/L Grands Travaux®: 400 g/L Grands Travaux plus®: 450 g/L
Experimental approach, Statistical design, test environment	Replicates per dose level: 4 x 3 replicates Statistics: All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01 or 0.05.
Test organisms	Cell cultures: Hepatoma cell line HepG2, breast cancer cell line MDA-MB453-kb2
Biological effects	All parameters were disrupted at sub-agricultural doses with all formulations within 24h: - Human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. - Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Yes, partly
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Clair et al. (2012)

glyphnosubm_242	**CLAIR, E., MESNAGE, R., TRAVERT, C., SERALINI, G.E.**	**2012**	**A GLYPHOSATE-BASED HERBICIDE INDUCES NECROSIS AND APOPTOSIS IN MATURE RAT TESTICULAR CELLS *IN VITRO*, AND TESTOSTERONE DECREASE AT LOWER LEVELS**	**TOXICOLOGY IN VITRO (26)2:269-279**

Reliability	
Purpose of the study Description of endpoints	To test glyphosate and its formulation on mature rat fresh testicular cells from 1 to 10000 ppm EP: Citotoxicity (adenylate kinase activities); measurements of caspases 3 and 7 (key-caspases of apoptosis) in cell cultures by means of bioluminescence-based method; study of chromatin condensation by DAPI-labelling; measurement of 3β-HSD activity; changes in testosterone production secreted from Leydig cells in medium
Test compound, application procedure, exposure period, protocol	Test item: Roundup Bioforce® and glyphosate Purity: Glyphosate: not reported; Roundup Bioforce®: 360 g/L acid glyphosate (corresponding to 100%)
Experimental approach, Statistical design, test environment	Experimental approach: please refer to the study Statistics: All data are present as means ± SEM. Statistically significant differences from controls were determined by an ANOVA test followed by Bonferroni post-test with $p<0.001$ (****), $p<0.005$ (***), $p<0.01$ (**) and $p<0.05$ (*).
Test organisms	Rat: Cell Culture: Leydig, Sertoli and germ cells
Biological effects	- From 1 to 48 h of Roundup exposure Leydig cells are damaged. - Within 24–48 h this formulation is also toxic on the other cells, mainly by necrosis, by contrast to glyphosate alone which is essentially toxic on Sertoli cells - Later it induces apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. - At lower non toxic concentrations of Roundup and glyphosate (1 ppm), the main endocrine disruption is a testosterone decrease by 35%.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	Concentrations from 1 to 10000 ppm (from the range in some human urine and in environment to agricultural levels)
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Daruich et al. (2001)

glyphnosub m_245	**DARUICH, J. ZIRULNIK, F. GIMENEZ, M. S.**	**2001**	**EFFECT OF THE HERBICIDE GLYPHOSATE ON ENZYMATIC ACTIVITY IN PREGNANT RATS AND THEIR FOETUSES**	**ENVIRONMENTAL RESEARCH (85)226-231**

Reliability	
Purpose of the study Description of endpoints	To study the effects of the herbicide glyphosate on several enzymes of pregnant rats EP: Enzymatic activity of three cytosolic enzymes : isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. Organ weights: Liver, hearts and brains of maternal females
Test compound, application procedure, exposure period, protocol	Test item: Herbycigon Active substance(s): Glyphosate
Experimental approach, Statistical design, test environment	Dose levels: 0 (tap water), glyphosate solution 0.5% w/v in tap water (0.2 ml glyphosate/ml water) glyphosate solution 1% w/v in tap water (4 ml glyphosate/ml water) Animals per test substance group: 8 Animals per control group: Tap water control group: 8 Low water and low food control group: 6 Administration: The test substance was prepared as solution in tap water. 35 mL of the test substance preparations were provided in water bottles per day and animal The treatment was administered during the 21 days of pregnancy
Test organisms	Wistar rats
Biological effects	Please refer to the study
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity (sub-chapter Rabbit) in the Vol.1 of the BfR report
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Dallegrave et al. (2003)

glyphnosubm_247	**DALLEGRAVE, E. MANTESE, F. D. COELHO, R. S. PEREIRA, J. D. DALSENTER, P. R. LANGELOH, A..**	**2003**	**THE TERATOGENIC POTENTIAL OF THE HERBICIDE GLYPHOSATE-ROUNDUP® IN WISTAR RATS**	**TOXICOLOGY LETTERS (142)45-52**

Reliability		
Purpose of the study Description of endpoints	To assess the teratogenicity of the herbicide glyphosate-Roundup(R) (as commercialized in Brazil) to Wistar rats.	
Test compound, application procedure, exposure period, protocol	Test item: Roundup ® Active substance: Glyphosate Concentration: 360 g/L Surfactant Class: Polyoxyethyleneamine (POEA) Concentration: 18% (w/v) (POEA)	
Experimental approach, Statistical design, test environment	Study type: Developmental toxicity study Guideline: Refers to the EPA (Environmental Protection Agency), 1996.Guidelines for Reproductive Toxicity Risk Assessment- EPA/630/R-96/009, Washington, USA, pp. 1-163.(reproductive toxicity protocols; segment II).	
Test organisms	Wistar rats	
Biological effects	Results showed: - a 50% mortality rate for dams treated with 1000 mg/kg glyphosate - Skeletal alterations in 15.4, 33.1, 42.0 and 57.3% of fetuses from the control, 500, 750 and 1000 mg/kg glyphosate groups, respectively. The authors conclude that glyphosate-Roundup(R) may toxic to the dams and induces developmental retardation of the fetal skeleton.	
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints		
Biological Relevance		
1 Is an appropriate test species/ life-stage(s) studied?		yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?		yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?		yes
Environmental Relevance		
1 Is the substance tested representative and relevant for the substance being assessed?		yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?		
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?		yes
Concluding weight of evidence	Please refer to chapter 2.6.7.2 Developmental toxicity and teratogenicity in the Vol.1 of the BfR report	
Type of info. (Critical, supporting, low weight)		
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**	

Dallegrave et al. (2007)

glyphnosub m_248	**DALLEGRAVE, E. MANTESE, F. D. OLIVEIRA, R. T. ANDRADE, A. J. M. DALSENTER, P. R. LANGELOH, A.**	**2007**	**PRE- AND POSTNATAL TOXICITY OF THE COMMERCIAL GLYPHOSATE FORMULATION IN WISTAR RATS**	**ARCHIVES OF TOXICOLOGY (81):665-673**

Reliability	
Purpose of the study Description of endpoints	To elucidate whether glyphosate-Roundup® (commercial formulation) poses reproductive hazards to male and female offspring of rats exposed during pregnancy and lactation
Test compound, application procedure, exposure period, protocol	Test item: Roundup ® Active substance(s): Glyphosate Concentration: 360 g/L Surfactant: Polyoxyethyleneamine (POEA) Concentration: 18% (w/v) POEA
Experimental approach, Statistical design, test environment	Duration of study: 21-23 days during pregnancy; 21 days during lactation Dose levels: 0 (water), 50, 150, 450 mg/kg glyphosate-Roundup® Administration: Test substance preparations were prepared by diluting the Roundup-formulation with appropriate volumes of distilled water. Applications were done once daily by oral gavage Dosing volume: 10 mL/kg bw Statistics: Parametric data, expressed as mean ± standard error (SEM), were analyzed by repeated measure ANOVA or one-way ANOVA, followed by the Bonferroni test when appropriate. The nonparametric data, expressed as proportion or percentage, were analyzed by the chi-square test. Differences were considered statistically significant when $P < 0.05$.
Test organisms	Wistar rats
Biological effects	Glyphosate-Roundup (R) did not induce maternal toxicity but induced adverse reproductive effects on male offspring rats: a decrease in sperm number per epididymis tail and in daily sperm production during adulthood, an increase in the percentage of abnormal sperms and a dose-related decrease in the serum testosterone level at puberty, and signs of individual spermatid degeneration during both periods. There was only a vaginal canal-opening delay in the exposed female offspring.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	These findings suggest that in utero and lactational exposure to glyphosate-Roundup (R) may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood.
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

Hokanson et al. (2007)

glyphnosubm_263	**HOKANSON, R., FUDGE, R., CHOWDHARY, R., BUSBEE, D.**	**2007**	**ALTERATION OF ESTROGEN-REGULATED GENE EXPRESSION IN HUMAN CELLS INDUCED BY THE AGRICULTURAL AND HORTICULTURAL HERBICIDE GLYPHOSATE**	**HUMAN & EXPERIMENTAL TOXICOLOGY (26) 747-752**

Reliability	
Purpose of the study Description of endpoints	To examine the toxicity of glyphosate as a function of its capacity to alter gene expression in the presence or absence of E2 (17ß-estradiol). The authors present data resulting from an investigation of the potential endocrine disruptive activities of a commercially available, unregulated, glyphosate herbicide. EP: In vitro DNA microarray analysis, quantitative real-time PCR (qrtPCR)
Test compound, application procedure, exposure period, protocol	Test item: Glyphosate formulation Source: Unknown retail supplier Purity: Not reported Concentration: 15% home use preparation Dose levels: 0.1, 0.01, 0.001 or 0.0001% dilutions of the glyphosate stock solution containing 15% glyphosate. Duration of exposure: 18 h
Experimental approach, Statistical design, test environment	Please refer to the study
Test organisms	MCF-7
Biological effects	DNA microarray analysis indicated that a large number of genes, 680 out of 1550 on the chip, were dysregulated by in vitro exposure to the commercial glyphosate herbicide For example: three genes – HIF1, CXCL12 and EGR1 –determined by DNA microarray analysis and quantitative real-time PCR to be dysregulated by exposure to glyphosate, combine to give a bewildering array of potential altered gene regulation effects. These include initiation of apoptosis in cells of cerebral and myocardial tissues, increased angiogenesis in tumors, retinal ischemia, hypertension, pre-eclampsia, fetal growth retardation and inactivation of tumor repressor genes. Altered EGR1 levels in response to glyphosate salts are less clear than for HIF1 and CXCL12, but appear to potentially impact rates of apoptosis initiation and alter the levels of vascularization associated with tumor formation.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	partly
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	There remains an unclear pattern of very complex events following exposure of human cells to low levels of glyphosate, but events surrounding the altered levels of expression of only three genes – EGR1, CXCL12 and HIF1 – out of the entire battery tested, are both complicated and potentially damaging to adult and fetal cells.
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA3**

Mesnage R. et al. (2012)

glyphnosub m_243	**MESNAGE R., BERNAY B., SERALINI G.-E.**	**2012**	**ETHOXYLATED ADJUVANTS OF GLYPHOSATE-BASED HERBICIDES ARE ACTIVE PRINCIPLES OF HUMAN CELL TOXICITY**	**TOXICOLOGY,** IN PRESS, CORRECTED PROOF, AVAILABLE ONLINE 20 SEPTEMBER 2012

Reliability	
Purpose of the study Description of endpoints	To study potential active principles for toxicity on human cells for 9 glyphosate-based formulations. As controls a major adjuvant (the polyethoxylated tallowamine POE-15), glyphosate alone, and a total formulation without glyphosate were used. EP: mitochondrial activities, membrane degradations, and caspases 3/7 activities
Test compound, application procedure, exposure period, protocol	Glyphosate (CAS: 1071-83-6; Sigma–Aldrich) POE-15 (CAS: 61791-26-2; ChemService) Formulating agents without Glyphosate: Genamin T200 (60–80% of POE-15) 9 Formulating agents with Glyphosate: Bayer GC (12.5% of G, 1–5% of POE-15) Clinic EV (42% of G, 11% of POE-15) Glyphogan (39–43% of G, 13–18% of POE-15) Roundup Grand Travaux (400 g/L of G, R GT) Roundup Grand Travaux plus (450 g/L of G, 90 g/L of EtO-EA, R GT+) Roundup Ultra (41.5% of G, 16% surfactant) Roundup Bioforce (360 g/L of G) Roundup 3plus (170 g/L of G, 8% surfactant) Topglypho 360 (360 g/L of G)

Experimental approach Statistical design, test environment	Experiments were repeated at least 3 times in different weeks on 3 independent cultures (n = 9). LC_{50} values were calculated by a nonlinear regression using sigmoid (5-parameters) equation with the GraphPad software Statistical differences were determined by Student's t-test using significant levels with $p < 0.01$ (**) and $p < 0.05$ (*).
Test organisms	hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines
Biological effects	**Mitochondrial respiration (SD activity):** All chemicals are cytotoxic, inducing similar dose-dependent patterns on HEK293, HepG2, and JEG3 in 24 h. 3 groups of differentially toxic formulations: The most toxic : adjuvants alone POE-15 (LC_{50} ~ 1–2 ppm; agricultural dilutions: 1–2% of the herbicide formulation containing adjuvants) and Genamin, themselves around 100-fold more toxic than a middle group. Middle group: the majority of formulations (6, with among them R GT and GT+). This middle group is again 100-fold more toxic than the third one which includes R Ultra, R Bioforce, R 3plus and finally G alone POE-15 diluted to the concentration at which it is present in Clinic E.V. (a formulation from the middle group) presented a similar toxicity than this GBH and to the middle group in general. It thus appears to be the toxic principle in human cells. Two formulations claiming a similar concentration of G (360 g/L) and different adjuvants (16% of POEA or other adjuvants), Glyphogan and R Ultra respectively, exhibited very different toxicities, 150- fold stronger on average for Glyphogan on the 3 cell lines **Cytotoxicity**: results obtained with all cell lines: The cytotoxicity induced by GBH is not linear to G concentrations The cytotoxicity induced by GBH is only linear to the 3 ethoxylated adjuvants. a The cytotoxicity induced by GBH is not linear to the non-ethoxylated formulations => Ethoxylated adjuvants can thus be considered as the active principle of the toxicity of GBH in human cells **Critical micelle concentration (CMC) of POE-15** Disruptions of the cellular membranes by micellization were observed **Membrane disruption / caspases activation**: POE-15 and R GT+ (containing also an ethoxylated adjuvant) induced more necrosis by membrane alterations rather than apoptosis G induced only apoptosis at higher levels. Ethoxylated adjuvants are thus not inert at all but cell membrane disruptors, and then induce severe mitochondrial alterations.

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	
3 Is the ecotoxicological manifestation level appropriate for the assessment?	
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes: tested concentrations between 1 and 3 ppm and at environmental/occupational doses.
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes

Concluding weight of evidence	All formulations appeared more toxic than glyphosate, and 3 groups of differentially toxic formulations were experimentally separated according to their concentrations in ethoxylated adjuvants. Ethoxylated adjuvants alone and in formulations appeared as active principles for human cell toxicity
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

Walsh et al (2000)

glyphnosubm_067	**WALSH, L.P. MCCORMICK, C. MARTIN, C. STOCCO, D.M.**	**2000**	**ROUNDUP INHIBITS STEROIDOGENESIS BY DISRUPTING STEROIDOGENIC ACUTE REGULATORY (StAR) PROTEIN EXPRESSION.**	**ENVIRONMENTAL HEALTH PERSPECTIVES (108)769-776**

Reliability	
Purpose of the study Description of endpoints	To screen 8 currently used pesticide formulations for their ability to disrupt steroid hormone biosynthesis. EP: steroidogenic acute regulatory (StAR) protein expression in MA-10 cells; levels and activities of the P450scc and the 3p-hydroxysteroid dehydrogenase (3P-HSD) enzymes (conversion of cholesterol to pregnenolone and pregnenolone to progesterone; respectively)
Test compound, application procedure, exposure period, protocol	Roundup (180 g/L glyphosate): N-(phosphonomethyl) glycine
Experimental approach Statistical design, test environment	Please refer to the study: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1638308/pdf/envhper00309-0125.pdf
Test organisms	Mouse MA-10 Leydig tumor cell line
Biological effects	**Progesterone production and total cellular protein synthesis:** - Roundup decreased progesterone production in a dosage-dependent manner without inducing a parallel decrease in total protein synthesis (*indicating that this herbicide did not cause acute cellular toxicity or a general disruption in translation*). Roundup also significantly disrupted steroidogenesis over time without inducing a parallel decrease in total protein synthesis. - The active ingredient in Roundup, glyphosate, did not alter steroidogenesis or total protein synthesis at any dose tested (0-100 gg/mL) **P450scc and -30-HSD enzyme activity, expression, and steroidogenesis** - Although Roundup significantly reduced (Bu)2cAMPstimulated steroidogenesis by 84%, effects were completely reversible. - Roundup also significantly reduced 22R-HC-driven steroidogenesis by 71%, indicating that it inhibited P450scc and/or 3,B-HSD enzyme activity. - Although Roundup did not alter 30-HSD enzyme activity, indicating that the herbicide was not acutely toxic to cells or mitochondria, it significantly reduced P450scc activity by 61%. **StAR protein and mRNA levels** Northern blot analysis revealed that Roundup did not alter StAR mRNA levels, indicating that Roundup disrupted StAR protein expression post-transcriptionally.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	

1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	The results indicate that the commercial formulation of the herbicide glyphosate Roundup® might affect reproductive function in animals
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA2 for assessment of surfactand effects (POEA)**

McDaniel at al. (2008)

glyphecotox_496	**MCDANIEL, T.V., MARTIN, P.A., STRUGER, J., SHERRY, J., MARVIN, C.H., MCMASTER, M.E., CLARENCE, S., TETREAULT, G.**	**2008**	**POTENTIAL ENDOCRINE DISRUPTION OF SEXUAL DEVELOPMENT IN FREE RANGING MALE NORTHERN LEOPARD FROGS (*RANA PIPIENS*) AND GREEN FROGS (*RANA CLAMITANS*) FROM AREAS OF INTENSIVE ROW CROP AGRICULTURE**	**AQUATIC TOXICOLOGY 88 (4):230-42**

Reliability	
Purpose of the study Description of endpoints	To assess whether amphibians that inhabit wetlands in areas of IRCA in southern Ontario show evidence of exposure to endocrine disrupting substances EP: altered gonad histology, altered or abnormal plasma steroid levels, or Vtg expression in male To test for possible associations with any observed health effects or biomarker responses in the amphibians EP: measurements of concentrations of a suite of pesticides and nutrients in farm ponds and agricultural drains in the area of the frog collection sites and from the surrounding watersheds
Test compound, application procedure, exposure period, protocol	In-situ measurements: Glyphosate concentrations were analyzed using gas chromatography with nitrogen/phosphorus detection. Glyphosate was detected in trace amounts (>5,000 ng/L) at several agricultural sites

Experimental approach Statistical design, test environment	<u>Study sites:</u> Agricultural sites were located in two regions of southwestern Ontario within the Thames River watershed; an area north of the city of London in Middlesex county and an area west of the city of Chatham in Chatham/Kent county <u>Statistical analysis</u> Data were log transformed, where necessary, to meet normality and homogeneity of variance requirements for parametric tests. If those criteria could not be met then non-parametric tests were used. Biological endpoints (circulating sex steroids, gonadosomatic index, diameter of TOFS) were compared amongst regions and between males with and without TOFS using one-way analysis of variance (ANOVA). The particular associations between concentrations of circulating sex steroids or the proportion of males with TOFS and atrazine concentrations were assessed using Pearson product moment. Goodness of fit tests were used to test the hypothesis that the frequency of TOFS were equal amongst regions, for this test, the sites within regions were pooled in order to increase sample sizes. In order to look at variation within region, individual sites with sample sizes greater than 20 animals in Chatham Region were compared for frequency of TOFS (Testicular Ovarian Follicles).
Test organisms	*Rana pipiens* and *Rana clamitans*
Biological effects	- Glyphosate was detected in several agricultural sites. - Occurrence of testicular ovarian follicles (TOFS) in male *R. pipiens* was significantly higher (42%; $p < 0.05$) at agricultural sites - There was no difference in circulating sex steroid levels between frogs from agricultural and reference sites and sex steroid levels did not correlate with pesticide concentrations in the environment - No differences were detected in the gonadosomatic indices or stage of spermatogenesis between frogs from agricultural and non-agricultural regions ($p > 0.05$). - Plasma Vtg-lpwas detected in only one male *R. pipiens* from an agricultural site. - Neither gonad size, gonad maturity nor sex steroid levels differed between normal males and those with testicular oocytes. - Proportion of testicular oocytes correlate with a mixture of pesticides and nutrients

Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes
Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	Monitoring study
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	-/-
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	-/-s
Concluding weight of evidence	No relevant for the risk assessment of glyphosate, however the study showed that mixtures of pesticides potentially have endocrine effects in amphibians inhabiting farm ponds and agricultural drains in intensive row crop agriculture
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA3**

Quassinti et al. (2009)

glyphecotox_235	**Quassinti, L., Maccari, E., Murri, O., Bramucci, M.**	**2009**	**EFFECTS OF PARAQUAT AND GLYPHOSATE ON STEROIDOGENESIS IN GONADS OF THE FROG RANA ESCULENTA IN VITRO**	**A Pesticide Biochemistry and Physiology 93 (2):91-95.**

Reliability	
Purpose of the study Description of endpoints	To assess how paraquat and glyphosat affect reproduction in amphibians EP: 17b-estradiol and testosterone levels
Test compound, application procedure, exposure period, protocol	Glyphosate (Sigma–Aldrich) was solubilized at 100 mM concentration in Krebs Ringer Bicarbonate buffer Diluted solutions of herbicides were added to each cells culture well to reach the final concentrations of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M.
Experimental approach Statistical design, test environment	Experimental approach: ovarian tissue and testis of the water frog *Rana esculenta* were incubated in vitro in presence of different concentrations of the two herbicides Statistics: Data represent the mean ± S.D. of 4 determinations. Data were subjected to Levene's test for assay homogeneity of variance. Significant differences between groups were established by use Mann–Whitney U nonparametric test. The minimum level of significance considered was $P < 0.05$. All statistical analysis used SPSS version 13.0 for Windows.
Test organisms	*Rana esculenta* (water frog)
Biological effects	Glyphosate showed no effect on gonadal steroidogenesis even at high concentrations. Glyphosate does not exert a significant inhibition on testosterone production at the highest tested concentrations. Treatment with glyphosate showed no evidence of specific activity on 17b-estradiol production by frog ovary.
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	

Biological Relevance	
1 Is an appropriate test species/ life-stage(s) studied?	yes
2 Is the magnitude of effects of biological significance, e.g. is a very small statistically significant effect able to cause a (population) relevant effect?	yes
3 Is the ecotoxicological manifestation level appropriate for the assessment?	yes

Environmental Relevance	
1 Is the substance tested representative and relevant for the substance being assessed?	yes
2 Do the tested concentrations relate to measured or predicted environmental concentrations (if available)?	yes
3 Have parameters influencing the endpoints been considered (e.g. pH, temperature, light conditions)?	yes
Concluding weight of evidence	Glyphosate showed no effect on gonadal steroidogenesis
Type of info. (Critical, supporting, low weight)	
Consideration/concluding score	**UBA1 for assessment of surfactand effects (POEA)**

B.9.13 16.1 Summary of the relevant literature on surface active substances in glyphosate-based formulations

In glyphosate-based formulations –as in almost all plant protection products (PPP) –, a varing amount of co-formulants are added to improve the handling and efficacy of the product. A great amount of the co-formulants might consist of water to which substances with antifoaming or surface active properties are added.
Surfactant do have a mode of action that attack membranes, so to permit the active substance to enter cells and reach the target. A class of non-ionic surfactants, the so called alkylamine ethoxylates (ANEO), exert an (eco)toxicological effect that can be detected in glyphosate- based formulations. Polyethoxylated alkylamine (POEA) are non-ionic surfactants belonging to the alkylamine ethoxylates.
The lead formulation for the assessment of glyphosate as active substance for PPP in the European Union does not contain alkylamine ethoxylates as surfactant. Nevertheless, since several glyphosate-based products are formulated with alkylamine ethoxylates, RMS considers it adequate to provide general background informations to other Member States in the European Union to facilitate the assessment of the risk arising from glyphosate-based PPP other than the lead formulation.
The toxicity of glyphosate-based products is greatly enhanced if the active substance is formulated with alklyamine ethoxylates (e.g. Figure B.9.13-2).

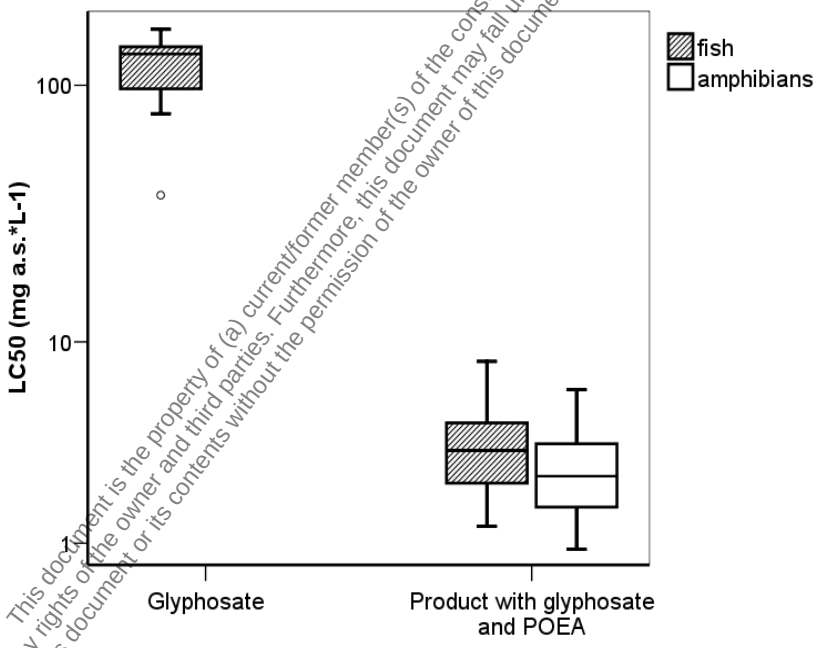


Figure B.9.13-2: LC50 values determined for fish and amphibians: exposed to glyphosateor to glyphosate-based products containing polyethoxylated alkylamines. Data submitted for authorization of different products. Box gives median and 50 %, whiskers 75 % values

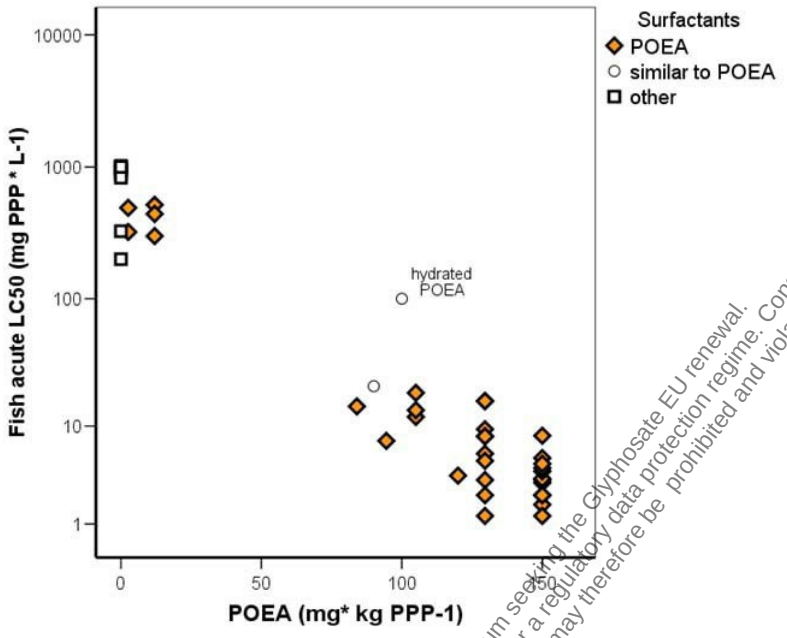


Figure B. 9.13-3: LC_{50} for fish exposed to several glyphosate-based PPPs with different surfactants as a function of product surfactant content. POEA: polyethoxylated alkylamines;similar to POEA: other alkylamine ethoxylates. Other: other surfactant classes.

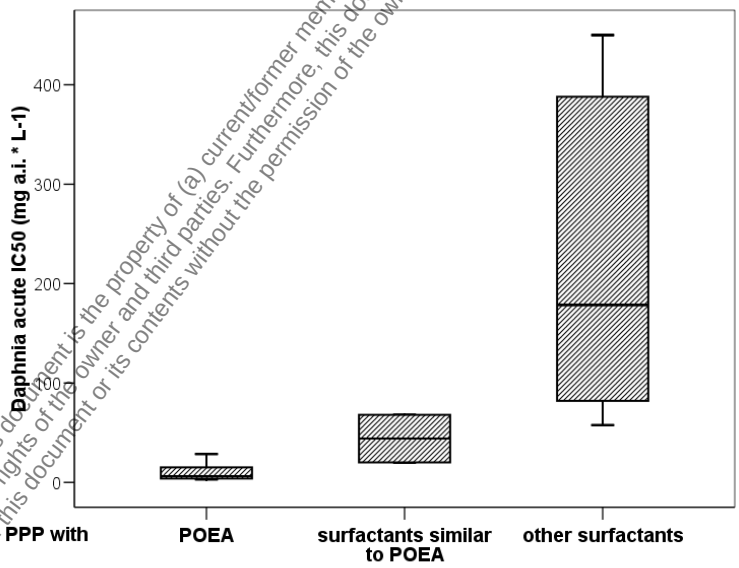


Figure B. 9.13-4: LC_{50} for Daphnia exposed to several glyphosate-based PPPs with different surfactants. POEA: polyethoxylated alkylamines; similar to POEA: other alkylamine ethoxylates. Other: other classes. Data submitted for authorization of different products. Box: median/ 50 %, whiskers 75 % values

As can be seen in Figure B.9.13-2, the acute toxicity of PPP with glyphosate and POEA for fish and amphibians (stage Gosner 25, see Gosner 1960) is comparable, as was discussed in the respective chapter.

The clearly higher toxicity of some PPP with glyphosate as active substances can be predicted from the

surfactant 'class' in the formulation and can be depicted as relative to the content of the surfactant and not of the amount of active substance in the product (Figure B. 9.13-3).

Similar data showing enhanced toxicity of glyphosate-based PPP when formulated with POEA are available for Daphnia (Figure B. 9.13-4) and algae (data not shown).

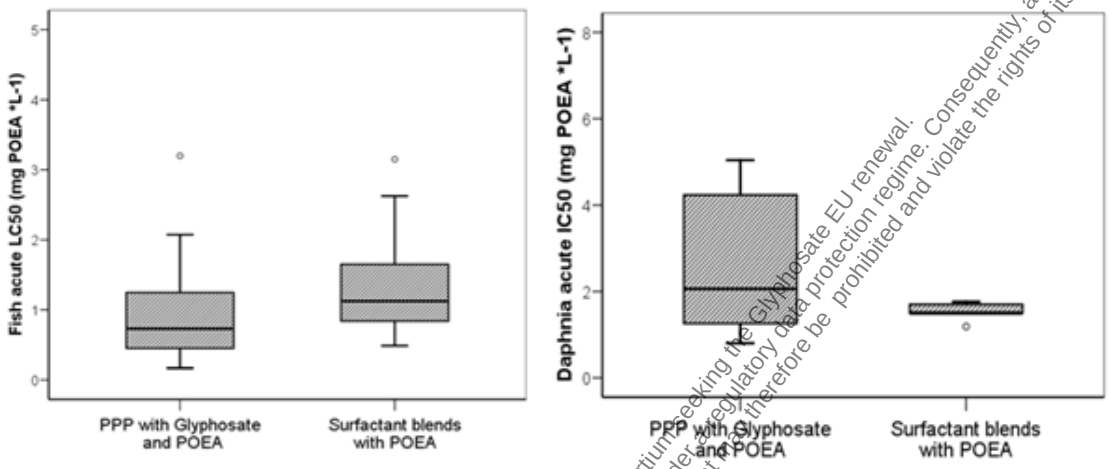


Figure B. 9.13-5: LC50 data for fish and daphnids exposed either to glyphosate-based products containing alkylamine ethoxylates or to the surfactants alone. Data submitted for authorization of different products. Box gives median and 50 %, whiskers 75 % values

During authorization processes for different glyphosate-based products, several toxicity data were generated with fish, daphnid and algae exposed to the surfactant blend alone (Figure B. 9.13-5). All data show that the toxicity of the surfactant does drive the toxicity of the product, in part because glyphosate itself is only moderately acutely toxic to the tested organisms.

Summarizing the data available, the acute risk for non-target organisms exposed to glyphosate-based products containing alkylamine ethoxylates can be assessed in the opinion of RMS based on the product tests submitted with the registration dossiers.

Hovewer, several studies published and peer reviewed that were submitted with this dossier and allocated to different themes (e.g. fish, aquatic invertebrates, algae, amphibians) do rise concerns on the effects of glyphosate and glyphosate-based formulation on endpoints regarding genotoxicity, mutagenicity, development or reproduction of non target organisms (Paganelli et al., 2010; Romano et al., 2010 and 2012; Gasnier et al., 2009; Dallegrave et al., 2003 and 2007; Hockanson et al., 2007; Walsh et al., 2000, McDaniel et al., 2008).
For the evaluation of studies with glyphosate and glyphosate-based products in in-vitro and in-vivo experimental set-ups with the aim to detect possible endocrine, genotoxic, carcenogenetic effect please refer also to the specific chapters assessing human toxicity (Vol.1, chapter 2.6.7.2, developmental toxicity and teratogenicityand respective Vol. 3 of this report).

RMS considers several of the observed effects to be mediated by the surfactants included in the formulatios. In different studies, the effects were clearly more pronounced in treatments where the tested products contained alkylamine exthoxylated surfactants (e.g. Benachour et al., 2007 and 2009); glyphosate acid treatment (when tested alone) did extremely seldom show any effects on biomarkers or higher endpoints (e.g. Quassinti, 2009) . In the paper of Mesnage et al. (2012), the direct actue toxic effects of co-formulants belonging to the alkylamine ethoxylates class has been identified and very well characterized.
In chapter Vol 3 of this report, chapter B.6.13, an evaluation of the potential chronic toxicity,

carcinogenicity reproduction and developmental toxicity of POEA is performed.

All (eco)toxicological data available give strong evidence that the toxicity of glyphosate-base formulation with POEA arisises from the surfactant. Nevetheless, even if this evidence relieves for the time being the active substance glyphosate from the suspect of being potentially carcenogenetic, endocrine disruptive and mutagenic, it does not tell the same for the surfactant class of the alkylamine ethoxylates.

Walsh et al. (2000) report that glyphosate based formulations, but not glyphosate alone, might affected the steroidogenesis pathway by inhibiting the progesterone production. Levine et al. (2007) determined that Roundup® branded formulation and a Roundup blank formulation without glyphosate decreased the hCG-stimulated increase in progesterone production. These findings indicate that the effect on progesterone is largely attributable to the surfactant, insofar as it decreases progesterone production upon mitochondrial membrane disruption.

Other finding (e.g. Dallegrave et al., 2007, Knapp, 2007 and 2008) give indication on reproductive toxicity of a commercial Roundup® formulation and the surfactant formulation MON 0818 (POEA) on reproduction. As stated in Vol 3, chapter B.6.13.3 "(...) Nonetheless, the published findings suggest that offspring development was in fact a particularly sensitive target of Roundup and the POE-tallowamine. The findings in young male rats might indicate impairment of spermatogenesis (...)".

More indications exisits that glyphosate-based products with ethoxylated alkylamines might interfere with the endocrine system of vertebrates (e.g. estrogen synthesis, aromatase activity Soso et al., 2007; Richard et al., 2005). Moreover, it is not clear how glyphosate-based PPP with alkylamine exthoxylated affect the process of amphibian methamorphosis (please refer to the respective chapter B.9.13 10.1).

Therefore, the authorization of glyphosate-based products with alkylamine exthoxylated surfactants might require the generation of further data. The requests should cover the clarification of the effect of POEA on endocrine enpoints (e.g by a fish screening assay) and thyroid mediated processes (e.g. by extended amphibian metamorphosis tests) with representative POEA.

References

Benachour, N. Sipahutar, H. Moslemi, S. Gasnier, C. Travert, C. Seralini, G. E. (2007): Time- and dose-dependent effects of roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology 53, 126-133

Benachour, N., Seralini, G. E. (2009): Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chemical Research in Toxicology 22, 97-105

Dallegrave, E. Mantese, F. D. Coelho, R. S. Pereira, J. D. Dalsenter, P. R. Langeloh, A. (2003): The teratogenic potential of the herbicide glyphosate-Roundup® in Wistar rats. Toxicology letters 142, 45-52

Dallegrave, E. Mantese, F. D. Oliveira, R. T. Andrade, A. J. M. Dalsenter, P. R. Langeloh, A. (2007): Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Archives of Toxicology 81, 665-673

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M. C., Seralini, G. E (2009): Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262/3, 184-191

Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae. Herpetologica 16, 183-190

Hokanson, R., Fudge, R., Chowdhary, R., Busbee, D. (2007): Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Human & Experimental Toxicology 26, 747-752

Knapp, J.F. (2007): A reproduction/developmental toxicity screening study of MON 0818 in rats – Revised final report. WIL Research Labs., Ashland, Ohio, USA, on behalf of Monsanto. Project no. WIL-50282, Sponsor no. WI-2003-103, unpublished; ASB2010-365

Knapp, J.F. (2008): A combined 28-day repeated dose oral (dietary) toxicity study with the reproduction/developmental toxicity screening test of MON 8109 and MON 0818 in rat. WIL Research Labs., Ashland, Ohio, USA, on behalf of Monsanto. Project no. WIL-50337, Sponsor no. WI-2007-013, unpublished; ASB2010-364

Levine SL, Han Z, Liu J, Farmer DR, Papadopoulos V. (2007): Disrupting mitochondrial function with surfactants inhibits MA-10 Leydig cell steroidogenesis. Birds Cell Biol Toxicol. 23/6, 385-400

McDaniel, T.V., Martin, P.A., Struger, J., Sherry, J., Marvin, C.H., McMaster, M.E., Clarence, S., Tetreault, G. (2008): Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) from areas of intensive row crop agriculture. Aquatic Toxicology 88/4, 230-42

Paganelli, A., Gnazzo, V., Acosta, H., Lopez, S.L., Carrasco, A.E. (2010): Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signalling. Chemical Research in Toxicology 23, 1586-1595

Quassinti, L., Maccari, E., Murri, O., Bramucci, M. (2009): Effects of paraquat and glyphosate on steroidogenesis in gonads of the frog *Rana esculenta* in vitro. Pesticide Biochemistry and Physiology 93/2, 91-95

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE. (2005): Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ Health Perspect. 113, 716-20

Romano, M.A., Romano, R.M., Santos, L.D., Wisniewski, P., Campos, D.A., de Souza, P.B., Viau, P., Bernardi, M.M., Nunes, M.T., de Oliviera, C.A. (2012): Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. Archives of Toxicology 86/4, 663-673

Romano, R.M. Romano, M.A. Bernardi, M.M. Furtado, P.V. Oliveira, C.A. (2010): Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Archives of Toxicology 84, 309-317

Soso, A.B., Barcellos, L.J.G., Ranzani-Paiva, M.J., Kreutz, L.C., Quevedo, R.M., Anziliero, D., Lima, M., da Silva, L.B., Ritter. F., Bedin, A.C., Finco, J.A. (2007): Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundia (*Rhamdia quelen*). Environmental Toxicology and Pharmacology 23/3, 308-313

Walsh, L.P. McCormick, C. Martin, C. Stocco, D.M (2000): Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environmental Health Perspectives 108, 769-776

Glyphosate

Annex M-CA 8-02: Endpoint Selection Considerations for Refinement of the Mammalian Risk Assessment

Annex to the Documen M of the technical section[18]:

ECOTOXICOLOGY

[18] Annex to the Doc ID: 110054-MCA7_GRG_Jun_2020

Justification for a refined acute oral toxicity endpoint for mammalian risk assessment

In the Toxicology Section document M-CA Section 5, there are multiple rodent acute oral toxicity studies that have been re-evaluation for this dossier. In the following evaluation, 27 studies out of 39 available studies are considered (with 28 individual endpoints; marked in bold in the table below). Of the 39 studies, 8 studies were considered supportive and 4 studies were not considered (see footnote to **Table 5.2.1-1** in document M-CA Section 5).

For the acute environmental mammal risk assessment, at the screening level of the assessment, the lowest available acute oral endpoint (>2000 mg/kg bw) is considered.

The acute oral rodent toxicity endpoints are presented below (reproduced from document M-CA Section 5).

Table 1: Acute oral toxicity studies for glyphosate acid in rats and mice

Annex Point	Study	Study type	Substance(s)	Status	Result [LD_{50}]
CA 5.2.1/001	2014	*in vivo:* RccHanTM:Wistar rats, ♀ (fixed dose method)	Glyphosate technical (Batch: 04062014, Purity: 85.79 %)	valid, Category 2a	**>2000 mg/kg bw (females)**
CA 5.2.1/002	2011	*in vivo:* RjHan:WI rats, ♀ (up and down procedure)	Glyphosate technical (Batch: 569753(BX20070911), Purity: 96.3 %)	valid, Category 2a	**>5000 mg/kg bw (females)**
CA 5.2.1/003	2010	*in vivo:* CD / Crl:CD(SD) rats, ♀ (ATC method)	Glyphosate technical (Batch: 2009051501, Purity: 96.4 %)	valid[#], Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/004	2010	*in vivo:* CD / Crl:CD(SD) rats, ♀ (ATC method)	Glyphosate technical (Batch: 20090506, Purity: 97.3 %)	valid[#], Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/005	2009	*in vivo:* CD / Crl:CD(SD) rats, ♀ (ATC method)	Glyphosate technical (Batch: 20080801, Purity: 98.8 %)	valid[#], Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/006	2009	*in vivo:* HanRcc: WIST (SPF) rats, ♀ (ATC method)	Glyphosate technical (Batch: GI-1045, Purity: 96.66 %)	valid, Category 2a	**>2000 mg/kg bw (females)**
CA 5.2.1/007	2009	*in vivo:* Sprague-Dawley rats, ♀ (up and down procedure)	Glyphosate tech grade mixed 5-batch (Batch: 080704-1 thru 5, Purity: 96.40 %)	valid, Category 2a	**>5000 mg/kg bw (females)**
CA 5.2.1/008	2008	*in vivo:* Wistar Hannover rats, ♀ (ATC method)	Glyphosate technical (Batch: 20070606, Purity: 98.05 %)	valid, Category 2a	**>2000 mg/kg bw (females)**
CA 5.2.1/009	, 2007	*in vivo:* HanRcc: WIST (SPF) rats, ♀ (up and down procedure)	Glyphosate technical material (Batch: 0507, Purity: 96.1 %)	valid, Category 2a	**>5000 mg/kg bw (females)**
CA 5.2.1/010	2007	*in vivo:* HanRcc: WIST (SPF) rats, ♀ (ATC method)	Glyphosate technical (Batch: 200609062, Purity: 95.1 %)	valid, Category 2a	**>2000 mg/kg bw (females)**
CA 5.2.1/011	, 2005	*in vivo:* Sprague-Dawley derived rats, ♀ (up and down procedure)	Glyphosate acid technical (Batch: 040205, Purity: 97.23 %)	valid, Category 2a	**>5000 mg/kg bw (females)**

Table 1: Acute oral toxicity studies for glyphosate acid in rats and mice

Annex Point	Study	Study type	Substance(s)	Status	Result [LD_{50}]
CA 5.2.1/012	, 1999	*in vivo:* Sprague-Dawley derived, albino rats, ♂ / ♀	NUP5a99 (Batch: Drum Sample E, Purity: 62 %) IPA salt	supportive Category 2a	>5000 mg/kg bw
CA 5.2.1/013	1996	*in vivo:* Alpk:AP_fSD (Wistar-derived) rats, ♂ / ♀	Glyphosate acid, (Batch: P24, Purity: 95.6 %)	valid, Category 2a	**>5000 mg/kg bw**
CA 5.2.1/014	, 1995	*in vivo:* Crj:CD-1(ICR) mice, ♂ / ♀	MON 0139 (Batch: LBRV-11092, Purity: 62.34 %) IPA salt	valid, Category 2a	**>5000 mg/kg bw**
CA 5.2.1/015	, 1995	*in vivo:* Sprague-Dawley (Crj:CD), SPF, albino rats, ♂ / ♀	Glyphosate technical, HR-001 (Batch: 940908-1, Purity: 95.68 %)	valid, Category 2a	**>5000 mg/kg bw**
CA 5.2.1/016	1995	*in vivo:* ICR (Crj:CD-1), SPF mice, ♂ / ♀	Glyphosate technical, HR-001 (Batch: 940908-1, Purity: 95.68 %)	valid, Category 2a	**>5000 mg/kg bw**
CA 5.2.1/017	1995	*in vivo:* rats (limit test)	Glyphosate acid technical (Batch: 1073, Purity: 97.6 %)	valid, Category 2a	**>2000 mg/kg bw**
CA 5.2.1/018	1995	*in vivo:* rats (limit test)	Glyphosate (Batch: 940950, Purity: 62 % IPA)	supportive, Category 2a	>2000 mg/kg bw
CA 5.2.1/019	1994	*in vivo:* rats	Not applicable	valid, Category 4a	Not applicable
CA 5.2.1/020	1994	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate Premix (Batch: 290-JaK-146-4, Purity: 46.1 % (Glyphosate), 62.2 % (IPA salt)	supportive, Category 2a	>5000 mg/kg bw
CA 5.2.1/021	1994	*in vivo:* rats (limit test)	Glyphosate	valid, Category 4a	**>2000 mg/kg bw**
CA 5.2.1/022	1994	*in vivo:* Wistar rats, ♂ / ♀	Glyphosate Technical (Batch: 36300892, Purity: 99.6 %)	valid, Category 2a	**>5000 mg/kg bw**
CA 5.2.1/023	1994	*in vivo:* rats	Glyphosate technical	valid, Category 4a	**>2000 mg/kg bw**
CA 5.2.1/024	1992	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate (Batch: L3258; purity: not specified)	valid, Category 2a	**>2000 mg/kg bw**
CA 5.2.1/025	1991	*in vivo:* Bom:NMRI mice, ♂ / ♀	Glyphosate Technical (PMG) (Batch: 206-JaK-25-1, Purity: 98.6 %)	valid, Category 2a	**>2000 mg/kg bw**
CA 5.2.1/026	, 1991	*in vivo:* Wistar rats, ♂ / ♀	Glyphosate Technical (Batch: 60, Purity: 96.80 %)	valid, Category 2a	**>7500 mg/kg bw**
CA 5.2.1/027	1991	*in vivo:* Swiss albino mice, ♂ / ♀	Glyphosate Technical (Batch: 60, Purity: 96.80 %)	valid, Category 2a	**>7500 mg/kg bw**

Table 1: Acute oral toxicity studies for glyphosate acid in rats and mice

Annex Point	Study	Study type	Substance(s)	Status	Result [LD_{50}]
CA 5.2.1/028	, 1990	*in vivo:* CD rats, ♂ / ♀	Glyphosate Technical (Batch: 0190 A, Purity: 98.1 %)	valid, Category 2a	**>8000 mg/kg bw**
CA 5.2.1/029	, 1989	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate Technical (PMG) (Batch: 206-JaK-25-1, Purity: 98.6 %)	supportive, Category 2a	>5000 mg/kg bw
CA 5.2.1/030	1989	*in vivo:* rats	Glyphosate technical (IPA salt 62 %)	valid, Category 4a	**>2000 mg/kg bw**
CA 5.2.1/031	1988	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate (Batch: XLI-55, Purity: 97.76 %)	valid, Category 2a	**>5000 mg/kg bw**
CA 5.2.1/032	1987	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate (MON8750) (Batch: XLG-255, Purity: 90.8 %, ammonium salt)	valid, Category 2a	**5904 mg/kg bw (males) >2222 mg/kg bw (females)**
CA 5.2.1/033	1987	*in vivo:* Sprague-Dawley rats	MON8722	valid, Category 4a	**4613 mg/kg bw**
CA 5.2.1/034	, 1987	*in vivo:* mice	SN750721 (Purity: 64 %) IPA salt	valid, Category 4a	**4373 mg/kg bw**
CA 5.2.1/035	1987	*in vivo:* mice	SN750721 (Purity: 41 %) IPA salt	valid, Category 4a	**3669 mg/kg bw**
CA 5.2.1/036	1983	*in vivo:* Kasauli mice, ♂ / ♀	Glyphosate Technical (Batch: R&D sample (9-7-83), Purity: 95 %)	supportive, Category 3a	4000 mg/kg bw
CA 5.2.1/037	1983	*in vivo:* rats	Glyphosate (tech.)	supportive, Category 4a	Not applicable
CA 5.2.1/038	, 1981	*in vivo:* Sprague-Dawley (Crl:CD® (SD)BR) rats, ♂ / ♀	Glyphosate (MON 0139) (Batch: SSRT-11012, Purity: 65%) IPA salt	supportive, Category 2a	>5000 mg/kg bw
CA 5.2.1/039	1979	*in vivo:* Wistar rats, ♂ / ♀	Glyphosate technical (Batch: XHI-180, Purity: 99 %)	supportive, Category 2a	5600 mg/kg bw

This study was performed at the Laboratory of Pharmacology and Toxicology (LPT) in Hamburg, Germany

From the toxicology Section (document M-CA Section 5.2) a range of oral gavage dosing vehicles were used across the oral acute studies, yet most were dosed using water as the dosing vehicle. Other dosing vehicles, included peanut oil, CMC and arachis oil. However, despite the differences in the dosing vehicles, the endpoints achieved are similar with all endpoints being unbounded and >5000 mg/kg bw.

Where clinical sub-lethal effects were observed in the acute studies, they were similar in nature and extent. In all cases the observed clinical effects were transient, and all animals appeared normal at the end of the study - for both species tested.

As the endpoint required for use in the acute mammalian risk assessment is an acute lethality endpoint, transient symptomology observed in these studies is not relevant to an acute wild mammal risk assessment, especially where considering that it was not sustained for the duration of the studies.

Therefore, it is considered relevant and appropriate, to fully consider all of the available information when

selecting an endpoint for use at the refinement step of the risk assessment. In accordance with the EFSA (2009) guidance document, Section 2.4.2, a geometric mean endpoint has been calculated by firstly considering endpoints according to species (mouse or rat), which have then been combined to give an overall geometric mean endpoint.

The endpoints used in the geometric mean calculation are presented in bold in the above table.
For the 21 rat acute oral studies, the overall geometric mean acute endpoint value was determined to be 3578.9 mg/kg bw.

For the six mouse acute oral studies, the overall geometric mean acute endpoint value was determined to be 3809.4 mg/kg bw.

When combined, the overall geometric mean value is **3694.1 mg/kg bw.** This value will be used in the refined acute mammalian risk assessment.

Additional points concerning the equivalence of the acute rodent study test design.
In the information below, a comparison of the available rat and mouse acute oral toxicology studies conducted using glyphosate is presented. This aimed to demonstrate equivalence in the study designs used to generate the acute oral gavage toxicity endpoints, and to enable grouping of the endpoints to generate a geometric mean endpoint for use in the risk assessment. Compared elements included;

- Test guideline used
- Test design
- Animal strain,
- Numbers of animals used per group,
- Influence of dosing vehicle on the result
- Nature and duration of the clinical observations
- Endpoints

Based on the tabulated comparison presented for each of the studies, it was clear that the acute mammalian toxicity endpoints were essentially achieved in studies performed using equivalent test designs, with all achieved acute rat and mouse study endpoints being at the limit dose tested or higher.

Mammalian reproductive Endpoint Refinement Considerations

In the Final Addendum to the glyphosate RAR (Volume 3, Annex B.9; 31 March 2015), the RMS proposed an overall NOAEL of 50 mg/kg bw/d (from Brooker et al. (1991) for maternal and developmental effects – rabbit developmental toxicity study) to be considered for use in the mammalian long-term reproductive risk assessment. This endpoint was selected from all available developmental toxicity studies performed in rabbit (lagomorph) dosed via the oral gavage route rather than via the dietary exposure route, the expected exposure route in the field.

However, on analysis of these data - presented in detail below, the selection of this endpoint is considered overly-conservative, due to dose spacing in Brooker et al. (1991) study, as there are higher NOAELs in other studies that fall below the lowest LOAEL (considering all the available rabbit developmental toxicity data). A more appropriate approach to selecting the NOAEL for use in the risk assessment is to consider all available rabbit developmental toxicity study data together, as if derived in a single study, and then, from the larger dataset, to select the highest NOAEL value that falls below all LOAEL values. This approach for handling data from several studies is detailed in section 2.4.3 of the EFSA Guidance on Risk Assessment for Birds and Mammals (2009). Based on this procedure, refined endpoints from rabbit developmental toxicology studies are considered as follows;

For maternal effects;
- the lowest LOAEL value is **150 mg/kg bw/d** (., 1991)
- the highest NOAEL value below the lowest LOAEL is **100 mg/kg bw/d**

- achieved in two studies, (1995) and (1996)

For developmental or offspring effects;

- the lowest LOAEL value is **200 mg/kg bw/d (** , 1996)
- the highest NOAEL value below the lowest LOAEL is **175 mg/kg bw/d (** , 1996)

a refined NOAEL of 100 mg/kg bw/d and a refined LOAEL of 150 mg/kg bw/d are derived for the most sensitive test species rabbit.

The refined endpoint value still represents a conservative estimate of expected NOAEL and LOAEL levels relevant for consideration in the mammalian long-term reproductive risk assessment. Whilst the developmental toxicity data informs about human risk assessment, for the higher tier refinement of the mammalian reproductive risk assessment – from an ecotoxicological perspective – more representative exposure data should be considered, that excludes dosing via the oral gavage route. The latter exposure route is not considered representative of dietary exposure expected in the field that is addressed in an ecotoxicological risk assessment. The use of gavage dosing can result in high systemic levels that may induce adverse findings that cannot be reproduced when equivalent doses (in mg/kg bw/d) are given via the diet (see EFSA Guidance on Risk Assessment for Birds and Mammals (2009), section 2.3). In contrast to the oral gavage route of exposure, dietary exposure considers the opportunity for uptake and toxicokinetic processes (absorption, distribution, metabolism and excretion) to take place in a more realistic and gradual manner. Evidence of lower absorption of glyphosate was observed in an ADME (absorption, distribution, metabolism and excretion) study by (1973, TOX9552355; Final Addendum to the glyphosate RAR Volume 1, chapter 2.6.2; page 41; 31 March 2015) when results were compared with those achieved in ADME studies dosed using the standard oral gavage procedure.

Furthermore, these ADME studies also demonstrate rapid excretion of glyphosate from the body in urine (absorbed glyphosate) and predominantly in faeces (unabsorbed glyphosate), with no evidence of accumulation of glyphosate in mammals (Final Addendum to the glyphosate RAR Volume 1, chapter 2.6.2; pages 37-41; 31 March 2015).

A further position on the relevance of rabbit developmental toxicity studies to risk assessment is also presented in the Toxicology Section (document M-CA Section 5).

Further Mammalian Endpoint Refinement Considerations

The EFSA Guidance on Risk Assessment for Birds and Mammals (2009) recommends the use of an ecotoxicologically relevant endpoint for the higher tier risk assessment. The decision on ecotoxicological relevance is a case-by-case decision which is not only dependent on the already addressed relevance of exposure route and absorption, but also by consideration of the mammalian species for which acceptable risk could not be shown in Screening and Tier 1 steps.

As an acceptable long-term risk for lagomorphs like the rabbit, can already be demonstrated using the most conservative NOAEL of 50 mg/kg bw/d at the Screening and Tier 1 steps, it should be considered that a higher tier risk assessment for the protection of wild mammals, as being only required for rodents.

When deriving a higher tier endpoint for rodents, the available dataset on rodents also provides information from more extended study designs, such a 2-generation rat reproduction studies, compared to developmental studies – which are considered not to fully inform on relevant endpoints for the survival of wild mammal populations. It is therefore relevant to consider additional effects data on parameters such as pup development, exposure via lactation, reproductive success of offspring, which are considered in 2-generation rat reproduction studies, when deriving a higher tier endpoint for use in the risk assessment.

Based on the review of all available information on maternal/adult toxicity and developmental, reproductive and offspring effects of glyphosate on rodents an overall reproductive NOAEL for rats of 300 mg a.s./kg bw/d (as stated in the EU list of endpoints for Glyphosate, toxicology section, page 12) is considered relevant for use in the higher tier refinement of the long-term mammalian risk assessment.

There is a large toxicology dataset available from which endpoints may be selected for use in the mammalian ecotoxicological risk assessment. Since the last renewal, all toxicology studies from the existing toxicological dataset have undergone a re-evaluation to determine their relevance and reliability for use in risk assessment. This available list of toxicology endpoints is presented in the Toxicology Section B5 of the dossier.

For the chronic mammalian risk assessment, endpoints that inform on long term effects on reproduction developmental and / or maternal effects are considered relevant to the risk assessment. Most notably has been the use of endpoints from rabbit developmental toxicology studies. There are also rodent multi-generational reproduction studies available within the toxicological dataset, that may also be considered. In accordance with the EFSA (2009) bird and mammal guidance document, typically the most sensitive endpoint has been selected for use in the risk assessment.

In the rabbit developmental toxicology studies, growth and development of pregnant dams / rabbits from conception through to off-spring delivery is monitored, with both maternal and developmental endpoints recorded. In the rodent multi-generational studies, rodents (rats and mice) are exposed over multiple generations, with exposure via the diet with their growth, development and reproductive success monitored.

In the toxicology Section (document M-CA Section 5), there is a position on the relevance of the rabbit developmental toxicity study to risk assessment. There is strong evidence that maternal toxicities noted in these studies dosed via oral gavage are related to general gastro-intestinal disturbance (to which rabbits are especially sensitive, as noted above), rather than systemic toxicity following repeated exposure (– as is the case in multi-generation studies with rats or mice), is the complete absence of systemic toxicity noted in three repeat-dose dermal toxicity assays up to equivalent systemic exposures in the same species (document M-CA Section 5). Reported gross necropsy observations, organ weights, organ pathology, hematology and clinical chemistry in these repeat-dose studies confirm an absence of specific target organ toxicity following repeated exposure in rabbits. An important consideration is that glyphosate is essentially unmetabolized in mammals and therefore systemic doses provide the opportunity to evaluate for specific target organ toxicity following repeated exposure, irrespective of the route of exposure.

Further details on the relevance of the rabbit developmental toxicity study for use in risk assessment is presented in the toxicology section of the dossier.

Given the uncertainty associated with the use of the rabbit endpoints in risk assessment, three positions on endpoint selection are presented here.

The first position considers chronic endpoints from six rabbit development toxicology studies. The second position considers endpoints from seven rat developmental studies and the third position considers endpoints from nine multi-generational studies performed using rats.

Rabbit Developmental Toxicity Endpoints
There are six rabbit developmental toxicity studies considered in the following endpoint selection evaluation.

The previous RAR (2015) concluded the most relevant chronic endpoint for use in risk assessment was the NOAEL of 50 mg/kg bw/d (1991). This is considered overly conservative and is a function of large spacing factors between consecutive doses in this study. By comparing all the available rabbit developmental toxicology studies ‘side-by-side’ it is possible to determine the most relevant endpoint for use in risk assessment (NOAEL). This is the approach as stated in section 2.4 of the EFSA (2009) guidance document.

Further details on the studies (study summaries) and their relevance for use in risk assessment is presented in the Toxicology Section (document M-CA Section 5).

Table 2: Endpoints from developmental studies with rabbits exposed by oral gavage

Strain	Dose levels (mg/kg bw/d)	Developmental NOEL (mg/kg bw/d)	Developmental LOAEL (mg/kg bw/d)	Maternal NOEL (mg/kg bw/d)*	Maternal LOAEL (mg/kg bw/d	Reference
NZW	50 200 400	**50****	**200**	**50***	200	1996 RAR B.5.6.11/02
NZW	50 150 450	**150****	450	**50***	**150**	1991[1] IIA, 5.6.11/05
NZW	75 175 350	350	>350	**75***	175	1980 IIA, 5.6.11/04
NZW	100 175 300	**175****	300	**100***	175	1996 RAR B.5.6.11/03
Japanese White rabbits Kbl:JW, SPF	10 100 300	300	>300	**100***	300	1995 RAR B.5.6.11/01
NZW	125 250 500	250	500	250	500	1989[1] IIA, 5.6.11/07

[1] Glyphosate Monograph B.5
* Highlighted maternal NOEL values are all below the lowest LOAEL of 150 mg/kg bw/d, in the (1991) study
** Highlighted developmental NOEL values are all below the lowest LOAEL of 200 mg/kg bw/d, in the (1996) study

For maternal effects;

- the lowest LOAEL value is **150 mg/kg bw/d (** 1991)
- the highest NOAEL value below the lowest LOAEL is **100 mg/kg bw/d**
  - achieved in two studies, (1995) and (1996)

For developmental or offspring effects;

- the lowest LOAEL value is **200 mg/kg bw/d (** , 1996).
- the highest NOAEL value below the lowest LOAEL is **175 mg/kg bw/d (** 1996)

The endpoint NOAEL value of **100 mg/kg bw/d** based on maternal effects, is considered the highest relevant NOAEL from the rabbit developmental toxicology studies to be used in the chronic mammalian risk assessment.

A further observation of this data is that despite maternal effects observed at 150 mg/kg bw/d, there were no developmental effects observed at the same rate. Developmental effects based on all available data occurred at 200 mg/kg bw/d.

The nature and severity of the clinical observations in the rabbit studies are considered relevant to understanding what the impact of maternal and / or developmental effects would be at the population level. To evaluate this, the protection goals from the EFSA (2009) guidance are considered.

The 'surrogate' protection goals at the 1st tier of the chronic mammalian assessment indicates that *'..surrogate protection goal of making mortality or reproductive effects unlikely.'*

Based on the observed effects in the rabbit studies, the individual mortality (maternal or offspring) and effects that lead to effects on reproduction / recruitment into subsequent generation, such as maternal implantation losses, reduced offspring numbers and non-viable offspring. Both of these effects would have an impact on both abundance and diversity.

In the studies by (1996) and . (1991), the maternal LOAEL and the developmental NOAEL occur at the same dose level. The maternal effects observed in these studies were related to appetite loss, reduced bodyweight gain and soft stools or liquid faeces. The clinical observations are not thought to be due to systemic exposure as discussed in the Toxicology Section B5. The effects are considered due to gastro-intestinal tract irritation caused by the dosing route / test design used.

In these two studies, at the maternal LOAEL, there were no mortalities (maternal nor offspring) and there were no developmental effects. In the RAR (2015) it was stated that the 50 mg/kg bw/day endpoint was selected, to be protective of implantation losses seen at higher doses. From the current dataset, implantation losses were first observed at the 200 mg/kg bw/d dose rate in the (1996) study.

Considering the 'actual' protection goal from the EFSA (2009) applicable at the refinement step of the risk assessment, EFSA states *'...no visible mortality or long-term repercussions on abundance and diversity.'*

Based on the available data and considering the protections goals as stated in the EFSA (2009) guidance, an endpoint NOAEL of 150 mg/kg bw/day is considered protective of both maternal and developmental effects at the population level for wild mammals.

Rat Developmental Toxicity Endpoints
There are seven rat developmental toxicity studies considered in the following endpoint selection evaluation.

By comparing all the available rat developmental toxicology studies 'side-by-side' it is possible to determine the most relevant endpoint for use in risk assessment (NOAEL). This is the approach as stated in section 2.4 of the EFSA (2009) guidance document. This is presented in the next Table, and graphically in the following figure.

Further details on the studies (study summaries) is presented in the Toxicology Section (document M-CA Section 5) of this dossier.

Table 3: Endpoints from developmental studies with rat exposed by oral gavage

Strain	Dose levels (mg/kg bw/d)	Developmental NOEL (mg/kg bw/d)	Developmental LOAEL (mg/kg bw/d)	Maternal NOEL (mg/kg bw/d)*	Maternal LOAEL (mg/kg bw/d	Reference
Sprague-Dawley	0 250 500 1000	1000	No effects	1000	No effects	, 1996 CA 5.6.2/001
Sprague-Dawley	0 30 300 1000	1000	No effects	300	Slightly loose stool	1995 CA 5.6.2/002
Sprague-Dawley	0 300 1000 3500	300	3500	300	3500	1991 CA 5.6.2/003
Wistar	0 1000	1000	No effects	1000	No effects	, 1991 CA 5.6.2/004 & 5.6.2/005

Strain	Dose levels (mg/kg bw/d)	Developmental NOEL (mg/kg bw/d)	Developmental LOAEL (mg/kg bw/d)	Maternal NOEL (mg/kg bw/d)*	Maternal LOAEL (mg/kg bw/d	Reference
Wistar	0 100 500	500	No effects	500	No effects	, 1986 CA 5.6.2/006
CFY	0 22 103 544	544	No effects	544	No effects	Anonymous, 1981 CA 5.6.2/007
CD	0 300 1000 3500	1000	3500	1000	3500	, 1980 CA 5.6.2/008

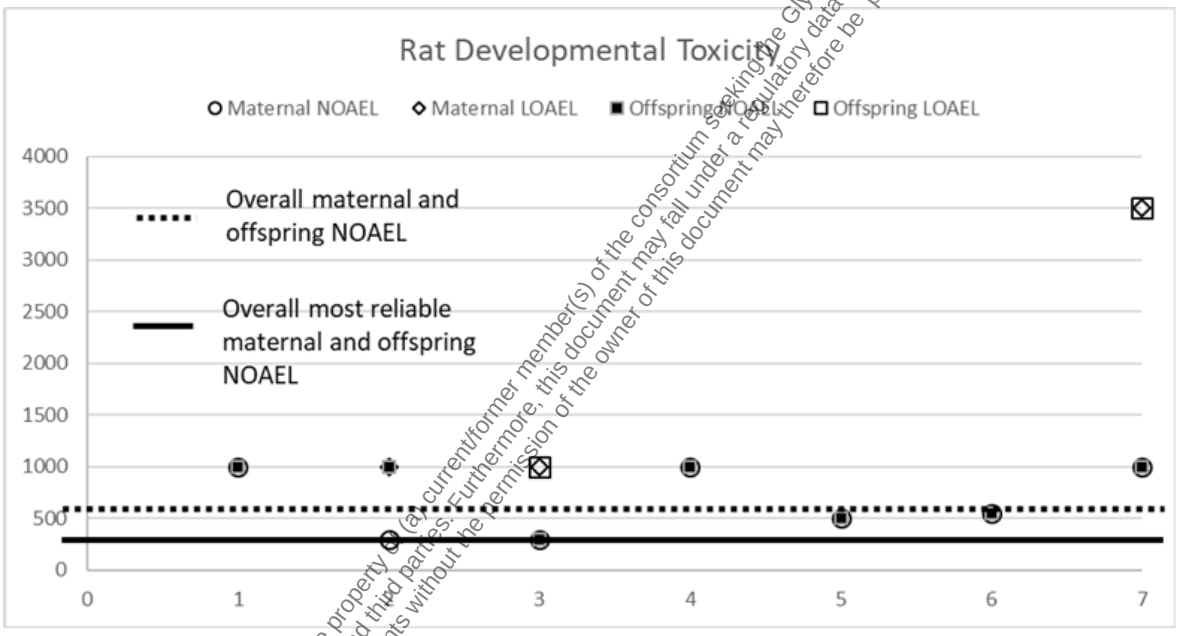


Figure M-CA 8-02-01: Rat developmental toxicology endpoints comapring maternal andoffspring NOAEL and LOAEL values. The numbers of the x-axis relate directly to the study numbers presented in the Table above. The y-axis is endpoint value in mg/kg bw/d.

For maternal effects;

- the lowest LOAEL value is **3500 mg/kg bw/d** (., 1991 and , 1980).
- the highest NOAEL value below the lowest LOAEL is **1000 mg/kg bw/d**
  - achieved in three studies, (1996), (1980) and (1991)

For developmental or offspring effects;

- the lowest LOAEL value is **3500 mg/kg bw/d** (Brooker et al., 1991 and , 1980)
- the highest NOAEL value below the lowest LOAEL is **1000 mg/kg bw/d**
  - achieved in four studies, (1996), a (1995), (1991) and (1980)

In the toxicology section B5.6, further evaluation of the study findings are presented. Overall, the available studies on developmental toxicity in rats consistently revealed that *in utero* exposure to glyphosate did not

result in teratogenicity in rats. If observed, test substance-related effects, including maternal toxicity and developmental effects occur at 1000 mg/kg bw/day. Thus, based on the available data a NOEL of 300 mg/kg bw/day was derived for both maternal and developmental toxicity in rats.

Rodent Multi-generational Toxicology study Endpoints.
Developmental toxicity data generated in the laboratory also do not fully inform on relevant endpoints for the survival of wild mammal populations, such as pup development, exposure via lactation, reproductive success of offspring. These endpoints are considered more adequately addressed via more extended study designs, such as the 2-3 generation rat reproduction studies, with dosing via the dietary route.

In the Toxicology Section (document M-CA Section 5), there are nine relevant and reliable rat multi-generation studies that are considered relevant to the risk assessment. Full details of the studies (study summaries) are presented in the Toxicology Section of the dossier.

Long-term exposure studies using a dietary route of exposure and that have a reproductive element, are placed to reflect the typical exposure route and likely effects, expected for wild mammals in the field compared to oral gavage exposure studies.

Therefore, in addition to the refined approach on endpoint selection presented above for the rabbit an alternative endpoint selection approach based on the available rat multi-generational data is presented.

Selection of such an endpoint from a multi-generation study is still considered very conservative, as dietary exposure is maintained throughout the study duration at an artificially high and continuous level of dietary residues, with no alternate food choice, compared to a varied dietary component choice (residue dilution) expected in the field.

Furthermore, multi-generation studies create a more comprehensive set of endpoints that evaluates effects relevant at the community and population level compared to developmental studies. Available endpoints achieved in multi-generation reproduction study using rats are therefore relevant to the assessment, as effects on reproduction, pup development, exposure via lactation and reproductive success of offspring (all endpoints considered relevant at the population success level) are included.

A further point to note – which is relevant to the metabolism and excretion routes of glyphosate from the body, dietary versus the oral gavage route of exposure (as in developmental studies) considers the opportunity for absorption, distribution, metabolism and excretion (ADME) to take place in a more realistic and gradual manner, which completely contrasts with the expectations from a single high dose approach as used in oral gavage studies.

There are nine multi-generational reproduction studies available, listed in the Table below.

Table 4: Multi- Generational Rreproduction Studies in Rat

Study type	Strain	Dose levels (mg/kg diet)	Reproductive effects NOEL	Parental & offspring toxicity NOEL	Reference
2-generation feeding	Sprague-Dawley Crl:CD (SD) IGS BR	0 1500 5000 15000	5000 mg/kg diet 351 mg/kg/bw/d	5000 mg/kg diet 351 mg/kg/bw/d	2007 IIA, 5.6.1/01
2-generation feeding	Alpk:AP$_f$SD	0 1000 3000 10000	3000 mg/kg diet 293 mg/kg bw/d	3000 mg/kg diet 293 mg/kg bw/d	2000 IIA, 5.6.1/02
2-generation feeding	Sprague-Dawley; Crj:CD (SD)	0 1200 6000	30000 mg/kg bw/d >2000 mg/kg	6000 mg/kg diet 417 mg/kg	1997 IIA, 5.6.1/03

Study type	Strain	Dose levels (mg/kg diet)	Reproductive effects NOEL	Parental & offspring toxicity NOEL	Reference
		30000	bw/d	bw/d	
2-generation feeding	Wistar	0 100 1000 10000	10000 mg/kg diet 700-800 mg/kg bw/d	10000 mg/kg diet 700-800 mg/kg bw/d	, 1993[1,2] IIA, 5.6.1/04
2-generation feeding	Sprague-Dawley	0 1000 3000 10000	10000 mg/kg diet 668 mg/kg bw/d	3000 mg/kg diet 197 mg/kg bw/d	1992[1] IIA, 5.6.1/06
2-generation feeding	Sprague-Dawley	0 2000 10000 30000	10000 mg/kg diet 722 (M)/757 (F) mg/kg bw/d	10000 mg/kg diet 722 (M)/757 (F) mg/kg bw/d	1990[1] IIA, 5.6.1/07
3-generation feed	Wistar	0 75 150 300	300 mg/kg bw/d	300 mg/kg bw/d	, 1988a CA 5.6.1/011
1-generation feed	Wistar	0 5 10	10 mg/kg bw/d	10 mg/kg bw/d	1988b CA 5.6.1/012
3-generation feed	Sprague-Dawley	0 3 10 30	30 mg/kg bw/d	30 mg/kg bw/d	198 CA 5.6.1/014

[1] Glyphosate Renewal Assessment Report
[2] Study considered supplementary

From the Toxicology section, a comparison of the achieved endpoints in the multi-generational studies has been conducted.

Table 5: Rat Mulit-generational endpoints for consideration in risk assessment

Study No.	Reference	Offspring NOAEL [mg/kg bw/day]	Offspring LOAEL [mg/kg bw/day]
1	2007	351	1000
2	2000	322	1063
3	, 1997	417	2150
4	, 1993	700	-
5	1992	668	-
6	, 1990	666	1983
7	1988a	15	-
8	1988b	10	-
9	1981	30	-

It is again possible to compare the NOAEL with the LOAEL values and to determine the highest NOAEL below the lowest LOAEL. From the table, the lowest LOAEL for offspring effects was 1000 mg/kg bw/d (2007), whilst the highest NOAEL below the lowest LOAEL was 700 mg/kg bw/d, achieved in the (1993) study.

For those studies where there is no LOAEL value, the offspring NOAEL was achieved at the highest dose tested in the study.

This comparison is presented graphically in the next figure.

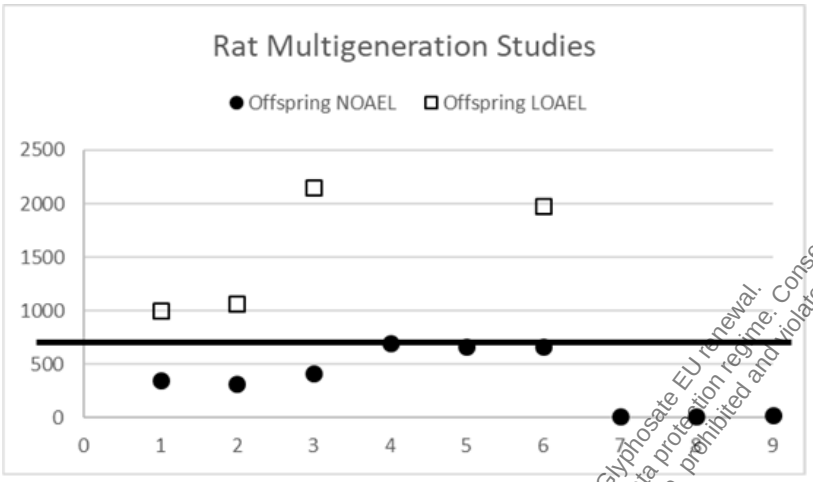


Figure 2: Rat multigeneration endpoints comapring offspring NOAEL and LOAEL. The numbers of the x-axis relate directly to the study numbers presented in the Table above. The y-axis is endpoint value in mg/kg bw/d

The availability of a large number of relevant multi-generational studies with rats (9) and developmental toxicity studies with rats (7) and the multiple rabbit developmental toxicity studies (6) is considered to reduce the uncertainty associated with endpoint selection / extrapolation to wild rodent species in the field.

TER trigger values are established to address the uncertainty associated with extrapolation of effects observed in model test species in the laboratory to effects at the field level. Where multiple toxicity studies are available for the same (or similar) study type / organism, the uncertainty associated with the selected endpoint may be reduced and a lower trigger value is considered a relevant option. However, through pragmatic selection of an appropriate endpoint, an acceptable risk assessment is achievable in all cases.

Overall assessment of data for derivation of an ecotoxicologically relevant endpoint for the higher-tier refinement of the mammalian reproductive risk assessment

Refinement of the endpoint for the higher-tier risk assessment is only necessary where achieved TER values based on the Tier 1 exposure assessment are below the trigger value (5) for reproductive risk.

Therefore, for the higher tier risk assessment, an ecotoxicologically relevant endpoint refinement considering the available rabbit developmental toxicological endpoints is presented achieving a refinement endpoint **NOAEL of 100 mg/kg bw/d**. A further position is presented for the rabbit developmental toxicology endpoints that considers the nature oand severity of the achieved endpoints in those studies within the context of the EFSA surrogate and actual protection goals.

Where the use of the rabbit developmental toxicology study is not considered relevant to the wild mammal risk assessment - based on the positioning presented in the Toxicology (B5) section of the dossier, two alternate endpoint approaches are also considered.

The first considers the available rat developmental toxicology endpoints and achieves an endpoint **NOAEL of 300 mg/kg bw/d**, as being relevant for use in the risk assessment.
The second considers a more appropriate route of exposure via the diet, considering the multi-generational rodent study endpoints, which achieves a **NOAEL of 700 mg/kg bw/d.**

The available information on maternal / adult toxicity and developmental, reproductive and offspring effects of glyphosate on rodents were reviewed and an overall reproductive NOAEL values based on the

available study types were proposed. The approach taken and the achieved endpoints are considered appropriate and relevant for use in the higher tier refinement of the long-term mammalian risk assessment.

In terms of the order in which these endpoints should be used. The screening level assessment is based on current chronic mammal endpoint of 50 mg/kg bw/d as presented in the ESFA (2015) conclusion report. This ensures that all relevant exposure scenarios are considered at Tier I. At the refinement step of the Tier I assessment, the revised endpoints of 100 and 300 mg/kg bw/d should be considered. The endpoint achieved for the multi-generational rodent studies should be considered as being supportive of a lack of effects when considering appropriate exposure via the diet in the field.