Document Title

Tier 2 Summary
of Fate and Behaviour in the Environment
for the active substance Fenhexamid (KBR 2738)
(Specification No.: 102000006806)

Substance(s)

FENHEXAMID
(Annex I renewal)

Data Requirements

Regulation EC/1141/2010
on the renewal of the inclusion of AIR2 active substances
in conjunction with
Directive 91/414/EEC and Regulation EC/1107/2009

According to OECD format guidance for industry data submissions
(SANCO/10387/2010 rev. 8 - on the renewal of active substances included in Annex I)

Annex II
Document M
Section 5, Point 7

Date

2012-01-31

Author(s)

[redacted]

Bayer CropScience

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer CropScience. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

TABLE OF CONTENTS

IIA 7 Fate and Behaviour in the Environment

Introduction

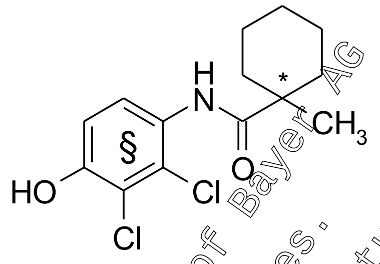
Additional information is provided in this document on the fate and behaviour of the fungicidal active substance fenhexamid (KBR 2738) in soil and water using the ^{14}C-cyclohexane label besides the ^{14}C-phenyl label of fenhexamid. In case where the new studies were not specifically labeled, ^{14}C-phenyl label was used for fenhexamid. The active substance belongs to the chemical class of hydroxyanilides for use on fruits and vegetables in Europe and other regions.

The chemical structures and report names of metabolites are given in the List of Metabolites as part of document N of this submission.

Physico-Chemical Properties of KBR 2738:

Parameter	Values
Molecular Weight	302.2 g/mol
Molecular Formula	C14 H17 Cl2 N O2
Water solubility (at 20°C)	pH 5 → 14 mg/L pH 7 → 24 mg/L pH 9 → 412 mg/L
Vapour pressure	25°C → 9 x 10^{-9} hPa
Henry's Law Constant	20°C → 9 x 10E-6 Pa m^3/mol (pH 5), 5 x 10E-6 Pa m^3/mol (pH 7), 3 x 10E-7 Pa m^3/mol (pH 9)
UV absorption (peak maxima)	204, 206, 237, 313 nm
pK_a (ambient temperature, pH 2-11)	7.3
Partition coefficient oct.-wat. P_{ow} / log P_{ow} (20°C)	pH 4 → 3.62 pH 7 → 3.51 pH 9 → 2.23
Relative Density (20°C)	1.34 g/mL
Melting Point	153°C
Stability of compound at room temperature	Stable

The positions of radiolabel are illustrated in the structure below:



Positions of radiolabel:

*: [cyclohexyl-1-^{14}C]KBR 2738

§: [phenyl-UL-^{14}C]KBR 2738

Route of degradation in aerobic soil

The degradation of [cyclohexyl-1-^{14}C]KBR 2738 was investigated under aerobic conditions at 20 °C and a soil moisture of 55% of MWHC[1] in four soils by incubation in the dark for 120 days in maximum.

One metabolite, a [C-C]biphenyl-KBR 2738 with BayerCropScience code BCS-CQ88719 (M24) was identified as a major compound formed in a range from 4.1-8.8% AR in maximum during 120 days of incubation (Table 7-1). A total of eight other minor components were observed in the course of the study, none of them accounting for more than 5.0% AR for an individual component. The total of other unidentified components which were not quantified individually amounted up to 14.9% in maximum.

A second transformation product which was frequently detected was tentatively identified as a [C-O-C] dimer of KBR 2738 (M24) with BayerCropScience code BCS-CQ88720 via HPLC-co-chromatography and occurred with maximum amounts up to 5.0% of AR.

The microbial conversion of KBR 2738 under aerobic conditions in soil thus includes the following steps:

(1) Formation of the major metabolite [C-C]biphenyl-KBR 2738

(2) Formation of non-extractable residues and

(3) Mineralization (formation of CO_2).

---

[1] Maximum water holding capacity

High amounts of non-extractable residues were found which reached their maximum values after 14 or 21 days of incubation (max. 67.0% of AR). The amounts declined then slowly and continuously up to the end of the study.

Experiments with sterilized soil samples showed no mineralization (< 0.1%) over eight days. Despite that, the same general metabolite pattern was observed in the sterilized as in the non-sterilised samples. These results indicate that the metabolism of fenhexamid, and in particular the formation of bound residues, are partly abiotic processes which are however enhanced by the activity of soil microorganisms.

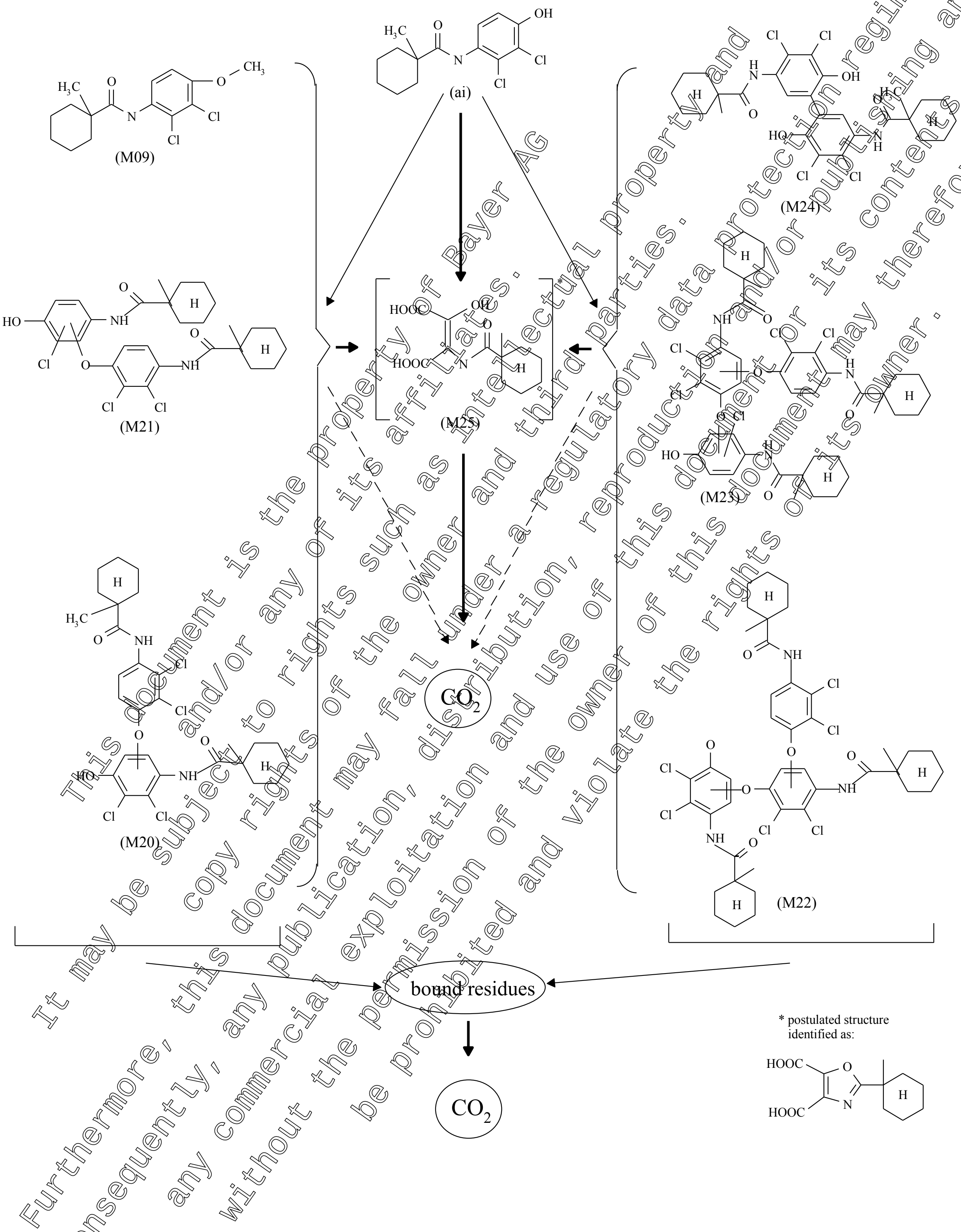
Table 7-1: Occurrence of [C-C]-dimer of KBR 2738 (BCS-CQ88719 (M24)) metabolite in four EU soils under aerobic laboratory conditions

Compound	Soil	Maximum occurrence (% AR)	Maximum occurrence at day	Occurrence at study end (% AR)
BCS-CQ88719:	[redacted]	8.8	1	1.7
	[redacted]	7.6	1	1.1
	[redacted]	4.7	1	1.4
	[redacted]	4.1	3	0.4

All degradation products observed were of transient nature which is indicated by the high amounts of CO_2.

When compared to the results of the old aerobic soil degradation study (with phenyl moiety radiolabel for fenhexamid), no significant changes were observed with the exception that the [C-C]biphenyl-KBR 2738 was observed in higher amounts in the new stud. Therefore this metabolite was then considered as a major metabolite of fenhexamid. However, the proposed pathway of fenhexamid has not changed and is shown in Figure 7-1.

Figure 7-1: Proposed route of degradation of KBR 2738 in aerobic soil



* postulated structure identified as:

Rate of degradation in aerobic soil

The route and rate of degradation in aerobic soil was investigated under defined conditions in the laboratory after application of [cyclohexyl-1-^{14}C]-labelled KBR 2738 (new study) and [phenyl-UL-^{14}C]-labelled KBR 2738 (old study) to four European each reflecting a range of soil types with respect to their texture, organic carbon content and pH.

The investigations resulted in the detection of metabolite BCS-CQ88719 (M24), a [C-C]biphenyl-KBR 2738 at a significant level to be addressed as compound in environmental risk assessment. This finding resulted in additional rate of degradation test with ^{14}C-labelled BCS-CQ88719 (M24) applied to four European soils.

The laboratory tests performed on route and rate of degradation in aerobic soil were kinetically evaluated following the criteria as set out by FOCUS guidance. The evaluation resulted in reliable and robust data sets for aerobic half-life and DT$_{90}$ of degradation in soil for comparison with trigger endpoints and input parameters for use in environmental modelling.

For comparison with EU trigger endpoints in soil, the data sets derived are summarized for the parent compound KBR 2738 and metabolite BCS-CQ-88719 (M24) in Tables 7-2 to 7-3, respectively.

Table 7-2: Best-fit DT$_{50}$- and DT$_{90}$-values for parent compound KBR 2738 in 8 soils under aerobic laboratory conditions for comparison with EU trigger endpoints

Soil, Test Temperature	**Label**	**DT$_{50}$ (days)**	**DT$_{90}$ (days)**	**Model**
[redacted]	1	0.16	1.37	FOMC
[redacted]	1	0.21	1.84	FOMC
[redacted]	1	0.08	1.19	FOMC
[redacted]	1	0.76	2.52	SFO
[redacted]	2	0.12	1.37	**FOMC**
[redacted]	2	0.27	0.96	**SFO**
[redacted]	2	1.06	9.93	**SFO**
[redacted]	2	0.41	1.63	**SFO**
[redacted]		0.27		

Label 1 = cyclohexyl-1-^{14}C, Label 2 = phenyl-UL-^{14}C

Table 7-3: Best-fit DT$_{50}$- and DT$_{90}$-values for metabolite BCS-CQ88719 (M24) in four soils under aerobic laboratory conditions for comparison with EU trigger endpoints

Soil, Test Temperature	**DT$_{50}$ (days)**	**DT$_{90}$ (days)**	**Model**
[redacted]	5.84	130.73	**FOMC**
[redacted]	4.33	85.73	**FOMC**
[redacted]	1.18	> 1000	**FOMC**
[redacted]	22.74	231.12	**FOMC**
[redacted]	5.10		

For the parent compound KBR 2738 a total set of eight DT_{50}-values was obtained to range from 0.08 days in soil [redacted] to a maximum of 1.06 days in soil [redacted]. Values of DT_{90} were calculated to range from 0.96 days in soil [redacted] to 9.93 days in soil [redacted].

For metabolite BCS-CQ88719 (M24) evaluations resulted in a total set of four DT_{50}-values ranging from 1.18 days in soil [redacted] to a maximum of 22.74 days in soil [redacted]. Values of DT_{90} were calculated to range from 85.73 days in soil [redacted] to >1000 days in soil [redacted].

Rates of aerobic degradation of KBR 2738 and its soil metabolite were found to be very fast under conditions of the laboratory. Therefore field studies were not taken into account for the investigation of the degradation behavior of fenhexamid nor its major degradation product BCS-CQ88719 (M24).

The results of kinetic evaluation for use as modelling input parameters in environmental risk assessments are summarized for the parent compound KBR 2738 both radio labels and metabolite BCS-CQ88719 (M24) in Tables 7-4 to 7-6, respectively.

Table 7-4: Optimised DT_{50}-values for parent compound [cyclohexyl-1-^{14}C] KBR 2738 from four soils under aerobic laboratory conditions for use as modelling input parameters in environmental exposure assessments

Soil	Model	k-rate$_{SFO}$ [1/day]	DT50$_{SFO}$ [days]	FF [-]
[redacted]	FOMC	1.682	0.41)^a	0.11
[redacted]	FOMC	1.250	0.56)^a	0.09
[redacted]	FOMC	1.931	0.36)^a	0.06
[redacted]	SFO	0.915	0.76	0.05

)^a calculated from DT90 of bi-phasic model/3.32

Table 7-5: Optimised DT_{50}-values for parent compound [phenyl-UL-^{14}C] KBR 2738 from four soils under aerobic laboratory conditions for use as modelling input parameters in environmental exposure assessments

Soil	Model	k-rate$_{SFO}$ [1/days]	DT50$_{SFO}$ [days]
[redacted]	SFO	2.023	0.34
[redacted]	SFO	2.362	0.29
[redacted]	FOMC	0.232	2.99)^a
[redacted]	SFO	1.555	0.45

)^a calculated from DT90 of FOMC model/3.32

Table 7-6: Optimised DT_{50}-values for metabolite BCS-CQ88719 (M24) from four soils under aerobic laboratory conditions for use as modelling input parameters in environmental exposure assessments

Soil	Model	k-rate$_{SFO}$ [1/day]	DT50$_{SFO}$ [days]
[redacted]	FOMC	0.018	39.42)^{a}
[redacted]	FOMC	0.027	25.83)^{a}
[redacted]	DFOP	0.009)^{b}	75.34
[redacted]	DFOP	0.010)^{b}	72.20

)^{a} calculated from DT90 of FOMC model/3.32

)^{b} calculated from k-slow

For the parent compound fenhexamid a new study using the [cyclohexyl-1-^{14}C] labelled KBR 2738 and an old study using [phenyl-UL-14C] labeled KBR 2738 have been performed. For modeling endpoints total set of four optimised DT_{50}-values could be derived each by use of different kinetic model. Recalculations have been done to result in DT50$_{SFO}$ values. The DT50$_{SFO}$ values ranged from 0.29 days in soil [redacted] to 2.99 days in soil [redacted].

For metabolite BCS-CQ88719 (M24) a total set of four DT_{50}-values was derived by use of the FOMC and DFOP biphasic kinetic models. In order to evaluate the DT50 SFO values recalculations were done as mentioned in Table 7-6. The DT50$_{SFO}$ values ranged from 25.83 days in soil [redacted] to 75.34 days in soil [redacted].

The kinetic evaluation resulted in acceptable and reliable fits by use of different kinetic models and subsequently recalculated DT50$_{SFO}$ values. The resulted DT50$_{SFO}$ values and k-rates$_{SFO}$ served as conservative and robust input parameters in environmental risk assessments.

Soil adsorption

The sorption behaviour to soil was derived by standard batch equilibrium tests on four soils with the parent compound KBR 2738. Due to the insufficient water solubility of the metabolite BCS-CQ88719 (M24) the classical batch equilibrium method was not feasible. To derive reliable K_{OC} values a soil column leaching study with four soils was performed with BCS-CQ88719 (M24).

For the **parent compound fenhexamid (KBR 2738)** values for the coefficients of adsorption according to Freundlich ($K_{F,\ ads}$) ranged from 12.43 mL/g to 15.79 mL/g with corresponding values related to organic carbon ($K_{oc,\ ads}$) to range from 313 mL/g to 654 mL/g (arithmetic mean: 517 mL/g). Values for the Freundlich coefficient of adsorption 1/n ranged from 0.8604 to 0.8958. No significant dependence was observed for the adsorption behaviour from pH or the texture of investigated soils. According to Briggs, fenhexamid can be classified as low mobile to immobile for adsorption and as immobile for desorption.

For **metabolite BCS-CQ88719 (M24), a [C-C]-dimer of fenhexamid** values for the soil adsorption coefficients K_d calculated according to Lambert ranged from 15.2 to 19.8 mL/g (mean: 17.3 mL/g). The respective organic carbon normalized soil adsorption coefficients (K_{OC}) ranged from 353 to 893 mL/g (mean: 668 mL/g).

The soil adsorption coefficients K_d calculated according to Hamaker and McCall ranged from 20.5 to 27.5 mL/g (mean: 23.7 mL/g). The respective organic carbon normalized soil adsorption coefficients (K_{OC}) ranged from 489 to 1214 mL/g (mean: 912 mL/g).

No ^{14}C-radioactivity was detected in the leachates. Approximately the whole radioactivity applied was found in the first segment (0-3 cm) only amounts of less than 1% of AR were found in the other segments below.

Using the Briggs classifications for the estimation of the mobility of crop protection agents in soil based on K_{OC} values, BCS-CQ88719 (M24) can be classified as low mobile to immobile for adsorption.

Aqueous photolysis

In former photolysis study in aqueous buffer solution ([redacted], 1996a) the benzoxazole of KBR 2738, M10 (WAK 7004) which was formed in amounts of approximately 24 % of applied radioactivity was further metabolized very fast (DT50 << 1 d) and therefore not taken into consideration for modelling purposes.

In a phototransformation experiment with fenhexamid published in Chemosphere vol. 81, pp. 844-852 ([redacted] et al. 2010) another new aqueous photometabolite occurred in amounts up to 15% of AR and was identified as 1-methyl cyclohexane carboxamide (M40). Different photo sentitive additives like acetone, etc. and humic substances like humic acids, etc. were utilized in those phototransformation experiments. The metabolite is added into the aquatic degradation pathway shown in Figure 7-2.

Fate in water/sediment

The biotransformation of KBR 2738 using the [cyclohexyl-1-^{14}C] radiolabel was studied under conditions of sediment/water testing in two different systems. The route of degradation was shown to to result in the formation of two major metabolites KBR 2738-3-des-chloro (M12) and 1-methylcyclohexanecarboxylic acid (M39) and one minor metabolite the sulfate of KBR 2738 (M27) which was also detected in minor amounts in a formerly performed aerobic water/sediment metabolism study.

1-Methylcyclohexane carboxylic acid (M39) accounted for up to max. 8.9 % of the AR in water and up to max. 7.8% of AR in sediment (these amounts include a minor metabolite, KBR 2738-sulfate (M27), which accounted for a maximum of 4.2 % of AR in the total system).

KBR 2738-3-deschloro (M12) accounted for only up to 1.1 % of AR in the water phase and in the sediment extract it accounted to a maximum amount of 7.5 % of the AR.

Besides the formation of NER (max. occurrence 55.6% of AR) the degradation was accompanied by formation of significant amounts of ^{14}C-carbon dioxide occurring up to 36.4% AR in total systems in the course of the study.

Trigger endpoints

Following FOCUS kinetic guidance the dissipation half-life of KBR 2738 from water was estimated to range from 1.62 to 5.45 days using different radiolabels and water/sediment test systems. In sediment the dissipation half-life ranged between 10.58 and 22.81 days.

For the degradation of KBR 2738 in total systems half-lives were in the range of 6.77 to 14.60 days of AR when applying different kinetic models as best fit. The values serve for comparison with trigger endpoints in aquatic systems.

The parent compound fenhexamid dissipated relatively fast from water and sediment phase. The major metabolite 1-methylcyclohexanecarboxylic acid (M39) which was formed during incubation in significant amounts decreased to amounts below the LOD only. Whereas the major metabolite KBR 2738-3-des-chloro (M12) which occurred only in the sediment phase of one water/sediment test system in significant amounts is still present in low amounts (5.0 % of AR) at the end of the study but decreasing steadily.

[cyclohexyl-1-^{14}C] fenhexamid:

Table 7-7: Degradation and dissipation parameters for persistence endpoints of [cyclohexyl-1-^{14}C] fenhexamid

Evaluation level / type of parameter	Phase	--[redacted]--			-[redacted]-		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
PI / deg.	**Total system**	HS	10.96	45.60	DFOP	14.60	60.64
PI / diss.	**Water**	DFOP	5.45	19.74	DFOP	2.41	14.98
PI / diss.	**Sediment**	FOMC	22.20	102.68	FOMC	22.81	108.55

Table 7 -8: Degradation and dissipation parameters for persistence endpoints of M39 (1-methyl-cyclohexanecarboxylic acid)

Evaluation level / type of parameter	Phase	--[redacted]--			-[redacted]-		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
MI / deg.	**Total system**	SFO (parent HS)	6.42	21.32	SFO (parent DFOP)	7.97	26.48
MI / diss.	**Water**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	**Sediment**	FOMC	17.65	113.81	SFO	23.26	77.28

Table 7-9: Degradation and dissipation parameters for persistence endpoints of M12 (KBR 2738-3-des-chloro)

Evaluation level / type of parameter	Phase	--[redacted]--			-[redacted]-		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
MI / deg.	**Total system**	n.a.	n.a.	n.a.	SFO (parent DFOP)	73.79	245.12
MI / diss.	**Water**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	**Sediment**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[phenyl-UL-^{14}C] fenhexamid:

Table 7-10: Degradation and dissipation parameters for persistence endpoints of [phenyl-UL-^{14}C] fenhexamid

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
PI / deg.	Total system	DFOP	13.91	57.73	SFO	6.77	22.48
PI / diss.	Water	DFOP	1.62	15.32	DFOP	2.28	13.06
PI / diss.	Sediment	FOMC	14.98	116.37	SFO	10.58	35.16

Modelling endpoints

The dissipation and degradation data from tests of KBR 2738 in two water/sediment systems were additionally evaluated in order to derive best fits to measured data as input parameters for use by modelling within aquatic environmental exposure assessments.

[cyclohexyl-1-^{14}C] fenhexamid:

Table 7-11: Degradation and dissipation parameters for modelling endpoints of [cyclohexyl-1-^{14}C] fenhexamid

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	k-rate (1/days)	DT50 (days)	Model	k-rate (1/days)	DT50 (days)
PI / deg.	Total system	SFO	0.062	11.14	FOMC	0.037	18.49)^a
PI / diss.	Water	SFO	0.123	5.65	FOMC	0.127	5.47)^a
PI / diss.	Sediment	SFO	0.028	25.08	SFO	0.027	26.09

)^a calculated by DT90$_{FOMC}$/3.32

Table 7 -12: Degradation and dissipation parameters for modelling endpoints of M39 (1-methylcyclohexanecarboxylic acid)

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	k-rate (1/days)	DT50 (days)	Model	k-rate (1/days)	DT50 (days)
MI / deg.	Total system	SFO (parent FOMC)	0.111	6.23	SFO (parent FOMC)	0.083	8.33
MI / diss.	Water	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	Sediment	SFO	0.030	22.98	SFO	0.030	23.26

Table 7-13: Degradation and dissipation parameters for modelling endpoints of M12 (KBR 2738-3-des-chloro)

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	**k-rate (1/days)**	**DT50 (days)**	**Model**	**k-rate (1/days)**	**DT50 (days)**
MI / deg.	**Total system**	n.a.	n.a.	n.a.	SFO (parent FOMC)	0.010	68.90
MI / diss.	**Water**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	**Sediment**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[phenyl-UL-^{14}C] fenhexamid:

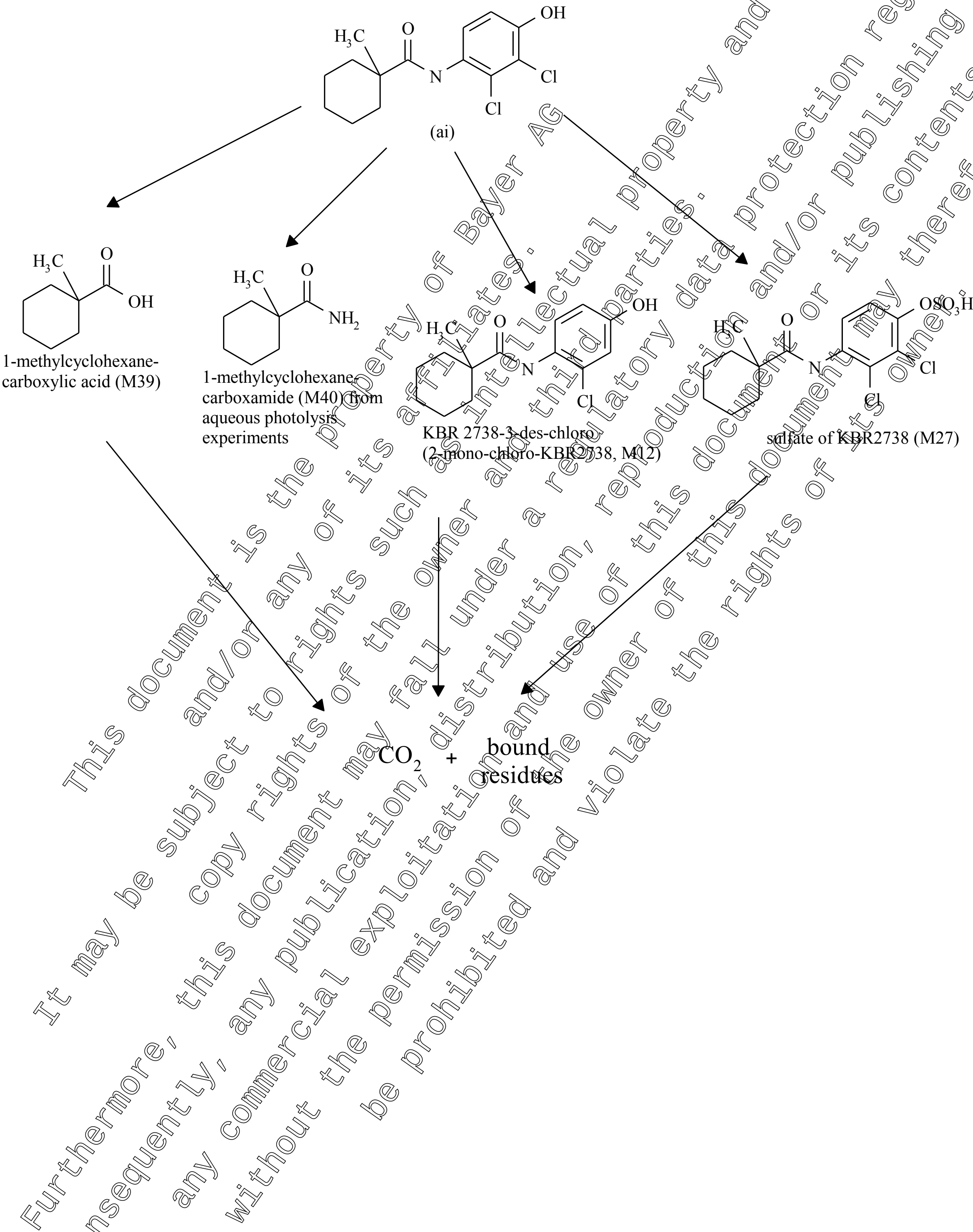
Table 7-14: Degradation and dissipation parameters for modelling endpoints of [phenyl-UL-^{14}C] fenhexamid

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	**k-rate (1/days)**	**DT50 (days)**	**Model**	**k-rate (1/days)**	**DT50 (days)**
PI / deg.	**Total system**	SFO	0.047	14.71	SFO	0.102	6.77
PI / diss.	**Water**	DFOP	0.150	4.61)[a]	DFOP	0.176	3.93)[a]
PI / diss.	**Sediment**	HS	0.023)[b]	29.88	SFO	0.066	10.58

)[a] calculated by DT90$_{DFOP}$/3.32
)[b] k-rate of slow phase

For evaluation of modelling endpoints often the SFO kinetic model was assayed. If a biphasic model (HS, DFOP, FOMC) was choosen as the kinetic model which will result in robust and reliable modelling evaluation the DT$_{50}$ values were recalculated. In case of bi-phasic fits a conservative SFO-type half-life was re-calculated from the DT$_{90}$ of the FOMC model or from the slower phase of DFOP decline curve. The kinetic parameters determined are compiled for Level P-I in Tables 7-11 to Table 7-14.

Figure 7-2: Proposed metabolic pathway for KBR 2738 under aerobic conditions of water/sediment and in addition one major metabolite occurring in an external aqueous photolysis study



IIA 7.1 Route of degradation in soil - laboratory studies

IIA 7.1.1 Aerobic degradation

Report:	**KIIA 7.1.1 /01; [redacted].; 2011**
Title:	[cyclohexyl-1-^{14}C]Fenhexamid (KBR 2738): Aerobic Degradation/Metabolism in Four European Soils
Report No & Document No	MEF-11/037 **M-415995-01-1**
Guidelines:	- OECD: Guideline 307, Aerobic and Anaerobic Transformation in Soil, 2002 - Commission Directive 95/36/EC amending Council Directive 91/414/EEC (Annexes I and II, Fate and Behaviour in the Environment), 1995 - Regulation (EC) No. 1107/2009
GLP	Yes

Executive Summary

The degradation of [cyclohexyl-1-^{14}C]KBR 2738 was investigated under aerobic conditions at 20 °C and a soil moisture of 55% of MWHC[2] in four soils by incubation in the dark for 120 days in maximum. The test substance ^{14}C-KBR 2738 was applied at a nominal test concentration of 2.7 mg a.s./kg soil, equivalent to a rate of 1000 g/ha in the field (2.5 cm depth, 1.5 g/cm^3 bulk density). In parallel sterilized soil samples were also utilized in degradation experiments. Sterilization was done using gamma radiation of Co-60 and in addition treatment with $HgCl_2$.
In the following those parts of the study are summarized which were performed to elucidate the route of degradation in soil. Parts concerning evaluation of rate of degradation are reported in sections IIA 7.2.1 of this document.

The applied radioactivity (AR) was completely recovered (93.0-102.6% AR). After 120 days of incubation, non-extractable radioactivity ranged from 51.7–60.7% AR. Mineralisation accounted for 10.4-19.6% AR as $^{14}CO_2$ by day 120. No significant amounts of other organic volatile components were observed (≤0.1% AR).

Metabolite BCS-CQ88719 (M24), a [C-C]biphenyl-KBR 2738 was identified as a major compound formed in a range from 4.1-8.8% AR in maximum during 120 days of incubation. A total of eight other minor components were observed in the course of the study, none of them accounting for more than 5.0% AR for an individual component. The total of other unidentified components which were not quantified individually amounted to up to 14.9% in maximum.
The microbial conversion of KBR 2738 under aerobic conditions in soil thus includes the following steps:
(1) Formation of the major metabolite BCS-CQ88719 (M24)
(2) Formation of non-extractable residues and
(3) Mineralization (formation of CO_2).

---

[2] Maximum water holding capacity

High amounts of non-extractable residues were found which reached their maximum values after 14 or 21 days of incubation (max. 67.0% of AR). Then, the amounts slowly declined towards the end of the study.

Experiments with sterilized soil samples showed no mineralization (< 0.1%) over eight days. Despite that, the same general metabolite pattern was observed in the sterilized as in the non-sterilised samples, and also significant amounts of bound residues were formed in the sterilized samples. The formation of metabolites and of bound residues was considerably slower, however, under sterile conditions.

Considering that the sterilized soil samples were not entirely sterile (as was shown by the determination of germ total), these results may indicate that the metabolism of fenhexamid, and in particular the formation of bound residues, are partly abiotic processes, which are however enhanced by the activity of soil microorganisms.

I. Material and Methods

A. Materials

1. Test Material: [cyclohexyl-1-^{14}C]KBR 2738
Specific radioactivity: 4.64 MBq/mg
Radiochemical purity / Chemical purity: > 99%
Sample ID: KATH 6639

2. Soil: Soil samples as characterised in Table 7.1.1-1 were collected freshly from the field. A few days before start of the test the air dried soils were sieved through a 2 mm sieve.

Table 7.1.1-1: Characteristics of test soils

Soil	[redacted]	[redacted]	[redacted]	[redacted]
Geographic Location (City / State / Country)	[redacted] / NRW / Germany	[redacted] / NRW / Germany	[redacted] / NRW / Germany	[redacted] / NRW / Germany
GPS coordinates	[redacted]	[redacted]	[redacted]	[redacted]
Pesticide use history	No pesticide use for previous 5 years			
Collection procedures	Sample taken with shovel and transport in plastic bag			
Sampling depth	0 – 20 cm			
Storage conditions	Room temperature			
Storage length	4-5 days after sieving before pre-incubation			
Soil preparation	Sieved (2 mm)			
Texture Class (USDA)	Loamy sand	Silt loam	Loam	Clay loam
Sand [50 µm - 2 mm] (%) Silt [2 µm - 50 µm] (%) Clay [< 2 µm] (%)	81 10 9	23 60 17	37 42 21	33 36 31
pH in Water pH in $CaCl_2$ (0.01 M) pH in KCl (1 M)	6.5 6.2 6.1	6.8 6.5 6.3	6.1 5.8 5.4	7.5 7.4 7.1
Organic Matter ^{A} (%)	3.3	4.1	5.0	7.8
Organic Carbon (%)	1.9	2.4	2.9	4.5
CEC (meq/100 g)	9.3	13.4	10.1	20.6
MWHC (g/100 g)	50.7	64.7	62.5	85.5
Moisture at 1/3 bar = pF 2.5 (g H_2O /100 g dry soil)	not reported			
Microbial biomass (mg $C_{biomass}$/100 g dry vital soil)				
Initial (Day 0) Final, Day 120 (-/+)	613 209/272	985 440/558	873 400/671	2892 1697/1892
Microbial biomass (mg $C_{biomass}$/100 g dry sterilized soil)				
Day 3 without solvent application; gamma irradiation only	203	259	323	481

[redacted]; ^{A}) % organic matter = % organic carbon × 1.724;
CEC: Cation exchange capacity; MWHC: Maximum Water Holding Capacity; n.d.: not determined; (-/+) with or without solvent application.

B. Study design

1. Experimental conditions: Four soils were incubated in the dark at 20°C (±1°C) with [cyclohexy-1-^{14}C]KBR 2738 for 120 days in maximum. The soils received a nominal test concentration of 2.7 mg/kg soil corresponding to a field rate of 1000 g/ha (assuming incorporation into the top 2.5 cm of soil and a bulk density of 1.5 g/mL). The test substance was dissolved in an acetone water mixture before a volume of 2 mL was applied dropwise to 100 g of each soil using an adjustable pipette. No evaporation procedure was performed. The application solution was prepared using water/acetone (6/1, v/v). The effect of the solvent on the microbial activity was investigated by the determination of microbial activity in biomass samples with solvent application.Soil moisture was adjusted to 55% MWHC and samples of 100 g dry weight of soil were filled into Erlenmeyer incubation flasks. The static test systems were completed by attachment with traps (soda lime and polyurethane foam) for the collection of ^{14}C-carbon dioxide and other volatiles.

2. **Sampling:** Duplicate samples were collected after 0, 0.1, 1, 3, 7, 14, 30, 44, 63, 90 and 120 days of incubation. The complete samples were immediately processed by extraction and concentration. The extracts were analysed by HPLC. Two-dimensional TLC-analysis confirmed the results of the HPLC measurements for the test item fenhexamid and metabolites BCS-CQ88719 (M24) and CQ88720 (M20) as well as the detection of numerous minor transformation products which were not identified due to their low amounts of AR.

3. **Analytical procedures:** The entire soil sample in each test vessel was processed by a stepwise extraction procedure. After separation by centrifugation the soil was extracted four times successively with aqueous acetonitrile (80:20, v/v) by shaking at ambient temperature for 30 min followed by centrifugation. Microwave extraction (1000 W, 10 min) with aqueous acetonitrile (80:20, v/v) was performed on the residual soil at enhanced temperature (70°C) twice. The ambient extracts were combined and analyzed for volume and radioactivity. The same was done for the extracts obtained from microwave extraction ("microwave" or "aggressive extracts").

Ambient extracts were concentrated prior to HPLC-analysis when necessary. The aggressive extracts were concentrated at each sampling interval. Following quantitation of radioactivity in each extract by LSC, analysis of extracts was performed by reversed-phase HPLC and ^{14}C-flow-through detection techniques as the primary analytical method. Extracts from representative samples were re-analysed by normal phase TLC followed by ^{14}C-detection with a Fuji BAS 2000 bio-imaging system as confirmatory method.

The determination of non-extractable residues (NER) was performed by combustion/LSC of aliquots of the air-dried extracted soil.

The LOD was in the range of 0.1% of AR. Based on a LOD of 0.1% of the applied radioactivity (for the sum of all extracts) the limit of quantification (LOQ) is estimated to be about 0.3% of AR (3 times x LOD = 0.3%).

II. Results and Discussion

A. Data: The results of aerobic biotransformation of [cyclohexyl-1-^{14}C]KBR 2738 and in four European soils are summarized in Tables 7.1.1-2 to 7.1.1-5.

Table 7.1.1-2: Degradation of [cyclohexyl-1-^{14}C]KBR 2738 in loamy sand soil [redacted] under aerobic conditions

Compound	Replicate No	Residues (% AR) on the following days after treatment (DAT)											
		0	0.1	1	3	7	14	21	30	44	63	90	120
Fenhexamid	(A)	95.7	63.3	12.4	3.5	2.7	2.4	2.3	2.0	1.7	1.6	2.2	2.6
	(B)	97.1	62.3	12.8	3.6	2.7	2.2	2.0	1.6	1.7	1.7	2.3	2.5
	Mean	96.4	62.8	12.6	3.5	2.7	2.3	2.2	1.8	1.7	1.6	2.3	2.6
BCS-CQ88719	(A)	0.5	5.7	8.8	5.5	3.7	2.3	1.9	1.5	1.2	1.1	1.3	1.7
	(B)	0.3	5.9	8.8	6.0	3.8	2.4	1.9	1.5	1.3	1.0	1.6	1.6
	Mean	0.4	5.8	8.8	5.8	3.7	2.4	1.9	1.5	1.2	1.1	1.4	1.6
CQ88720	(A)	0.4	2.7	5.0	3.8	2.5	1.5	1.4	1.2	0.9	1.2	1.5	1.5
	(B)	0.3	2.5	5.0	3.7	2.4	1.9	1.3	1.1	1.4	1.0	1.6	1.5
	Mean	0.3	2.6	5.0	3.8	2.5	1.7	1.4	1.2	1.2	1.1	1.6	1.5
RT 14	(A)	0.1	0.8	1.9	1.0	0.5	0.5	0.7	0.6	0.7	0.6	0.3	0.7
	(B)	<LOQ	1.1	2.1	1.2	0.6	0.4	0.4	0.6	0.6	0.7	0.3	0.7
	Mean	0.0	1.0	2.0	1.1	0.5	0.5	0.6	0.6	0.7	0.7	0.3	0.7
RT 17	(A)	<LOQ	0.7	2.8	0.7	1.2	0.9	1.1	0.8	0.9	0.9	1.4	2.0
	(B)	<LOQ	0.8	2.9	0.9	1.1	0.8	0.8	0.9	0.8	1.0	1.8	1.5
	Mean		0.8	2.8	0.8	1.2	0.9	0.9	0.9	0.9	1.0	1.6	1.7
RT 21	(A)	<LOQ	0.5	0.8	1.1	1.0	1.2	0.6	0.5	1.5	1.3	1.6	1.6
	(B)	<LOQ	0.5	1.0	1.2	0.9	0.8	0.8	1.1	1.2	1.1	1.7	1.8
	Mean		0.5	0.9	1.1	1.0	1.0	0.7	0.8	1.3	1.2	1.6	1.7
RT 36	(A)	n.d.	1.0	2.5	2.5	3.1	3.0	2.5	2.5	2.2	2.4	2.9	2.2
	(B)	n.d.	1.3	2.6	2.8	2.9	2.5	2.6	2.3	2.2	2.3	2.7	2.2
	Mean		1.1	2.5	2.7	3.0	2.7	2.6	2.4	2.2	2.3	2.8	2.2
RT 38	(A)	n.d.	0.9	2.4	2.5	2.1	2.1	2.5	2.2	2.2	2.7	2.6	1.6
	(B)	n.d.	1.5	2.3	2.3	2.5	2.6	2.4	2.4	2.2	2.7	1.7	1.8
	Mean		1.2	2.4	2.4	2.3	2.3	2.4	2.3	2.2	2.7	2.1	1.7
RT 40	(A)	n.d.	0.9	2.3	4.1	4.1	2.9	3.2	3.3	3.5	3.0	1.3	1.0
	(B)	n.d.	1.2	2.8	4.3	4.4	3.4	3.6	3.9	3.0	2.9	1.1	1.2
	Mean		1.1	2.5	4.2	4.3	3.1	3.4	3.6	3.2	2.9	1.2	1.1
RT 42	(A)	n.d.	0.4	1.8	2.7	3.0	3.4	2.2	2.6	3.2	2.2	0.7	0.4
	(B)	n.d.	0.7	1.5	2.6	2.6	3.6	2.4	1.7	2.2	2.5	0.5	0.4
	Mean		0.6	1.6	2.6	2.8	3.5	2.3	2.2	2.7	2.4	0.6	0.4
Unidentified radioactivity	(A)	0.6	3.2	12.4	14.8	12.9	14.1	13.1	12.6	11.0	11.6	10.5	9.5
	(B)	0.5	3.2	12.2	13.2	13.7	14.3	13.1	12.8	12.7	10.3	10.4	9.4
	Mean	0.5	3.2	12.3	14.0	13.3	14.2	13.1	12.7	11.9	11.0	10.4	9.5
Total extractable residues	(A)	97.2	80.1	53.0	42.1	36.8	34.2	31.5	29.8	29.2	28.6	26.2	24.8
	(B)	98.2	81.1	54.0	41.7	37.6	35.0	31.5	30.0	29.4	27.1	25.6	24.6
	Mean	97.7	80.6	53.5	41.9	37.2	34.6	31.5	29.9	29.3	27.8	25.9	24.7
$^{14}CO_2$	(A)	n.a.	<0.1	0.9	3.4	5.9	7.8	8.9	10.5	12.2	14.2	16.6	19.6
	(B)	n.a.	<0.1	0.9	3.4	5.3	7.5	8.9	10.3	12.0	13.7	17.2	19.7
	Mean			0.9	3.4	5.6	7.7	8.9	10.4	12.1	14.0	16.9	19.6
Volatile organics	(A)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	(B)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean												
Not-extractable residue	(A)	2.9	20.1	44.1	52.9	55.8	58.5	56.7	55.1	57.3	55.8	52.0	52.0
	(B)	2.8	20.3	43.6	53.1	54.1	55.8	56.7	56.0	54.9	56.9	53.4	52.3
	Mean	2.9	20.2	43.8	53.0	55.0	57.2	56.7	55.5	56.1	56.3	52.7	52.2
Total % recovery	(A)	100.1	100.2	97.9	98.4	98.5	100.5	97.1	95.5	98.7	98.6	94.8	96.4
	(B)	101.1	101.4	98.5	98.2	97.1	98.3	97.1	96.3	96.3	97.7	96.2	96.6
	Mean	100.6	100.8	98.2	98.3	97.8	99.4	97.1	95.9	97.5	98.1	95.5	96.5

n.d. : not detected, n.a. : not analyzed, DAT : days after treatment

Table 7.1.1-3: Degradation of [cyclohexyl-1-^{14}C]KBR 2738 in silt loam soil [redacted] [redacted] under aerobic conditions

Compound	Replicate No	Residues (% AR) on the following days after treatment (DAT) 0	0.1	1	3	7	14	21	30	44	63	90	120
Fenhexamid	(A)	96.5	68.9	17.6	3.5	3.0	2.4	1.7	1.6	1.5	1.4	2.0	1.4
	(B)	96.6	68.6	19.3	3.7	2.9	2.4	1.6	2.0	1.6	1.4	1.5	2.2
	Mean	96.5	68.7	18.4	3.6	2.9	2.4	1.6	1.8	1.5	1.4	1.7	1.8
BCS-CQ88719	(A)	0.5	4.1	7.7	5.4	3.5	2.6	1.6	1.4	1.0	0.9	0.9	1.1
	(B)	0.4	4.0	7.6	5.5	3.4	2.4	1.5	1.8	0.9	0.9	0.9	1.2
	Mean	0.4	4.0	7.6	5.4	3.5	2.5	1.6	1.6	1.0	0.9	0.9	1.1
CQ88720	(A)	0.3	1.5	4.2	3.1	2.0	1.3	1.0	0.9	0.7	0.6	1.0	0.9
	(B)	0.4	1.4	4.0	3.3	1.9	1.3	0.8	1.2	0.7	0.6	0.7	0.9
	Mean	0.4	1.4	4.1	3.2	2.0	1.3	0.9	1.0	0.7	0.6	0.9	0.9
RT 14	(A)	0.1	1.0	1.8	1.1	0.9	0.5	0.5	0.4	0.5	0.4	0.3	0.4
	(B)	0.1	1.1	2.0	1.2	0.9	0.5	0.5	0.5	0.5	0.5	0.3	0.6
	Mean	0.1	1.1	1.9	1.2	0.9	0.5	0.5	0.5	0.5	0.4	0.3	0.5
RT 17	(A)	<LOQ	1.0	3.0	0.7	1.2	0.8	0.5	0.5	0.5	0.5	0.7	1.0
	(B)	<LOQ	1.0	2.7	0.9	0.9	0.7	0.5	0.6	0.4	0.5	0.6	1.1
	Mean		1.0	2.8	0.8	1.0	0.7	0.5	0.6	0.5	0.5	0.6	1.0
RT 21	(A)	n.d.	0.3	1.2	1.2	1.6	1.0	0.6	0.5	0.5	0.5	0.5	0.9
	(B)	n.d.	0.3	0.8	1.2	1.2	0.7	0.7	0.8	0.5	0.5	0.6	0.7
	Mean		0.3	1.0	1.2	1.4	0.8	0.6	0.6	0.5	0.5	0.6	0.8
RT 36	(A)	n.d.	0.5	1.7	1.8	2.0	2.0	1.5	1.4	1.2	1.4	1.1	1.3
	(B)	n.d.	0.5	1.4	1.9	1.8	1.8	1.4	1.1	1.3	1.2	1.3	0.6
	Mean		0.5	1.5	1.8	1.9	1.9	1.5	1.3	1.2	1.3	1.2	1.0
RT 38	(A)	n.d.	0.4	1.1	1.5	1.6	1.7	1.4	1.3	1.0	1.1	1.2	0.8
	(B)	n.d.	0.5	1.4	1.4	1.5	1.5	1.2	1.1	1.1	1.1	1.0	0.6
	Mean		0.4	1.3	1.5	1.6	1.6	1.3	1.2	1.1	1.1	1.1	0.7
RT 40	(A)	n.d.	0.4	1.2	1.6	1.7	1.7	2.1	1.8	1.6	1.6	0.9	0.4
	(B)	n.d	0.6	1.4	1.6	1.6	1.4	1.6	1.0	1.6	2.0	0.8	0.3
	Mean		0.5	1.3	1.6	1.7	1.6	1.9	1.4	1.6	1.8	0.8	0.3
RT 42	(A)	n.d.	n.d.	0.9	1.2	1.3	0.8	1.6	1.7	1.3	1.5	0.4	n.d.
	(B)	n.d.	n.d.	0.8	1.3	1.6	0.7	1.7	0.9	1.3	1.3	0.4	n.d.
	Mean			0.9	1.2	1.5	0.8	1.6	1.3	1.3	1.4	0.4	
Unidentified radioactivity	(A)	0.7	1.3	6.6	9.6	6.3	6.3	6.8	6.0	6.2	5.5	4.7	4.7
	(B)	0.4	1.2	5.7	8.5	6.8	6.7	7.0	6.1	5.6	5.5	5.1	4.0
	Mean	0.5	1.2	6.1	9.1	6.5	6.5	6.9	6.0	5.9	5.5	4.9	4.4
Total extractable residues	(A)	98.0	79.4	46.9	30.6	25.2	21.0	19.3	17.5	16.0	15.3	13.7	12.9
	(B)	97.9	79.2	47.2	30.6	24.6	20.1	18.5	17.2	15.6	15.4	13.2	12.1
	Mean	97.9	79.3	47.0	30.6	24.9	20.6	18.9	17.3	15.8	15.4	13.4	12.5
$^{14}CO_2$	(A)	n.a.	<0.1	1.1	4.4	6.9	9.2	10.4	12.0	13.9	15.8	18.5	20.9
	(B)	n.a.	<0.1	1.1	4.2	7.1	9.4	10.8	12.2	14.1	16.0	18.7	20.9
	Mean			1.1	4.3	7.0	9.3	10.6	12.1	14.0	15.9	18.6	20.9
Volatile organics	(A)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	(B)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	<0.1	<0.1
	Mean									0.1	0.1		
Not-extractable residue	(A)	4.3	20.4	47.0	61.5	62.0	67.0	65.3	63.5	62.6	63.7	63.6	59.6
	(B)	4.2	21.7	47.1	60.4	64.3	66.6	66.6	64.1	64.6	65.4	64.3	61.9
	Mean	4.3	21.0	47.0	61.0	63.1	66.8	65.9	63.8	63.6	64.6	63.9	60.7
Total % recovery	(A)	102.3	99.8	95.0	96.4	94.1	97.3	95.0	93.1	92.5	94.9	95.8	93.4
	(B)	102.1	100.8	95.3	95.2	95.9	96.1	95.9	93.5	94.4	96.9	96.2	94.9
	Mean	102.2	100.3	95.2	95.8	95.0	96.7	95.4	93.3	93.4	95.9	96.0	94.2

n.d.: not detected; n.a.: not analyzed; DAT: days after treatment

Table 7.1.1-4: Degradation of [cyclohexyl-1-^{14}C]KBR 2738 in loam soil [redacted] under aerobic conditions

Compound	Replicate No	Residues (% AR) on the following days after treatment (DAT)											
		0	0.1	1	3	7	14	21	30	44	63	90	120
Fenhexamid	(A)	89.9	46.4	9.8	4.3	2.9	3.2	2.1	2.3	1.7	1.7	2.7	3.4
	(B)	89.0	45.8	10.1	4.3	3.2	3.1	2.1	1.7	1.8	1.5	2.7	1.8
	Mean	89.4	46.1	9.9	4.3	3.1	3.2	2.1	2.0	1.7	1.6	2.7	2.6
BCS-CQ88719	(A)	0.9	4.6	4.8	3.4	2.5	1.9	1.3	1.2	1.1	0.9	1.4	1.7
	(B)	0.8	4.6	4.6	3.6	2.2	1.9	1.4	1.2	1.0	0.8	1.4	1.1
	Mean	0.9	4.6	4.7	3.5	2.4	1.9	1.4	1.2	1.1	0.8	1.4	1.4
CQ88720	(A)	0.4	2.2	3.4	3.1	2.2	1.8	1.3	1.5	1.0	1.0	2.1	2.4
	(B)	0.4	2.2	3.5	2.8	2.0	1.8	1.5	1.4	1.2	1.0	2.4	1.4
	Mean	0.4	2.2	3.5	3.0	2.1	1.8	1.4	1.4	1.1	1.0	2.2	1.9
RT 14	(A)	0.2	2.4	2.9	1.6	1.0	1.0	0.6	0.7	0.7	0.6	0.8	0.9
	(B)	0.3	2.3	2.4	1.6	0.9	1.0	0.6	0.6	0.8	0.6	0.6	0.6
	Mean	0.3	2.3	2.7	1.6	0.9	1.0	0.6	0.7	0.8	0.6	0.7	0.8
RT 17	(A)	0.1	1.1	2.0	1.0	1.3	1.2	0.6	0.5	0.4	0.5	0.7	1.1
	(B)	0.1	1.1	2.0	0.9	1.6	1.0	0.6	0.6	0.6	0.5	0.9	0.7
	Mean	0.1	1.1	2.0	0.9	1.5	1.1	0.6	0.5	0.5	0.5	0.8	0.9
RT 21	(A)	0.4	0.5	0.7	1.1	0.8	1.1	0.8	0.5	0.7	0.6	0.9	1.1
	(B)	0.4	0.4	0.4	1.0	0.8	0.9	0.7	0.7	0.5	0.8	0.9	0.6
	Mean	0.4	0.4	0.6	1.0	0.8	1.0	0.7	0.6	0.6	0.7	0.9	0.9
RT 36	(A)	n.d.	1.4	2.1	2.4	2.7	2.6	2.1	2.1	2.5	1.9	2.4	1.9
	(B)	n.d.	1.6	2.6	2.5	2.4	2.2	2.0	2.2	2.0	2.1	3.2	2.6
	Mean		1.5	2.4	2.4	2.5	2.4	2.1	2.2	2.2	2.0	2.8	2.3
RT 38	(A)	n.d.	1.4	2.3	2.3	2.8	2.2	2.4	2.6	1.9	2.1	1.7	1.5
	(B)	n.d.	1.4	2.3	2.9	2.2	2.1	2.4	2.6	1.9	1.9	1.9	2.3
	Mean		1.4	2.3	2.6	2.5	2.1	2.4	2.6	1.9	2.0	1.8	1.9
RT 40	(A)	n.d.	1.7	2.4	3.4	4.7	2.9	4.0	3.6	2.6	3.1	1.1	0.6
	(B)	n.d.	2.2	2.9	2.9	4.1	3.2	3.5	2.9	2.7	2.2	1.6	2.6
	Mean		2.0	2.7	3.2	4.4	3.1	3.7	3.2	2.7	2.6	1.3	1.6
RT 42	(A)	n.d.	1.1	2.8	2.6	2.2	2.8	2.6	2.6	1.9	2.0	1.8	0.5
	(B)	n.d.	1.4	2.7	2.5	2.5	2.3	2.7	2.0	2.2	2.0	1.2	2.6
	Mean		1.2	2.7	2.6	2.4	2.5	2.7	2.3	2.1	2.0	1.5	1.5
Unidentified radioactivity	(A)	0.8	7.0	14.4	14.8	14.2	12.8	14.2	12.9	14.1	13.0	8.8	8.2
	(B)	0.7	6.0	14.3	15.0	13.8	14.3	14.0	14.0	13.7	12.5	8.5	8.2
	Mean	0.8	6.5	14.4	14.9	14.0	13.5	14.1	13.4	13.9	12.8	8.6	8.2
Total extractable residues	(A)	92.7	69.7	47.6	40.0	37.3	33.4	32.0	30.5	28.6	27.3	24.5	23.4
	(B)	91.8	69.0	47.9	40.1	35.8	33.8	31.5	29.9	28.5	25.9	25.4	24.4
	Mean	92.2	69.4	47.8	40.1	36.5	33.6	31.7	30.2	28.5	26.6	24.9	23.9
$^{14}CO_2$	(A)	n.a.	<0.1	1.3	3.8	5.8	7.4	8.4	9.8	11.3	13.4	16.3	18.7
	(B)	n.a.	<0.1	1.2	3.6	5.4	7.3	8.3	9.4	11.2	13.5	16.0	17.9
	Mean			1.2	3.7	5.6	7.3	8.3	9.6	11.3	13.5	16.1	18.3
Volatile organics	(A)	n.a.	<0.1	<0.1	<0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	(B)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean					0.1							
Not-extractable residue	(A)	8.8	30.3	45.2	52.8	53.1	57.5	55.4	55.5	55.2	54.2	54.2	51.5
	(B)	9.2	30.5	46.8	52.0	56.6	55.9	55.0	54.8	55.6	56.1	53.4	51.9
	Mean	9.0	30.4	46.0	52.4	54.8	56.7	55.2	55.2	55.4	55.2	53.8	51.7
Total % recovery	(A)	101.5	100.0	94.1	96.6	96.4	98.3	95.7	95.8	95.1	94.9	95.0	93.5
	(B)	100.9	99.5	95.9	95.7	97.7	97.0	94.8	94.2	95.4	95.6	94.8	94.3
	Mean	101.2	99.8	95.0	96.1	97.1	97.6	95.3	95.0	95.2	95.3	94.9	93.9

n.d.: not detected; n.a.: not analyzed; DAT: days after treatment

Table 7.1.1-5: Degradation of [cyclohexyl-1-^{14}C]KBR 2738 in clay loam soil [redacted] under aerobic conditions

Compound	Replicate No	Residues (% AR) on the following days after treatment (DAT)											
		0	0.1	1	3	7	14	21	30	44	63	90	120
Fenhexamid	(A)	98.0	82.3	41.5	4.2	2.4	1.8	1.4	1.6	1.3	1.1	0.9	0.8
	(B)	98.0	81.0	41.2	4.3	2.4	1.9	1.5	1.4	1.5	1.2	1.2	0.8
	Mean	98.0	81.6	41.4	4.2	2.4	1.9	1.5	1.5	1.4	1.2	1.0	0.8
BCS-CQ88719 ((M24) (M24)	(A)	0.2	1.3	3.6	4.4	2.9	2.0	1.6	1.9	1.1	0.7	0.6	0.4
	(B)	0.2	1.2	3.3	3.7	2.6	2.0	1.6	1.5	0.9	0.8	0.8	0.4
	Mean	0.2	1.2	3.4	4.1	2.7	2.0	1.6	1.7	1.0	0.8	0.7	0.4
CQ88720	(A)	n.d.	0.6	2.1	2.4	1.1	1.0	0.7	0.5	0.5	0.4	0.3	0.2
	(B)	n.d.	0.6	1.5	2.2	1.1	0.8	0.6	0.7	0.5	0.4	0.3	0.2
	Mean		0.6	1.8	2.3	1.1	0.9	0.7	0.6	0.5	0.4	0.3	0.2
RT 14	(A)	0.1	0.8	2.1	1.3	0.7	0.5	0.4	0.5	0.4	0.4	0.3	0.3
	(B)	0.1	1.1	1.9	1.5	0.7	0.4	0.5	0.3	0.4	0.3	0.4	0.3
	Mean	0.1	0.9	2.0	1.4	0.7	0.5	0.4	0.4	0.4	0.3	0.3	0.3
RT 17	(A)	<LOQ	1.0	2.7	1.4	1.2	1.0	0.6	0.8	0.5	0.4	0.4	0.3
	(B)	<LOQ	1.0	2.8	1.2	0.8	1.0	0.6	0.6	0.5	0.4	0.5	0.2
	Mean		1.0	2.7	1.3	1.0	1.0	0.6	0.7	0.5	0.4	0.4	0.3
RT 21	(A)	n.d.	0.3	1.5	2.9	2.8	1.0	0.7	0.8	0.4	0.3	0.5	0.3
	(B)	n.d.	0.5	1.7	3.0	2.5	1.0	0.7	0.6	0.3	0.4	0.5	0.3
	Mean		0.4	1.6	2.9	2.6	1.0	0.7	0.7	0.3	0.4	0.5	0.3
RT 36	(A)	n.d.	n.d.	0.5	0.8	0.7	0.9	0.7	0.3	0.8	0.6	0.5	0.5
	(B)	n.d.	n.d.	0.4	0.8	0.7	0.9	0.7	0.7	0.6	0.7	0.1	0.7
	Mean			0.5	0.8	0.7	0.9	0.7	0.5	0.7	0.7	0.3	0.6
RT 38	(A)	n.d.	n.d.	0.4	0.6	0.5	0.6	0.6	0.3	0.4	0.6	0.4	0.5
	(B)	n.d.	n.d.	0.4	0.5	0.6	0.6	0.5	0.6	0.3	0.4	0.1	0.6
	Mean			0.4	0.5	0.5	0.6	0.5	0.5	0.4	0.5	0.2	0.6
RT 40	(A)	n.d.	n.d.	0.3	0.6	0.5	0.8	0.6	0.3	0.5	0.6	0.4	0.9
	(B)	n.d.	n.d.	0.5	0.6	1.0	0.6	0.9	0.8	0.3	0.3	0.1	0.8
	Mean			0.4	0.6	0.8	0.7	0.8	0.6	0.4	0.5	0.2	0.9
RT 42	(A)	n.d.	n.d.	0.4	0.5	0.5	0.5	0.5	n.d.	0.5	0.5	0.2	0.5
	(B)	n.d.	n.d.	0.1	0.5	0.6	0.4	0.5	n.d.	0.7	0.5	0.1	0.5
	Mean			0.2	0.5	0.6	0.4	0.5		0.6	0.5	0.2	0.5
Unidentified radioactivity	(A)	0.8	1.5	2.3	4.8	4.7	3.6	3.9	3.5	3.3	3.1	3.0	2.7
	(B)	1.2	1.2	3.4	4.7	4.6	3.8	3.5	3.4	3.1	3.1	3.6	2.8
	Mean	1.0	1.3	2.9	4.7	4.7	3.7	3.7	3.4	3.2	3.1	3.3	2.8
Total extractable residues	(A)	99.0	87.7	57.3	23.8	18.0	13.7	11.6	10.5	9.7	8.7	7.6	7.4
	(B)	99.6	86.5	57.4	22.8	17.4	13.4	11.7	10.6	9.1	8.5	7.6	7.6
	Mean	99.3	87.1	57.3	23.3	17.7	13.5	11.6	10.6	9.4	8.6	7.6	7.5
$^{14}CO_2$	(A)	n.a.	<0.1	1.7	7.6	11.8	14.4	16.3	18.2	20.1	22.5	25.8	27.4
	(B)	n.a.	<0.1	1.8	7.8	11.9	14.6	16.3	18.5	20.7	22.7	25.7	27.4
	Mean			1.8	7.7	11.8	14.5	16.3	18.4	20.4	22.6	25.7	27.4
Volatile organics	(A)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	(B)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Mean												
Not-extractable residue	(A)	3.1	13.6	36.9	63.8	65.5	66.9	66.9	64.8	64.1	63.9	60.1	59.6
	(B)	3.4	14.2	35.3	65.0	65.6	66.5	67.1	65.3	64.0	62.8	60.8	59.2
	Mean	3.2	13.9	36.1	64.4	65.6	66.7	67.0	65.0	64.1	63.3	60.5	59.4
Total % recovery	(A)	102.2	101.3	95.9	95.2	95.3	95.0	94.9	93.5	94.0	95.1	93.6	94.4
	(B)	102.9	100.7	94.4	95.6	94.8	94.5	95.1	94.5	93.8	94.0	94.0	94.3
	Mean	102.5	101.0	95.2	95.4	95.0	94.8	95.0	94.0	93.9	94.5	93.8	94.3

n.d.: not detected; n.a.: not analyzed; DAT: days after treatment

B. Mass balance: The total radioactive material balances showed a complete recovery to range from 92.5 –102.9% AR for the four soils investigated. The results are summarised in more detail in Table 7.1.1-6. Conclusively there were no signs for losses of radioactivity from sample work-up and processing.

Table 7.1.1-6: Material balances of radioactivity in four European soils

Soil	[redacted]	[redacted]	[redacted]	[redacted]
Total Recovery (% AR)	94.8 – 101.4	92.5 – 102.3	93.5 – 101.5	93.5 – 102.9
Mean	98.0	96.1	96.4	95.8
Rel. standard deviation	1.8	2.8	2.3	3.0

C. Bound and extractable residues: Values of extractable radioactivity decreased with time accompanied by the formation of non-extractable residues as summarized in Table 7.1.1-7. Starting from a complete extractability by day zero (97.8% for [redacted] 98.0% for [redacted] [redacted] [redacted], 92.2% for [redacted] and 99.3% for [redacted]) values decreased to 24.7% ([redacted]), 12.5% ([redacted]), 23.9% ([redacted]) and 7.5% ([redacted]) after a maximum incubation period of 120 days.
In turn values for non-extractable radioactivity were low by day zero (2.9% for [redacted], 4.3% for [redacted], 9.0% for [redacted] and 3.2% for [redacted]) to increase to maximum amounts of 57.2, 66.8, 56.7 and 67.0% at DAT-14 or DAT-21 in soils [redacted], [redacted], [redacted] and [redacted], respectively. Towards the end of the study the amounts of bound residues slowly decline to 52.2, 60.7, 51.7 and 59.4% of AR in soils [redacted], [redacted], [redacted] and [redacted], respectively.

In the parallel experiments with sterilized soils (Co-60 gamma irradiation and treatment with $HgCl_2$) no mineralization was observed until DAT-8 (<0.1% $^{14}CO_2$, when the microbially active samples had 5.6% to 11.8% $^{14}CO_2$ on DAT-7). However, in the sterilized soils both dimers and numerous minor metabolites were formed, though slower than in the microbially active samples. The same is true for the formation of non-extractable residues: After 8 days of incubation, the amounts of bound residues in the sterilized samples were 43.4, 45.2, and 32.9% of AR for soils [redacted] and [redacted], respectively, i.e., considerably lower than the amounts of bound residues found in non-sterilized samples on DAT-7 (55.0, 63.1 and 65.6% of AR, respectively); for soil [redacted] similar amounts of bound residues were found in the $HgCl_2$ treated (52.7% of AR, DAT-8) and degradation rate test vessels (54.8% of AR, DAT-7).

Table 7.1.1-7: Extractable and non-extractable residues in four European soils

Soil	[redacted]	[redacted]	[redacted]	[redacted]
Extracted RA (%)	24.7 -97.8	12.5 – 98.0	23.9 – 92.2	7.5 - 99.3
Bound Residues (%)	2.9 – 57.2	4.3 – 66.8	9.0 – 56.7	3.2 – 67.0
Extraction Efficiency on DAT-0 (%)	97.8	98.0	92.2	99.3

All values given as percentages of initially applied radioactivity.

D. Volatile radioactivity: The maximum amount of 14CO2 was 27.4% of AR at the end of the study (soil [redacted], DAT-120). In soils [redacted] and [redacted] the maximum amounts of 14CO2 detected were 19.6, 20.9 and 18.3% of AR, respectively. No significant amounts of volatile organic compounds were detected in any soil (values being < 0.2% at all sampling intervals).

E. Transformation of parent compound: There was observed the formation of a single major component identified as [C-C]-dimer of KBR 2738: BCS-CQ88719 (M24). The amounts of BCS-CQ88719 (M24) reached maximum values of 8.8, 7.6, 4.7 and 4.1% of AR after one or three days of incubation in soils [redacted] and [redacted] respectively. After a decrease until DAT-14/DAT-21, the amounts of BCS-CQ88719 (M24) in the extracts remained more or less stable until the end of the study. On DAT-120, BCS-CQ88719 (M24) accounted for 1.7, 1.1, 1.4 and 0.4% of AR in soils [redacted] [redacted] and [redacted], respectively.
A second transformation product which was frequently detected was tentatively identified as the [C-O-C]-dimer CQ88720 (M20) via HPLC-co-chromatography. Maximum amounts of 5.0, 4.1, 3.5 and 2.3% of AR were detected after one or three days of incubation in soils [redacted] and [redacted], respectively. In addition, seven minor peaks or zones were frequently detected. They were characterized according to their retention times (RT) and reached maximum amounts of 2.7% (RT14), 2.8% (RT17), 2.9% (RT21), 3.0% (RT36), 2.7% (RT38), 4.4% (RT40) and 3.5% (RT42) of AR.
The profiles of identified compounds found in four soils tested are presented in Table 7.1.1-8.

Table 7.1.1-8: Occurrence of metabolites of [cyclohexyl-1-^{14}C]KBR 2738 in aerobic soil.

Compound	Soil	Maximum occurrence (% AR)	Maximum occurrence at day	Occurrence at study end (% AR)
[C-C]-dimer of KBR 2738: BCS-CQ88719 (M24)	[redacted]	8.8	1	1.7
	[redacted]	7.6	1	1.1
	[redacted]	4.7	1	1.4
	[redacted]	4.1	3	0.4

III. Conclusion

Based on the study results the route of aerobic degradation of KBR 2738 in soil is characterised by the following major processes:

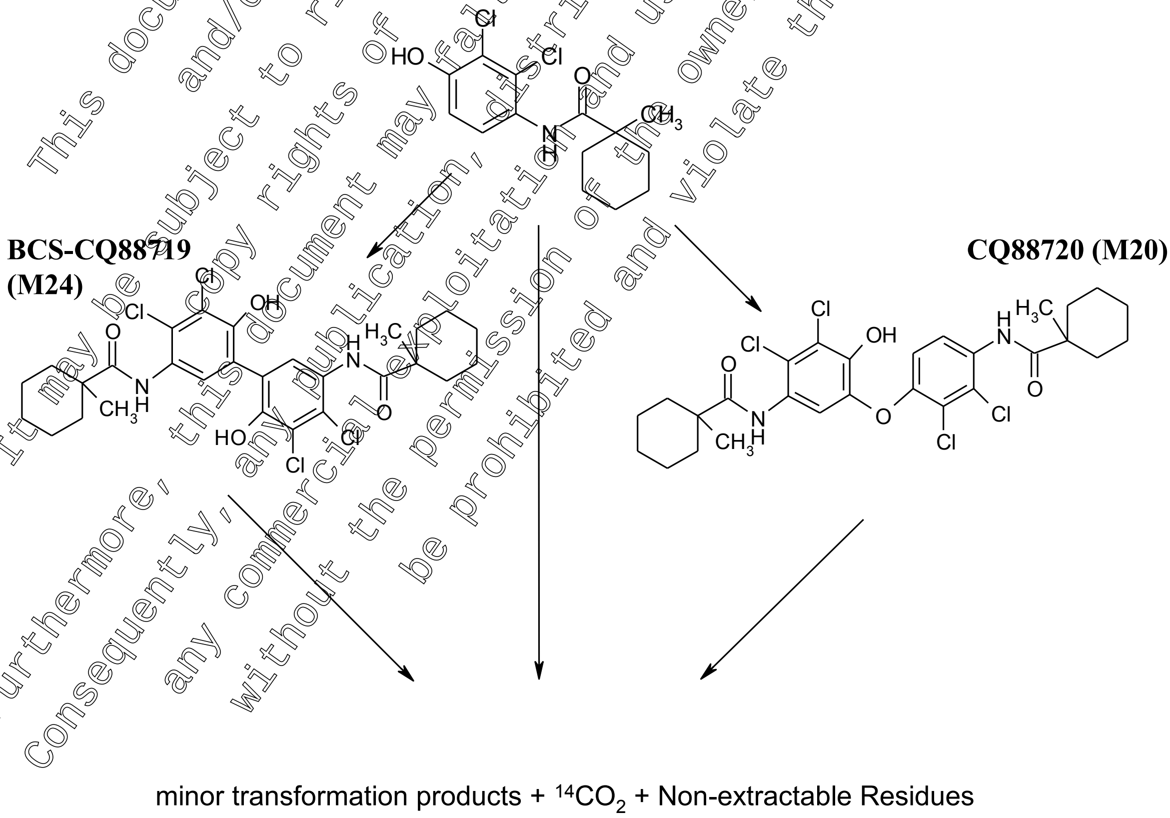
- Formation of the major metabolite BCS-CQ88719 (M24)
- Formation of non-extractable (bound) residues
- Mineralisation (formation of carbon dioxide)

Experiments with sterilized soil samples showed no mineralization (< 0.1%) over eight days. Despite that, the same general metabolite pattern was observed in the sterilized as in the non-sterilised samples, and also significant amounts of bound residues were formed in the sterilized samples. The formation of metabolites and of bound residues was considerably slower, however, under sterile conditions.

Considering that the sterilized soil samples were not entirely sterile (as was shown by the determination of germ total), these results indicate that the metabolism of fenhexamid, and in particular the formation of bound residues, are partly abiotic processes, which are however enhanced by the activity of soil microorganisms. All degradation products observed were of transient nature which is indicated by the high amounts of CO_2.

A summary of the proposed metabolic pathway of KBR 2738 in aerobic soil is presented in Figure 7.1.1-1.

Figure 7.1.1-1: **Proposed metabolic pathway for KBR 2738 in aerobic soil.**



IIA 7.1.2 Anaerobic degradation

Due to the proposed use patterns (application as a fungicide in fruits and vegetables) it can be justified that KBR 2738 will not be exposed to anaerobic conditions. Therefore, a study on anaerobic degradation is considered not relevant.
However, an anaerobic soil degradation was conducted, but not summarized here.

Report:	**KIIA 7.1.2 /01; [redacted] 2008**
Title:	[Phenyl-UL-14C] Fenhexamid: Anaerobic Soil Metabolism
Report No Document No	MEKBL007-1 **M-367218-02-1**
Guidelines:	- OECD Guidelines for Testing of Chemicals, Guideline 307, 2002 - US EPA Subdivision N, Section 162-2, Anaerobic soil metabolism, 1982

IIA 7.2 Rate of degradation in soil(s) - laboratory studies

IIA 7.2.1 Aerobic degradation of the active substance in soils at 20 °C

An evaluation of the kinetics of degradation was performed separately for two studies on route and rate of degradation in aerobic soil under laboratory conditions. The half-lives derived from evaluations serve for comparison with EU trigger endpoints.

Report:	**KIIA 7.1.1 /01; [redacted] 2011**
Title:	[cyclohexyl-1-^{14}C]Fenhexamid (KBR 2738): Aerobic Degradation/Metabolism in Four European Soils
Report No & Document No	MEF-11/037 **M-415995-01-1**
Guidelines:	- OECD: Guideline 307, Aerobic and Anaerobic Transformation in Soil, 2002 - Commission Directive 95/36/EC amending Directive 91/414/EEC (Annexes I and II, Fate and Behaviour in the Environment), 1995 - Regulation (EC) No. 1107/2009
GLP	Yes

Report:	**KIIA 7.2.1 /01; [redacted], 2012**
Title:	Kinetic Evaluation of the Aerobic Metabolism of [Cyclohexyl-1-14C]Fenhexamid (KBR 2738) in Soil for Modelling and Trigger Purposes
Report No & Document No	MEF-11/690 **M-421881-01-1**
Guidelines:	"Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration". Report of the FOCUS Work Group on Degradation Kinetics. EC Sanco/10058/2005 ver.2.0, 2006
GLP	No (calculation)

Executive Summary

The aerobic degradation of [Cyclohexyl-1-^{14}C]fenhexamid and its metabolite BCS-CQ88719 (M24) was kinetically evaluated based on one laboratory study ([redacted], 2011) on 4 soils in total (20 °C, 55 % of maximum water holding capacity).

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006) and can be considered as a basis for modelling and persistence endpoints.

For the parent compound fenhexamid the FOMC model was selected in all soils except [redacted] (SFO) to estimate modelling (Table 7.2.1-1) and persistence (Table 7.2.1-2) endpoints. For BCS-CQ88719 (M24) reliable conservative formation fractions were estimated.

Table 7.2.1-1: Optimised degradation parameters of fenhexamid for modelling endpoints and formation fractions (FF) of BCS-CQ88719 (M24).

Soil	Model	k-rate$_{SFO}$ [1/day]	DT50$_{SFO}$ [days]	FF (M24) [-]
[redacted]	FOMC	1.682	0.41)a	0.11
[redacted]	FOMC	1.250	0.56)a	0.09
[redacted]	FOMC	1.931	0.36)a	0.06
[redacted]	SFO	0.915	0.76	0.05

)a calculated from DT90 of bi-phasic model/3.32

Table 7.2.1-2: Optimised degradation parameters of fenhexamid for persistence endpoints.

Soil	Model	DT50 [days]	DT90 [days]
[redacted]	FOMC	0.16	1.37
[redacted]	FOMC	0.21	1.84
[redacted]	FOMC	0.08	1.19
[redacted]	SFO	0.76	2.52

I. Material and Methods

Details on the study conduct and its results are summarized under IIA 7.1.1/01. The degradation data for KBR 2738 and its metabolite BCS-CQ88719 (M24), a [C-C]dimer of KBR 2738 were kinetically evaluated following FOCUS guidance with the software KinGUI, version 1.1.

The measured values were taken into account as reported and thus treated as individual replicates. All sets with their data points were weighted equally. The concentration at time zero was included in the parameter optimisation with the initial value being allowed to be estimated by the model.

For all residues used in the kinetic evaluation, the following procedure was applied. The parent value at time 0 was set to the value of the total mass balance at this time point. The metabolite value at the same time point was set to "0". This procedure assumes that at time 0 no dissipation of the parent substance occurs.

The kinetic evaluation took into account the models simple first order (SFO), first order multi compartment (FOMC, Gustafson-Holden), Hockey-stick (HS) and double first order in parallel

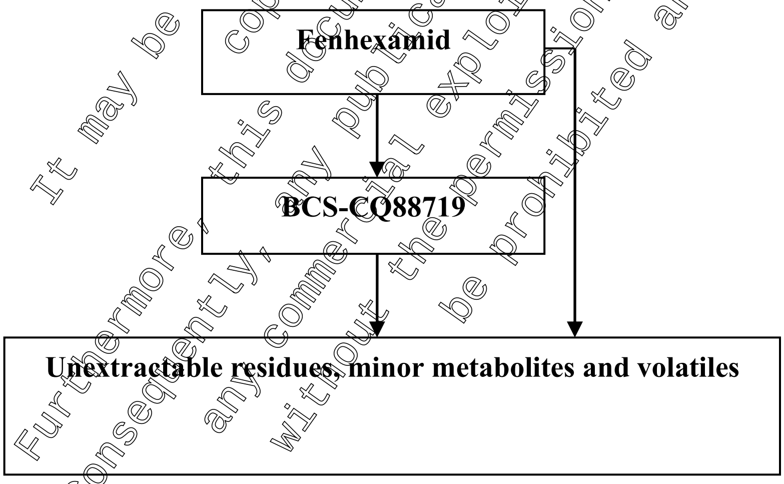
(DFOP). The parameters of the four models were estimated each by non-linear optimization to the measured data with KinGUI.
The identification of the appropriate kinetic model was based on visual assessment of fits and the chi^2 scaled-error (χ^2) to be a minimum. The visual inspection focused on a rather random distribution of residuals rather than to show a systematic distribution from the calculated curve. However, in case of small systematic deviations from the curve, fits could still be qualified as visually acceptable.

A single-sided T-test is used to identify the probability that a parameter is not significant, that is equal or smaller than zero. Generally, this probability is the higher the more uncertain a parameter is. Let p be the value of a parameter and s its standard deviation then its T-value is calculated as $T = p / s$. Finally the probability of this T (t-prob) is obtained from the Student's t-distribution where the degrees of freedom are defined as the number of measurements minus the numbers of model parameter. Normally, a probability t-prob of 0.05 is considered as sufficiently small. A t-prob up to 0.1 is deemed acceptable if it can be justified by the fit or weight of evidence from other data. The degradation parameters are required to be significant for modelling purposes.

Other than for the parent, it will be often neither feasible nor meaningful to differentiate between SFO and the bi-phasic models for metabolites because the number of free parameters for such systems assuming SFO is already at its limit. The use of bi-phasic models easily doubles the number of free parameters which results in more uncertain, i.e. non-significant parameters.

In a first step the parent is fitted without considering the metabolite data to identify the appropriate parent model. In a second step the metabolite is added to the selected parent model and all parameter are optimized simultaneously. If the metabolite can not be fitted adequately a decline fit is performed. The aerobic soil metabolism of fenhexamid can be characterised by the metabolic pathway shown in Figure 7.2.1-1.

Figure 7.2.1-1: Compartment model used for the kinetic analysis of [Cyclohexyl-1-14C] fenhexamid in aerobic soil systems.



II. Results and Discussion

Model selection for parent compound KBR 2738:

The best-fit models were selected according to visual assessment and lowest χ^2 error as it is summarized in Table 7.2.1-3. The fits from application of the SFO, FOMC and DFOP model were significantly different with regard to the visual assessment as well as for the difference in chi² error with values being as high as approximately 20% for the SFO model for some soils.

The bi-phasic model FOMC provided the best fits for the three soils [redacted] and [redacted]. The degradation rates were significant (t-prob <0.05) for all cases with the exception for soil [redacted].

For soil [redacted] degradation was described best by the SFO model. Only for this model the t-prob gave significant degradation rates.

Table 7.2.1-3: Chi2-error, visual assessment (VA) and significance of degradation rate (t-prob) for fenhexamid in the soils studied by [redacted] (2011). Kinetic evaluations accepted are given in bold for modelling endpoints and italic for persistence endpoints.

Soil	Model	Chi2 [%]	VA)[a]	t-prob)[b]
[redacted]	SFO	19.2	-	<0.001
	FOMC	***7.2***	+	***<0.001/<0.001***
	DFOP	9.3	+	0.002
[redacted]	SFO	19.4	-	<0.001
	FOMC	***6.3***	+	***<0.001/<0.001***
	DFOP	8.2	+	<0.001
[redacted]	SFO	19.9	-	<0.001
	FOMC	***6.9***	+	***<0.001/<0.001***
	DFOP	9.8	+	0.003
[redacted]	***SFO***	***12.0***	+	***<0.001***
	FOMC	12.5	+	0.231/0.248

)[a] Visual assessment: + good, o acceptable, - not acceptable

)[b] t-prob of k-rate for SFO, of Alpha/Beta for FOMC, and k-slow for DFOP

Model selection for metabolite [C-C]biphenyl of KBR 2738 (BCS-CQ88719 (M24)):

In Table 7.2.1-4 the results of the evaluation of the modelling and persistence endpoints of BCS-CQ88719 (M24) are summarized. No valid estimation was possible.

Table 7.2.1-4: Chi2-error, visual assessment (VA) and significance of degradation rate (t-prob) for BCS-CQ88719 (M24) in the soils studied by [redacted] (2011) modelled with decline fits. Kinetic evaluations accepted are given in bold for modelling endpoints and italic for persistence endpoints.

Soil	Model	Chi2 [%]	VA)[a]	t-prob)[b]
[redacted]	SFO (parent FOMC)	33.6	-	<0.001
[redacted]	SFO (parent FOMC)	29.0	-	<0.001
[redacted]	SFO (parent FOMC)	36.9	-	<0.001
[redacted]	SFO (parent SFO)	27.2	-	0.064

)[a] Visual assessment: + good, o acceptable, - not acceptable

---

)[b] t-prob of k-rate for SFO

Estimation of Formation Fractions

The degradation rate of the metabolite BCS-CQ88719 (M24) could not be evaluated appropriately. Therefore also the validity of the estimated values for the formation fraction (FF) from the fits is questionable, although the formation and maximum concentration of the metabolite was fitted very well and FF was determined with relatively small uncertainties (standard deviation << estimated value).

A further degradation study is available in which BCS-CQ88719 (M24) is applied directly to the same four soils as in the current study. The degradation rate could be conservatively evaluated with FOMC and DFOP.

In the following it is tested whether the substance parameter values (DT50 of parent and FF from current study and DT50 of BCS-CQ88719 (M24)) result in a conservative representation of the current degradation study. This would indicate that the leaching assessment based on these values will result in higher concentrations than expected from the studies and are therefore conservative.

For each of the four soils forward runs were performed with all parameters fixed to their estimated values. These parameters were fixed and forward runs calculated with KinGUI for all four soils.

It is demonstrated that for each soil the measured concentrations are usually widely overpredicted during the study period of 120 days. The parameters used for risk assessment, including the formation fractions, hence conservatively describe the experimental concentrations. The usage of the formation fraction therefore appears justified.

III. Conclusion

Values for half-lives and DT90 were determined for the parent compound KBR 2738. The values are suitable for comparison with trigger endpoints within the environmental risk assessment in soil.
The degradation of the parent fenhexamid could be described best by bi-phasic kinetic model FOMC for the soils [redacted] and [redacted]. Reliable formation fractions of the BCS-CQ88719 (M24), a [C-C]-dimer of KBR2738 could be estimated.

After selection of most appropriate kinetic models according to the results given in Tables 7.2.1-3 and 7.2.1-4 values for half-lives of degradation and their associated DT90-values were obtained as it is summarized for the parent compound KBR 2738 in Table 7.2.1-5.

Table 7.2.1-5: Optimised degradation parameters of fenhexamid for persistence endpoints.

Soil	Model	DT50 [days]	DT90 [days]
[redacted]	FOMC	0.16	1.37
[redacted]	FOMC	0.21	1.84
[redacted]	FOMC	0.08	1.19
[redacted]	SFO	0.76	2.52

All calculated half-lives for the parent fenhexamid were clearly below 1 day, even the DT_{90} did not exceed 3 days.

New kinetic evaluation of a former aerobic soil degradation study with the [phenyl-UL-^{14}C] labeled fenhexamid was done to derive half-lives of fenhexamid for use as input parameters in environmental exposure assessments by modelling.

Report:	**KIIA 7.2.1 /01; [redacted]**
Title:	Aerobic degradation and metabolism of KBR 2738 in soil
Report No & Document No	PF4195 // MR 434/96 **M-002410-02-1**
Guidelines:	- US EPA Pesticide Assessment Guidelines, Subdivision N Chemistry: Environmental Fate, § 162-1 Aerobic Soil Metabolism Studies, 1982 - BBA, Guidelines for Official Testing of Plant Protectants, Part IV, 4-1,1986
GLP	Yes

Report:	**KIIA 7.2.1 /02; [redacted] 2012**
Title:	Kinetic Evaluation of the Aerobic Metabolism of [phenyl-UL-^{14}C]Fenhexamid (KBR 2738) in Soil for Modelling and Trigger Purposes
Report No & Document No	MEF-11/689 **M-422395-01-1**
Guidelines:	"Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration". Report of the FOCUS Work Group on Degradation Kinetics. EC Document Reference Sanco/10058/2005 version 2.0, 2006
GLP	No (calculation)

Executive Summary

The aerobic degradation of [phenyl-UL-^{14}C] fenhexamid was kinetically evaluated based on one laboratory study ([redacted], 1996) on four soils (20 °C, 40 % of maximum water holding capacity, for one soil ([redacted]) 75% of 1/3 bar moisture).

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006) and can be considered as a basis for modelling and persistence endpoints.

As kinetic function to estimate the modelling endpoint in all cases the SFO model was selected except [redacted] (FOMC). As kinetic function to estimate the persistence endpoint in two cases the FOMC model ([redacted] and [redacted]) and in two cases the DFOP model ([redacted] and [redacted]) was selected.

Table 7.2.1-6: Optimised degradation parameters of fenhexamid for modelling endpoints.

Soil	Model	k-rate$_{SFO}$ [1/days]	DT50$_{SFO}$ [days]
[redacted]	SFO	2.023	0.34
[redacted]	SFO	2.362	0.29
[redacted]	FOMC	0.232	2.99)^{a}
[redacted]	SFO	1.555	0.45

)[a] calculated from DT90 of FOMC model/3.32

Table 7.2.1-7: Optimised degradation parameters of fenhexamid for persistence endpoints.

Soil	Model	DT50 [days]	DT90 [days]
[redacted]	FOMC	0.12	1.37
[redacted]	DFOP	0.27	0.96
[redacted]	FOMC	1.06	9.93
[redacted]	DFOP	0.41	1.63

I. Material and Methods

Details on the study conduct and its results are summarized in the old dossier submitted in 1997. The degradation data for KBR 2738 were kinetically evaluated following FOCUS guidance with the software KinGUI, version 1.1.

The measured values were taken into account as reported and thus treated as individual replicates. All sets with their data points were weighted equally. The concentration at time zero was included in the parameter optimisation with the initial value being allowed to be estimated by the model.

For all residues used in the kinetic evaluation, the following procedure was applied. The parent value at time 0 was set to the value of the total mass balance at this time point.

“Non-detects” (n.d.) at sampling intervals before or directly after the last detect were replaced by a concentration of 0.5 x LOQ (LOQ = 0.2% AR). All other sampling times without detectable amounts were not considered for the evaluation.

Sampling points at dates > 121 days after application were excluded from evaluation. This is justified when taking into account that viability of soil microbes is known to suffer during prolonged storage of soils under laboratory conditions.

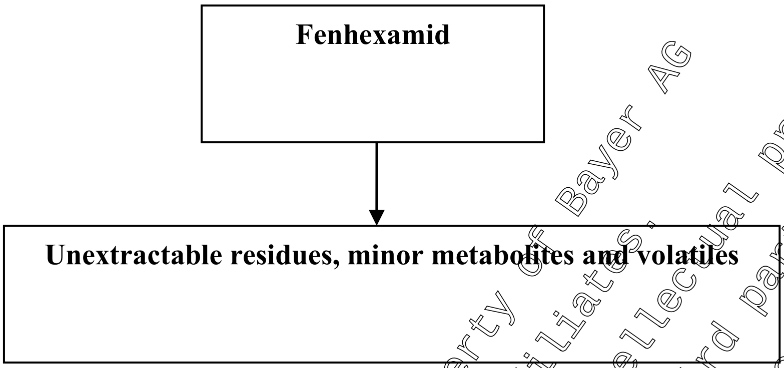
The kinetic evaluation took into account the models simple first order (SFO), first order multi compartment (FOMC, Gustafson-Holden), Hockey-stick (HS) and double first order in parallel (DFOP). The parameters of the four models were estimated each by non-linear optimization to the measured data with KinGUI.

The identification of the appropriate kinetic model was based on visual assessment of fits and the chi^2 scaled-error (χ^2) to be a minimum. The visual inspection focused on a rather random distribution of residuals rather than to show a systematic distribution from the calculated curve. However, in case of small systematic deviations from the curve, fits could still be qualified as visually acceptable.

A single-sided T-test is used to identify the probability that a parameter is not significant, that is equal or smaller than zero. Generally, this probability is the higher the more uncertain a parameter is. Let p be the value of a parameter and s its standard deviation then its T-value is calculated as T = p / s. Finally the probability of this T (t-prob) is obtained from the Student’s t-distribution where the degrees of freedom are defined as the number of measurements minus the numbers of model parameter. Normally, a probability t-prob of 0.05 is considered as sufficiently small. A t-prob up to 0.1 is deemed acceptable if it can be justified by the fit or weight of evidence from other data. The degradation parameters are required to be significant for modelling purposes.

The aerobic soil metabolism of fenhexamid can be characterised by the metabolic pathway shown in Figure 7.2.1-2.

Figure 7.2.1-2: Compartment model used for the kinetic analysis of fenhexamid in aerobic soil systems.



II. Results and Discussion

Model selection for [phenyl-UL-^{14}C]fenhexamid:

The best-fit models were selected according to visual assessment and lowest χ^2 error as it is summarized in Table 7.2.1-8.

For modelling endpoints the SFO kinetic model was taken for the soils [redacted] and [redacted], only for the soil [redacted] the FOMC kinetic model was choosen due to the visual assessment. For persistent endpoints the kinetic model FOMC was evaluated for the soils [redacted] and [redacted] and for the soils [redacted] and [redacted] the kinetic model DFOP was evaluated.

Table 7.2.1-8: Chi2-error, visual assessment (VA) and significance of degradation rate (t-prob) for fenhexamid in the soils studied by [redacted] (1996). Kinetic evaluations accepted are given in bold for modelling endpoints and italic for persistence endpoints.

Soil	Model	Chi2 [%]	VA)[a]	t-prob)[b]
[redacted]	**SFO**	**11.0**	**o**	**<0.001**
	FOMC	*3.0*	*+*	*<0.001/<0.001*
	HS	4.3	+	0.004
	DFOP	4.2	+	0.004
[redacted]	**SFO**	**6.5**	**o**	**<0.001**
	FOMC	2.2	+	<0.001/0.046
	HS	1.55	+	0.025
	DFOP	*1.52*	*+*	*0.025*
[redacted]	SFO	13.2	-	<0.001
	FOMC	***1.7***	***+***	***<0.001/<0.001***
	HS	5.5	o	0.005
	DFOP	4.9	o	0.017
[redacted]	**SFO**	**7.2**	**o**	**<0.001**
	FOMC	2.3	+	<0.001/0.014
	HS	2.3	+	0.062
	DFOP	*2.1*	*+*	*0.073*

)[a] Visual assessment: + good, o acceptable, - not acceptable

)[b] t-prob of k-rate for SFO, of Alpha/Beta for FOMC, and k-slow for DFOP and HS

III. Conclusion

Values for half-lives and DT90 were determined for the parent compound KBR 2738. The values are suitable for comparison with trigger endpoints within the environmental risk assessment in soil.

The degradation of the parent fenhexamid could be described best by bi-phasic kinetic model FOMC for the soils [redacted] and [redacted] and by the bi-phasic model DFOP for the soils [redacted] and [redacted].

Table 7.2.1-5: Optimised degradation parameters of [phenyl-UL-^{14}C] fenhexamid for persistence endpoints.

Soil	Model	DT50 [days]	DT90 [days]
[redacted]	FOMC	0.12	1.37
[redacted]	DFOP	0.27	0.96

[redacted]	FOMC	1.06	9.93
[redacted]	DFOP	0.41	1.63

Besides for soil [redacted] which has a half life of 1.06 days all other calculated half-lives for the parent fenhexamid were clearly below 1 day, even the DT_{90} did not exceed 10 days.

IIA 7.2.2 Aerobic degradation of the active substance in soils at 10 °C

No particular study was performed to investigate the aerobic degradation of KBR 2738 in soil at the lower temperature of 10 °C under laboratory conditions. Degradation rates may be extrapolated to lower temperatures by an Arrhenius type approach. For a conversion of DT_{50} values determined at 20 °C to rates at 10 °C a multiplication factor of 2.58 may be used. This procedure follows a proposal described by the EFSA: Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. The EFSA Journal (2007) 622, 1-32.

IIA 7.2.3 Aerobic degradation of relevant metabolites in soils at 20 °C

Route of degradation studies performed with the [cyclohexane-1-^{14}C] fenhexamid in aerobic soil (IIA 7.1.1/02) indicated [C-C]-biphenyl of fenhexamid (M24) to be significant transformation product formed under laboratory conditions.

The [C-C]-biphenyl of fenhexamid, BCS-CQ88719 (M24) was observed at maximum values of 8.8% AR in soil [redacted] ([redacted]) by day 1 and declined towards study end down to 1.7 % of AR. The potential formation at a significant level in aerobic soil thus triggers environmental risk assessments in soil, groundwater and surface water.

For metabolite BCS-CQ88719 (M24) the corresponding laboratory data on rate of degradation and their evaluations with regard to trigger evaluation and modelling input parameters is summarized in the following.

Report:	**KIIA 7.2.3 /01; [redacted]., 2011**
Title:	[Biphenyl-UL-^{14}C]BCS-BCS-CQ88719: Aerobic Degradation in Four European Soils
Report No & Document No	MEF-11/940 **M-422085-01-1**
Guidelines:	- OECD: Guideline 307, Aerobic and Anaerobic Transformation in Soil, 2002
GLP	Yes

Executive Summary

The degradation [biphenyl-UL-^{14}C]BCS-CQ88719 (M24) was investigated under aerobic conditions at 20 °C and a soil moisture of 55% of MWHC in four European soils by incubation in the dark for 120 days in maximum. The test substance was applied at a nominal test concentration of 235 µg BCS-CQ88719 (M24)/kg soil, equivalent to a rate of 1000 g a.s. fenhexamid/ha in the field and a maximum occurrence of 8.8% AR in studies on route of degradation (2.5 cm depth, 1.5 g/cm^3 bulk density).
The applied radioactivity (AR) was completely recovered (91.7- 98.3% AR). After 120 days of incubation, non-extractable radioactivity ranged from 58.9–65.9% AR. Mineralisation was moderate to account for 5.2-7.4% AR as ^{14}C-carbon dioxide by day 120. Formation of other organic volatile components was insignificant (≤ 0.1% AR).
The biotransformation in soil resulted in the formation of a bunch of minor metabolites. To facilitate the analyses of the radio-chromatograms the chromatograms were roughly devided into three regions of which each reagion contains a bunch of metabolites. Non of those metabolites exceed the trigger of 5% of AR compared to the parent fenhexamid. Therefore those regions were not further characterized.

The biotic character of degradation of BCS-CQ88719 (M24) in aerobic soil is indicated by the formation of non-extractable (bound) residues *via* minor metabolites and the formation of ^{14}C-carbon dioxide to a moderate, but marked extent.
Investigations of the degradation behaviour of the [C-C]-biphenyl of fenhexamid (BCS-CQ88719 (M24)) in four aerobic soils resulted in half-lives of 1.18 to 22.74 days (geometric mean: 5.10 days) for best fits following FOCUS kinetic guidance.

I. Material and Methods

A. Materials

1. **Test Material:** [Biphenyl-UL-^{14}C] BCS-CQ88719 (M24)
   Specific radioactivity: 3.33 MBq/mg
   Radiochemical purity: >98% (Radio-HPLC)
   Chemical purity: > 99% (HPLC-UV)
   Sample ID: KML 9120
2. **Soil:** Soil samples as characterised in Table 7.2.3-1 were collected freshly from the field. A few days before start of the test the air dried soils were sieved through a 2 mm sieve.

Table 7.2.3-1: Characteristics of test soils

Soil	[redacted]	[redacted]	[redacted]	[redacted]
Geographic Location (City / State / Country)	NRW/ Germany	[redacted] NRW Germany	[redacted]/ NRW Germany	[redacted]/ NRW / Germany
GPS coordinates	[redacted]	[redacted]	[redacted]	[redacted]
Pesticide use history	No pesticide use for previous 5 years			
Collection procedures	Sample taken with shovel and transport in plastic bag			
Sampling depth	0 – 20 cm			
Storage conditions	Room temperature			
Storage length	4-5 days after sieving before pre-incubation			
Soil preparation	Sieved (2 mm)			
Texture Class (USDA)	Sandy loam	Silt loam	Loam	Clay loam
Sand [50 µm - 2 mm] (%) Silt [2 µm - 50 µm] (%) Clay [< 2 µm] (%)	77 14 9	23 60 17	33 50 17	33 36 31
pH in Water pH in $CaCl_2$ (0.01 M) pH in KCl (1 M)	5.9 5.7 5.5	6.4 6.2 5.9	5.2 4.9 4.5	7.2 7.1 6.9
Organic Matter ^{A} (%)	3.3	3.1	5.0	9.7
Organic Carbon (%)	1.9	1.8	2.9	5.6
CEC (meq/100 g)	9.8	12.1	10.0	22.0
MWHC (g/100 g)	47.1	52.0	60.6	81.6
Moisture at 1/3 bar = pF 2.5 (g H_2O /100 g dry soil)	not reported			
Microbial biomass (mg $C_{biomass}$/100 g dry vital soil)				
Initial (Day 0) Final, Day 120 (-/+)	675 312/151	840 407/169	587 250/110	2926 1999/2123

[redacted] $^{A)}$ % organic matter = % organic carbon × 1.724;
CEC: Cation exchange capacity; MWHC: Maximum Water Holding Capacity; n.d.: not determined

B. Study design

1. Experimental conditions: Samples of 100 g dry weight of soil each were filled into Erlenmeyer incubation flasks and pre-equilibrated for 3-4 days (darkness, 20 °C, moisture content of 55% MWHC). At application each sample received a dose of [biphenyl-UL-^{14}C] BCS-CQ88719 (M24) equivalent to a nominal test concentration of 235 µg BCS-CQ88719 (M24)/kg soil, corresponding to a field rate of 1000 g a.s. fenhexamid/ha and a maximum occurrence of 8.8% AR in study IIA 7.1.1/02. For dose calculation incorporation into the top 2.5 cm of soil and a bulk density of 1.5 g/mL was assumed. For application the targeted amount of test item dissolved in DMSO (dimethyl sulfoxide) was dispensed drop-wise to the soil surface as uniformly as possible by an adjustable pipette.

The actual dose applied per test vessel was 25.1 µg of [biphenyl-UL-^{14}C]BCS-CQ88719 (M24) for all soils. Following application the static test systems were completed by attachment with traps (soda lime and polyurethane foam) for the collection of ^{14}C-carbon dioxide and other volatiles. Samples were incubated at 20 ± 1 °C and a moisture content of 55% MWHC in the dark for 120 days in maximum.

In addition, samples containing untreated soil were incubated under the same conditions for determination of soil microbial activity at selected time points.

2. Sampling: Duplicate samples were removed for work-up after 0, 0.1, 1, 3, 8, 14, 21, 30, 44, 63, 91 and 120 days of incubation. Samples for determination of soil microbial biomass were investigated after 0 and 120 days of incubation. The complete samples were immediately processed by extraction and HPLC analysis was usually performed within three days. After analysis, the extracts were stored in a freezer. In addition to the HPLC-analysis, representative extracts were subjected to TLC-analysis within about 5 month after sampling. The trap attachments containing soda lime were processed within a maximum of 78 days. The PU-foam was extracted and analyzed within 58 days. Bound residues were analyzed by combustion and subsequent LSC measurements within up to 68 days after sampling.

3. Analytical procedures: The entire soil sample in each test vessel was processed by a stepwise extraction procedure. After separation by centrifugation the soil was extracted four times successively with aqueous acetonitrile (80:20, v/v) by shaking at ambient temperature for 30 min followed by centrifugation (cold extract). An additional extraction was performed using microwave for 10 min at approximately 70°C with aqueous acetonitrile (80:20, v/v) on the residual soil as the last extraction step (hot extract). The different extraction fractions from each extraction "cold" or "hot" were combined and if necessary extracts were concentrated to a small volume prior to analysis.

A ^{14}C-material balance was established for each sample by extraction, analysis of volatiles and combustion of non-extractable residues. Following quantitation of radioactivity in each extract by LSC, analysis of extracts was performed by reversed phase HPLC and ^{14}C-flow-through detection techniques. The determination of non-extractable residues (NER) was performed by combustion/LSC of aliquots of the air-dried extracted soil.

Radioactivity was determined by LSC measurements with an LOQ of up to 0.6% of AR depending on the extraction solvent, etc. A single peak of 2.7 Bq could be detected which corresponded to 0.4% and 0.2% of applied radioactivity for the ambient and microwave soil extracts, respectively. Values between maximum LOD (0.4% of applied radioactivity) and LOQ (three times maximum LOD = 1.1% of applied radioactivity) were taken into account in the tables and for calculations.

C. Determination of degradation kinetics: Degradation data were kinetically evaluated according to FOCUS guidance by use of the software KinGui, version 1.1. Following calculations of fits with kinetic models SFO, FOMC and DFOP, the best fit was evaluated by visual assessment and the error of chi-square (χ^2) to be a minimum in the significance test.

II. Results and Discussion

A. Data: The results of aerobic biotransformation of [biphenyl-UL-^{14}C]BCS-CQ88719 (M24) after incubation in four European soils are summarized in Tables 7.2.3-2 to 7.2.3-5.

Table 7.2.3-2: Degradation of [biphenyl-UL-14C]BCS-CQ88719 (M24) in sandy loam soil [redacted] [redacted] under aerobic conditions

Compound	Replicate No	Residues (% AR) on the following days after treatment (DAT)											
		0	0.1	1	3	8	14	21	30	44	63	91	120
BCS-CQ88719 (M24)	(A)	88.0	84.6	71.5	58.5	41.1	31.1	23.5	21.3	17.8	15.3	9.5	7.0
	(B)	89.3	84.9	73.9	59.2	41.0	30.7	24.0	21.4	18.9	14.5	8.9	7.5
	Mean	88.6	84.7	72.7	58.9	41.0	30.9	23.8	21.4	18.4	14.9	9.2	7.3
Region 1	(A)	2.7	2.9	3.1	3.5	4.0	5.4	5.7	6.2	8.0	8.2	9.2	10.2
	(B)	2.6	2.8	2.7	3.8	4.8	5.9	5.8	7.6	8.0	9.2	9.2	9.9
	Mean	2.7	2.9	2.9	3.6	4.4	5.6	5.8	6.9	8.0	8.7	9.2	10.0
Region 2	(A)	2.0	2.0	1.9	1.8	1.4	1.6	1.5	1.6	1.5	1.8	1.0	0.7
	(B)	2.0	2.2	1.9	1.7	1.6	1.6	1.6	1.8	2.4	1.6	1.3	0.7
	Mean	2.0	2.1	1.9	1.7	1.5	1.6	1.6	1.7	1.9	1.7	1.1	0.7
Region 3	(A)	n.d.	1.0	3.1	3.4	4.7	5.7	6.5	7.1	7.4	7.7	8.7	8.2
	(B)	n.d.	0.6	2.3	3.4	4.3	5.9	6.8	7.3	6.5	8.2	8.8	8.9
	Mean	n.d.	0.8	2.7	3.4	4.5	5.8	6.6	7.2	6.9	7.9	8.7	8.5
Unidentified radioactivity	(A)	4.7	6.0	8.1	8.6	10.1	12.7	13.8	14.8	16.9	17.7	18.9	19.1
	(B)	4.6	5.6	6.9	8.8	10.7	13.4	14.2	16.6	16.8	19.0	19.2	19.4
	Mean	4.7	5.8	7.5	8.7	10.4	13.0	14.0	15.7	16.9	18.3	19.1	19.2
Total extractable Residues*	(A)	92.7	90.6	79.6	67.2	51.2	43.8	37.3	36.2	34.8	32.9	28.4	26.1
	(B)	93.9	90.4	80.7	68.0	51.6	44.1	38.2	38.0	35.7	33.5	28.2	26.9
	Mean	93.3	90.5	80.2	67.6	51.4	44.0	37.8	37.1	35.3	33.2	28.3	26.5
$^{14}CO_2$#	(A)	n.a.	< 0.1	0.1	0.2	0.5	1.1	1.8	2.4	3.2	3.9	4.7	5.5
	(B)	n.a.	< 0.1	0.1	0.2	0.7	1.2	1.8	2.4	3.2	4.0	5.0	6.0
	Mean	n.a.	< 0.1	0.1	0.2	0.6	1.1	1.8	2.4	3.2	4.0	4.8	5.8
Volatile Organics#	(A)	n.a.	< 0.1	< 0.1	< 0.1	0.1	n.d.	n.d.	n.d.	< 0.1	n.d.	< 0.1	< 0.1
	(B)	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	n.d.	< 0.1	n.d.	n.d.	< 0.1
	Mean	n.a.	< 0.1	< 0.1	< 0.1	0.1	< 0.1	n.d.	n.d.	< 0.1	n.d.	< 0.1	< 0.1
Not-Extractable# Residue#	(A)	2.0	6.3	15.3	26.1	40.5	49.2	52.4	54.1	59.5	58.9	60.1	61.2
	(B)	2.0	6.4	14.9	25.4	40.6	47.0	53.0	53.5	57.2	57.5	58.7	59.0
	Mean	2.0	6.3	15.1	25.7	40.5	48.1	52.7	53.8	58.4	58.2	59.4	60.1
Total % Recovery*	(A)	94.7	96.8	95.0	93.5	92.3	94.2	91.5	92.7	97.6	95.7	93.2	92.8
	(B)	95.9	96.8	95.8	93.6	92.9	92.3	93.0	93.8	96.2	95.0	91.8	92.0
	Mean	95.3	96.8	95.4	93.5	92.6	93.2	92.3	93.3	96.9	95.3	92.5	92.4

n.d. : not detected, n.a. : not analyzed, DAT : days after treatment
values taken from material balance
* differences to material balance values may occur due to rounding errors

Table 7.2.3-3: Degradation of [biphenyl-UL-14C]BCS-CQ88719 (M24) in solt loam soil [redacted] under aerobic conditions

Compound	Replicate No	Residues (% AR) on the following days after treatment (DAT)											
		0	0.1	1	3	8	14	21	30	44	63	91	120
BCS-CQ88719 (M24)	(A)	88.7	84.0	70.9	53.9	37.1	26.0	20.7	17.2	14.3	11.0	8.8	7.0
	(B)	89.7	84.4	70.8	55.2	36.8	25.8	20.5	14.3	13.6	10.5	9.5	7.4
	Mean	89.2	84.2	70.9	54.6	37.0	25.9	20.6	15.7	14.0	10.8	9.2	7.2
Region 1	(A)	3.0	3.2	3.5	4.4	3.8	5.3	5.9	5.7	7.1	7.1	6.8	5.5
	(B)	3.4	4.0	3.3	4.0	4.5	4.9	5.4	6.3	7.2	7.3	6.0	5.8
	Mean	3.2	3.6	3.4	4.2	4.1	5.1	5.7	6.0	7.1	7.2	6.4	5.7
Region 2	(A)	2.5	2.4	2.1	2.3	1.8	1.4	1.4	1.6	1.5	1.9	0.8	1.0
	(B)	2.7	2.4	2.3	2.2	2.0	1.8	1.2	1.8	1.5	1.2	0.5	1.0
	Mean	2.6	2.4	2.2	2.2	1.9	1.6	1.3	1.7	1.5	1.6	0.7	1.0
Region 3	(A)	n.d.	0.8	2.2	2.7	3.6	3.7	5.2	6.7	6.3	6.2	6.7	6.9
	(B)	n.d.	0.8	1.9	2.9	3.4	4.0	5.9	7.5	7.4	6.5	7.6	6.5
	Mean	n.d.	0.8	2.0	2.8	3.5	3.9	5.5	7.1	6.8	6.4	7.1	6.7
Unidentified radioactivity	(A)	5.5	6.4	7.8	9.3	9.2	10.4	12.5	14.0	14.9	15.3	14.3	13.4
	(B)	6.1	7.3	7.5	9.1	9.9	10.7	12.6	15.6	16.1	15.1	14.1	13.2
	Mean	5.8	6.8	7.7	9.2	9.5	10.5	12.5	14.8	15.5	15.2	14.2	13.3
Total extractable Residues*	(A)	94.2	90.4	78.7	63.2	46.3	36.4	33.2	31.1	29.3	26.2	23.1	20.4
	(B)	95.8	91.6	78.4	64.3	46.7	36.5	33.1	29.9	29.7	25.6	23.6	20.6
	Mean	95.0	91.0	78.5	63.8	46.5	36.5	33.1	30.5	29.5	25.9	23.4	20.5
$^{14}CO_2$#	(A)	n.a.	< 0.1	0.1	0.4	1.1	1.7	2.5	3.4	4.2	5.2	6.6	7.5
	(B)	n.a.	< 0.1	0.1	0.4	1.0	1.7	2.5	3.5	4.4	5.6	6.4	7.3
	Mean	n.a.	< 0.1	0.1	0.4	1.0	1.7	2.5	3.4	4.3	5.4	6.5	7.4
Volatile Organics#	(A)	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	n.d.	n.d.	< 0.1	< 0.1	n.d.	n.d.
	(B)	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	n.d.	< 0.1	n.d.	< 0.1	n.d.
	Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	n.d.	< 0.1	< 0.1	< 0.1	n.d.
Not-Extractable# Residue#	(A)	2.0	6.8	17.3	31.4	46.1	54.3	57.8	57.3	62.4	63.0	63.6	65.8
	(B)	2.0	6.3	16.9	30.9	45.7	53.6	56.8	58.4	61.7	64.6	64.2	65.1
	Mean	2.0	6.6	17.1	31.1	45.9	53.9	57.3	57.8	62.0	63.8	63.9	65.5
Total % Recovery*	(A)	96.2	97.2	96.2	95.0	93.5	92.4	93.4	91.8	95.8	94.5	93.4	93.7
	(B)	97.8	98.0	95.4	95.6	93.4	91.9	92.4	91.7	95.8	95.8	94.3	93.1
	Mean	97.0	97.6	95.8	95.3	93.4	92.1	92.9	91.7	95.8	95.2	93.8	93.4

n.d. : not detected, n.a. : not analyzed, DAT : days after treatment
values taken from material balance
* differences to material balance values may occur due to rounding errors

Table 7.2.3-4: Degradation of [biphenyl-UL-14C]BCS-CQ88719 (M24) in loam soil [redacted] under aerobic conditions

Compound	Replicate No	Residues (% AR) on the following days after treatment (DAT)											
		0	0.1	1	3	8	14	21	30	44	63	91	120
BCS-CQ88719 (M24)	(A)	78.5	65.8	56.2	47.8	35.5	27.0	23.7	20.7	19.0	16.7	16.2	15.3
	(B)	79.0	65.3	56.6	47.9	36.4	28.9	24.3	22.3	18.8	16.7	15.9	15.3
	Mean	78.7	65.5	56.4	47.8	36.0	28.0	24.0	21.5	18.9	16.7	16.0	15.3
Region 1	(A)	2.3	1.9	2.6	3.4	3.8	3.7	4.9	4.9	5.9	6.0	6.6	6.5
	(B)	2.4	2.3	2.9	3.1	3.4	3.4	4.7	4.5	5.7	6.0	6.1	6.7
	Mean	2.3	2.1	2.7	3.3	3.6	3.5	4.8	4.7	5.8	6.0	6.3	6.6
Region 2	(A)	1.6	1.4	1.9	1.5	1.3	1.2	1.2	1.4	2.0	1.6	0.9	0.7
	(B)	1.6	1.5	1.5	1.1	1.7	1.7	0.9	1.7	1.4	1.2	1.3	1.2
	Mean	1.6	1.4	1.7	1.3	1.5	1.4	1.0	1.5	1.7	1.4	1.1	0.9
Region 3	(A)	0.9	4.4	7.0	6.9	7.7	8.1	9.2	10.6	9.8	8.8	8.1	9.0
	(B)	1.4	3.9	5.6	6.7	7.3	6.9	9.5	9.3	9.4	9.6	8.3	8.4
	Mean	1.2	4.1	6.3	6.8	7.5	7.5	9.4	9.9	9.6	9.2	8.2	8.7
Unidentified radioactivity	(A)	4.8	7.7	11.4	11.9	12.9	12.9	15.3	16.8	17.7	16.4	15.6	16.1
	(B)	5.4	7.6	10.0	11.0	12.4	12.0	15.1	15.5	16.5	16.8	15.7	16.4
	Mean	5.1	7.6	10.7	11.4	12.6	12.4	15.2	16.2	17.1	16.6	15.6	16.2
Total extractable Residues*	(A)	83.3	73.5	67.6	59.7	48.4	39.9	39.0	37.6	36.7	33.1	31.7	31.5
	(B)	84.4	72.9	66.6	58.8	48.8	40.9	39.4	37.8	35.3	33.5	31.6	31.6
	Mean	83.9	73.2	67.1	59.3	48.6	40.4	39.2	37.7	36.0	33.3	31.7	31.5
$^{14}CO_2$#	(A)	n.a.	< 0.1	0.1	0.3	0.8	1.4	2.0	2.9	3.0	4.0	4.9	5.3
	(B)	n.a.	< 0.1	0.1	0.3	0.8	1.4	2.0	2.2	3.1	3.9	4.8	5.2
	Mean	n.a.	< 0.1	0.1	0.3	0.8	1.4	2.0	2.5	3.1	3.9	4.9	5.2
Volatile Organics#	(A)	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	n.d.	n.d.	< 0.1	n.d.	n.d.	n.d.
	(B)	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	n.d.	< 0.1	< 0.1	n.d.	n.d.	< 0.1
	Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	n.d.	< 0.1	< 0.1	n.d.	n.d.	< 0.1
Not-Extractable# Residue#	(A)	14.6	23.1	29.1	35.7	45.1	51.5	51.8	53.4	56.0	57.7	57.4	59.5
	(B)	14.1	23.1	29.7	35.4	44.8	50.8	52.1	53.8	56.9	57.3	56.4	58.3
	Mean	14.4	23.1	29.4	35.5	45.0	51.1	52.0	53.6	56.4	57.5	56.9	58.9
Total % Recovery*	(A)	98.0	96.6	96.9	95.6	94.3	92.9	92.8	93.8	95.7	94.8	94.1	96.3
	(B)	98.5	96.0	96.4	94.5	94.4	93.1	93.6	93.8	95.3	94.7	92.8	95.1
	Mean	98.2	96.3	96.6	95.1	94.3	93.0	93.2	93.8	95.5	94.7	93.4	95.7

n.d. : not detected, n.a. : not analyzed, DAT : days after treatment

values taken from material balance

* differences to material balance values may occur due to rounding errors

Table 7.2.3-5: Degradation of [biphenyl-UL-14C]BCS-CQ88719 (M24) in clay loam soil [redacted] under aerobic conditions

Compound	Replicate No	Residues (% AR) on the following days after treatment (DAT)											
		0	0.1	1	3	8	14	21	30	44	63	91	120
BCS-CQ88719 (M24)	(A)	92.0	89.7	84.8	78.6	68.2	57.3	49.3	40.7	31.2	24.5	18.1	16.0
	(B)	91.4	90.6	84.7	79.9	67.4	56.4	49.9	39.9	31.9	24.1	19.7	15.7
	Mean	91.7	90.1	84.8	79.2	67.8	56.8	49.6	40.3	31.5	24.3	18.9	15.8
Region 1	(A)	1.6	2.4	3.1	3.8	7.6	9.0	9.6	9.1	9.6	8.6	7.1	5.4
	(B)	2.4	2.6	3.5	3.5	7.6	9.3	9.6	10.6	9.1	8.4	6.4	5.7
	Mean	2.0	2.5	3.3	3.7	7.6	9.1	9.6	9.8	9.3	8.5	6.8	5.6
Region 2	(A)	2.1	2.1	1.8	2.1	1.8	1.3	1.4	1.8	1.8	1.2	1.0	0.9
	(B)	2.2	1.8	1.7	2.3	1.4	1.4	1.0	1.4	1.7	1.0	1.0	0.8
	Mean	2.1	1.9	1.7	2.2	1.6	1.3	1.2	1.6	1.7	1.1	1.0	0.9
Region 3	(A)	n.d.	n.d.	0.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	(B)	n.d.	n.d.	0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean	n.d.	n.d.	0.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unidentified radioactivity	(A)	3.7	4.5	5.6	5.9	9.3	10.3	11.0	10.9	11.3	9.8	8.1	6.3
	(B)	4.5	4.3	5.7	5.8	9.0	10.7	10.5	12.0	10.7	9.4	7.4	6.5
	Mean	4.1	4.4	5.7	5.8	9.1	10.5	10.8	11.4	11.0	9.6	7.8	6.4
Total extractable Residues*	(A)	95.7	94.1	90.5	84.5	77.6	67.6	60.3	51.6	42.5	34.4	26.2	22.3
	(B)	96.0	94.9	90.4	85.7	76.4	67.1	60.5	51.9	42.6	33.5	27.1	22.2
	Mean	95.8	94.5	90.4	85.1	77.0	67.3	60.4	51.7	42.6	33.9	26.6	22.2
$^{14}CO_2$#	(A)	n.a.	< 0.1	0.1	0.2	0.5	0.9	1.4	2.0	2.8	3.8	5.4	6.5
	(B)	n.a.	< 0.1	0.1	0.2	0.5	0.9	1.4	2.0	2.9	4.0	5.2	6.7
	Mean	n.a.	< 0.1	0.1	0.2	0.5	0.9	1.4	2.0	2.9	3.9	5.3	6.6
Volatile Organics#	(A)	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1	< 0.1
	(B)	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1	n.d.	n.d.	< 0.1	< 0.1	< 0.1
	Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Not-Extractable# Residue#	(A)	1.4	2.8	6.3	10.1	18.0	26.2	31.4	40.3	50.5	56.2	60.6	66.2
	(B)	1.2	2.6	6.0	10.3	18.7	26.8	32.2	39.9	50.2	56.9	60.6	65.7
	Mean	1.3	2.7	6.2	10.2	18.4	26.5	31.8	40.1	50.4	56.5	60.6	65.9
Total % Recovery*	(A)	97.1	96.9	96.8	94.8	96.1	94.7	93.1	94.0	95.8	94.3	92.1	95.0
	(B)	97.2	97.5	96.5	96.2	95.6	94.8	94.0	93.8	95.7	94.4	92.9	94.5
	Mean	97.1	97.2	96.7	95.5	95.9	94.8	93.6	93.9	95.8	94.3	92.5	94.8

n.d. : not detected, n.a. : not analyzed, DAT : days after treatment
values taken from material balance
* differences to material balance values may occur due to rounding errors

B. **Mass balance:** The total material balances of radioactivity showed a complete recovery to range from 91.7 – 98.3% AR for the four soils investigated. The results are summarised in more detail in Table 7.2.3-6. Conclusively there were no signs for losses of radioactivity from sample work-up and processing.

Table 7.2.3-6: Total material balances of radioactivity in four European soils

Soil	[redacted]	[redacted]	[redacted]	[redacted]
Total Recovery (% AR)	92.3 – 96.9	91.7 – 97.6	93.0 – 98.3	92.5 – 97.2
Mean (% AR)	94.1	94.5	95.0	95.2
Rel. standard deviation	1.8	1.9	1.6	1.5

Values given as percentages of initially applied radioactivity

C. Bound and extractable residues: Values of extractable radioactivity decreased fast with time accompanied by the formation of non-extractable residues as summarized in Table 7.2.3-7. Starting from a complete extractability given by day zero (93.3% for soil [redacted] 95.0% for [redacted] [redacted], 83.9% for [redacted] and 95.8% for [redacted]) values decreased to 26.5% ([redacted]), 20.5% ([redacted]), 31.5% ([redacted]) and 22.2% ([redacted]) after a maximum incubation period of 120 days.

In turn values for non-extractable radioactivity were low by day zero (2.0% for soil [redacted] [redacted] 2.0% for [redacted] [redacted] and 1.3% for [redacted]) with the exception of soil [redacted] with 14.4 to increase to 60.1% ([redacted]), 65.5% ([redacted] [redacted] [redacted]), 58.9% ([redacted]) and 65.9% ([redacted]) at the last sampling interval (day 120).

Table 7.2.3-7: Extractable and non-extractable residues in four European soils (mean ± SD)

Soil	**Extractable residues (%)**		**Non-extractable residues (%)**	
	Day 0	**Day 120**	**Day 0**	**Day 120**
[redacted]	93.3 ±0.6	26.5 ±0.4	2.0 ±0.0	60.1 ±1.1
[redacted]	95.0 ±0.8	20.5 ±0.1	2.0 ±0.0	65.5 ±0.3
[redacted]	83.9 ±0.5	31.5 ±0.1	14.4 ±0.3	58.9 ±0.6
[redacted]	95.8 ±0.1	22.2 ±0.1	1.3 ±0.1	65.9 ±0.2

Values given as percentages of initially applied radioactivity.

D. Volatile radioactivity: The extent of mineralisation to ^{14}C-carbon dioxide was moderate to account for 5.8% AR (soil [redacted]), 7.4% ([redacted] [redacted]), 5.2% ([redacted] [redacted]) and 6.6% ([redacted]) at study end (day 120). Formation of other volatile radioactivity was insignificant for all soils at any sampling interval (≤ 0.1% AR).

E. Transformation of parent compound: There was observed the formation of a bunch of minor metabolites which were grouped to region 1, 2 and 3 for simplification. No single peak of those regions exceeded the trigger of 5% of AR compared to the parent compound fenhexamid. The maximum radioactivity measured in those regions was 10% of AR (region 1) at day 120 in soil [redacted].

The maximum occurrence of further not identified or diffuse radioactivity was 19.2 % of AR in soil [redacted] at day 120. However, compared to the parent fenhexamid this amount is far below 5% and therefore no further investigestions were made.

The biotic character of degradation of the [C-C]-biphenyl of fenhexamid (BCS-CQ88719 (M24)) in aerobic soil is indicated by the formation of non-extractable (bound) residues *via* minor metabolites and the formation of ^{14}C-carbon dioxide to a moderate, but marked extent.

F. Degradation kinetics: The evaluation of degradation kinetics was performed by fitting of data to the three kinetic models SFO, FOMC (Gustafson-Holden) and DFOP[3] and the quality of fits was assessed according to FOCUS kinetic guidance. The initial concentration at time zero was included in the parameter optimisation. All data points were weighted equally. For optimal goodness of fit, the initial value was also allowed to be estimated by the model. The best-fit kinetic model was selected by applying the criteria for chi-square (χ^2) scaled-error to be a minimum and on the basis of visual assessment. The results of the kinetic evaluation are provided in Table 7.2.3-8.

The fits describing degradation of BCS-CQ88719 (M24) in the four soils resulted in different chi-square (χ^2) errors for SFO or biphasic models. When including results of visual assessment best fits were found to follow the FOMC and thus bi-phasic kinetic model for all soils.
The degradation half-lives of BCS-CQ88719 (M24) were estimated to 5.84 days (soil [redacted]), 4.33 days ([redacted] [redacted]), 1.18 days ([redacted]) and 22.74 days ([redacted]) on the basis of FOMC kinetics. The corresponding DT_{90}-values were estimated to 131 days (soil [redacted]), 86 days ([redacted] [redacted]), >1000 days ([redacted]) and 231 days ([redacted]).

Degradation kinetics and their fits were also re-visited in a supplemental report to derive input parameters for modeling purposes in environmental exposure assessments with results presented in IIA 7.2.3/02.

Table 7.2.3-8: Kinetics of aerobic degradation of BCS-CQ88719 (M24) in four soils at 20°C

Soil	Kinetic model	BCS-CQ88719 (M24) DT_{50}	DT_{90}	Chi² value
[redacted]	SFO	11.66	38.74	17.9
	FOMC	**5.84**	**130.73**	**4.5**
	DFOP	5.68	94.44	5.6
[redacted]	SFO	8.55	28.41	18.4
	FOMC	**4.33**	**85.73**	**4.9**
	DFOP	4.48	78.81	7.4
[redacted]	SFO	14.40	47.84	28.5
	FOMC	**1.18**	**> 1000**	**10.2**
	DFOP	2.75	144.56	16.4
[redacted]	SFO	30.43	101.07	8.5
	FOMC	**22.74**	**231.12**	**3.0**
	DFOP	22.68	167.58	3.4
[redacted]	Best fit	5.10		

Best fits according to the criteria set are marked bold.

III. Conclusion

---

[3] SFO = Single first order; FOMC = First order multi compartment; DFOP = Double first order in parallel

Investigations of the degradation behaviour of the [C-C]-biphenyl of fenhexamid (BCS-CQ88719 (M24)) in four aerobic soils resulted in half-lives of 1.18 to 22.74 days (geometric mean: 5.10 days) for best fits following FOCUS kinetic guidance.
Apart from CO2 which accounted for up to 7.4% of AR, numerous minor transformation products were detected, as well as bound residues which reached their maximum values at the end of the study.

Results of kinetic evaluation within study IIA 7.2.3 /01 were re-visited to result in report IIA 7.2.3/02 in order to derive input parameters for modelling use in environmental exposure assessments.

Report:	**KIIA 7.2.3 /02; [redacted] 2011**
Title:	Kinetic Evaluation of the Aerobic Metabolism of BCS-CQ88719 in Soil for Modelling Purposes
Report No & Document No	MEF-11/886 **M-422686-01-1**
Guidelines:	"Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration". Report of the FOCUS Work Group on Degradation Kinetics. EC Document Reference Sanco/10058/2005 version 2.0, 2006
GLP	No (calculation)

Executive Summary

The data on degradation of [biphenyl-UL-^{14}C]BCS-CQ88719 (M24) in four soils were reported and evaluated kinetically for comparison with trigger endpoints in IIA 7.2.3/01. The data were re-visited in order to derive input parameters for modelling use in environmental exposure assessments.

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006) and can be considered as a basis for modelling endpoints.
For BCS-CQ88719 (M24) the FOMC and the DFOP model were selected each in two soils. Degradation parameters relevant for modelling are given in (Table 7.2.3-10). The values from the four EU soils investigated are regarded as suitable and reliable for use as modelling endpoints in environmental exposure assessments.
Half-lives were calculated to range from 25.83 to 75.34 days.

Table 7.2.3-10: Optimised degradation parameters of BCS-CQ88719 (M24) for modelling endpoints.

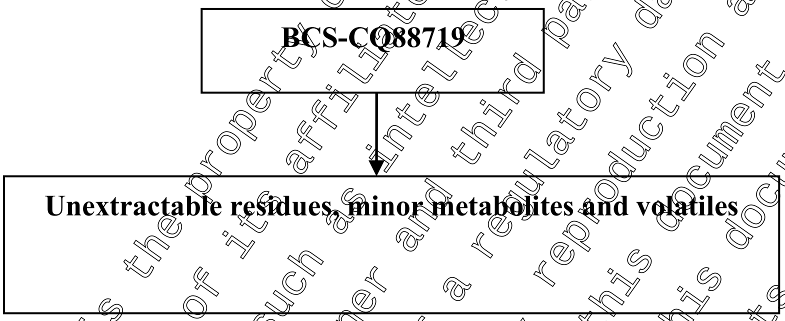
Soil	Model	k-rate$_{SFO}$ [1/day]	DT50$_{SFO}$ [days]
[redacted]	FOMC	0.018	39.42)^{a}
[redacted]	FOMC	0.027	25.83)^{a}
[redacted]	DFOP	0.009)^{b}	75.34
[redacted]	DFOP	0.010)^{b}	72.20

)^{a} calculated from DT90 of FOMC model/3.32
)^{b} calculated from k-slow

I. Material and Methods

Experimental details of the study and its kinetic evaluation are described under IIA 7.2.3/01.
Data were pre-processed as followed: The parent value at time 0 was set to the value of the total mass balance at this time point.
The aerobic soil metabolism of BCS-CQ88719 (M24) can be characterised by the metabolic pathway shown in Figure 7.2.3-1.

Figure 7.2.3-1: Compartment model used for the kinetic analysis of [biphenyl-UL-^{14}C]BCS-CQ88719 (M24) in aerobic soil systems.



The kinetic evaluation was performed following the recommendations of FOCUS (2006) to derive degradation parameters for fate modelling.
The initial amount of the parent was fitted together with the degradation parameters. All data were equally weighted which corresponds to an absolute error model.
The identification of the appropriate kinetic model was based on visual inspection of the fit and the scaled error chi^2. The simple first-order (SFO) model was tested first, because SFO is the simplest of the kinetic models and the one almost exclusively used in environmental exposure models.
If the SFO fit is visually not acceptable or the chi2-value exceeds significantly a value of 15 %, then the bi-phasic models are tested. Finally the model is chosen which is visually acceptable and provides a significantly better fit in terms of chi2. Thus it is avoided to use an over-parameterised model based on a marginally better fit only.
Ideally, the chi^2 value should be below 15 %. However, this value should only be considered as guidance and not absolute cut-off criterion. There might be cases where chi^2 for a metabolite is higher, but the fit still represents a reasonable description of its formation and degradation behaviour. Higher values for metabolites are a consequence of low mean residue levels to which chi^2 is inversely proportional. For example, = 30 % AR (e.g. for parent data) and relative small mean deviation of 1.5 % AR leads to chi^2 = 5 %, whereas the same deviation and = 6 % AR (more typical for a metabolite) leads to chi2 = 25 %.
The bi-phasic models recommended are FOMC, DFOP and HS, where FOMC should not be used in cases where the final residue is more than 10% of its initial value, DFOP and HS should not be used if the residues fall below 10% of its initial value during the study period. For modelling purposes, an equivalent SFO half life ($DT50_{SFO}$) value can be calculated as $DT90_{FOMC}/3.32$. By this method the equivalent SFO-curve meets the bi-phasic curve at the time $DT90_{FOMC}$ and consequently over predicts

the residue values at former times. In cases where DFOP or HS is the most appropriate model, $DT50_{SFO}$ can be calculated by $\ln(2)/k_2$ where k_2 is the slow phase degradation rate estimated by the bi-phasic model.

II. Results and discussion

Soil [redacted]

BCS-CQ88719 (M24) can not be modelled adequately with the SFO model. The initial mass is largely underestimated (82% instead of measured 97%) and degradation in the first days is too slow. After 20 days the measured degradation slows down so that the SFO-curve underestimates the measured concentrations. This results in a systematic deviation between measurements and model fit. The SFO-model is not acceptable. Because the residues fall below 10% of the initial mass within the study period, the FOMC model is applied in the next step. The FOMC model yields an almost perfect fit which is also expressed by the low chi^2-error value of 4.7%. The FOMC model is selected as modelling endpoint.

Soil [redacted]

BCS-CQ88719 (M24) can not be modelled adequately with the SFO model. The initial mass is underestimated (83% instead of measured 98%) and degradation in the first days is too slow. After 20 days the measured degradation slows down so that the SFO-curve underestimates the measured concentrations. This results in a systematic deviation between measurements and model fit. The SFO-model is not acceptable. Because the residues fall below 10% of the initial mass within the study period, the FOMC model is applied in the next step. The FOMC model yields an almost perfect fit which is also expressed by the low chi2-error value of 5.2%. The FOMC model is selected as modelling endpoint.

Soil [redacted]

BCS-CQ88719 (M24) can not be modelled adequately with the SFO model. The initial mass is largely underestimated (69% instead of 100%) and degradation in the first days is too slow. After 20 days the measured degradation slows down so that the SFO-curve underestimates the measured concentrations. This results in a systematic deviation between measurements and model fit. The SFO-model is not acceptable. Because the residues remain above 10% of the initial mass within the study period, the HS- and DFOP models are applied in the next step. Both models yield in satisfying descriptions of the measurements with chi^2-error values of 19.4% (HS) and 16.7% (DFOP). By both models the estimated M0-value is underestimated (77% and 83%, respectively, instead of measured 98%). This underestimation is conservative because the degradation of 20-25% of the substance is not covered by the degradation parameters. This is proved by applying the DFOP-parameters to a M0 of 100% (as would be done in PEC-calculations) and performing a forward calculation. This lead to a slightly more conservative representation of the concentrations than the original fit (with M0 = 83%). The DFOP model is chosen as modelling endpoint, because of the lowest chi^2-error value and a reliable k-slow (t-prob <0.05).

Soil [redacted]

BCS-CQ88719 (M24) can not be modelled adequately with the SFO model. After 60 days the measured concentrations are systematically underestimated by the SFO-curve. The SFO-model is not acceptable. Because the residues remain above 10% of the initial mass within the study period, the HS- and DFOP models are applied in the next step. Both models result in an acceptable fit with low chi^2-error values (HS = 4.7%, DFOP = 3.4%). Due to the lower chi^2-error the DFOP model is selected as modelling endpoint.

In Table 7.2.3-11 the results of the model selection for the modelling endpoints of BCS-CQ88719 (M24) are summarized. For modelling endpoints the SFO model was substituted by the FOMC model in two cases and by the DFOP model in two cases.

Table 7.2.3-11: Chi2-error, visual assessment (VA) and significance of degradation rate (t-prob) for BCS-CQ88719 (M24) in the soils studied by [redacted] 2012). Kinetic evaluations accepted are given in bold for modelling endpoints.

Soil	Model	chi2 [%]	VA)^a	t-prob)^b
[redacted]	SFO	17.9	-	<0.001
	FOMC	**4.7**	**+**	**<0.001/<0.001**
[redacted]	SFO	18.4	-	<0.001
	FOMC	**5.2**	+	**<0.001/<0.001**
[redacted]	SFO	28.5	-	<0.001
	HS	19.4	o	0.068
	DFOP	**16.7**	**o**	**0.005**
[redacted]	SFO	8.5	-	<0.001
	HS	4.7	o	<0.001
	DFOP	**3.4**	**+**	**<0.001**

)^a Visual assessment: + good, o acceptable, - not acceptable
)^b t-prob of k-rate for SFO, of Alpha/Beta for FOMC, and k-slow for DFOP and HS

III. Conclusion

Values for optimized half-lives were determined for metabolite BCS-CQ88719 (M24) using the corresponding model FOMC or DFOP and recalculations according to FOCUS kinetic guidance to result in DT50$_{SFO}$ values. The values from the four EU soils investigated are regarded as suitable and reliable for use as modelling endpoints in environmental exposure assessments.

DT50$_{SFO}$ values ranged from 25.83 to 75.34 days.

IIA 7.3 Field studies

Degradation tests of KBR 2738 in aerobic soil under laboratory conditions resulted in very short half-lives of less than 1 day. Therefore field studies were not taken into account for the investigation of the degradation behavior of fenhexamid nor its major degradation product BCS-CQ88719 (M24).

IIA 7.4 Mobility studies

Due to insufficient parental mass data during former adsorption/desorption study a new study was performed. The sorption behaviour to soil was investigated for the active substance KBR 2738 and its soil metabolites BCS-CQ88719 (M24), a [C-C]-dimer of KBR 2738 using batch equilibrium or soil column leaching experiments with soils covering a distinct range of pH, texture and organic carbon.

IIA 7.4.1 Adsorption and desorption of the active substance

Report:	**KIIA 7.4.1 /01; [redacted] 2011**
Title:	[phenyl-UL-^{14}C]Fenhexamid (KBR 2738): Adsorption/Desorption on Four Soils
Report No & Document No	MEF-11/248 **M-417746-01-1**
Guidelines:	- OECD Guideline for Testing of Chemicals No. 106, Adsorption/Desorption - Commission Directive 95/36/EC amending Council Directive 91/414/EEC (Annexes II, Fate and Behaviour in Environment) July 14, 1995
GLP	Yes

Executive Summary

The adsorption/desorption characteristics of KBR 2738 were determined for four soils in a concentration range of two orders of magnitude. In the definitive test the overall mean values of recoveries for all concentrations were in the range of 94.4 to 100.3% (mean: 97.5%) and thus in an acceptable range.

Values for the coefficients of adsorption according to Freundlich ($K_{F\ ads}$) ranged from 12.43 mL/g to 15.79 mL/g with corresponding values related to organic carbon ($K_{oc\ ads}$) to range from 313 mL/g to 654 mL/g (arithmetic mean: 517 mL/g). Values for the Freundlich coefficient of adsorption 1/n ranged from 0.8604 to 0.8958.

No significant dependence was observed for the adsorption behaviour from pH or the texture of investigated soils.

According to Briggs, fenhexamid can be classified as low mobile to immobile for adsorption and as immobile for desorption.

I. Material and Methods

A. Material

1. **Test Material:** [Phenyl-UL-^{14}C]Fenhexamid (KBR 2738)
   Specific radioactivity: 3.33 MBq/mg (90 µCi/mg)
   Radiochemical purity: > 99% (HPLC, radioactivity-detector)
   Chemical purity: > 99%(HPLC, UV-detector, 210 nm)
   Sample ID: KATH 6488

2. **Soil:** Sorption test were performed with four soils covering a range of pH, organic carbon content and texture. The characteristics of soils originating from Germany are summarised in Table 7.4.1-1. The soils were also used for tests on route and rate of degradation in aerobic soil in the laboratory.

Table 7.4.1-1: Characteristics of test soils

Parameter	Results/Units [C]			
Soil ID/ Batch ID	[redacted] 20100308	[redacted] 20100308	[redacted] 20100308	[redacted] 20100308
Geographic Location (City / State / Country)	[redacted]/ North Rhine-Westphalia/ Germany	[redacted]/ North Rhine-Westphalia/ Germany	[redacted]/ North Rhine-Westphalia/ Germany	[redacted]/ North Rhine-Westphalia/ Germany
Soil Series	N/A	N/A	N/A	N/A
Texture Class [A]	Loamy Sand	Silt Loam	Loam	Clay Loam
Sand [A] Silt [A] Clay [A]	81% 10% 9%	23% 60% 17%	37% 42% 21%	33% 36% 31%
pH (0.01 M $CaCl_2$, 1:2) pH (Water, 1:1) pH (Saturated Paste) pH (1 N KCl, 1:1)	6.2 6.5 6.6 6.1	6.5 6.8 6.8 6.3	5.8 6.1 6.1 5.4	7.4 7.5 7.5 7.1
Organic Matter [B]	3.3%	4.1%	5.0%	7.8%
Organic Carbon	1.9%	2.4%	2.9%	4.5%
Cation Exchange Capacity (CEC)	9.3 meq/100 g	13.4 meq/100 g	10.1 meq/100 g	20.6 meq/100 g
Water Holding Capacity 0.1 bar (pF 2.0)	14.4%	30.8%	31.4 %	37.5%
Water Holding Capacity 0.33 bar (pF 2.5)	11.6%	22.3%	22.5%	32.7%
Bulk Density Particle Density	1.19 g/cm³ N/A	1.05 g/cm³ N/A	1.08 g/cm³ N/A	1.00 g/cm³ N/A
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic, Typic Cambudolls	Loamy, mixed, mesic, Typic Argudalfs	N/A	N/A
Soil Mapping Unit	N 51°04.6' E 006°53.5'	N 51° 04.0' E 007°06.3'	N 51° 04.5' E 007° 06.4'	N 50° 22.9' E 006° 43.0'

[A] according to USDA classification
[B] % organic matter = % organic carbon × 1.724
[C] Analyses performed at [redacted], USA.

B. Study design

1. **Experimental conditions:** Samples of 0.5 g dry weight of soil (2 mm) were weighed each into centrifuge tubes to which a solution of 0.01 M aqueous calcium chloride containing 50 mg HgCl2/L was added to result in a final volume of 18 mL. The slurry was pre-equilibrated for at least one day followed by the addition of 2 mL of the corresponding application solution to result in a final volume of 20 mL and a soil/solution ratio of 1:40. As part of pre-tests control samples containing no soil were prepared the same way for determination of stability of the test item in calcium chloride solution and for testing of adsorption to the walls of the test vessels. Initial nominal concentrations of the ^{14}C-test substance in the aqueous phase were 1, 0.3, 0.1, 0.03 and 0.01 mg/L thus covering two orders of magnitude.

Adsorption and desorption took place in the dark at 20 ± 1 °C for 24 hours each (soils [redacted] and [redacted]) using an overhead shaker at approximately 30 rpm. Due to insufficient stability of the test item in the soil samples [redacted] and [redacted] adsorption was limited to 9 hours and desorption to 15 hours.

The highest concentration tested was therefore significantly below than the water solubility of 19-38 mg/L as determined at different pH (3-9). Acetonitrile was present as an organic co-solvent below 0.1% during the tests from preparation of the application solutions. An initial pH of 6.0 was determined for the aqueous 0.01 M calcium chloride solution in the absence of soil.

Due to the insufficient stability of the test item, it was not possible to establish a plateau concentration. Therefore, 24 hours was chosen as adsorption equilibration time (i.e. shaking duration) for soil samples [redacted] and [redacted] in the definitive test, whereas the adsorption equilibration time for soil samples [redacted] and [redacted] was limited to 9 hours. With regard to the stability of the test item, 24 hours was considered appropriate for desorption equilibrium of the soil samples [redacted] and [redacted] and 15 hours for the soil samples [redacted] and [redacted].

For work-up the aqueous supernatant was separated from soil by decantation and centrifugation (10 min, 4200 rpm). Radioactivity in water and soil extracts was determined by liquid scintillation counting (LSC). Non-extractable radioactivity in soil was determined by combustion followed by LSC to establish a full material balance.

Finally the adsorption parameters were calculated using the Freundlich adsorption isotherm.

2. **Analytical procedures:** Radio-labeled KBR 2738 was determined by liquid scintillation counting (LSC) in the definitive test. HPLC analyses with ^{14}C Detector were used for the parental mass balance in the pre tests. The limit of detection (LOD) was set to 0.3% of applied radioactivity, the limit of quantification (LOQ) to three times the LOD, i.e. approximately 1% of the applied radioactivity. Values between LOD and LOQ are used for calculation just as given.

II. Results and Discussion

A. Mass balance and results of preliminary tests:

Preliminary tests performed on solubility and stability of the test substance in aqueous 0.01 M calcium chloride solution confirmed stability under the conditions of the test. Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil for up to 96 hours showed no adsorption as it is documented by a constant concentration during the total testing period.

Pre-tests on adsorption equilibrium to soil resulted in mass balances ranging from 92.9% to 96.7% of AR (mean values of duplicates) for all soils (Table 7.4.1-2). In parallel, the stability of the test substance was confirmed by parental mass balances of more than 90% of AR.

For the definitive tests the overall mass balance for individual samples at all concentrations and for all soils investigated ranged from 94.4% to 100.3% of AR. The resulting mean recoveries (Table 7.4.1-3) were found to range from 95.6% (soil [redacted]) to 99.3% (soil [redacted]) with standard deviations ranging from ± 0.4% AR (soil [redacted] to ± 1.0% AR (soil [redacted]).

Table 7.4.1-2: Parental mass balance after incubation for 24 and 48 hours, respectively, calculated as percentage of applied radioactivity in solution and soil extract (mean of duplicates).

Matrices	**Soil 1**	**Soil 2**	**Soil 3**	**Soil 4**
Soil ID	[redacted]	[redacted]	[redacted]	[redacted]
Recovery radioactivity in supernatant [% AR]	69.9	71.5	64.2	70.1
Test item in supernatant [Area %] *	99.1	100.0	99.4	99.2
Recovery test item in supernatant [% AR]	**69.3**	**71.5**	**63.8**	**69.5**
Recovery radioactivity in solid phase [% AR]	25.9	26.0	31.1	25.5
Test item in solid phase [Area %] *	95.8	97.1	93.7	96.6
Recovery test item in solid phase [% AR]	**24.9**	**25.3**	**29.1**	**24.6**
Non-extractable residues	N/A	N/A	N/A	N/A
Total recovery of radioactivity [% AR]	95.8	97.5	95.2	95.6
Total recovery of test item [% AR]	**94.1**	**96.7**	**92.9**	**94.1**

Mean for all soils: 94.5 %
* % of Regions of Interest from HPLC
Soils [redacted] and [redacted]: incubation for 48 hours
Soils [redacted] and [redacted]: incubation for 24 hours
N/A = Not available

Table 7.4.1-3: Overall material balance for soils after adsorption, desorption, soil extraction and combustion expressed as percentage of applied radioactivity (one replicate).

Description	**Soil 1**	**Soil 2**	**Soil 3**	**Soil 4**
Soil ID	[redacted]	[redacted]	[redacted]	[redacted]
Test concentration (mg/L)	**Recovery [% AR]**	**Recovery [% AR]**	**Recovery [% AR]**	**Recovery [% AR]**
1.0	99.8	97.2	98.7	96.5
0.3	99.7	95.5	99.3	96.6
0.1	98.7	96.9	100.3	94.4
0.03	99.1	95.8	98.9	94.4
0.01	99.0	95.1	98.6	95.8
Mean recovery	99.3	96.1	99.1	95.6
SD	± 0.4	± 0.8	± 0.6	± 1.0

Data are calculated based on sum of radioactivity in removed supernatants after adsorption and desorption steps, soil extraction and final soil combustion.

B. Transformation of parent compound: The stability of the test substance was confirmed by HPLC analysis to result in parental mass balances of more than 90% of AR.

C. Findings: Based on the results of pre-tests for an adequate soil-to-solution ratio the definitive tests were performed at a ratio of 1:40 for all soils. The equilibration time for adsorption was 24 hours for soils [redacted] and [redacted] and 9 hours for soils [redacted] [redacted] and [redacted], respectively. The equilibration time for desorption was 24 hours for soils [redacted] [redacted] and [redacted] and 15 hours for soils [redacted] [redacted] and [redacted] respectively.

Within definitive tests, the portion of ^{14}C-KBR 2738 adsorbed to soil after 24 hours was found to be 23.7% to 33.8% AR for soil [redacted] 24.7% to 36.6% AR for soil [redacted], 28.5% to 41.9% AR for soil [redacted] and 26.3% to 38.8% AR for soil [redacted] (Table 7.4.1-4).

Following 24/15 hours of desorption the corresponding values for radioactivity desorbed amounted to 47.1% to 54.9% (% of initially adsorbed radioactivity) for soil [redacted] 38.4% to 49.4% for soil [redacted], 36.8% to 49.0% for soil [redacted] and 35.0% to 49.3% for soil [redacted] (Table 7.4.1-5).

The adsorption behaviour of [phenyl-UL-^{14}C]KBR 2738 could be accurately described within a nominal concentration range of 0.01 mg/L to 1.0 mg/L by the Freundlich equation for all soils (Table 7.4.1-6). The adsorption constants $K_{F(ads)}$ of the Freundlich isotherms ranged from 12.43 to 14.07 mL/g with associated Freundlich exponents 1/n to be below 1 for all soils (0.8604 to 0.8958). The adsorption behaviour to soil was thus to some extent affected by the concentration of the test item. The corresponding correlation coefficients of adsorption isotherms ranged from 0.9957 to 0.9996 therefore indicating a good linear fit to the measured data. When being normalized for organic carbon content of soil values of $K_{OC(ads)}$ varied from 313 mL/g (soil [redacted]) to 654 mL/g in maximum (soil [redacted]) with an arithmetic mean of 517 mL/g. Using the Briggs classifications for the estimation of the mobility of crop protection agents in soil based on KF and/or KOC-values, fenhexamid can be classified as low mobile to immobile for adsorption and as immobile for desorption

Desorption constants $K_{F(des)}$ according to Freundlich ranged from 29.23 mL/g (soil [redacted]) to 34.92 mL/g (soil [redacted]). The corresponding values for $K_{OC(des)}$ ranged from 715 mL/g (soil [redacted]) to 1538 mL/g (soil [redacted]) to result in an arithmetic mean of 1225 mL/g.

$K_{OC(des)}$ values were thus slightly higher than the corresponding values of $K_{oc(ads)}$, indicating a strengthening of binding of KBR 2738 once adsorbed to soil particles.

Table 7.4.1-4: Definitive test: Concentration of [phenyl-UL-^{14}C]KBR 2738 in aqueous and soil phase at the end of adsorption equilibrium (mean ± s.d.)

Description			
Concentration of a.i.	**Soil* (mg/kg)**	**Solution (mg/L)**	**Percentage adsorbed**
Soil	[redacted] **(Soil ID:** [redacted]**)**		
Control	N/A	N/A	
0.011 mg/L	0.145	0.007	33.8 ± 0.7
0.031 mg/L	0.390	0.021	31.4 ± 1.3
0.11 mg/L	1.269	0.073	30.2 ± 0.6
0.31 mg/L	3.340	0.231	26.6 ± 0.4
1.04 mg/L	9.843	0.793	23.7 ± 0.2
Soil	[redacted] **(Soil ID:** [redacted]**)**		
Control	N/A	N/A	
0.011 mg/L	0.157	0.007	36.6 ± 0.4
0.031 mg/L	0.431	0.020	34.7 ± 0.3
0.11 mg/L	1.304	0.072	31.0 ± 0.0
0.31 mg/L	3.727	0.221	29.7 ± 0.4
1.04 mg/L	10.278	0.782	24.7 ± 0.2
Soil	[redacted] **(Soil ID:** [redacted]**)**		
Control	N/A	N/A	
0.011 mg/L	0.179	0.006	41.9 ± 0.1
0.031 mg/L	0.489	0.019	39.4 ± 0.2
0.11 mg/L	1.507	0.067	35.9 ± 0.6
0.31 mg/L	4.131	0.211	32.9 ± 0.7
1.04 mg/L	11.824	0.743	28.5 ± 0.3
Soil	[redacted] **(Soil ID:** [redacted]**)**		
Control	N/A	N/A	
0.011 mg/L	0.166	0.007	38.8 ± 0.0
0.031 mg/L	0.501	0.019	40.3 ± 1.1
0.11 mg/L	1.525	0.067	36.3 ± 1.9
0.31 mg/L	3.636	0.223	28.9 ± 0.5
1.04 mg/L	10.921	0.766	26.3 ± 0.6

* The amount of test item adsorbed to the soil was calculated by subtracting the equilibrium concentration in the solution from the initial concentration (applied concentration).

Table 7.4.1-5: Definitive test: Concentration of [phenyl-UL-^{14}C]KBR 2738 in aqueous and soil phase at the end of desorption equilibrium (mean ± s.d.)

Description			
Concentration of a.i.	**Soil (mg/kg)**	**Solution (mg/L)**	**Percentage desorbed***
Soil	[redacted] **(Soil ID: [redacted])**		
Control	N/A	N/A	
0.011 mg/L	0.077	0.002	47.1 ± 0.6
0.031 mg/L	0.200	0.005	48.7 ± 2.2
0.11 mg/L	0.638	0.016	49.8 ± 0.8
0.31 mg/L	1.600	0.043	52.1 ± 0.8
1.04 mg/L	4.435	0.135	54.9 ± 0.0
Soil	[redacted] **(Soil ID [redacted])**		
Control	N/A	N/A	
0.011 mg/L	0.097	0.002	38.4 ± 0.3
0.031 mg/L	0.257	0.004	40.3 ± 0.1
0.11 mg/L	0.736	0.014	43.5 ± 0.4
0.31 mg/L	2.073	0.041	44.4 ± 0.5
1.04 mg/L	5.203	0.127	49.4 ± 0.3
Soil	[redacted] **(Soil ID: [redacted])**		
Control	N/A	N/A	
0.011 mg/L	0.113	0.002	36.8 ± 0.2
0.031 mg/L	0.309	0.005	36.9 ± 0.0
0.11 mg/L	0.873	0.016	42.1 ± 0.9
0.31 mg/L	2.319	0.045	43.9 ± 0.7
1.04 mg/L	6.038	0.145	49.0 ± 1.2
Soil	[redacted] **(Soil ID: [redacted])**		
Control	N/A	N/A	
0.011 mg/L	0.108	0.001	35.0 ± 1.3
0.031 mg/L	0.337	0.004	32.8 ± 1.1
0.11 mg/L	0.969	0.014	36.5 ± 1.9
0.31 mg/L	2.009	0.041	44.8 ± 0.9
1.04 mg/L	5.545	0.134	49.3 ± 1.8

* Expressed as a percentage of the initially adsorbed material, one desorption step for all concentrations.

Table 7.4.1-6: Adsorption and desorption constants of KBR 2738 in soil.

Soil	Soil type	Adsorption					Desorption			
		pH*	K_F [mL/g]	1/n	R^2	K_{OC}** [mL/g]	K_F [mL/g]	1/n	R^2	K_{OC}*** [mL/g]
[redacted]	loamy sand	6.2	12.43	0.8958	0.9991	654	29.23	0.9303	0.9988	1538
[redacted]	silt loam	6.5	13.37	0.8849	0.9993	557	34.92	0.9042	0.9994	1455
[redacted]	loam	5.8	15.79	0.8770	0.9996	544	34.54	0.8846	0.9987	1191
[redacted]	clay loam	7.4	14.07	0.8604	0.9957	313	32.18	0.8504	0.9922	715
Mean (arithmetic)			13.91	0.8795	0.9984	517	32.72	0.8924	0.9973	1225

pH: Value given as determined with 0.01 M calcium chloride solution
K_F: Freundlich coefficients of adsorption (**) and after first desorption (***)
1/n : Slope of the Freundlich adsorption/desorption isotherms
K_{oc}: Adsorption coefficient per organic carbon (K x 100/% organic carbon)
R^2: Regression coefficient of Freundlich equation

III. Conclusion

Overall medium values of adsorption were determined for the active substance KBR 2738. Except for organic carbon content, the Freundlich coeffients K_F for adsorption and desorption did not correlate with other physico-chemical properties of soil. There was also no obvious dependence of adsorption or desorption on pH of soil. This is in good agreement with the chemical structure showing no structural elements that could obviously be a cause for such dependence within the range of pH being relevant for environmental conditions.

The desorption constants $K_{F(des)}$ were two to three times higher than the respective adsorption constants, indicating enhanced sorption of the compound once adsorbed to soil.

IIA 7.4.4 Column leaching studies rel. metabolites, degr. & react. products

Column leaching studies with the soil metabolite BCS-CQ88719 (M24), a [C-C]-dimer of fenhexamid were performed in order to evaluate reliable values for the K_{OC}-value. The classical batch equilibrium method was not feasible due to the insufficient water solubility of the test item.

Report:	**KIIA 7.4.4 /01; [redacted]., 2012**
Title:	[Biphenyl-UL-^{14}C]BCS-CQ88719: Soil Column Leaching
Report No & Document No	MEF-11/991 **M-422470-01-1**
Guidelines:	- OECD Guideline - Testing Chemicals, No. 312, Leaching in Soil Columns, 2004 - US EPA Fate, Transport and Transformation Test Guidelines, OPPTS 835.1240, Leaching Studies, 2008
GLP	Yes

Executive Summary

The soil adsorption behavior of [biphenyl-UL-^{14}C]BCS-CQ88719 (M24) was investigated by a column leaching experiment performed on four test soils from Germany. About 16 µg test item BCS-CQ88719 (M24) was applied onto the surface of bulk-packed soil columns (dimension 30 cm length, 5 cm inner diameter), which had been saturated with 0.01 M aqueous $CaCl_2$ solution before.

The columns were eluted with $CaCl_2$ solution at a constant flow rate of approximately 8.2 mL/h for three days, which equals a simulated constant rainfall of about 100 mm per day, or 300 mm in total. Column elution was conducted under saturated flow conditions throughout the experiment. The column eluates were collected in constant time intervals of 12 hours (approx. 100 mL/fraction) and analyzed by LSC.

No ^{14}C-radioactivity was detected in the leachates. Approximately the whole radioactivity applied was found in the first segment (0-3 cm), only amounts of less than 1% of AR were found in the other segments below.

After draining, the soil columns were deep-frozen and cut into 6 segments. The radioactivity content in each segment was determined by extraction/combustion, followed by LSC. The material balance of each soil column was in the range of 96.2 to 100.8% of the applied test item radioactivity. The complete material balance found for all test columns demonstrated that no significant radioactivity dissipated or was lost during processing.

The evaluation of the numeric soil distribution coefficient (Kd) and the corresponding organic carbon normalized distribution coefficient (KOC) was based on mathematics derived from chromatographic theory.

The following Table 7.4.4-1 summarizes the key data of this study:

Table 7.4.4-1: Summary of key data and results of soil column leaching

Soil origin:	[redacted] **Germany**	[redacted] **Germany**	[redacted] **Germany**	[redacted] **Germany**
Soil type (USDA)	Sandy loam	Silt loam	Loam	Clay loam
pH ($CaCl_2$, soil characterization)	5.7	6.2	4.9	7.1
Organic carbon [%]	1.9	1.8	2.9	5.6
K_d [mL/g]	15.3^1 / 20.7^2	16.1^1 / 21.9^2	18.0^1 / 24.8^2	19.7^1 / 27.4^2
K_{OC} [mL/g]	806^1 / 1090^2	893^1 / 1214^2	621^1 / 856^2	353^1 / 489^2

(Mean K_{OC}: 6681 / 9122 mL/g)

1 calculation according to [redacted], Movement and sorption of chemicals applied to the soil, Weeds13, 185-190, 1965.

2 calculation according to [redacted] "The interpretation of soil leaching experiments", in: Environmental dynamics of pesticides, (Eds. [redacted]), pp.135-172, Plenum Press, N. Y., 1975.

According to the BRIGGS classification system for mobility of crop protection agents in soil, BCS-CQ88719 (M24) would be classified as immobile in soils [redacted] and [redacted], low to immobile in soil [redacted] and low mobile in soil [redacted].

I. Material and Methods

A. Materials

1. Test Material: [Biphenyl-UL-^{14}C]BCS-CQ88719 (M24)
Specific radioactivity: 3.33 MBq/mg (90 µCi/mg)
Radiochemical purity: > 98% (HPLC, TLC)
Chemical purity: > 98% (HPLC)
Sample ID: KML 9120 (original KML 9103)

2. Soil: Soil column leaching test were performed with four soils from Germany covering a range of pH, organic carbon content and texture. The characteristics of four soils originating from Germany are summarised in Table 7.4.4-2. A number of soil types were used for testing route and rate of degradation in aerobic soil in the laboratory.

Table 7.4.4-2: Characteristics of test soils

Parameter	Results/Units^C			
Soil ID/ Batch ID	[redacted] 20110523	[redacted] 20110524	[redacted] 20110524	[redacted] 20110524
Geographic Location (City / State / Country)	[redacted]/ North Rhine-Westphalia/ Germany	[redacted] North Rhine-Westphalia/ Germany	[redacted]/ North Rhine-Westphalia/ Germany	[redacted]/ North Rhine-Westphalia/ Germany
Soil Series	N/A	N/A	N/A	N/A
Texture Class^A	Sandy Loam	Silt Loam	Loam	Clay Loam
Sand^A	77%	23%	33%	23%
Silt^A	14%	60%	50%	44%
Clay^A	9%	17%	17%	33%
pH (0.01 M $CaCl_2$, 1:2)	5.7	6.2	4.9	7.1
pH (Water, 1:1)	5.9	6.4	5.2	7.2
pH (Saturated Paste)	5.9	6.4	5.2	7.1
pH (1 N KCl, 1:1)	5.5	5.9	4.5	6.9
Organic Matter^B	3.3%	3.1%	5.0%	9.7%
Organic Carbon	1.9%	1.8%	2.9%	5.6%
Cation Exchange Capacity (CEC)	9.8 meq/100 g	12.1 meq/100 g	10.0 meq/100 g	22.0 meq/100 g
Water Holding Capacity 0.1 bar (pF 2.0)	18.3%	38.8%	37.0%	43.6%
Water Holding Capacity 0.33 bar (pF 2.5)	10.8%	22.9%	24.9%	34.3%
Bulk Density	1.24 g/cm^3	1.16 g/cm^3	1.04 g/cm^3	0.99 g/cm^3
Particle Density^D	2.58 g/cm^3	2.51 g/cm^3	2.59 g/cm^3	2.52 g/cm^3
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic, Typic Camboudolls	Loamy, mixed, mesic, Typic Argudalfs	N/A	N/A
Soil Mapping Unit	N 51°04.6' E 006°53.5'	N 51° 04.0' E 007°06.3'	N 51° 04.5' E 007° 06.4'	N 50° 22.9' E 006° 43.0'

[A] according to USDA classification
[B] % organic matter = % organic carbon × 1.724
[C] Analyses performed at [redacted] USA.
[D] long term average

B. Study design

1. **Experimental conditions:** The study was performed with cylindrical glass tubes (inner diameter 5 cm, length ca. 45 cm) filled with sieved air-dry soil to a height of approximately 30 cm. During the study the columns were eluted with 0.01 M aqueous CaCl2 solution. Two peristaltic pumps were used for solution delivery to the column top and for active effluent drainage from the column bottom. The set-up allowed for adjustable constant flow, and maintenance of approximately 10-20 mm ponding solution above the top soil layer, such ensuring saturated flow conditions as required for the calculation of Kd/KOC values according to the chromatographic theory.

The outlet of the glass tube was covered with quartz wool. Then, the conical bottom was filled with a layer of washed quartz sand up to a level just reaching the cylindrical part of the glass tube. Small portions of the sieved, air-dry test soil were successively poured into the glass column under gentle vibration to obtain a uniformly, dry-packed soil layer. For a final height of 30 cm, 628 to 775 g of soil were used per column.

The packed soil columns were slowly saturated with 0.01 M CaCl2 solution by reverse flow from bottom to top, to displace the air in the soil pores by water. The columns were equilibrated for approximately 8 hours, before the excess solution was drained and the solution level was brought to soil surface level. Saturation and excess volumes were determined. 15.8 µg of test item BCS-CQ88719 (M24) were applied to the surface of bulk-packed soil columns (30 cm long, 5 cm inner diameter; duplicate columns for each soil) that had been saturated with 0.01 M aqueous CaCl2 solution before. The experiments were performed in duplicates.

For a total of three days, eluent solution was delivered automatically to the column tops by a multichannel peristaltic pump. A ponding solution of 10-20 mm above soil level was maintained, to ensure saturated flow conditions. After gravitational passage through the soil columns, constant flow drainage was achieved by a second pump.

The total irrigation volume that was applied over the 72 hours of the experiment was 589 mL. The eluent flow rates were adjusted to 8.2 mL/h, which equals a simulated constant rainfall of about 100 mm per day. The study was conducted in a walk-in climatic chamber at controlled temperature (20 ± 1 °C). The column eluates were collected in constant time intervals of 12 hours (approximately 100 mL per fraction) by a time-controlled automatic fraction collector and analyzed by LSC. No 14C-radioactivity was detected in the leachates. After the irrigation was finished, the columns were allowed to drain. The leachate fractions were analyzed daily by LSC (2 x 2 mL), and the eluent volumes and pH values were determined.

After draining, the soil columns were deep-frozen. For further analysis, the frozen soil columns were pushed out of the glass tubes and cut into segments:

- columns 1 and 2 of each soil (treated with test item): 6 segments (2 segments of 3 cm length each, 4 segments of 6 cm length each)
- columns 3 and 4 of each soil (treated with reference substance): 5 segments of 6 cm length each

The quartz sand was combined with the bottom segment.

2. **Analytical procedures:** The radioactivity in solutions was determined by liquid scintillation counting (LSC). The LOQ was estimated to 1.2 Bq per 500 µL aliquot measured. The radioactivity in

soil was determined by combustion of the air- and freeze-dried samples after extraction.Extraction procedures are summarized in Table 7.4.4-3:

Table 7.4.4-3: Characteristics of test soils

Samples	Solvent	Volume	Duration	Temperature	Cycles
Column 1*, segments 1-6; Column 2*, segment 1	Acetonitrile	80 mL	30 min shaking	ambient	3
Column 1*, segment 1	Acetonitrile	80 mL	10 min. microwave	70 °C	2
Column 3, segments 1-5**	Acetonitrile/water 4/1 (v/v)	80 mL	30 min shaking	ambient	3

* treated with test item
** treated with reference substance

No particular LOQ for this analytical method was estimated or evaluated in the study.

II. Results and Discussion

A. Mass balance

The material balance of test item was in the range of 96.2 to 100.8% of the applied radioactivity (Table 7.4.4-4 - Table 7.4.4-7). The good material balance in all columns demonstrates that no significant amount of radioactivity dissipated or was lost during processing.
Radioactivity of [^{3}H]-labeled water detected in the leachate fractions accounted for 91.7 to 100.2% of AR. The material balance of atrazine was in the range of 91.7 to 96.7% of the applied radioactivity.

B. Transformation of parent compound: In the soil extracts only parent could be detected. No major degradation products were observed.

C. Findings: Extractable radioactivity in the top segments of soil [redacted] and [redacted] accounted for 56.7, 53.9, 64.0 and 76.9% of AR (mean), respectively (Table 7.4.4-4 - Table 7.4.4-7). No radioactivity was lost during concentration of the extracts (recoveries >94%). The radioactivity assigned to the test item by HPLC was >93%. Extractable radioactivity detected in segments 2-6 was <0.5%. Non-extractable radioactivity in the top segments of soil [redacted] and [redacted], determined by combustion, accounted for 41.1, 42.1, 34.5 and 22.0% of AR (mean), respectively (Table 7.4.4-4 - Table 7.4.4-7). Non-extractable radioactivity detected in segments 2-6 was <1%.

Table 7.4.4-4: Material balance of BCS-CQ88719 (M24) in soil column packed with soil [redacted] in % of applied radioactivity

Extr. Radioactivity	Replicate No.	**Soil Segments 1**	**2**	**3**	**4**	**5**	**6**
Ambient extract	1	54.88	0.23	0.02	0.00	0.00	0.00
	2	58.04	n.a.	n.a.	n.a.	n.a.	n.a.
	Mean	**56.5**					
Microwave	1	0.55	n.a.	n.a.	n.a.	n.a.	n.a.
	2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Mean						
Total	1	55.43	0.23	0.02	0.00	0.00	0.00
	2	58.04	n.a.	n.a.	n.a.	n.a.	n.a.
	Mean	**56.7**					
Bound Residue	1	39.90	0.26	0.10	0.06	0.09	0.09
	2	42.36	0.07	0.09	0.08	0.06	0.10
	Mean	**41.1**	**0.2**	**0.1**	**0.1**	**0.1**	**0.1**
Total Bound Residues	1	40.50					
	2	42.76					
	Mean	**41.6**					
Segments Subtotal	1	95.33	0.49	0.12	0.06	0.09	0.09
	2	100.40	0.07	0.09	0.08	0.06	0.10
	Mean	**97.9**	**0.3**	**0.1**	**0.1**	**0.1**	**0.1**
Segments Total	1	96.18					
	2	100.80					
	Mean	**98.5**					
Leachate	No.	**Fractions 1**	**2**	**3**	**4**	**5**	**6**
	1	n.d.	n.d.	n.d.	n.d.	<0.1	<0.1
	2	n.d.	n.d.	n.d.	n.d.	<0.1	<0.1
	Mean						
Leachates Total	1	<0.1					
	2	<0.1					
	Mean						
Column Total	1	96.18					
	2	100.80					
	Mean	**98.5**					

n.a. : not analyzed

Table 7.4.4-5: Material balance of BCS-CQ88719 (M24) in soil column packed with soil [redacted] in % of applied radioactivity

Extr. Radioactivity		Replicate No.	**Soil Segments**						
			1	**2**	**3**	**4**	**5**	**6**	
	Ambient extract	1	54.17	0.37	0.07	0.05	0.02	0.02	
		2	53.19	n.a.	n.a.	n.a.	n.a.	n.a.	
		Mean	**53.7**						
	Microwave	1	0.34	n.a.	n.a.	n.a.	n.a.	n.a.	
		2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
		Mean							
	Total	1	54.51	0.37	0.07	0.05	0.02	0.02	
		2	53.19	n.a.	n.a.	n.a.	n.a.	n.a.	
		Mean	**53.9**						
Bound Residue		1	41.67	0.47	0.08	0.06	0.03	0.05	
		2	42.49	0.77	0.31	0.06	0.04	0.02	
		Mean	**42.1**	**0.6**	**0.2**	**0.1**	**0.0**	**0.0**	
Total Bound Residues		1	42.37						
		2	43.69						
		Mean	**43.0**						
Segments Subtotal		1	96.18	0.85	0.14	0.11	0.05	0.07	
		2	95.68	0.77	0.31	0.06	0.04	0.02	
		Mean	**95.9**	**0.8**	**0.2**	**0.1**	**0.0**	**0.0**	
Segments Total		1	97.41						
		2	96.88						
		Mean	**97.1**						
Leachate			**Fractions**						
		No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
		1	n.d.	n.d.	n.d.	n.d.	n.d.	<0.1	n.d.
		2	n.d.	n.d.	n.d.	n.d.	n.d.	<0.1	n.d.
		Mean							
Leachates Total		1	<0.1						
		2	<0.1						
		Mean							
Column Total		1	97.41						
		2	96.88						
		Mean	**97.1**						

Table 7.4.4-6: Material balance of BCS-CQ88719 (M24) in soil column packed with soil [redacted] in % of applied radioactivity

Extr. Radioactivity	Replicate No.	**Soil Segments**						
		1	**2**	**3**	**4**	**5**	**6**	
Ambient extract	1	65.26	0.04	0.02	0.11	0.02	0.02	
	2	62.13	n.a.	n.a.	n.a.	n.a.	n.a.	
	Mean	**63.7**						
Microwave	1	0.68	n.a.	n.a.	n.a.	n.a.	n.a.	
	2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
	Mean							
Total	1	65.94	0.04	0.02	0.11	0.02	0.02	
	2	62.13	n.a.	n.a.	n.a.	n.a.	n.a.	
	Mean	**64.0**						
Bound Residue	1	33.31	0.06	0.02	0.00	0.02	0.00	
	2	35.76	0.24	0.15	0.09	0.09	0.10	
	Mean	**34.5**	**0.1**	**0.1**	**0.0**	**0.1**	**0.0**	
Total Bound Residues	1	33.41						
	2	36.43						
	Mean	**34.9**						
Segments Subtotal	1	99.25	0.10	0.04	0.11	0.04	0.02	
	2	97.89	0.24	0.15	0.09	0.09	0.10	
	Mean	**98.6**	**0.2**	**0.1**	**0.1**	**0.1**	**0.1**	
Segments Total	1	99.57						
	2	98.56						
	Mean	**99.1**						
Leachate		**Fractions**						
	No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean							
Leachates Total	1	n.d.						
	2	n.d.						
	Mean							
Column Total	1	99.57						
	2	98.56						
	Mean	**99.1**						

n.a. : not analyzed

Table 7.4.4-7: Material balance of BCS-CQ88719 (M24) in soil column packed with soil [redacted] in % of applied radioactivity

Extr. Radioactivity	Replicate No.	Soil Segments						
		1	**2**	**3**	**4**	**5**	**6**	
Ambient extract	1	74.95	0.14	0.02	0.02	0.02	0.02	
	2	78.36	n.a.	n.a.	n.a.	n.a.	n.a.	
	Mean	**76.7**						
Microwave	1	0.45	n.a.	n.a.	n.a.	n.a.	n.a.	
	2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
	Mean							
Total	1	75.40	0.14	0.02	0.02	0.02	0.02	
	2	78.36	n.a.	n.a.	n.a.	n.a.	n.a.	
	Mean	**76.9**						
Bound Residue	1	22.46	0.13	0.11	0.03	0.04	0.12	
	2	21.50	0.09	0.12	0.14	0.08	0.10	
	Mean	**22.0**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	
Total Bound Residues	1	22.88						
	2	22.02						
	Mean	**22.5**						
Segments Subtotal	1	97.86	0.26	0.13	0.05	0.06	0.14	
	2	99.86	0.09	0.12	0.14	0.08	0.10	
	Mean	**98.9**	**0.2**	**0.1**	**0.1**	**0.1**	**0.1**	
Segments Total	1	98.51						
	2	100.39						
	Mean	**99.4**						
Leachate		**Fractions**						
	No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	1	<0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2	<0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean							
Leachates Total	1	<0.1						
	2	<0.1						
	Mean							
Column Total	1	98.51						
	2	100.39						
	Mean	**99.4**						

n.a. : not analyzed

The evaluation of the numeric soil distribution coefficient (K_d) and the corresponding organic carbon normalized distribution coefficient (K_{OC}) was based on mathematics derived from chromatographic theory.

Table 7.4.4-8: K_d/K_{OC} Values of Test Item BCS-CQ88719 (M24):

Soil	K_d[1]	K_{OC}[1]	K_d[2]	K_{OC}[2]
[redacted] (columns [redacted]/[redacted])	15.2 / 15.4	799 / 813	20.5 / 20.9	1079 / 1101
[redacted] (columns [redacted])	15.9 / 16.2	883 / 902	21.6 / 22.1	1198 / 1230
[redacted] (columns [redacted])	18.1 / 17.9	624 / 618	25.0 / 24.6	861 / 850
[redacted] (columns [redacted])	19.6 / 19.8	351 / 354	27.3 / 27.5	487 / 491
Mean	17.3	668	23.7	912

1 calculation according to Lambert
2 calculation according to Hamaker / McCall

Table 7.4.4-9: K_d/K_{OC} Values of ^{14}C-Atrazine:

Soil	K_d[1]	K_{OC}[1]	K_d[2]	K_{OC}[2]
[redacted] (mean of [redacted])	4.8	254	6.4	337
[redacted] (mean of [redacted])	2.3	130	3.0	168
[redacted] (mean of [redacted])	8.9	304	12.1	415
[redacted] (mean of [redacted])	9.4	167	12.9	230

1 calculation according to Lambert
2 calculation according to Hamaker / McCall

III. Conclusion

The soil adsorption coefficients K_d calculated according to Lambert ranged from 15.2 to 19.8 mL/g (mean: 17.3 mL/g). The respective organic carbon normalized soil adsorption coefficients (K_{OC}) ranged from 353 to 893 mL/g (mean: 668 mL/g).
The soil adsorption coefficients K_d calculated according to [redacted] ranged from 20.5 to 27.5 mL/g (mean: 23.7 mL/g). The respective organic carbon normalized soil adsorption coefficients (K_{OC}) ranged from 489 to 1214 mL/g (mean: 912 mL/g).
Using the Briggs classifications for the estimation of the mobility of crop protection agents in soil based on K_{OC} values, BCS-CQ88719 (M24) can be classified as low mobile to immobile for adsorption.

IIA 7.6 Direct phototransformation of relevant metabolites in water

In former photolysis study in aqueous buffer solution ([redacted], 1996a) the benzoxazole of KBR 2738, M10 (WAK 7004) which was formed in amounts of approximately 24 % of applied radioactivity was further metabolized very fast (DT50 << 1 d) and therefore not taken into consideration for modelling purposes.
Additional to the direct photolysis mentioned under point IIA 2.9, a further phototransformation experiment published in Chemosphere vol. 81, pp. 844-852 from 2010 was taken into account. In this study performed by [redacted] et al. a new major aqueous photometabolite occurred in amounts up to 15% of AR and was identified as the 1-methyl cyclohexane carboxamide (M40). In those

phototransformation experiments different photo sentitive additives like acetone, etc. and humic substances like humic acids, etc. were utilized in the experiments. The metabolite is added into the aquatic degradation pathway shown in Figure 7-2.

IIA 7.8 Degradation in aquatic systems

IIA 7.8.1 Aerobic biodegradation in aquatic systems

Studies on the aerobic biodegradation in aquatic systems are not a data requirement of Directive 91/414/EEC. However, data of a water-sediment study allow for a comprehensive description of the fate and behaviour of KBR 2738 in biotic aquatic systems.

IIA 7.8.3 Water/sediment studies

Report:	**KIIA 7.8.3 /01; [redacted] 2011**
Title:	[Cyclohexyl-1-^{14}C] Fenhexamid: Aerobic Aquatic Metabolism
Report No & Document No	MEF-10/637 **M-417147-01-1**
Guidelines:	- OECD Guideline for the Testing of Chemicals No. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, adopted April 24, 2002 - Commission Directive 95/36/EC amending Council Directive 91/414/EEC (Annexes II and III, Fate and Behavior in the Environment), July 14, 1995 - US EPA Fate, Transport and Transformation Test Guidelines, OPPTS 835.4300 and OPPTS 835.4400, Aerobic and Anaerobic Aquatic Metabolism, 2008
GLP	Yes

Executive Summary

The aerobic biotransformation of [cyclohexyl-1-^{14}C]fenhexamid (KBR 2738) in two different water-sediment systems [redacted] and [redacted] at 20 ± 2 °C in the dark for 100 days.

The total recovery of radioactivity in individual test vessels of the [redacted] systems ranged from 92.9 to 102.0 % (mean of duplicates, overall mean 96.3 %, RSD 2.6 %). The radioactivity in individual test vessels of the [redacted] test systems ranged from 91.9 to 100.8 % (mean of duplicates, overall mean 95.1 %, RSD 3.0 %).

A full material balance was established for all samples.

The radioactivity in test systems from [redacted] water decreased steadily from 93.6 % of the AR at DAT-0 to 1.2 % of the AR at study termination (Day 100). The radioactivity in test systems from [redacted] water decreased from 95.7 % of the AR at DAT-0 to 6.0 % of the AR towards the end of the study. The extractable radioactivity in test systems from [redacted] increased from 3.0 % of the AR at DAT-0 to 52.2 % of the AR at DAT-7 and declined to 14.2 % of the AR at study termination. Extractable ^{14}C residues in sediments from [redacted] increased from 1.5 % of the AR at DAT-0 to 27.6 % of the AR at DAT-14 and dropped to 6.9 % of the AR at study termination. The non extractable residues for test systems from [redacted] were 0.2 % of the AR at DAT-0 and increased to 55.6 % of the AR at study termination. For [redacted] water/sediment systems, the amounts of bound residues were 0.1 % of the AR at DAT-0 and increased to 42.7 % of the AR towards the end of the incubation period, with a maximum of 45.4 % of the AR at DAT-30. At termination of the study, the $^{14}CO_2$ recovery (mean values of duplicates) in systems from [redacted] was 22.0 % of the AR. In [redacted] water/sediment systems, $^{14}CO_2$ accounted for 36.4 % of the AR at study termination while formation of other organic volatile components was negligible (<0.1% of AR).

1-Methylcyclohexane carboxylic acid (M39) was observed as a major degradation product of [cyclohexyl-1-^{14}C]KBR 2738 in the water phases and in the sediment extracts of both water/sediment systems. In the water phases it accounted for up to of 6.4 % ([redacted]) and 8.9 % ([redacted]) of the AR. In the sediment extracts, 1-methylcyclohexane carboxylic acid accounted for 7.8 % and 4.1 % of the AR, respectively (these amounts include a minor metabolite, KBR 2738-sulfate (M27), which accounted for a maximum of 4.2 % of the AR in the total system of [redacted]).

KBR 2738-3-deschloro (M12) was observed as a major degradation product of fenhexamid in the water/sediment system of [redacted] only: In the water phase it accounted for only up to 1.1 % of the AR and in the sediment extract it accounted to a maximum amount of 7.5 % of the AR. In the water/sediment system of [redacted] residues of KBR 2738-3-deschloro (M12) were detected only in small amounts: In the water phase residues above the LOD were not found and in the sediment extract it accounted to a maximum amount of 1.4 % of the AR.

KBR 2738-sulfate (M27) was observed as a minor degradation product of fenhexamid only in the water/sediment system of [redacted] in amounts of up to 4.2 % of the AR (calculated value for the total system).

The half-life for the dissipation from the water was calculated to 5.6 days (system [redacted]) and 2.7 days ([redacted]), both determined on the basis of a best fit to measured data by applying the bi-phasic kinetic model DFOP.

For the degradation in total systems half-lives were calculated to 10.8 days (system [redacted]) and 14.5 days ([redacted]) from best fits applying the bi-phasic kinetic model DFOP.

I. Material and Methods

A. Materials

1. Test Material: [cyclohexyl-1-^{14}C]KBR 2738
Specific radioactivity: 4.64 MBq/mg
Radiochemical purity: >99% (HPLC)
Sample ID: KML 9212

2. Test System: The study was carried out with two contrasting water/sediment systems collected at two locations in Germany. Water and sediment of each system were collected from the same area. While system "[redacted]" was collected from the standing water of a pond, system "[redacted]" was collected from an artificially dammed creek and thus originating from flowing water. No pesticide was applied to or around either area prior to collection. Sediments and water were collected from the top 0 to 20 cm and stored at ambient temperature overnight prior to further processing. Before set-up of samples, the wet sediment was passed through a 2 mm sieve by use of the associated water. The characteristics of the sediments and their associated water are summarised in Table 7.8.3-1 and Table 7.8.3-2.

Table 7.8.3-1: Physico-chemical characteristics of associated sediments

Properties of Sediments		
Parameter	[redacted])	[redacted]
Geographic Location	close to [redacted] North Rhine-Westphalia Germany	[redacted] North Rhine-Westphalia Germany
Soil Taxonomic Classification (USDA)$^{\#}$	Silt Loam	Sand
Sand (2000 – 50 µm) [%]$^{\#}$	35	99
Silt (< 50 – 2 µm) [%]$^{\#}$	57	0
Clay (< µm) [%]$^{\#}$	8	1
pH$^{\#}$	5.0 ($CaCl_2$); 5.2 (H_2O)	6.1 ($CaCl_2$); 6.5 (H_2O)
Organic Carbon [%]^{*,2}	4.94 / n.a. / 4.83	0.29 / n.a. / 0.49
Soil Microbial Activity [mg CO_2 /hr/kg sediment (dry wt)]^2	n.a. / 44.2, 36.7 / 14.6, 21.3	n.a. / 2.7, 3.3 / 2.5, 2.5
Cation Exchange Capacity [meq/100 g]$^{\#}$	10.9	2.4
Redox Potential E_h [mV]^{1,3}	+101	+212

Table 7.8.3-2: Physico-chemical characteristics of water

Properties of Waters		
Parameter	[redacted]	[redacted]
Temperature [°C]^1	6.4	11.5
pH^1	7.6	7.5
Hardness [°dH]^{*,2}	n.a. / 3.5 / 4.4	n.a. / 9.7 / 10.3
Oxygen Concentration (saturation) [%]^1	96.8	92.2
Total Organic Carbon (TOC) [mg/L]^{*,2}	< 2 / 4 / 9	2 / 2 / 4
Dissolved Organic Carbon (DOC) [mg/L]^{*,2}	< 2 / 4 / 9	< 2 / 2 / 3
Total Nitrogen [mg/L]^{*,2}	3.7 / n.a. / 1.2	4.0 / n.a. / < 1.0
Total Phosphorus [mg/L]^{*,2}	< 0.03 / n.a. / 0.2	< 0.03 / n.a. / 0.08
Redox Potential E_H [mV]^1	+108	+229

B. Study design

1. Experimental conditions: The tests were performed in individual glass cylinders as test vessels which were filled with sieved sediment to a depth of 2 cm. Associated water was added to each test vessel up to a depth of 6 cm above the sediment to result in a sediment-to-water ratio of 1:3 (v/v). The test vessels were pre-incubated at 20 ± 2 °C in the dark for 5 days for equilibration of systems with regard to oxgen content, pH, redox potential and phase separation. During periods of acclimation and incubation the water phase was aerated by gentle agitation of the water phase by an orbital shaker.

Each of the static test vessels was attached to a trap for volatile components formed, *i.e.* soda lime for ^{14}C-carbon dioxide and polyurethane foam plug for other volatiles. Two replicates were prepared for each sampling date.

The nominal application rate of 52 µg/batch corresponds to about 100 µg/L water and was orientated on the projected maximum field application rate of 1000 g/ha. The actual test concentrations was 53 µg a.s/L for both, [redacted] and [redacted].

Each sample was dosed by applying an methanol: water (1:1, v:v) solution of ^{14}C-KBR 2738 dropwise onto the water surface to result in average amount of 53.1 µg KBR 2738 applied per test vessel.

Non-sterile, untreated samples were prepared in parallel for each water/sediment system for monitoring of total organic carbon (TOC) in the water and the microbial biomass in the sediment phase, respectively. One sample per water/sediment system was analyzed at time zero and after 2, 100 days of incubation taken each two aliquots out of one test system.

The water/sediment samples were incubated under static conditions (gentle shaking of test vessels, test flasks permeable to air) at 20 ± 2 °C for a maximum period of 100 days.

2. Sampling: Duplicate samples of were removed for analysis after 0, 1, 2, 3, 7, 14, 30, 62 and 100 days of incubation for [redacted] and [redacted] systems.

3. Analytical procedures: At each sampling interval, the pH and dissolved oxygen content were measured in the water phase. In addition, the redox potential was determined in the water phase and the sediment.

Water and sediment were separated by decantation which was followed by extraction of the sediment at the same day. Sediment was extracted exhaustively with aqueous acetonitrile under ambient and aggravated conditions (3 x 80 mL acetonitrile/water (4/1, v/v) extractions at ambient temperature and 1 x 80 mL acetonitrile/water (4/1, v/v) using microwave-accelerated solvent extraction).

Water samples were analysed by LSC and HPLC directly without concentration. Sediment samples were in general concentrated prior to HPLC analysis.

Extracted sediment was air-dried prior to quantification of radioactivity *via* combustion and LSC.

Volatile radioactivity in soda lime was determined by addition of 18% hydrochloric acid and absorption of the liberated of $^{14}CO_2$ in a mixture of Carbosorb E/Permafluor E+ for LSC measurement. The potential presence of $^{14}CO_2$ in the water phase was checked the same way following the removal of a 50 mL aliquot directly after decantation.

For determination of other volatile radioactivity polyurethane foam plugs were extracted with ethyl acetate followed by LSC assay of the extracts.

The radioactivity in liquid samples was directly determined by LSC while extracted sediment was combusted to adsorb ^{14}C-carbon dioxide formed prior to the determination by LSC.

Chromatographic investigations were performed by reversed phase HPLC with ^{14}C-flow-through detection as the primary analytical method for quantification. Identification of transformation products was carried out by co-elution with authentic reference material. Normal-phase TLC using a bio-radioimaging system as ^{14}C-detector was utilized as the confirmatory analytical method for selected water and sediment samples. The identity of the ^{14}C-test material was confirmed by HPLC/MS- and HPLC/proton-NMR coupling techniques.

The LOD for the HPLC method was about 0.9 % of the AR for the water phase and about 0.1 % of the AR for the sediment extracts.

C. Determination of degradation kinetics: Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1. The kinetic evaluation included the fitting of data with kinetic models SFO, FOMC and DFOP to the experimental data and their assessment according to FOCUS guidance to result in vlaues for comparison with trigger endpoints.

Results of a separate kinetic analysis in order to derive input data for modelling within aquatic environmental risk assessments are presented in another report, under point IIA 7.8.3/02.

II. Results and Discussion

A. Findings

After having reached equilibrium between water phase and the sediment the anticipated test conditions were maintained througout the whole incubation period after application of the test substance.

The pH-values in the water phases from [redacted] ranged from pH 6.2 – 7.3. For the water phases of [redacted], pH values ranged from 8.0 - 8.4.

The pH-values in the sediment from [redacted] ranged from pH 6.1 – 7.1. For the sediment of [redacted], pH values ranged from 6.8 – 8.3.

Measurements of the redox potential in water and sediment and the oxygen content in the water indicated aerobic conditions for both water/sediment systems during incubation (Table 7.8.3-3 and Table 7.8.3-4).

The results of microbial biomass determinations in sediments showed that biological activity of the test systems was given during the entire incubation period. From lower values of biomass after 100 days of incubation some trend for a reduction could be derived for both systems. The decrease may be regarded as a typical situation within laboratory tests on soils and sediments with microbia suffering from a lack of nutrients when being held artificially in test flasks and separated from the outdoor environment.

Table 7.8.3-3: Measurements of dissolved oxygen, pH and redox potential in system [redacted]

Sampling interval *	**Water**			**Sediment**	
(day)	**O_2-Sat.** (%)	**pH**	**E_{obs}** (mV)	**pH**	**E_{obs}** (mV)
0	90	8.1	176	7.4	173
	92	8.1	164	7.3	175
1	90	8.1	157	7.5	163
	90	8.2	172	7.4	176
2	89	8.1	160	7.4	158
	89	8.1	146	7.5	167
3	84	8.0	178	7.4	188
	84	8.0	182	7.3	154
7	82	8.1	172	7.4	136
	80	8.0	166	7.3	159
14	81	8.1	168	6.8	162
	78	8.2	164	6.9	159
30	92	8.0	124	7.6	117
	93	8.1	127	6.9	105
62	98	8.2	180	7.4	177
	97	8.3	181	7.5	150
100	90	8.4	180	7.8	194
	93	8.4	183	8.3	185

E_{obs} = Redox potential as measured with reference electrode (Ag/AgCl). The redox potential referring to the hydrogen standard electrode (E_h) results from the sum of the measured value (Eobs) and a fixed value of +197 mV for the potential of the reference electrode used (E_{ref}), i.e. $E_h = E_{obs} + E_{ref}$.

Table 7.8.3-4: Measurements of dissolved oxygen, pH and redox potential in system [redacted]

Sampling interval * (day)	Water			Sediment	
	O_2-Sat. (%)	pH	E_{obs} (mV)	pH	E_{obs} (mV)
0	78	7.2	170	7.0	143
	79	7.3	168	7.0	129
1	79	7.3	157	7.1	157
	77	7.3	166	7.1	107
2	78	7.3	155	6.9	42
	69	7.3	156	7.1	18
3	70	7.2	178	7.1	54
	67	7.3	177	7.1	58
7	41	7.1	135	7.1	8
	48	7.2	137	7.1	3
14	65	7.2	159	7.0	48
	64	7.2	151	6.9	32
30	83	6.2	142	6.7	89
	80	6.4	134	6.7	45
62	87	6.5	185	6.2	169
	90	6.4	186	6.3	147
100	91	6.6	180	6.1	185
	83	6.4	180	6.2	140

E_{obs} = Redox potential as measured with reference electrode (Ag/AgCl). The redox potential referring to the hydrogen standard electrode (E_h) results from the sum of the measured value (Eobs) and a fixed value of +197 mV for the potential of the reference electrode used (E_{ref}), i.e. $E_h = E_{obs} + E_{ref}$.

A. Data

A summary of key data on total recovery and the distribution of radioactivity into the various components formed in water and sediment is given for system [redacted] in Table 7.8.3-5 and in Table 7.8.3-6 for system [redacted].

Table 7.8.3-5: Biotransformation of [cyclohexyl-1-^{14}C] KBR 2738 in water/sediment system [redacted] at 20 °C

		Sampling interval (day)								
Component/Matrix		**0**	**1**	**2**	**3**	**7**	**14**	**30**	**62**	**100**
Fenhexamid	Water Layer	95.7	80.6	72.1	65.0	39.4	19.6	1.3	< LOD	n.d.
		± 0.2	± 4.1	± 1.3	± 2.9	± 6.0	± 2.6	± 0.0		
	Sediment	1.3	9.2	10.2	16.5	21.1	21.2	12.9	5.4	3.0
		± 0.0	± 1.8	± 0.6	± 1.5	± 3.0	± 0.9	± 1.9	± 0.6	± 0.0
	Entire System	97.0	89.8	82.2	81.4	60.6	40.8	14.1	5.4	3.0
		± 0.2	± 2.3	± 0.7	± 1.4	± 3.0	± 1.6	± 1.9	± 0.6	± 0.0
U2*	Water Layer	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	3.2	2.3	2.2
								± 0.2	± 0.0	± 0.1
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	3.2	2.3	2.2
								± 0.2	± 0.0	± 0.1
1-methyl-cyclohexane carboxylic acid**	Water Layer	n.d.	1.4	2.4	3.2	6.0	8.9	1.0	< LOD	< LOD
			± 0.2	± 0.3	± 0.7	± 0.1	± 0.8	± 0.0		
	Sediment	n.d.	< LOD	< LOD	1.3	2.6	4.1	2.1	1.1	< LOD
					± 0.1	± 0.2	± 0.4	± 0.2	± 0.0	
	Entire System	n.d.	1.4	2.4	4.4	8.6	12.9	3.1	1.1	< LOD
			± 0.2	± 0.3	± 0.8	± 0.1	± 0.4	± 0.2	± 0.0	
KBR 2738-3-des-chloro	Water Layer	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Sediment	n.d.	n.d.	n.d.	< LOD	n.d.	< LOD	1.4	< LOD	< LOD
								± 0.0		
	Entire System	n.d.	n.d.	n.d.	< LOD	n.d.	< LOD	1.4	< LOD	< LOD
								± 0.0		
Unidentified/ Diffuse radioactivity	Water Layer	n.d.	n.d.	n.d.	< LOD	< LOD	4.4	5.2	4.4	3.8
							± 0.7	± 0.4	± 0.2	± 0.2
	Sediment	< LOD	< LOD	1.6	1.0	1.3	< LOD	1.0	1.0	< LOD
				± 0.2	± 0.0	± 0.2		± 0.1	± 0.1	
	Entire System	< LOD	< LOD	1.6	1.4	1.8	4.4	6.2	5.4	4.3
				± 0.2	± 0.5	± 0.3	± 0.7	± 0.5	± 0.0	± 0.7
Total extractable residues	Water Layer	95.7	82.0	74.5	68.6	46.0	32.8	7.5	4.4	3.8
		± 0.2	± 3.9	± 1.0	± 2.7	± 6.6	± 1.0	± 0.4	± 0.2	± 0.2
	Sediment	1.3	9.2	11.7	18.7	25.1	25.3	17.3	7.5	3.4
		± 0.0	± 1.8	± 0.8	± 1.6	± 3.3	± 0.5	± 3.4	± 0.5	± 0.5
	Entire System	97.0	91.2	86.2	87.3	71.0	58.1	24.9	11.9	7.3
		± 0.2	± 2.0	± 0.2	± 1.0	± 3.2	± 0.5	± 3.0	± 0.6	± 0.7
$^{14}CO_2$		n.a.	0.6	0.8	0.6	2.0	4.0	17.9	32.3	36.4
			± 0.0	± 0.0	± 0.0	± 0.2	± 0.3	± 1.3	± 1.7	± 1.9
Organic volatiles		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
			± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Non-extract. residues		0.1	4.3	7.5	11.9	20.6	26.0	45.4	44.7	42.7
		± 0.0	± 1.9	± 0.3	± 0.9	± 1.4	± 0.1	± 2.0	± 6.4	± 1.2
Material balance***		97.3	97.3	96.2	100.8	94.6	92.6	92.8	92.6	91.9
		± 0.4	± 0.5	± 0.2	± 0.1	± 1.8	± 0.4	± 9.1	± 6.1	± 3.8

Mean values (mean ± standard deviation; n = 2) given as percentages of applied radioactivity (% AR)

Entire system = water + sediment; n.a. = not analyzed; n.d. = not detected

* besides U2 two other region of interest above the LOD were detected but not shown here

**sum of 1-methylcyclohexane carboxylic acid and max. occurrence of 4.2% of AR KBR 2738-sulfate

*** Difference of this values taken from material balance values in comparison to an addition of the fraction listed here are due to rounding errors in this special case due to the fact that the amounts of peaks occurred in the HPLC chromatograms but were below the LOD were divided into different regions of interest

Table 7.8.3-6: Biotransformation of [cyclohexyl-1-^{14}C] KBR 2738 in water/sediment system [redacted] [redacted] at 20 °C

Component/Matrix		Sampling interval (day) 0	1	2	3	7	14	30	62	100
Fenhexamid	Water Layer	93.6 ± 0.1	62.6 ± 0.6	48.4 ± 2.9	47.0 ± 2.8	27.8 ± 0.4	9.7 ± 0.1	< LOD	n.d.	n.d.
	Sediment	3.0 ± 0.4	28.2 ± 0.5	36.5 ± 2.2	42.8 ± 2.3	46.3 ± 0.6	37.8 ± 1.0	22.6 ± 1.6	11.3 ± 0.1	6.0 ± 0.1
	Entire System	96.7 ± 0.3	90.8 ± 0.0	85.0 ± 0.6	89.8 ± 0.5	74.2 ± 0.2	47.5 ± 0.9	22.6 ± 1.6	11.3 ± 0.1	6.0 ± 0.1
U6	Water Layer	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.
	Sediment	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	n.d.	n.d.	1.1 ± 0.0
	Entire System	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD	n.d.	1.1 ± 0.0
1-methyl-cyclohexane carboxylic acid	Water Layer	n.d.	1.1 ± 0.0	2.1 ± 0.1	2.3 ± 0.0	4.5 ± 0.1	6.4 ± 0.2	< LOD	n.d.	n.d.
	Sediment	n.d.	< LOD	< LOD	1.5 ± 0.1	3.6 ± 0.3	7.8 ± 0.1	6.0 ± 0.0	1.3 ± 0.1	< LOD
	Entire System	n.d.	1.1 ± 0.0	2.6 ± 0.6	3.7 ± 0.1	8.1 ± 0.1	14.2 ± 0.3	6.6 ± 0.6	1.3 ± 0.1	< LOD
KBR 2738-3-des-chloro	Water Layer	n.d.	n.d.	n.d.	n.d.	< LOD	1.1 ± 0.2	< LOD	n.d.	n.d.
	Sediment	n.d.	< LOD	< LOD	< LOD	2.2 ± 0.0	5.1 ± 0.1	7.1 ± 0.5	7.5 ± 0.3	5.0 ± 0.2
	Entire System	n.d.	< LOD	< LOD	< LOD	2.2 ± 0.0	6.2 ± 0.1	7.1 ± 0.5	7.5 ± 0.3	5.0 ± 0.2
Unidentified/ Diffuse radioactivity	Water Layer	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.4 ± 0.0	1.2 ± 0.0
	Sediment	n.d.	< LOD	n.d.	n.d.	< LOD	< LOD	< LOD	< LOD	1.1 ± 0.0
	Entire System	n.d.	< LOD	n.d.	n.d.	< LOD	< LOD	< LOD	2.0 ± 0.6	2.3 ± 0.1
Total extractable residues	Water Layer	93.6 ± 0.1	63.7 ± 0.5	50.5 ± 2.7	49.3 ± 2.8	32.4 ± 0.5	17.2 ± 0.3	< LOD	1.4 ± 0.0	1.2 ± 0.0
	Sediment	3.0 ± 0.4	28.2 ± 0.5	37.0 ± 2.7	44.7 ± 2.6	52.1 ± 0.9	51.2 ± 0.5	36.2 ± 1.6	20.7 ± 0.9	12.1 ± 0.1
	Entire System	96.7 ± 0.3	92.0 ± 0.0	87.5 ± 0.6	94.0 ± 0.2	84.4 ± 0.4	68.5 ± 0.8	36.7 ± 1.1	22.1 ± 0.9	13.3 ± 0.1
$^{14}CO_2$		n.a.	< 0.1 ± 0.0	0.1 ± 0.0	< 0.1 ± 0.0	0.1 ± 0.0	1.2 ± 0.2	11.2 ± 3.6	17.7 ± 0.7	22.0 ± 0.8
Organic volatiles		n.a.	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0
Non-extract. residues		0.2 ± 0.0	4.5 ± 0.2	8.2 ± 0.2	7.6 ± 0.8	11.8 ± 0.0	23.6 ± 0.1	40.7 ± 1.1	53.6 ± 2.7	55.6 ± 1.7
Material Balance**		96.9 ± 0.5	97.2 ± 0.3	96.6 ± 0.2	102.0 ± 0.4	97.1 ± 0.8	95.7 ± 0.7	93.4 ± 1.4	94.7 ± 1.9	92.9 ± 1.5

Mean values (mean ± standard deviation; n = 2) given as percentages of applied radioactivity (% AR)

Entire system = water + sediment

n.a. = not analyzed; n.d. = not detected

**Difference of this values taken from material balance values in comparison to an addition of the fraction listed here are due to rounding errors in this special case due to the fact that the amounts of peaks occurred in the HPLC chromatograms but were below the LOD were divided into different regions of interest

B. Material balance: For system [redacted] the total recovery of radioactivity in the individual test vessels ranged from 91.9% of AR to 100.8% (mean 95.1 ± 3.0%). The recovery in individual test vessels of system [redacted] ranged from 92.9% to 102.0% AR (mean 96.3 ± 2.6%). The balances of radioactivity were therefore in an acceptable range for all sampling intervals indicating no significant losses of radioactivity during incubation and processing of samples.

C. Residues in water, bound and extractable residues in sediment:

The radioactivity in test systems from [redacted] water decreased steadily from 93.6 % of the AR at DAT-0 to 1.2 % of the AR at study termination. The radioactivity in test systems from [redacted] water decreased from 95.7 % of the AR at DAT-0 to 6.0 % of the AR towards the end of the study.

Extractable ^{14}C sediment residues in test systems from [redacted] increased from 3.0 % of the AR at DAT-0 to 52.2 % of the AR at DAT-7 and declined to 14.2 % of the AR at study termination. Extractable ^{14}C residues in sediments from [redacted] increased from 1.5 % of the AR at DAT-0 to 27.6 % of the AR at DAT-14 and dropped to 6.9 % of the AR at study termination.

The maxima of non-extractable ^{14}C residues (mean values of duplicates) in the sediments were 55.6 % of the AR for [redacted] and 45.4 % of the AR for [redacted].

At the end of the study periods, 22.0 % of the AR was present as $^{14}CO_2$ in systems from [redacted] and 36.4 % of the AR was present as $^{14}CO_2$ in systems from [redacted]. The total amount of $^{14}CO_2$ accounted for $^{14}CO_2$ trapped in soda lime as well as for the amount of $^{14}CO_2$ present in the water phases (all sampling intervals) and sediments (only DAT-100). Organic volatile compounds amounted to < 0.1% of the AR in both systems.

In the water phase from [redacted], the amount of fenhexamid decreased from 93.6 % of the AR at DAT-0 to < LOD at DAT-30. In the [redacted] water, the amount of fenhexamid decreased from 95.7 % of the AR at DAT-0 to < LOD at DAT 62.

In the sediment phase from [redacted], the amount of fenhexamid increased from 3.0 % of the AR at DAT-0 to a maximum amount of 46.3 % of the AR at DAT-7 and then declined to 6.0 % of the AR towards study termination. In the [redacted] sediment the amount of fenhexamid increased from 1.3 % of the AR at DAT 0 to a maximum amount of 21.2 % of the AR at DAT-14 and then declined to 3.0 % of the AR at study termination.

1-Methylcyclohexane carboxylic acid was observed as a major degradation product of fenhexamid in the water phases and in the sediment extracts of both water/sediment systems. In the water phases it accounted for up to of 6.4 % ([redacted]) and 8.9 % ([redacted]) of the AR. In the sediment extracts, 1-methylcyclohexane carboxylic acid accounted for 7.8 % and 4.1 % of the AR, respectively (these amounts include a minor metabolite, KBR 2738-sulfate, which accounted for a maximum of 4.2 % of the AR in the total system of [redacted]).

In addition KBR 2738-3-deschloro was observed as a major degradation product of fenhexamid in the water/sediment system of [redacted] only: In the water phase it accounted for only up to 1.1 % of the AR and in the sediment extract it accounted to a maximum amount of 7.5 % of the AR. In the water/sediment system of [redacted] residues of KBR 2738-3-deschloro were detected only in small amounts: In the water phase residues above the LOD were not found and in the sediment extract it accounted to a maximum amount of 1.4 % of the AR.

KBR 2738-sulfate was observed as a minor degradation product of fenhexamid only in the water/sediment system of [redacted] in amounts of up to 4.2 % of the AR (calculated value for the total system. Minor transformation products were detected in both water/sediment systems tested. None of the components exceeded 3.2% of AR in total systems in the course of the study.

D. Volatilisation: Formation of 14CO2 was observed in all water/sediment systems. At termination of the study, the 14CO2 recovery (mean values of duplicates) in systems from [redacted] was 22.0 % of the AR. In [redacted] water/sediment systems, 14CO2 accounted for 36.4 % of the AR at study termination. From these data it can be concluded that a high amount of fenhexamid is mineralized in water/sediment systems. The formation of other volatile components was insignificant by accounting for less than 0.1% AR at any sampling interval in the course of the study. KBR 2738 and its residues were therefore subject to further transformation in water/sediment systems till mineralisation.

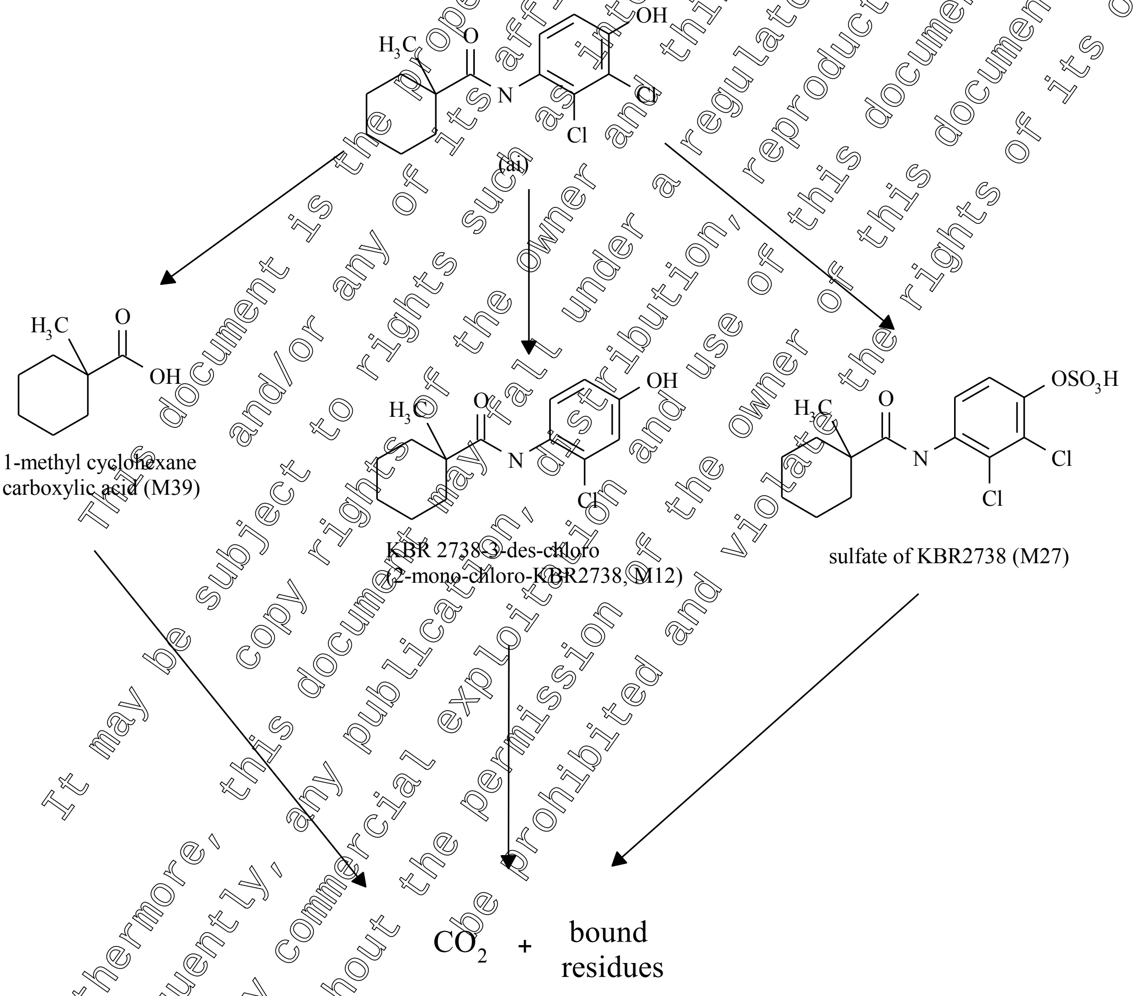
E. Transformation of parent compound: KBR 2738 was transformed by a cleavage of the carboxamide moiety, dechlorination and conjugation processes in both water/sediment test systems (for structures, see Figure 7.8.3-1).
1-Methylcyclohexane carboxylic acid (M39) was found up to 14.2 % of the AR and up to 12.9 % of the AR in the total systems of [redacted] and [redacted] at DAT-14, respectively. In case of the [redacted] system, the amount includes a minor metabolite, KBR 2738-sulfate (M27), which accounted for a maximum of 4.2 % of the AR in the total system.
KBR 2738-3-deschloro (M12) was detected up to 7.5 % of the AR at DAT-62 and up to 1.4 % of the AR at DAT-30 in the total systems of [redacted] and [redacted], respectively.
Metabolites as well as their maximum occurrence are shown in Table 7.8.3-9.
The mechanisms of dissipation from the water body to the sediment as well as degradation therefore contributed to the elimination of the parent substance from the total systems to result in the formation of NER and ^{14}C-carbon dioxide as terminal products of biotransformation.

Table 7.8.3-9 Metabolites of [cyclohexyl-1-14C] KBR 2738 formed under conditions of water/sediment testing; maximum values used for calculation of PECSW marked bold

Component	Maximum occurrence (% AR)			System	Day
	Water	Sediment	Total		
KBR 2738-3-des-chloro (M12)	n.d.	1.4	1.4	[redacted]	30
	1.1	7.5	**7.5**	[redacted]	62 (sediment) 14 (water)
1-methyl-cyclohexane carboxylic acid (M39)*	8.9	4.1	12.9	[redacted]	14
	6.4	7.8	**14.2**	[redacted]	14

* in [redacted] only: sum of 1-methylcyclohexane carboxylic acid (M39) and KBR 2738-sulfate (M27) with max. amounts of 4.2% AR of KBR 2738-sulfate (M27) in entire system

Figure 7.8.3-1: Proposed metabolic pathway for KBR 2738 under aerobic conditions of water/sediment testing



F. Degradation kinetics: The evaluation of degradation kinetics in the water phase and the total water/sediment systems was performed by use of the software KinGui (Version 1.1). After fitting of data to the three kinetic models SFO, FOMC (Gustafson-Holden) and DFOP[4] the quality of fits was assessed according to FOCUS kinetic guidance. The data sets were treated as replicate data. The initial concentration at time zero was included in the parameter optimisation. All data points were weighted equally. For optimal goodness of fit, the initial value was also allowed to be estimated by the model. For the detrmination of the best fit preference was given to the error of chi-square (χ^2) to be a minimum. The results of the kinetic evaluation are provided in Table 7.8.3-10.

With regard to degradation of KBR 2738 in total water/sediment systems the chi-square (χ^2) errors were found to be below 6 for all models applied with overall ranges of χ^2-errors being marginal for a given system. For the dissipation of KBR 2738 from the water phase differences in the overall range of χ^2-error was more pronounced ending up in a bi-phasic kinetic behaviour for the best fit for both systems (DFOP for both systems).

The dissipation half-life of KBR 2738 from water was estimated to 5.6 days (system [redacted]) and 2.7 days (system [redacted]) on the basis of DFOP kinetics. The application of the DFOP kinetic model resulted in a DT$_{90}$-value of 20.0 for system [redacted]. The DT$_{90}$-value for system [redacted] was estimated to 15.2 days.

For the degradation of KBR 2738 in total systems half-lives of 10.8 days (system [redacted]) and 14.5 days (system [redacted]) were estimated when applying a DFOP kinetic model as best fit.

Degradation kinetics was also evaluated in a separate report to derive input parameters for modeling purposes in environmental exposure assessments. These results are presented under IIA 7.8.3/02.

Table 7.8.3-10: Best-fit kinetics for the dissipation from water and degradation in total water/sediment systems of [cyclohexyl-1-^{14}C] KBR 2738 after incubation at 20°C

System	Matrix	Kinetic model	DT$_{50}$ (days)	DT$_{90}$ (days)	Chi^2 Err (%)
[redacted] Germany	Water phase	SFO	5.7	19.1	2.8
		FOMC	5.5	20.3	2.7
		DFOP	**5.6**	**20.0**	**2.7**
	Total system	SFO	11.1	37.0	2.9
		FOMC	10.7	41.2	2.6
		DFOP	**10.8**	**37.0**	**2.3**
[redacted] [redacted], Germany	Water phase	SFO	3.5	11.5	13.5
		FOMC	2.6	17.3	9.5
		DFOP	**2.7**	**15.2**	**3.9**
	Total system	SFO	15.4	51.2	5.2
		FOMC	14.6	60.1	5.0
		DFOP	**14.5**	**60.3**	**4.8**

Best fits according to the criteria set are marked bold.

III. Conclusion

Once applied to water surfaces KBR 2738 is eliminated from the water phase *via* sorption processes to the sediment. The processes are paralleled by a microbial degradation to result in KBR 2738-3-des-

---

[4] SFO = Single first order; FOMC = First order multi compartment; DFOP = Double first order in parallel

chloro (M12) and 1-methyl-cyclohexane carboxylic acid (M39) as major metabolites in water/sediment systems. Further microbial conversion proceeds result in non-extractable residues and large amounts of ^{14}C-carbon dioxide as terminal products of metabolic transformation under conditions of water/sediment testing.

Sorption of KBR 2738 to the sediment proceeded slowly in sandy water/sediment systems with low organic matter while elimination was pronounced in systems with a sandy loam texture and higher organic carbon content. Following FOCUS kinetic guidance optimum fits resulted in half-lives of 5.6 days ([redacted]) and 2.7 days ([redacted]) for the dissipation from water. For the degradation in total systems, the corresponding half-lives were 10.8 days for the sandy system [redacted]and 14.5 days for the sandy loam system [redacted].

KBR 2738 is therefore expected to degrade relatively fast in aquatic systems of the environment.

Report:	**KIIA 7.8.3 /02;** [redacted] **2012**
Title:	Kinetic Evaluation of the Aerobic Aquatic Metabolism of [Cyclohexyl-1-^{14}C]-Fenhexamid (KBR 2738) and its metabolites for modelling and persistence endpoints
Report No & Document No	MEF-11/757 **M-422393-01-1**
Guidelines:	“Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration”. Report of the FOCUS Work Group on Degradation Kinetics. EC Document Reference Sanco/10058/2005 version 2.0, 2006
GLP	No (calculation)

Executive Summary

The degradation and dissipation behaviour of [cyclohexyl-1-^{14}C]fenhexamid (KBR 2738) and its metabolites 1-methylcyclohexanecarboxylic acid (M39) and N-(2-chloro-4-hydroxyphenyl)-1-methyl-cyclohexanecarboxamide (M12) in an aquatic environment was investigated by kinetic evaluation of two aerobic water-sediment systems ([redacted] and [redacted], at 20 °C in the dark ([redacted], 2011).

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006) and considered modelling endpoints.

It includes an analysis of the kinetics of fenhexamid (parent = P) and its metabolites (M) considering the total system (Level I, degradation) and the single phases separately (Level I, dissipation).

Parameters of the selected models for fenhexamid are shown in Table 7.8.3-11, Table 7.8.3-13, Table 7.8.3-15 (modelling endpoints) and Table 7.8.3-12, Table 7.8.3-14, and Table 7.8.3-16 (persistence endpoints) for the total system (Level I, degradation) and for the single phases (Level I, dissipation) of fenhexamid, M39, and M12.

Table 7.8.3-11: SFO degradation and dissipation parameters for modelling endpoints of fenhexamid

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	**k-rate (1/days)**	**DT50 (days)**	**Model**	**k-rate (1/days)**	**DT50 (days)**
PI / deg.	**Total system**	SFO	0.062	11.14	FOMC	0.037	18.49)^{a}
PI / diss.	**Water**	SFO	0.123	5.65	FOMC	0.127	5.47)^{a}
PI / diss.	**Sediment**	SFO	0.028	25.08	SFO	0.027	26.09

)^{a} calculated by DT90$_{FOMC}$/3.32

Table 7.8.3-12: **Degradation and dissipation parameters for persistence endpoints of fenhexamid**

Evaluation level / type of parameter	Phase	-- [redacted] --			-- [redacted] --		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
PI / deg.	Total system	HS	10.96	45.60	DFOP	14.60	60.64
PI / diss.	Water	DFOP	5.45	19.74	DFOP	2.41	14.98
PI / diss.	Sediment	FOMC	22.20	102.68	FOMC	22.81	108.55

Table 7.8.3-13: **SFO degradation and dissipation for modelling endpoints of M39 (1-methylcyclohexane-carboxylic acid)**

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	k-rate (1/days)	DT50 (days)	Model	k-rate (1/days)	DT50 (days)
MI / deg.	Total system	SFO (parent SFO)	0.111	6.23	SFO (parent FOMC)	0.083	8.33
MI / diss.	Water	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	Sediment	SFO	0.030	22.98	SFO	0.030	23.26

Table 7.8.3-14: **Degradation and dissipation for persistence endpoints of M39 (1-methyl-cyclohexane-carboxylic acid)**

Evaluation level / type of parameter	Phase	-- [redacted] --			- [redacted] -		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
MI / deg.	Total system	SFO (parent HS)	6.42	21.32	SFO (parent DFOP)	7.97	26.48
MI / diss.	Water	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	Sediment	FOMC	17.65	113.81	SFO	23.26	77.28

Table 7.8.3-15: **SFO degradation and dissipation for modelling endpoints of M12 (KBR 2738-3-des-chloro)**

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	k-rate (1/days)	DT50 (days)	Model	k-rate (1/days)	DT50 (days)
MI / deg.	Total system	n.a.	n.a.	n.a.	SFO (parent FOMC)	0.010	68.90
MI / diss.	Water	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	Sediment	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table 7.8.3-16: Degradation and dissipation for persistence endpoints of M12 (KBR 2738-3-des-chloro)

Evaluation level / type of parameter	Phase	-- [redacted] --			- [redacted] -		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
MI / deg.	Total system	n.a.	n.a.	n.a.	SFO (parent DFOP)	73.79	245.12
MI / diss.	Water	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	Sediment	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

I. Material and Methods

The kinetic evaluation was based on data of a water-sediment study (IIA 7.8.3/01) conducted with [cyclohexyl-1-^{14}C]-labeled KBR 2738 in a sandy ([redacted]) and a silt loam sediment ([redacted]) and their associated water at 20°C in the dark for a maximum of 100 days. Measured and reported duplicates were taken into account singularly. There was no fit for non extractable residues (NER) and volatile radioactivity formed (open system).

Four kinetic models, the simple first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the hockey-stick model (HS), and the bi-exponential model (DFOP, double first order in parallel) were considered in this study. No weighting of the data was performed in the kinetic analysis.

A Level I evaluation was performed for the total system (degradation), and the single phases (dissipation, using decline fit from the maximum occurrence onwards). In contrast to degradation, the dissipation parameters in water-sediment studies include not only degradation but also transfer into the other phase (water or sediment).

Degradation parameters are useful for exposure models which explicitly consider the transfer of compound between different phases. Dissipation parameters can be used for exposure models which consider water and sediment phase separately.

The choice of the appropriate kinetic model is based on visual inspection of the fit and the scaled Chi²-error. For modelling endpoints it is additionally checked by the t-test whether degradation or dissipation parameters are sufficiently reliable to be used for exposure modelling.

For the derivation of modelling endpoints, simple first-order (SFO) kinetics are tested first, because SFO is the simplest of the kinetic models and the one almost exclusively used in environmental exposure models. If the SFO fit is visually not acceptable or Chi²-error significantly exceeds a value of 15 %, then the bi-phasic models are tested. Finally the model is chosen which is visually acceptable and provides a significantly better fit in terms of Chi²-error. This avoids using an over-parameterised model based on a marginally better fit only.

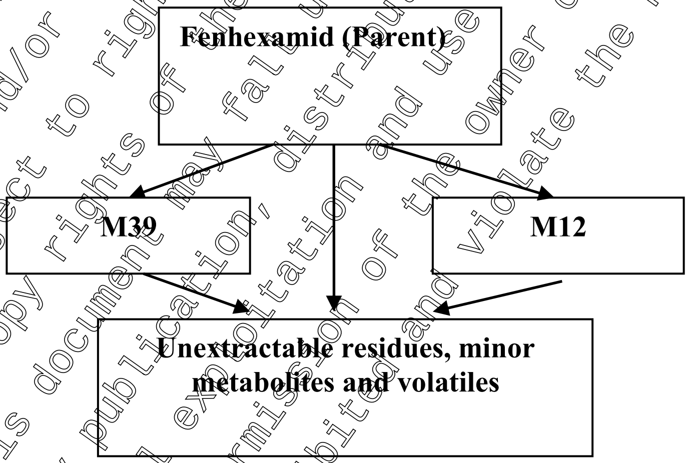
Ideally, the Chi²-error value should be below 15 %. However, this value should only be considered as guidance and not as an absolute cut-off criterion. There will be cases where the Chi²-error is higher, but the fit still represents a reasonable description of the degradation behaviour.

The bi-phasic models recommended are FOMC, DFOP and HS, where FOMC should not be used in cases where the final residue is more than 10 % of its initial value. For modelling purposes, an equivalent SFO half-life is calculated as DT90$_{\text{bi-phasic}}$/3.32, when the final residues are below 10% of

the initial value. By this method the equivalent SFO-curve meets the bi-phasic curve at the time $DT90_{bi-phasic}$ and consequently over predicts the residue values at earlier times. When the final residues remain above 10% of the initial value, the $DT50_{SFO}$ is calculated from the slow k-rate (k2) by $DT50_{SFO} = \ln(2)/k2_{HS,DFOP}$, and the $DT90_{SFO} = \ln(10)/k2_{HS,DFOP}$.
For the derivation of persistence endpoints, the SFO model is tested against the FOMC model. If the SFO model gives an acceptable fit and the Chi²-error is smaller than that of the FOMC model, the SFO model is selected. If the FOMC model is better, the other bi-phasic models HS and DFOP are tested, and the most appropriate in terms of visual fit and Chi²-error is selected.
For pathway fits in a first step the appropriate model for the parent is identified. In a second step the pathway is implemented with the parent model as identified and the SFO model for the metabolite.

In accordance with the experimental data [redacted] (2011) the compartment model shown in Figure 7.8.3-2 was developed. Between compartments transformation reactions were assumed to proceed only one-way. The data pre-processing was done in accordance with FOCUS (2006). The initial amount of the parent compound was free fitted and the initial amount for metabolites was fixed to a value of zero. All data were weighted equally thus corresponding to an absolute error model.
Following this scheme the parent fenhexamid degrades to M39 and M12. All compounds degrade to unextractable residues and volatiles. Minor metabolites are neglected.

Figure 7.8.3-2: Compartment model for the total water-sediment system kinetic evaluation of KBR 2738 (Level I)



For Level I evaluations of dissipation, the decline of a compound in either phase is fitted to measured data from their maximum onwards, M*(t*), using a simple differential equation as the first one of the system above. The time axis is shifted by the time tmax where the maximum occurs, $t^* = t - t_{max}$, and the residue values are chosen accordingly $M^*(t^*) = M(t - t_{max})$, $t \geq t_{max}$. This procedure is called in the following decline- or dissipation fit.

II. Results and Discussion

A. Model selection

1. Total System

Parent

For the [redacted] system the SFO fit was visually and statistically well acceptable. The last data points were slightly underestimated. However, according to FOCUS (2006) this is acceptable when the residuals decreased below 90% of the initial concentration until this date. This is the case (about 95% degradation at day 62). The k-rate is significant (with t-test <0.001) and the Chi²-errors are well below 15%. Thus the SFO model was selected for [redacted] for the modelling endpoint.

For persistence endpoints additionally the FOMC model was calculated. It achieved a very good fit with a smaller Chi²-error value. This triggered additional calculations with the HS and DFOP model to determine the best fit bi-phasic model. Both models yielded an excellent fit. The HS model was selected for persistence endpoints, because the Chi²-error (2.2%) is slightly smaller than the error of the FOMC and DFOP model.

For the [redacted] system the SFO fit looks similar to the [redacted] system, but the residuals at day 62 are >10%, hence too high to neglect deviations between model and measurements at that data point. Therefore additionally the FOMC model was calculated. The fit to the later data points is very good, and the Chi²-error value is sufficiently small (4.9%). Hence the FOMC model was selected for the modelling endpoint.

For persistence endpoints additionally the HS and DFOP models were calculated to determine the best fit bi-phasic model. Both models yielded a visually acceptable fit. The DFOP model was selected for persistence endpoints, because the Chi²-error (4.7%) is smaller than the error of the FOMC and HS model.

Table 7.8.3-17: Scaled Chi²-error , visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of fenhexamid for different kinetic models applied to the total system values. Values in bold indicate the kinetic model chosen for modelling endpoints, in italics for persistence endpoints.

	--- [redacted] ---			--- [redacted] ---		
Model	Chi²-error (%)	VA)ᵃ	t-prob)ᵇ	Chi²-error (%)	VA)ᵃ	t-prob)ᵇ
SFO	**3.0**	+	**<0.001**	5.2	-	<0.001
FOMC	2.7	+	0.027/0.042	**4.9**	+	**0.036/0.061**
HS	*2.2*	+	*0.105*	4.9	+	<0.001
DFOP	2.3	+	0.475	*4.7*	+	*0.500*

)ᵃ +/-: yes/no
)ᵇ for SFO t-test of k-rate; for FOMC of Alpha/Beta; for HS and DFOP of k-slow
t-prob = t-probability test

Metabolites

In the system [redacted] M12 occurred only at one time point. Therefore it is not included in the kinetic evaluation. The metabolite M39 for the system [redacted] was calculated with the SFO model in the metabolic pathway together with fenhexamid (using the identified models SFO for modelling endpoints and HS for persistence endpoints). In both pathway fits the model was very well able to reproduce both the formation and the degradation of the measured concentrations, although the maximum concentration is slightly underestimated. The chi2-error is higher than recommended. However, the fitted curve describes the measurements adequately and no systematic deviation between model and measurements can be observed. Therefore, the SFO-model is accepted as modelling and and the HS-model as persistence endpoint.

For [redacted] the visual fit of M39 is similarly good, representing the formation and the decline of the measured concentrations very well, and only slightly underestimating the maximum concentrations (parent FOMC (modelling endpoints) and parent DFOP (persistence endpoints)). The chi2-error is higher than recommended. However, the fitted curve describes the measurements adequately and no systematic deviation between model and measurements can be observed. Therefore, the SFO-model is accepted as modelling and and the DFOP-model as persistence endpoint.

For M12 the SFO fits are excellent and lead to statistically acceptable fits (chi^2-error <15% and t-prob <0.05). Both fits were selected for modelling and persistence endpoints, respectively.

Table 7.8.3-18: Scaled Chi^2-error , visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of M39 in the total system of [redacted] for the complete metabolic pathway (CP). Values in bold indicate the kinetic model chosen for modelling endpoints (mod), in italics for persistence endpoints (per).

Evaluation scheme	Model	Chi^2-error (%)	VA)^a	t-prob)^b
CP (mod)	**SFO-SFO**	**31.5**	+	**<0.001**
CP (per)	*HS-SFO*	*30.4*	+	*<0.001*

)^a +/-: yes/no

)^b for SFO t-test of k-rate; for FOMC of Alpha/Beta

Table 7.8.3-19: Scaled Chi^2-error , visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of M39 and M12 in the total system of [redacted] for the complete metabolic pathway (CP) and using decline fit (DC). Values in bold indicate the kinetic model chosen for modelling endpoints (mod), in italics for persistence endpoints (per).

Evaluation scheme	Substance	Model	Chi^2-error (%)	VA)^a	t-prob)^b
CP (mod)	**M39**	**FOMC-SFO**	**26.3**	**-**	**<0.001**
CP (per)	*M39*	*DFOP-SFO*	*24.9*	-	*<0.001*
CP (mod)	**M12**	**FOMC-SFO**	**14.9**	**+**	**0.035**
CP (per)	*M12*	*DFOP-SFO*	*13.7*	+	*0.028*

)a +/-: yes/no

)b for SFO t-test of k-rate; for FOMC of Alpha/Beta

2. Water Phase

Parent (PI)

For the [redacted] system the single phase fit for the water phase with SFO model was visually well acceptable. The statistical parameter Chi^2-error and t-prob are small (3.2% and <0.001, respectively). Hence the SFO model is selected for modelling endpoints.
For persistence endpoints additionally the FOMC model was calculated. It achieved a very good fit with a smaller Chi^2-error value. This triggered additional calculations with the HS and DFOP model to determine the best fit bi-phasic model. Both models yielded an excellent fit. The DFOP model was selected for persistence endpoints, because the Chi^2-error (2.1%) is smaller than the error of the FOMC and HS model.
For the [redacted] system the SFO model tends to underestimate the initial mass and leads to systematic deviations (overestimation of early data points, underestimation of late points). Hence, the FOMC model is calculated. It leads to an acceptable fit with a Chi^2-error of 8.7%. The last data point at day 30 is slightly overestimated which is conservative, because it underestimates degradation. The FOMC model was selected for modelling endpoints.

For the persistence endpoints, the HS and DFOP models were calculated additionally to determine the best fit bi-phasic model. Both models yielded a visually excellent fit. The DFOP model was selected for persistence endpoints, because the Chi^2-error (3.6%) is smaller than the error of the FOMC and HS model.

Table 7.8.3-20: Scaled Chi^2-error , visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of fenhexamid for different kinetic models applied to the water phase. Values in bold indicate the kinetic model chosen for modelling endpoints, in italics for persistence endpoints.

	--- [redacted]----			--- [redacted]---		
Model	Chi^2-error (%)	VA)^a	t-prob)^b	Chi^2-error (%)	VA)^a	t-prob)^b
SFO	**3.2**	+	**<0.001**	13.0	-	<0.001
FOMC	3.0	+	0.179/0.200	**8.7**	+	**0.002/0.017**
HS	3.6	+	0.497	9.9	+	<0.001
DFOP	*2.1*	+	*<0.001*	*3.6*	+	*<0.001*

)a +/-: yes/no
)b for SFO t-test of k-rate; for FOMC of Alpha/Beta; for HS and DFOP of k-slow

Metabolites (MI)

In both systems the maximum concentration of M39 is reached three sampling dates before complete dissipation of M39. Hence, only three data points are available for a decline fit. With the SFO model the last data point is underestimated in both systems. Hence, the SFO model can not be used for modelling or persistence endpoints. No other model is applicable for cases with such small numbers of data points. No modelling and persistence endpoints can be derived for the water phase.

Table 7.8.3-21: Scaled Chi^2-error , visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of M39 for the SFO kinetic model applied to the water phase values.

--- [redacted]----	--- [redacted]---

	Chi²-error (%)	VA)a	t-prob)b	Chi²-error (%)	VA)a	t-prob)b
SFO	5.7	-	0.004	7.9	-	0.002

)a +/-: yes/no
)b for SFO t-test of k-rate

3. Sediment

Parent

In the [redacted] system the maximum concentration of fenhexamid is reached four sampling dates before complete dissipation of fenhexamid. The visual fit of the SFO model is very good. This is also indicated by the small Chi²-error value of 4.8%. The SFO model was selected as modelling endpoint. For persistence endpoints additionally the FOMC model was calculated. It resulted in a slightly better fit (Chi²-error value = 2.1%). The HS and DFOP model can not be used due to the limited number of data points. Therefore, the FOMC model was selected as persistence endpoints.

For the [redacted] system 5 data points are available for the decline fit. The SFO model resulted in a very good fit with a Chi²-error of 4.6 and a t-prob of <0.001. It was selected for modelling endpoints.

For persistence endpoints additionally the FOMC model was calculated. The FOMC model resulted in an excellent fit and improved the Chi²-error to 1.8%. This triggered calculations with the HS and DFOP models. Both resulted also in excellent fits, but with slightly higher Chi²-error values (2.7% and 1.9%, respectively). The FOMC model was selected for persistence endpoints.

Table 7.8.3-22: Scaled Chi²-error, visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of fenhexamid for different kinetic models applied to the sediment phase values. Values in bold indicate the kinetic model chosen for modelling endpoints, in italics for persistence endpoints.

	--- [redacted] ----			--- [redacted] ---		
	Chi²-error (%)	VA)a	t-prob)b	Chi²-error (%)	VA)a	t-prob)b
SFO	**4.8**	+	**<0.001**	**4.6**	+	**<0.001**
FOMC	*2.1*	+	*0.159/0.209*	*1.8*	+	*0.007/0.020*
HS				2.7	+	<0.001
DFOP				1.9	+	0.320

)a +/-: yes/no
)b for SFO t-test of k-rate; for FOMC of Alpha/Beta; for HS and DFOP of k-slow

Metabolites

In the [redacted] system the maximum concentration of M39 is reached four sampling dates before complete dissipation of M39. The SFO model resulted in an excellent fit with a Chi²-error (9.2%) and small t-prob (0.001). The SFO model was selected as modelling endpoint.

For persistence endpoints the FOMC model is calculated. It resulted in a slightly better fit with lower Chi²-error (6.0%). Due to the limited data points the HS and DFOP models could not be used. The FOMC model was selected for persistence endpoints.

Also for the [redacted] system four data points are available. The SFO model resulted in a very good fit with an acceptable Chi²-error (12.6%) and small t-prob (<0.001). It was selected as modelling endpoint.

For persistence endpoints additionally the FOMC model was calculated, but it did not improve the fit (Chi²-error = 18.2%). Therefore the SFO model was also selected for persistence endpoints.

Table 7.8.3-23: Scaled Chi²-error, visual acceptability (VA; +/- yes/no) and significance of degradation rate (t-prob) of M39 for different kinetic models applied to the sediment phase values. Values in bold indicate the kinetic model chosen for modelling endpoints, in italics for persistence endpoints.

	--- [redacted] ----			--- [redacted] ---		
	Chi²-error (%)	VA)[a]	t-prob)[b]	Chi²-error (%)	VA)[a]	t-prob)[b]
SFO	**9.2**	+	**0.001**	***12.6***	+	***<0.001***
FOMC	*6.0*	+	*0.087/0.168*	18.2	+	0.370/0.383

)[a] +/-: yes/no

)[b] for SFO t-test of k-rate; for FOMC of Alpha/Beta

B. Kinetic parameters

Parameters of the selected models for fenhexamid are shown in Table 7.8.3-11, Table 7.8.3-13, Table 7.8.3-15 (modelling endpoints) and Table 7.8.3-12, Table 7.8.3-14, and Table 7.8.3-16 (persistence endpoints) for the total system (Level I, degradation) and for the single phases (Level I, dissipation) of fenhexamid, M39, and M12.

III. Conclusion

Once applied to water surfaces KBR 2738 is relatively fast eliminated from total water/sediment aquatic model systems.

Evaluation of persistent endpoints for the parent fenhexamid and its metabolites M39 and M12 using the best fit assessment resulted in different kinetic models as shown in Table 7.8.3-24 for Level I.

Table 7.8.3-24: Degradation and dissipation parameters for persistence endpoints of fenhexamid (parent) and its metabolites M39 and M12.

Evaluation level / type of parameter	Phase	-- [redacted] --			-[redacted]-		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
fenhexamid							
PI / deg.	**Total system**	HS	10.96	45.60	DFOP	14.60	60.64
PI / diss.	**Water**	DFOP	5.45	19.74	DFOP	2.41	14.98
PI / diss.	**Sediment**	FOMC	22.20	102.68	FOMC	22.81	108.55
M39							
MI / deg.	**Total system**	SFO (parent HS)	6.42	21.32	SFO (parent DFOP)	7.97	26.48
MI / diss.	**Water**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	**Sediment**	FOMC	17.65	113.81	SFO	23.26	77.28
M12							
MI / deg.	**Total system**	n.a.	n.a.	n.a.	SFO (parent DFOP)	73.79	245.12
MI / diss.	**Water**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MI / diss.	**Sediment**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a.: not applicable

New kinetic evaluation of a former aerobic aquatic water/sediment degradation study with the [phenyl-UL-^{14}C] labeled fenhexamid was done to derive half-lives of fenhexamid for use as input parameters in environmental exposure assessments by modelling.

Report:	**KIIA 7.8.3 /02;** [redacted]
Title:	Degradation and metabolism of KBR 2738 in the System Water/Sediment
Report No & Document No	PF 4202 // MR 913/96 **M-003782-01-1**
Guidelines:	- BBA-Guidelines for Testing of Plant Protectants (Registration) Part IV, 5-1, Degradability and Fate of Plant Protectants in the Water/Sediment System, 1990
GLP	Yes

Report:	**KIIA 7.8.3 /03;** [redacted] **2012**
Title:	Kinetic Evaluation of the Aerobic Aquatic Metabolism of [Phenyl-UL-^{14}C] Fenhexamid (KBR 2738) for modelling and persistence endpoints
Report No & Document No	MEF-11/057 **M-422394-01-1**
Guidelines:	"Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration". Report of the FOCUS Work Group on Degradation Kinetics. EC Document Reference Sanco/10058/2005 version 2.0, 2006
GLP	No (calculation)

Executive Summary

The degradation and dissipation behaviour of [phenyl-UL-^{14}C] fenhexamid (KBR 2738) in an aquatic environment was investigated by kinetic evaluation of two aerobic water-sediment systems ([redacted] and [redacted] [redacted], at 20-21 °C in the dark; [redacted] (1997)).

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006) and considered modelling and persistence endpoints.

It included an analysis of the kinetics of fenhexamid considering the total system (Level PI, degradation) and the single phases separately (Level PI, dissipation), which yielded the results shown in Table 7.8.3-25 for modelling endpoints and Table 7.8.3-26 for persistence endpoints.

Table 7.8.3-25: SFO degradation and dissipation parameters for modelling endpoints of [phenyl-UL-^{14}C] fenhexamid

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	k-rate (1/days)	DT50 (days)	Model	k-rate (1/days)	DT50 (days)
PI / deg.	**Total system**	SFO	0.047	14.71	SFO	0.102	6.77
PI / diss.	**Water**	DFOP	0.150	4.61)^{a}	DFOP	0.176	3.93)^{a}
PI / diss.	**Sediment**	HS	0.023)^{b}	29.88	SFO	0.066	10.58

)^{a} calculated by DT90$_{DFOP}$/3.32
)^{b} k-rate of slow phase

Table 7.8.3-26: Degradation and dissipation parameters for persistence endpoints of [phenyl-UL-^{14}C] fenhexamid

Evaluation level / type of parameter	Phase	--- [redacted] ---			--- [redacted] ---		
		Model	DT50 (days)	DT90 (days)	Model	DT50 (days)	DT90 (days)
PI / deg.	**Total system**	DFOP	13.91	57.73	SFO	6.77	22.48
PI / diss.	**Water**	DFOP	1.62	15.32	DFOP	2.28	13.06
PI / diss.	**Sediment**	FOMC	14.98	116.37	SFO	10.58	35.16

I. Material and Methods

The kinetic evaluation was based on data of a water-sediment study (IIA 7.8.3/01) conducted with [phenyl-UL -^{14}C]-labeled KBR 2738 in a loam sediment ([redacted]) and a silty clay loam sediment ([redacted]) and their associated water at 20°C in the dark for a maximum of 100 days.

Measured and reported mean of two replicates were taken into account singularly.

Four kinetic models, the simple first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the hockey-stick model (HS), and the bi-exponential model (DFOP, double first order in parallel) were considered in this study.

A Level I evaluation was performed for the total system (degradation), and the single phases (dissipation, using decline fit from the maximum occurrence onwards). In contrast to degradation, the dissipation parameters in water-sediment studies include not only degradation but also transfer into the other phase (water or sediment).

Degradation parameters are useful for exposure models which explicitly consider the transfer of compound between different phases. Dissipation parameters can be used for exposure models which consider water and sediment phase separately.

The choice of the appropriate kinetic model is based on visual inspection of the fit and the scaled Chi^{2}-error. For modelling endpoints it is additionally checked by the t-test whether degradation or dissipation parameters are sufficiently reliable to be used for exposure modelling.

For the derivation of modelling endpoints, simple first-order (SFO) kinetics are tested first, because SFO is the simplest of the kinetic models and the one almost exclusively used in environmental exposure models. If the SFO fit is visually not acceptable or Chi²-error significantly exceeds a value of 15 %, then the bi-phasic models are tested. Finally the model is chosen which is visually acceptable and provides a significantly better fit in terms of Chi²-error. This avoids using an over-parameterised model based on a marginally better fit only.

Ideally, the Chi²-error value should be below 15 %. However, this value should only be considered as guidance and not as an absolute cut-off criterion. There will be cases where the Chi²-error is higher, but the fit still represents a reasonable description of the degradation behaviour.

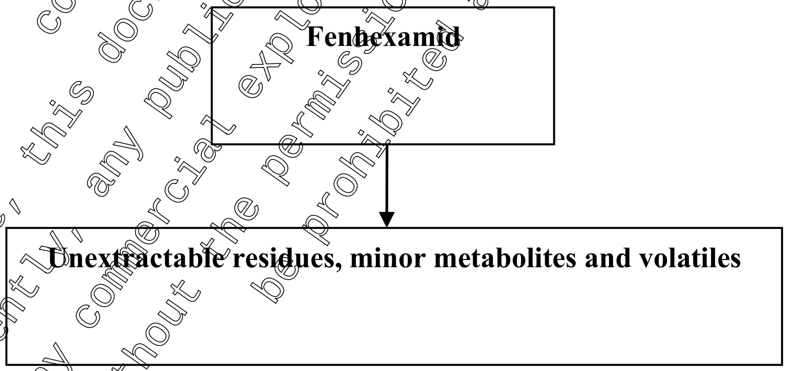
The bi-phasic models recommended are FOMC, DFOP and HS, where FOMC should not be used in cases where the final residue is more than 10 % of its initial value. For modelling purposes, an equivalent SFO half-life is calculated as $DT90_{bi\text{-}phasic}/3.32$, when the final residues are below 10% of the initial value. By this method the equivalent SFO-curve meets the bi-phasic curve at the time $DT90_{bi\text{-}phasic}$ and consequently over predicts the residue values at earlier times. When the final residues remain above 10% of the initial value, the $DT50_{SFO}$ is calculated from the slow k-rate (k2) by $DT50_{SFO} = \ln(2)/k2_{HS,DFOP}$, and the $DT90_{SFO} = \ln(10)/k2_{HS,DFOP}$.

For the derivation of persistence endpoints, the SFO model is tested against the FOMC model. If the SFO model gives an acceptable fit and the Chi²-error is smaller than that of the FOMC model, the SFO model is selected. If the FOMC model is better, the other bi-phasic models HS and DFOP are tested, and the most appropriate in terms of visual fit and Chi²-error is selected.

For pathway fits in a first step the appropriate model for the parent is identified. In a second step the pathway is implemented with the parent model as identified and the SFO model for the metabolite.

In accordance with the experimental data ([redacted], 1997) the compartment model shown in Figure 7.8.3-3 was developed. Following this scheme the parent fenhexamid degrades to unextractable residues, minor metabolites, and volatiles.

Figure 7.8.3-3: Compartment model for the total water-sediment system kinetic evaluation of [phenyl-UL-^{14}C]KBR 2738 (Level I)



For Level I evaluations of dissipation, the decline of a compound in either phase is fitted to measured data from their maximum onwards, M*(t*), using a simple differential equation as the first one of the system above. The time axis is shifted by the time tmax where the maximum occurs, t* = t - tmax, and the residue values are chosen accordingly M*(t*) = M (t - tmax), t ≥ tmax. This procedure is called in the following decline- or dissipation fit.

II. Results and Discussion

A. Model selection

1. Total System

For the parent the SFO fits are visually and statistically well acceptable for the [redacted] system. The last data point for [redacted] is slightly underestimated. However, according to FOCUS (2006) this is acceptable when the residuals decreased below 90% of the applied mass until this date. This is the case already at day 60. The k-rate is significant (with t-test <0.001) and the chi²-errors are well below 15%. Thus the SFO model was selected for the modelling endpoint.

For the persistence endpoint additionally the FOMC model is tested. The visual fit and chi²-error values are slightly better for the FOMC than for the SFO model. Hence, additionally the HS and DFOP model are tested. Both yield acceptable visual fits (for HS the last data point is underestimated similarly to the SFO model). The chi²-error value is also similar, though slightly better for the DFOP model than for the others. Therefore, the DFOP model is used to estimate the persistence endpoint.

For [redacted] the SFO model gives excellent results. The SFO fit is very good, with a chi²-error value of only 6.3 and a highly reliable k-rate. The SFO model is selected for modelling endpoints.

For persistence endpoints additionally the FOMC model is calculated. The fit is not better than the SFO model (chi²-error = 6.7). Hence, also for persistence endpoints the SFO model is selected.

Table 7.8.3-27: Scaled Chi²-error , visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of fenhexamid for different kinetic models applied to the total system values. Values in bold indicate the kinetic model chosen for modelling endpoints, in italics for persistence endpoints.

	--- [redacted] ---			--- [redacted] ---		
	chi²-error (%)	VA)[a]	t-prob)[b]	chi²-error (%)	VA)[a]	t-prob)[b]
SFO	**6.3**	**+**	**<0.001**	***6.3***	**+**	***<0.001***
FOMC	6.2	+	0.182/0.217	6.7	+	0.390/0.394
HS	7.1	+	0.006			
DFOP	*6.2*	+	*0.500*			

)[a] +/-: yes/no
)[b] for SFO t-test of k-rate; for FOMC of Alpha/Beta; for HS and DFOP of k-slow

t-prob = t-probability test

2. Water Phase

Evaluation of the dissipation from the water phase in the system [redacted] is performed with the SFO model in a first step. In the [redacted] the rapid decline within the first 6 hours followed by the slower dissipation can not be described appropriately with the SFO model (indicated also by the high chi²-error of 24.5% in Table 4). The initial mass is reduced to 77%, which is not acceptable. Fixing the initially applied mass to 100%, results in an unacceptable underestimation of the measured values from day 7 on. Hence, the SFO model is not accepted. In the next step the FOMC model is run,

which yields an unacceptable fit by underrepresenting early data points and overestimating later points. The clearly bi-phasic behaviour can be adequately described by both HS and DFOP model. The obvious breakpoint at 6 hours is met very well. Of course the fast k-rate (most probably induced by rapid sorption to the sediment) is not significant (p = 0.49 for both models) because it is based on only two time points. Since the slow phase is highly reliable, both models are acceptable. The DFOP model is chosen for modelling and persistence endpoints because it led to a slightly lower chi²-error.
In the [redacted] system the situation is similar, though the bi-phasic behaviour at the first day is not as pronounced as in the [redacted] system. Hence, the SFO and FOMC fit might be acceptable. However, the initial mass at SFO is again underestimated. To be consistent with [redacted], the HS and DFOP models are calculated and yield excellent fits. Again, the fast rate is not reliable (based on only few data points), which is deemed acceptable, since the slow rate is highly reliable and the breaking points are fitted well. The DFOP model yielded the lowest chi²-error value and is selected for both modelling and persistence endpoints.

Table 7.8.3-28: Scaled Chi²-error , visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of fenhexamid for different kinetic models applied to the water phase. Values in bold indicate the kinetic model chosen for modelling endpoints, in italics for persistence endpoints.

	--- [redacted] ----			--- [redacted] ---		
	chi²-error (%)	VA)[a]	t-prob)[b]	chi²-error (%)	VA*)[a]	t-prob)[b]
SFO	24.5	-	0.008	17.7	+	0.001
FOMC	20.3	-	0.014/0.168	17.1	+	0.123/0.215
HS	3.9	+	<0.001	6.8	+	<0.001
DFOP	***2.6***	+	***<0.001***	***2.3***	+	***<0.001***

)[a] +/-: yes/no
)[b] for SFO t-test of k-rate; for FOMC of Alpha/Beta; for HS and DFOP of k-slow

3. Sediment

In the [redacted] system with the SFO model (Figure 17) the residues at the last data point are underestimated. Since the dissipation is not larger than 90% (of the observed maximum) the SFO model is not acceptable. For the same reason only the HS (Figure 18) and DFOP models (Figure 19), but not the FOMC model, are applied in the next step to derive modelling endpoints. Both models yield a reasonable visual fit and a slightly improved chi²-error. As seen in the water phase, the fast rate is not really well identifiable, while the slow rate is sufficiently reliable for the HS model (t-prob = 0.090). The HS model is chosen for modelling endpoints due to the slightly smaller chi2-error.
For persistence endpoints additionally the FOMC model (Figure 20) is calculated, yielding an excellent fit and a larger reduction of the chi²-error. The FOMC model is chosen for persistence endpoints.
In the [redacted] system the SFO fit (Figure 21) is acceptable. The deviation at the last data point is very small and at a time point, where 90% of the initial mass already dissipated. Therefore the SFO model was accepted for modelling endpoints.
For persistence endpoints additionally the FOMC model (Figure 22) is calculated. No improvement in the visual fit or the chi²-error is observed. Hence, the SFO model is selected for calculating persistence endpoints.

Table 7.8.3-29: Scaled Chi^2-error, visual acceptability (VA: +/- yes/no) and significance of degradation rate (t-prob) of fenhexamid for different kinetic models applied to the sediment phase values. Values in bold indicate the kinetic model chosen for modelling endpoints, in italics for persistence endpoints.

	--- [redacted]----			--- [redacted]---		
	chi^2-error (%)	VA)[a]	t-prob)[b]	chi^2-error (%)	VA*)[a]	t-prob)[b]
SFO	10.9	-	0.008	***8.4***	+	***<0.001***
FOMC	*6.7*	+	*0.084/0.160*	9.8	+	0.441/0.443
HS	**8.0**	**+**	**0.090**			
DFOP	8.0	+	0.127			

)a +/-: yes/no
)b for SFO t-test of k-rate; for FOMC of Alpha/Beta; for HS and DFOP of k-slow

III. Conclusion

Once applied to water surfaces KBR 2738 is relatively fast eliminated from total water/sediment aquatic model systems (Table 7.8.3-24 for Level I).

Table 7.8.3-30: Degradation and dissipation parameters for persistence endpoints of [phenyl-UL-^{14}C] fenhexamid.

Evaluation level / type of parameter	Phase	--- [redacted]---			--- [redacted]---		
		Model	**DT50 (days)**	**DT90 (days)**	**Model**	**DT50 (days)**	**DT90 (days)**
PI / deg.	**Total system**	DFOP	13.91	57.73	SFO	6.77	22.48
PI / diss.	**Water**	DFOP	1.62	15.32	DFOP	2.28	13.06
PI / diss.	**Sediment**	FOMC	14.98	116.37	SFO	10.58	35.16